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ABSTRACTS

Abstracts of 52nd EASD Annual Meeting

OP 01 GLP-1 receptor agonists: combinations, type 1 diabetes and long-term use

1

Switching from sitagliptin to liraglutide in subjects with type 2 diabetes: analysis of composite endpoints from the LIRA-SWITCH randomised trial

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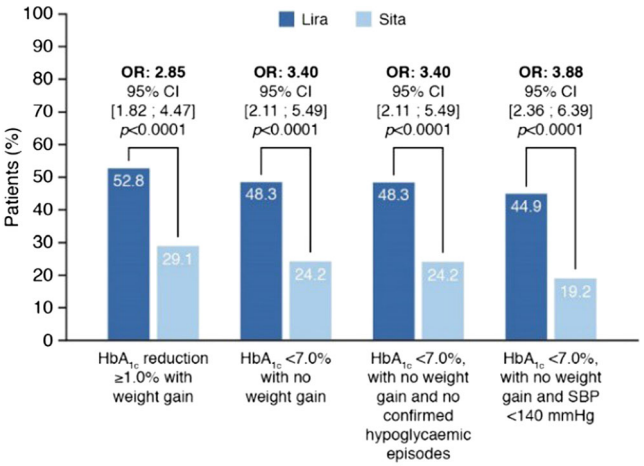
Background and aims: There is limited clinical evidence to guide treatment choices beyond the addition of another drug to achieve glycaemic target when second-line therapy is inadequate for patients with type 2 diabetes (T2D). The randomised, parallel-group, double-blind, double-dummy, active-controlled LIRA-SWITCH trial compared the efficacy and safety of switching from sitagliptin (sita) to liraglutide (lira) as add-on to metformin (MET) in subjects with T2D not achieving adequate glycaemic control with sita + MET. The aim of this analysis was to compare the proportion of subjects meeting four composite endpoints at 26 weeks, relating to glycaemia, body weight, systolic blood pressure (SBP) and hypoglycaemia outcomes.
Materials and methods: Eligible subjects (≥18 years, HbA_{1c} 7.5-9.5% [58-80 mmol/mol], BMI ≥20 kg/m^2), previously treated with stable doses of sita (100 mg/day) and MET (≥1500 mg/day or maximum tolerated dose ≥1000 mg/day) for ≥90 days, were randomised 1:1 to switch to lira 1.8 mg or continue sita 100 mg once daily, both + MET. A series of composite endpoints at week 26 were pre-defined: HbA_{1c} <7.0% (53 mmol/mol) with no weight gain; HbA_{1c} <7.0%, with no weight gain and SBP <140 mmHg; and HbA_{1c} reduction ≥1.0% with no weight gain. In addition, a *post hoc* analysis of a further endpoint was conducted: HbA_{1c} <7.0%, with no weight gain and no confirmed hypoglycaemic episodes. These dichotomous endpoints were analysed by logistic regression.
Results: In total, 407 subjects (male 60%, mean age 56 years, BMI 32 kg/m^2, HbA_{1c} 8.3% [67 mmol/mol], T2D duration 8 years) were randomised (lira: 203; sita: 204). At week 26, more subjects achieved each of the four composite endpoints by switching from sita to lira compared with continued sita. HbA_{1c} <7.0% with no weight gain: 48.3% vs. 24.2% (lira and sita, respectively), OR 3.40, 95% CI 2.11; 5.49, p<0.0001. HbA_{1c} <7.0%, with no weight gain and SBP <140 mmHg: 44.9% vs. 19.2%, OR 3.88, 95% CI 2.36; 6.39, p<0.0001. HbA_{1c} reduction ≥1.0% with no weight gain: 52.8% vs. 29.1%, OR 2.85, 95% CI 1.82; 4.47, p<0.0001. HbA_{1c} <7.0%, with no weight gain and no confirmed hypoglycaemic episodes: 48.3% vs. 24.2%, OR 3.40, 95% CI 2.11; 5.49, p<0.0001 (Figure).
Conclusion: Switching to lira resulted in more subjects achieving each of the composite endpoints analysed, compared with continued sita treatment. By switching from lira to sita, patients insufficiently controlled on sita and MET have higher probabilities of meeting clinically relevant composite endpoints relating to glycaemia, body weight, SBP and hypoglycaemia.



On-treatment analysis without rescue data. The binary endpoint was analysed using a logistic regression model with treatment, stratum and country as fixed factors and the baseline HbA_{1c} value as covariate. Missing data of the continuous parameters of the composite endpoints were imputed from an MMRM with treatment, country, stratum and baseline value, all nested within visit as a factor. Confirmed hypoglycaemic episodes were defined as episodes that were either: severe (requiring assistance of another person to administer carbohydrate, glucagon, or other resuscitative actions) or an episode biochemically confirmed by a plasma glucose value of <3.1 mmol/L, with or without symptoms consistent with hypoglycaemia. Missing data on hypoglycaemic episodes were not imputed. Lira, liraglutide; MMRM, mixed model for repeated measurement; SBP, systolic blood pressure, Sita, sitagliptin.

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2

Efficacy and safety of liraglutide added to insulin treatment in type 1 diabetes, the ADJUNCT ONE™ treat-to-target randomised trial

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Background and aims: To investigate if adjunct treatment with liraglutide, a glucagon-like peptide-1 analogue, improves glycaemic control and reduces insulin requirements and body weight in type 1 diabetes (T1D).
Materials and methods: A 52-week double-blinded multinational treat-to-target (TTT) trial in adults with T1D in suboptimal glycaemic control

(HbA1c 7-10%). Subjects (n=1398) were randomised in a 3:1 ratio to receive once-daily subcutaneous injections of liraglutide (1.8 mg, 1.2 mg or 0.6 mg) or placebo as adjunct to insulin. Endpoints, primary: change in HbA1c, fasting body weight, total insulin dose, and secondary: symptomatic hypoglycaemic episodes.

Results: At baseline, the mean age, T1D duration, HbA1c and body weight were 44 years, 21 years, 8.2% and 86.2 kg, respectively. There were 52% women, 28% on Continuous Subcutaneous Insulin (CSII) treatment, 7% had severe hypoglycaemia in the last year, 6% had hypoglycaemic unawareness and 17% had a fasting C-peptide ≥0.03 nmol/L. HbA1c was reduced 0.34-0.54% across groups at week 52. Despite the TTT design, reductions in HbA1c were significantly larger for liraglutide 1.8 mg and 1.2 mg compared with placebo (estimated treatment differences (ETD) 95% CI = 1.8 mg: -0.20% (-0.32;-0.07), 1.2 mg: -0.15% (-0.27;-0.03), 0.6 mg: -0.09% (-0.21;0.03)). Reductions in body weight were significantly larger for all liraglutide groups compared with placebo (ETD (95% CI) = 1.8 mg: -4.9 kg (-5.7;-4.2), 1.2 mg: -3.6 kg (-4.3;-2.8), 0.6 mg: -2.2 kg (-2.9;-1.5)). Reductions in total insulin dose were significantly larger for liraglutide 1.8 mg and 1.2 mg compared with placebo (estimated treatment ratios (95% CIs) = 1.8 mg: 0.92 (0.88;0.96), 1.2 mg: 0.95 (0.91;0.99), 0.6 mg: 1.00 (0.96;1.04)). Significantly more symptomatic hypoglycaemic episodes (=severe or by plasma glucose <56 mg/dL and hypoglycaemic symptoms) were seen for liraglutide 1.8 mg and 1.2 mg than for placebo (estimated rate ratio (95% CI) = 1.8 mg: 1.31 (1.07;1.59), 1.2 mg: 1.27 (1.03;1.55), 0.6 mg: 1.17 (0.97;1.43)). There were no significant differences for severe hypoglycaemic episodes (1.8 mg (45), 1.2 mg (31), 0.6 mg (40), placebo (57)). Significantly more hyperglycaemic episodes with ketosis >1.5 mmol/L were seen for 1.8 mg (77) than for placebo (37), but not for 1.2 mg (44) or 0.6 mg (54). There were 8 diabetic ketoacidosis episodes (1.8 mg (3), 1.2 mg (1), 0.6 mg (4), placebo (0)). The most frequently reported adverse events with liraglutide were nausea and vomiting.

Conclusion: Liraglutide 1.8 mg and 1.2 mg, as adjunct to insulin, led to greater reductions in HbA1c, body weight and total insulin dose compared with placebo, but the higher rates of symptomatic hypoglycaemia seem to limit the clinical utility for a broad T1D population as studied in this trial.

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Disclosure: **B. Zinman:** Employment/Consultancy; Abbott, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Merck, Novo Nordisk and Sanofi. Grants; Astra Zeneca, Boehringer Ingelheim, Novo Nordisk.

3

The differential and combined action of insulin glargine and lixisenatide on the fasting and post-prandial components of glucose control

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Background and aims: Impaired fasting and impaired postprandial glucose have different aetiologies and separate (albeit overlapping) influences on progression to diabetes. iGlarLixi is a once-daily titratable single injection of fixed-ratio combination of insulin glargine 100 U/mL (iGlar) and lixisenatide (Lixi), in development for type 2 diabetes treatment. The Phase 3, 30-week, LixiLan-O trial compared iGlarLixi, iGlar and Lixi in 1,170 patients previously uncontrolled on metformin ±1 other oral antidiabetes drug and showed that the improvement of HbA_{1c} with iGlarLixi (1.6%) was greater than the improvement with iGlar (1.3%) or Lixi (0.9%) alone.

Materials and methods: To assess the differential contributions of iGlar and Lixi to the overall action of iGlarLixi, we used 7-point glucose profiles from the LixiLan-O trial to deconstruct the improvement in glycaemic control observed on these agents into marginal improvements in: (i) fasting and postprandial plasma glucose (FPG, PPG) and (ii) fasting and postprandial glucose variability (FGV, PGV). FPG was assessed by the fasting reading of the profile; PPG was averaged across all meals for the day. FGV and PGV were assessed by the High Blood Glucose Index (HBGI) - a metric of the frequency and extent of glucose fluctuations above 6.25 mmol/L, which is particularly sensitive to hyperglycaemic excursions.

Results: As presented in Figure Panel A, iGlarLixi improved approximately equally FPG (by 3.47 mmol/L) and PPG (by 3.67 mmol/L). To a lesser degree, both components iGlar and Lixi improved PPG (2.62 mmol/L and 2.26 mmol/L). However, there was a significant 2-fold difference between FPG improvement on iGlar vs. Lixi (3.24 mmol/L vs. 1.43 mmol/L, $p<0.001$). When glucose variability (GV) was deconstructed along its FGV and PGV axis, similar effects emerged (Figure Panel B): iGlarLixi improved FGV (ΔHBGI = 6.40) and PGV (ΔHBGI = 10.0) significantly more than each of its components. Both iGlar and Lixi improved PGV (ΔHBGI = 7.7 and ΔHBGI = 6.9), but there was a significant difference between the FGV improvement on iGlar vs. Lixi (ΔHBGI = 5.7 vs. ΔHBGI = 3.5, $p<0.001$). Bivariate analyses showed that, for both average glycaemia and GV, the effect of iGlarLixi on fasting control was primarily due to its iGlar component and the relative contribution of Lixi along the fasting axis was more than twice lower, which is consistent with its mechanism of action; in contrast, the postprandial effect of iGlarLixi was due to the combined action of both iGlar and Lixi (Figure Panels A/B).

Conclusion: Glar and Lixi act selectively on FPG and PPG and GV. Clinically, their combination iGlarLixi offers more effective glucose control than the single components due to the cumulative effect of both iGlar and Lixi on PPG. The overall results are significantly improved HbA_{1c}, lower FPG compared to Lixi and improved absolute PPG concentrations compared to each compound, and reduced GV.

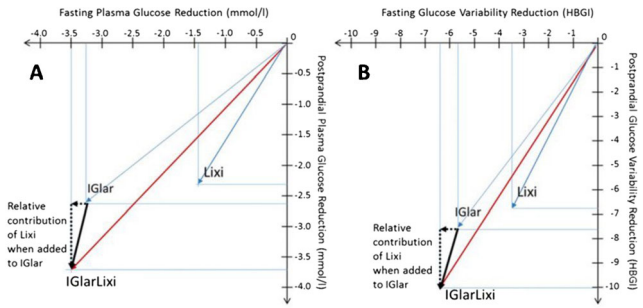

Figure: Improvement in Glucose Control (Panel A) and Glucose Variability (Panel B) on iGlar, Lixi, and iGlarLixi expressed as the vectors from the origin of the coordinate system to the centroids of each of these agent's treatment effect. The overall improvement on iGlarLixi reflects the compounded effect of iGlar and Lixi along the fasting and postprandial axis of glucose control. The black triangles represent the relative contribution of Lixi when added to iGlar, deconstructed along the fasting and prandial axis.

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4

Efficacy and safety of LixiLan, a fixed-ratio combination of insulin glargine plus lixisenatide in type 2 diabetes not adequately controlled on basal insulin: LixiLan-L trial

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Background and aims: LixiLan (iGlarLixi), a fixed-ratio combination of insulin glargine 100U (iGlar) and the glucagon-like peptide-1 receptor agonist lixisenatide (Lixi), is currently in development for the management of type 2 diabetes. This open-label trial compared the efficacy and safety of iGlarLixi with iGlar over 30 weeks.
Materials and methods: Patients were inadequately controlled on basal insulin, alone or with up to two oral antidiabetic drugs. In a 6-week run-in phase, iGlar was introduced and/or optimized. Patients whose HbA1c remained >7% (n=736), despite self-monitored plasma glucose ≤7.8 mmol/L after run-in, were then randomized to iGlarLixi or iGlar.
Results: HbA1c decreased from 8.5% to 8.1% during the run-in phase. At Week 30, the iGlarLixi group showed a statistically superior reduction from baseline HbA1c compared with iGlar (-1.1% versus -0.6%; p<0.0001), reaching an HbA1c level of 6.9% for iGlarLixi compared with 7.5% for iGlar. In total, 55% of iGlarLixi patients reached HbA1c <7% compared with 30% of iGlar patients. Mean body weight decreased by 0.7 kg in the iGlarLixi group and increased by 0.7 kg in the iGlar group (difference 1.4 kg; p<0.0001). The rate of documented (≤3.9 and ≤3.3 mmol/L) symptomatic hypoglycaemia was comparable between groups. Both treatments were well tolerated (Table).
Conclusion: iGlarLixi showed superior glycaemic control to iGlar, with a beneficial effect on body weight, no additional risk of hypoglycaemia, and a low rate of nausea and vomiting in patients with long-standing type 2 diabetes not adequately controlled on basal insulin and up to two oral glucose-lowering drugs.

Outcome	LixiLan (iGlarLixi, once daily) (n=366)	Insulin glargine 100U (iGlar, once daily) (n=365)
HbA_{1c} (%)		
Screening (Week –8)	8.5±0.7	8.5±0.7
Baseline	8.1±0.7	8.1±0.7
Week 30	6.9±0.9	7.5±0.9
LS mean±SE change from baseline*	–1.1±0.1	–0.6±0.1
LS mean±SE difference vs iGlar*	–0.5±0.1	
p value	<0.0001	
% patients reaching HbA_{1c} <7.0% at Week 30	55	30
p value	<0.0001	
FPG (mmol/L)		
Baseline (post run-in)	7.3±1.9	7.3±2.1
Week 30 (LOCF)	6.8±2.3	6.7±2.1
LS mean±SE change from baseline†	–0.4±0.1	–0.5±0.1
LS mean±SE difference vs iGlar†	0.1±0.2	
p value	0.4951	
2-hour PPG (mmol/L)‡		
Baseline	14.9±3.8	15.0±3.7
Week 30 (LOCF)	9.9±3.9	13.4±3.8
LS mean±SE change from baseline	–4.7±0.3	–1.4±0.3
LS mean±SE difference vs iGlar	–3.3±0.3	
Body weight (kg)		
LS mean±SE change from baseline*	–0.7±0.2	0.7±0.2
LS mean±SE difference vs iGlar*	–1.4±0.2	
p value	<0.0001	
Documented symptomatic hypoglycaemia§ (plasma glucose ≤3.9 mmol/L)		
Patients with event n (%)	146 (40.0)	155 (42.5)
Number of events per patient-year	3.0	4.2
Event rate ratio (95% CI) vs iGlar*	0.8 (0.6 to 1.1)	
Documented symptomatic hypoglycaemia§ (plasma glucose ≤3.3 mmol/L)		
Patients with event n (%)	89 (24.4)	83 (22.7)
Number of events per patient-year	1.1	1.1
Gastrointestinal disorders§		
Nausea n (%)	38 (10.4)	2 (0.5)
Leading to discontinuation	4 (1.1)	0 (0.0)
Vomiting n (%)	13 (3.6)	2 (0.5)
Leading to discontinuation	0 (0.0)	0 (0.0)

Data are mean±SD unless otherwise specified for modified intent-to-treat population
*Mixed-effect model with repeated measures
†Analysis of covariance model. 2-hour plasma glucose excursion = 2-hour PPG – PG 30 minutes prior to the meal and before IMP injection (only at Week 30)
‡During a standardized meal test
§n=365 for iGlarLixi group
FPG=fasting plasma glucose; iGlar=insulin glargine 100U; iGlarLixi=insulin glargine 100U/lixisenatide fixed-ratio combination; LOCF=last observation carried forward; LS=least squares; PPG=postprandial plasma glucose

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5

Factors associated with three years of response to HbA_{1c} goal with Exenatide QW or insulin glargine: retrospective analysis of DURATION-3

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Background and aims: Long-term HbA1c control with minimal medication change is a reasonable goal for patients with type 2 diabetes (T2D). 3y data from the DURATION-3 study were retrospectively analysed to characterise patients who sustained an HbA1c response to goal (<53 mmol/mol [7%]) over 3y.
Materials and methods: DURATION-3 was a 3-y, randomised, open-label trial of exenatide once weekly (QW; 2 mg sc) vs insulin glargine (titrated to fasting glucose [FG] of 4.0-5.5 mmol/L) in T2D patients on metformin ± sulfonylurea. Patients with a sustained response to therapy were defined as achieving HbA1c <53 mmol/mol (<7%) at 26 wks and maintaining HbA1c <53 mmol/mol (<7%) for 80% of the remaining visits, including 1 of 2 visits in the last 6 mo. 32 potential model parameters were tested iteratively and included if related to sustained success (P<0.05).
Results: The ITT population of DURATION-3 consisted of 456 patients, of whom 287 (63%) completed 3y. Of the 287 completers, 175 (61%) had an HbA1c <53 mmol/mol (<7%) at 26 weeks. Of the completers who reached the HbA1c goal, 84 (48%) demonstrated sustained control for ≥80% of visits over 3y. Responders with a sustained HbA1c response had a mean age of 57.7 years, a baseline body mass index (BMI) of 32.2 kg/m2, 50% were male, 81% were White; their mean baseline and Week 26 HbA1c were 62 mmol/mol (7.8%) and 44 mmol/mol (6.2%), respectively. Patients unable to sustain response to goal had a mean age of 58.6 years, a baseline BMI of 32.3 kg/m2, 53% were male, 92% were White; their mean baseline and Week 26 HbA1c were 65 mmol/mol (8.1%) and 48 mmol/mol (6.5%), respectively. Among patients consistently achieving HbA1c goal, significantly more were treated with exenatide QW (n=53) than insulin glargine (n=31; P=0.03). Other factors related to sustained treatment success included HbA1c and FG at 26 weeks (Table). The majority of factors tested including age, weight loss, waist to hip ratio, titre of anti-exenatide antibodies and sulfonylurea use were not related to sustained response.
Conclusion: This analysis demonstrates the difficulty of maintaining HbA1c to goal over 3y, even in a clinical trial setting, and suggests that lower HbA1c and FG values after 26 weeks of treatment increases a patient's likelihood of sustaining an HbA1c <53 mmol/mol (<7%) over 3y with minimal medication changes. Use of fixed dose exenatide QW supported long-term glycaemic control to a greater extent than use of titrated insulin glargine, although sustained success could be achieved with either therapy. The derived model indicates that greater response to exenatide QW treatment after 26 weeks increases the likelihood of sustained glycaemic control over 3y.

Table. Odds ratio estimates for a sustained response to treatment

Model result	Treatment with exenatide QW vs insulin glargine	Week 26 HbA1c	Week 26 FG
Point estimate	2.584	0.139	0.693
95% CI	1.288, 5.187	0.053, 0.366	0.541, 0.888
P-value	0.0075	<0.0001	0.0037
Interpretation*	Odds of stability increase by 158.4% with exenatide QW treatment vs insulin glargine	Odds of stability decrease by 86.1% for a 11 mmol/mol (1%) increase in Week 26 HbA1c	Odds of stability decrease by 30.7% for a 1 mmol/L increase in Week 26 FG

*Interpretation given all else held constant.
CI, confidence interval; FG, fasting glucose; QW, once weekly.

Clinical Trial Registration Number: NCT00641056
Supported by: AZ
Disclosure: **M.E. Trautmann:** Employment/Consultancy; Astra Zeneca, Lilly, BMS, Amylin. Lecture/other fees; Astra Zeneca. Stock/Shareholding; Lilly.

6

DURATION-1 extension in patients with type 2 diabetes: efficacy and tolerability of exenatide once weekly over 7 years

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Background and aims: Lifelong pharmacotherapy for type 2 diabetes (T2D) is anticipated, but efficacy and safety data from >1-2 years' follow-up are uncommon. Here, we report 7-year safety and efficacy data for DURATION-1.
Materials and methods: DURATION-1 compared exenatide once weekly (QW) and twice daily in 295 patients over 30 weeks, after which all patients received exenatide QW. Safety data are reported for the intent-to-treat (ITT) population and efficacy data for the completer population.
Results: Of the 295 patients in the ITT population, 122 (41%) patients completed 7 years of treatment. Withdrawal reasons included withdrawn consent (27%), adverse event (AE; 12%), investigator decision (7%), lost to follow-up (7%), and glucose control lost (4%). Baseline mean age was 56 years and T2D duration was 7 years. Concomitant medications included metformin (84%), sulfonylurea (59%), and thiazolidinedione (24%); 2% added long-acting insulin in years 2-5, 9% in year 6, and 12% in year 7. Sixty-five patients (53%) did not initiate new glucose-lowering medication. HbA1c, fasting plasma glucose (FPG), and body weight improved from baseline at 7 years (Table); 46% had HbA1c <53 mmol/mol (7.0%) and 30% had HbA1c ≤48 mmol/mol (6.5%). Mild gastrointestinal and injection site AEs primarily occurred in the initial 30 weeks. No major hypoglycaemia event was reported; most minor hypoglycaemia events occurred with concomitant sulfonylurea therapy. Serious AEs with incidence >1% included cholecystitis, cardiovascular disorders, and joint disorders. AEs of special interest included pancreatitis (n=2 events), pancreatic cancer (n=1), and acute renal failure (n=6).
Conclusion: Exenatide QW therapy for 7 years was associated with significant, sustained reductions in HbA1c and weight, with infrequent insulin initiation and no new relevant long-term safety findings.

Parameter	Baseline, Mean ± SE	7 Years, Mean ± SE	Change from Baseline, LS mean (95% CI)
HbA1c* (mmol/mol)	66.0 ± 1.1	54.0 ± 1.1	–16.4 (–19.7, –14.2)
HbA1c* (%)	8.2 ± 0.1	7.1 ± 0.1	–1.5 (–1.8, –1.3)
FPG (mmol/L)	9.2 ± 0.2	8.2 ± 0.2	–1.3 (–1.8, –0.8)
Body weight (kg)	101.2 ± 1.6	97.1 ± 1.6	–3.9 (–5.4, –2.4)
HOMA-B (%)	51.8 ± 3.0**	63.5 ± 3.6**	+26% (+10%, 44%)†
SBP (mmHg)	128.0 ± 13.4‡	129.1 ± 15.4‡	+1.2 ± 16.7‡
Heart rate (beats per min)	73.2 ± 9.1‡	74.4 ± 10.1‡	+1.2 ± 10.1‡

CI, confidence interval; HOMA-B, homeostatic model assessment of beta cell function; FPG, fasting plasma glucose; LS, least squares; SBP, systolic blood pressure; SD, standard deviation; SE, standard error. *Determined by ANOVA. Secondary endpoints determined by ANCOVA. **Geometric mean ± SE. †Percent increase from baseline based on geometric LS mean ratio of Year 7 to baseline (95% CI). ‡Mean ± SD.

Clinical Trial Registration Number: NCT00308139
Supported by: AZ
Disclosure: **P. Öhman:** Employment/Consultancy; AstraZeneca. Stock/Shareholding; AstraZeneca.

OP 02 Insulin insights

7

Ultra-rapid BioChaperone Lispro ameliorates postprandial blood glucose control compared to insulin lispro commercial formulation in subjects with type 1 diabetes mellitus

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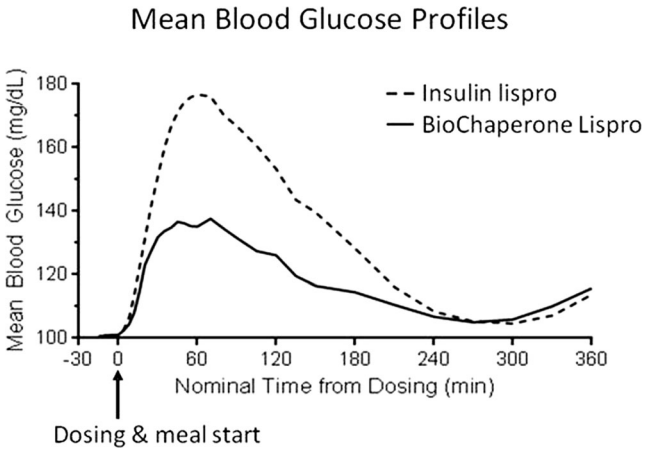
Background and aims: BioChaperone Lispro is an ultra-rapid insulin lispro formulation designed to better mimic the physiological timing of prandial insulin action than currently available prandial insulin treatments. This study is the first to compare the post prandial blood glucose control after injection of BioChaperone Lispro and insulin lispro.
Materials and methods: In a single center, double-blind, two-period, cross-over clinical trial, 38 subjects with type 1 diabetes [mean±SD duration: 22.8±9.3 yrs; age: 43.9±12.8 yrs; BMI: 24.96±1.84 kg/m^2; HbA1c: 7.38±0.87%], received a single subcutaneous dose (0.2 U/kg) of BioChaperone Lispro or insulin lispro commercial formulation at the start of a standardized liquid meal ingestion (600 kcal; 80 g carbohydrates; 25 g proteins; 20 g fat). Baseline blood glucose was controlled at 100 mg/dL and basal insulin treatment was suspended for meal test sessions.
Results: Compared to insulin lispro, BioChaperone Lispro exhibited a statistically significant higher early insulin exposure post-dosing (Least Square Mean ratio [95% confidence interval] $AUC_{0\text{-}30min}$: 2.68 [2.18; 3.30]; $AUC_{0\text{-}1h}$: 1.52 [1.37; 1.68]) and a lower late exposure ($AUC_{2\text{-}8h}$: 0.79 [0.72; 0.87]). Time to early and late 50% Cmax was reached earlier with BioChaperone Lispro ($T_{0.5\ max\ early}$: 19 vs. 30 min, ratio 0.63 [0.57; 0.71]; $T_{0.5\ max\ late}$: 142 vs. 167 min, ratio 0.85 [0.79; 0.91]) as was Tmax (49 vs. 65 min, ratio 0.75 [0.69;0.83]). Postprandial blood glucose was significantly better controlled with BioChaperone Lispro than with insulin lispro (Figure). The incremental blood glucose AUC over the two first hours was reduced by 61% ($AUC_{BG\_0\text{-}2h}$: 0.39 [0.38; 0.52]), and the mean blood glucose value 1h (BG_{1h}) and 2h (BG_{2h}) after meal start were reduced by -42 and -27 mg/dL respectively (BG_{1h}: 0.76 [0.69; 0.83]; BG_{2h}: 0.82 [0.74; 0.91]). The number of hypoglycemic events after each medication was similar and there were no safety or local tolerance issues.
Conclusion: This study confirms the ultra-rapid pharmacokinetic characteristics of BioChaperone Lispro and indicates that this novel insulin lispro formulation significantly improves postprandial blood glucose control.



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Disclosure: **G. Andersen:** Employment/Consultancy; Tim Heise: Member of advisory panels for Novo Nordisk A/S, Tim Heise's institute received research funds from Adocia, Astra Zeneca, Becton Dickinson, Biocon, Boehringer Ingelheim, Dance Pharmaceuticals, Eli Lilly, Grünenthal, Gulf Pharmaceuticals, Johnson&Johnson, Marvel, Medimmune, Medtronic, Novartis, Novo Nordisk, Roche Diagnostics, Sanofi, Senseonics, Zealand Pharma. Honorarium; Tim Heise received speaker honoraria and travel grants from Eli Lilly, Mylan and Novo Nordisk.

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BioChaperone Combo, a co-formulation of lispro and glargine, demonstrates both prandial and basal insulin time-action profiles in a single injection in type 2 diabetes

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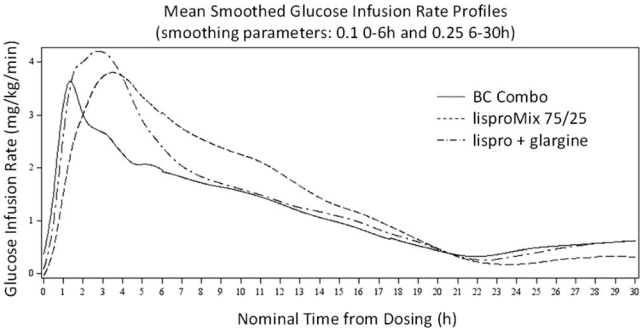
Background and aims: BC Combo is a co-formulation of 25% lispro (LIS) and 75% glargine (GLA) at neutral-pH using BioChaperone technology. BC Combo is being developed to provide rapid-acting prandial and long-acting basal insulin in a once-daily single injection. We characterized the insulin time-action profile of BC Combo over 30 hours compared to the profiles of the components injected separately or to 75% lispro protamine suspension (LMx).
Materials and methods: Twenty-four subjects with T2DM (mean age [± SD]: 57± 4 [years], BMI: 29±3 [kg/m2], HbA1c: 7.39 ±0.76 [%] received a single dose of 0.8 U/kg BC Combo, 0.8 U/kg LMx or separate simultaneous injections of 0.2 U/kg LIS and 0.6 U/kg GLA in a double-blind, randomized, double-dummy, cross-over treatment sequence. Pharmacodynamic profiles were investigated in a 30-hour (h) euglycaemic automated glucose clamp setting (ClampArt), with blood glucose stabilized at a target of 5.5 mmol/L (100 mg/dL).
Results: The insulin time-action profile of a single injection of BC Combo was comparable to the simultaneous separate injections of the components LIS and GLA. BC Combo showed a clear separation between the LIS and GLA components. The early or "prandial" component of BC Combo was comparable to LIS+GLA (mean AUCGIR 0-2h, [± SD]: 294±227 vs. 277±184 mg/kg, respectively, P=0.52 (NS)) but greater than LMx (174±126, p=0.0001). Time to peak insulin action (median GIRtmax) was faster with BC Combo (1.3 h) and LIS+GLA (2.9 h, p=0.006 vs. BC Combo) compared to LMx (3.8 h, p=0.0001 vs BC Combo), consistent with a more rapid and pronounced "prandial" glucose lowering effect. Late insulin action (mean AUCGIR 24-30h, [± SD]), extending beyond 24 hours, was evident with BC Combo (186±133 mg/kg) and LIS+GLA (174± 139, p=0.67) compared to LMx (99±102 mg/kg, p=0.01 vs. BC Combo). Total activity of BC Combo (AUCGIR 0-30h) over the testing interval (30 hours) was 81-82% of those of LIS+GLA and LMx.
Conclusion: BC Combo demonstrated both "prandial" and "basal" insulin time-action profiles comparable to the effects of LIS and GLA given separately. Compared to LMx, BC Combo showed a significantly faster and larger early (prandial) metabolic effect, as well as a stronger prolonged late (basal) metabolic effect when administered to subjects with T2DM. These results confirm and extend prior data in T1DM and support the development of BC Combo as a simple once-daily injection with the potential to improve fasting glucose and additionally control PPG, for example at the largest meal of the day.



Clinical Trial Registration Number: NCT02514850
Disclosure: **G. Meiffren:** Employment/Consultancy; Adocia.

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Double-blind mealtime faster-acting insulin aspart improves glycaemic control with superior reduction in postprandial glucose excursions vs insulin aspart in type 1 diabetes: onset® 1

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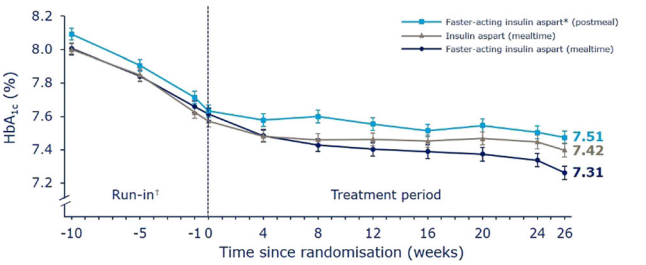
Background and aims: Limiting excursions of postprandial glucose (PPG) is desirable in people with diabetes. This multicentre, treat-to-target, phase 3 trial evaluated the efficacy and safety of faster-acting insulin aspart (faster aspart; an ultra-fast-acting mealtime insulin) vs insulin aspart (IAsp) in T1D. The primary endpoint was change from baseline in HbA_{1c} after 26 weeks of treatment.
Materials and methods: Following an 8-week run-in, adult subjects were randomised to double-blind mealtime faster aspart (n=381), or IAsp (n=380), or open-label postmeal faster aspart (n=382); each with insulin detemir. Pre-randomisation, subjects completing the run-in period underwent a standardised liquid meal test (80 g carbohydrate) to assess their 1−4 hour PPG levels; the meal test was repeated at Week 26.
Results: After 26 weeks of treatment, HbA_{1c} was reduced for faster aspart and IAsp (Figure), confirming non-inferiority to IAsp for both mealtime and postmeal dosing (estimated treatment difference [ETD], % [95% CI]: mealtime, -0.15 [-0.23; -0.07]); postmeal, 0.04 [-0.04; 0.12]); HbA_{1c} reduction was significantly greater for mealtime faster aspart vs IAsp. Superiority to IAsp for the 2 h PPG increment during a meal test was confirmed for mealtime faster aspart (ETD: -0.67 mmol/L [-1.29; -0.04]; P=0.0187). The 1 h PPG increment was also reduced (ETD: -1.18 mmol/L [−1.65; -0.71]). The body weight increase in all three treatment arms was similar, and <1 kg. There were no significant differences in the overall rate of severe or blood glucose (BG) confirmed hypoglycaemic episodes (BG <3.1 mmol/L). The overall safety profiles for faster aspart and insulin aspart were similar.
Conclusion: Faster aspart effectively improved glycaemic control with superior PPG control for mealtime faster aspart vs IAsp, and subjects randomised to dosing faster aspart postmeal for all meals maintained overall glycaemic control non-inferior to that obtained with mealtime IAsp. This may represent a clinically relevant advance in treating T1D.

Figure: Mean HbA_{1c} (%) over time.



*Faster-acting insulin aspart dosed 20 min after the start of a meal. †After initial screening, an 8-week run-in period was allowed for the optimisation of basal insulin detemir. Full analysis set; observed data. Error bars: ± standard error (mean).

Clinical Trial Registration Number: NCT01831765
Supported by: Novo Nordisk A/S
Disclosure: **D. Russell-Jones:** Employment/Consultancy; AstraZeneca, Lilly, Novo Nordisk, Sanofi. Grants; AstraZeneca, Lilly, Novo Nordisk, Sanofi. Honorarium; AstraZeneca, Lilly, Novo Nordisk, Sanofi. Lecture/other fees; AstraZeneca, Lilly, Novo Nordisk, Sanofi, Boehringer Ingelheim.

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Hypoglycaemia as a function of HbA_{1c} in type 2 diabetes: insulin glargine 300 U/ml in a patient-level meta-analysis of EDITION 1, 2 and 3

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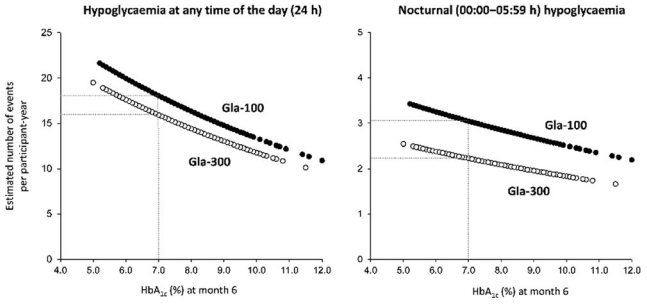
Background and aims: Basal insulin therapy can be a compromise between achieving glycaemic targets and avoiding hypoglycaemia, dependent on how intensively insulin is titrated. In the phase 3a EDITION 1, 2 and 3 studies, insulin glargine 300 U/ml (Gla-300) provided equivalent glycaemic control to insulin glargine 100 U/ml (Gla-100) with less hypoglycaemia in people with T2DM. The objective of the current analysis was to evaluate rates of confirmed (≤3.9 mmol/l) or severe hypoglycaemia over 6 months of treatment with Gla-300 or Gla-100 in these EDITION studies, as a function of HbA_{1c}.
Materials and methods: Meta-analysis was performed on patient-level data, and annualised hypoglycaemia rate as a function of HbA_{1c} at month 6 was fitted using a negative binomial regression model.
Results: Adding a treatment-by-HbA_{1c} interaction term to the model did not significantly improve the goodness of fit (interaction p-value 0.937 and 0.829 for anytime [24 h] and nocturnal [00:00-05:59 h] hypoglycaemia, respectively). Therefore the model without interaction describes the data accurately: people treated with Gla-300 experienced a consistently lower rate of confirmed (≤3.9 mmol/l) or severe hypoglycaemia vs those treated with Gla-100, regardless of HbA_{1c} at month 6 (Figure).
Conclusion: These results suggest that treatment with Gla-300 vs Gla-100 could allow people with T2DM to achieve equivalent glycaemic control with less hypoglycaemia.

Figure. Estimated annualised rates of confirmed (≤3.9 mmol/l) or severe hypoglycaemia over 6 months of treatment with Gla-300 or Gla-100 in the EDITION 1, 2 and 3 studies, as a function of HbA_{1c} at month 6
Grey dotted lines demonstrate the between-treatment difference in estimated number of events per participant-year when HbA_{1c} is 7.0 %



Clinical Trial Registration Number: NCT01499082, NCT01499095, NCT01676220
Supported by: Sanofi
Disclosure: **P. Choudhary:** Employment/Consultancy; Abbott, Cellnovo, Eli Lilly, Johnson & Johnson, Medtronic, MSD, Novo Nordisk, Roche, Sanofi. Grants; Beta-O2, Medtronic.

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Cost-effectiveness of switching to insulin degludec from other basal insulins: evidence from Swedish real-world data

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Background and aims: A health economic analysis was conducted to assess the cost-effectiveness of insulin degludec after switching from other basal insulins in people with T1D in a real life, clinical setting.
Materials and methods: A prospective, open-label, single arm, observational follow-up from August 2013—October 2015 of 476 consecutive patients with T1D at Danderyd Hospital [Stockholm, Sweden] who switched to insulin degludec from a prior basal insulin due to unacceptable HbA_{1c} levels, repeated hypoglycaemia/unstable glucose, need to administer the previous basal insulin twice daily or having difficulties with taking insulin at specific time points, among others. Information on HbA_{1c}, frequency of hypoglycaemia, and doses were collected before switching and after 4−6 mths of treatment. The IMS CORE Diabetes Model (CDM) was used to predict cost-effectiveness of life-long treatment with insulin degludec vs other basal insulins in terms of healthcare costs compared to quality-adjusted life years (QALYs). Treatment costs, costs of complications, and baseline characteristics were based on a Swedish setting.
Results: Mean (SD) age was 46.3 (16.1) years, duration of diabetes (SD) was 22.5 (14.2) years, 56% of patients were male, and duration of follow-up (SD) was 21.7 (6.0) weeks. Prior basal insulin included insulin glargine U100 (64%), insulin detemir (35%) and NPH (1%). After switching to insulin degludec, mean HbA_{1c} decreased by 2.7mmol/mol ($p<0.001$) from 68.2mmol/mol, and mean insulin doses decreased by 13.1% (from 31.3U to 26.7U; $p<0.001$) for basal insulin and by 7.5% (from 22.7U to 20.7U; $p<0.001$) for bolus insulin. The frequency of hypoglycaemia in the 4 wks prior to end of follow-up vs the 4-wk period immediately prior to switching decreased by 12% ($p=0.0127$) for non-severe daytime events and by 53% ($p<0.0001$) for non-severe nocturnal events. Severe hypoglycaemia was reduced by 62% ($p=0.0225$) during comparator periods of 4 months immediately prior to switching and prior to the end of follow-up. The CDM predicted a gain in life expectancy of 0.33 years for patients switching to insulin degludec. In addition, estimated costs of treating complications were lower for insulin degludec. Discounted direct cost represented a saving of SEK22,757 and a discounted gain in QALYs of 0.54 with insulin degludec over a lifetime. The Incremental Cost-Effectiveness Ratio (ICER) showed insulin degludec as dominant (i.e. higher effectiveness with a lower cost). Sensitivity analyses confirmed the robustness of the results.
Conclusion: In this clinical follow-up, the improvement in HbA_{1c} was accompanied by a reduction in frequency of hypoglycaemic events and lower insulin doses for patients in real-life clinical practice. The CDM estimated that switching to insulin degludec from other basal insulins translates into QALY gains and improved health-related quality-of-life as well as cost-savings for the health care system.

Table: Cost-effectiveness of switching to insulin degludec from other basal insulins: Evidence from Swedish real-world data

Cost effectiveness estimate	Insulin degludec	Previous basal insulin	Difference
Life expectancy (years)	16.58	16.44	0.14
Undiscounted Life expectancy (years)	26.16	25.83	0.33
Quality-Adjusted Life expectancy (years)	10.51	9.97	0.54
Undiscounted Quality-Adjusted Life expectancy (years)	16.19	15.27	0.92
Direct Costs, SEK	968,089	990,846	−22,757
Indirect Costs, SEK	464,591	480,986	−16,395
Combined Costs, SEK	1,432,680	1,471,832	−39,152
ICER (Direct costs/QALY), SEK			Dominant[a]
ICER (Combined costs/QALY), SEK			Dominant[a]

[a]Dominant, higher effectiveness combined with lower cost

Supported by: Novo Nordisk
Disclosure: **J. Gundgaard:** Employment/Consultancy; Employed: Novo Nordisk.

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Obese patients gain less weight than non-obese patients when treated with insulin, with similar HbA_{1c} reduction: new evidence from real-world data in type 2 diabetes

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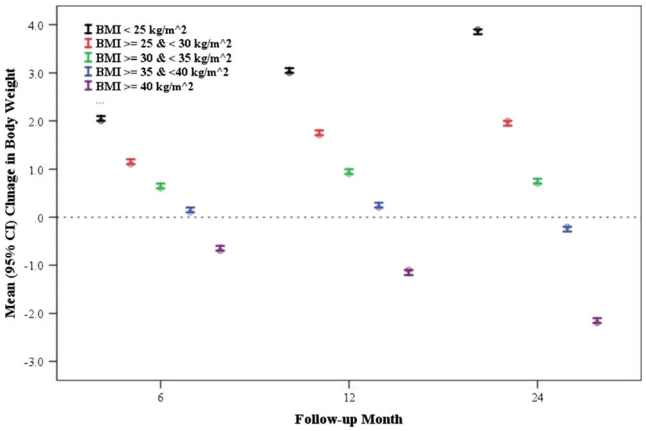
Background and aims: Barriers to insulin initiation in obese patients with type 2 diabetes (T2DM) include fear of weight gain. However, the weight gain that occurs in different adiposity classes, is not well studied. In particular, the weight gain associated with a specific reduction in HbA1c is not known. The objective was to evaluate the body weight and HbA1c changes over 2 years post insulin initiation in T2DM, in relation to BMI at the time of insulin initiation, and the possible association of glycaemic control with weight gain.
Materials and methods: From a primary / ambulatory care database from the USA, a cohort of 155,917 T2DM patients, who commenced insulin therapy and continued it for at least 6 months, was selected. Longitudinal changes in body weight and HbA1c by BMI categories from insulin initiation were estimated using robust multivariate regression models.
Results: The numbers of patients with at least 6, 12 and 24 months of insulin treatment were 155,917, 151,220 and 144,857 respectively. Patients had a mean age of 59 years, HbA1c of 9.5%, and BMI 35 kg/m2 at insulin initiation. The HbA1c levels at insulin initiation were significantly lower (9.2% to 9.4%) in the obese patients, than in patients with normal body weight (10%). However, the proportions of patients with HbA1c > 7.5% or 8% were similar across the BMI categories. Adjusted for age, sex, other anti-diabetes medications, and weighted by baseline body weight, mean changes in weight at 2 years were 3.9 / 2.0 / 0.7 / -0.2 / -2.0 kg in Normal / Over Weight/ Grade 1 Obese/ Grade 2 Obese / Grade 3 Obese groups respectively (Figure 1). The adjusted weight gain

progressively fell with increasing baseline BMI category over all 3 follow-up periods (p <0.01). The adjusted changes in HbA1c at 2 years were -1.4 / -1.3 / -1.2 / -1.2 / -1.1% in Normal / Over Weight/ Grade 1 Obese/ Grade 2 Obese / Grade 3 Obese groups respectively. A 1% decrease in HbA1c was associated with less weight gain as pre-treatment BMI rose, ranging from a 1.24 kg gain in those with BMI 40 kg/m2.

Conclusion: This study provides the first real-world evidence on three crucial aspects in insulin treated type 2 diabetes patients: (1) weight gain associated with insulin treatment is significantly and consistently lower in obese patients over 2 years post insulin initiation, compared to that observed in patients with normal body weight; (2) the glycaemic control over 2 years of treatment with insulin is similar among patients with different BMI levels; and (3) the weight gain associated with a 1% decrease in HbA1c falls progressively, as pre-treatment BMI increases. These findings provide important reassurance that, among obese patients with T2DM in routine clinical practice, meaningful improvements in glycaemic control can be achieved with only small increases in weight.



Supported by: NCRIS from Australian Government
Disclosure: **S. Paul:** None.

OP 03 Milk, olive oil or paleo: how to feed the person with type 2 diabetes

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Whey protein at breakfast, induces greater reduction of postprandial glycaemia and HbA_{1c}, weight loss and satiety, compared to other protein sources in type 2 diabetes

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Background and aims: Substantial evidence supports that a diet with high energy and protein breakfast (B) with reduced dinner is a successful strategy for reduction of overall postprandial glycemia (PPG), HbA1c and weight loss (WL) in obese and type 2 diabetes (T2D). Some sources of protein however have greater glucose lowering effects than others. Particularly whey protein exerts potent insulinotropic effect through enhancing plasma amino acids and glucagon-like peptide 1 (GLP-1) reducing postprandial glycemia in T2D. Therefore the study was undertaken to search whether high protein B containing whey protein has a greater impact on overall PPG, HbA1c, weight loss and satiety than B with different protein sources.

Materials and methods: 48 participant with T2D since 10.8 ± 2.5 years, treated with diet or diet plus metformin, aged 58.9± 4.5 yr, BMI 32.1±0.9 kg/m2 and HbA1c 7.8 ± 0.1%, were randomized to 12 weeks on one of 3 isocaloric diets, with B (660 kcal), lunch (560 kcal) and dinner (280 kcal), with the same composition at lunch and dinner but with different protein (P) composition at breakfast: 1) 42 g P namely from whey (WBd), n=17; 2) 42 g P of different sources (PBd), n=16; 3) High-carbohydrate breakfast (CBd), n=15, with 17 g P at breakfast. All patients also underwent 3 all day WBd, PBd and CBd meal tests.

Results: After 12 weeks, WL was -3.5 ± 0.3 kg (-3.8%) in CBd, -6.1 ± 0.3 kg (- 6.8%) in PBd and the greater WL was found in WBd, -7.6 ± 0.3 kg (- 8.4%) ($p<0.0001$). Compared to CBd, the % of WL in PBd was higher by 44%, and in WBd was greater by 55% ($p<0.0001$). The reduction of HbA1c was lower in CBd by -0.36 ± 0.04%, in PBd was -0.6 ± 0.04% and the greater reduction was found in WBd, 0.89 ± 0.05% ($p<0.0001$). The % of change for HbA1c was 4.6% in CBd, 7.7% in PBd and the greatest reduction in HbA1c occurred in WBd by 11.5% ($p<0.0001$). Compared to CBd the % of the reduction of HbA1c was greater by 41% in PBd, and by 64% in WBd ($p<0.0001$). Overall AUC for PPG was 95522 ± 565 mg/dl*min in CBd, 84065 ± 299 mg/dl*min in PBd and 77452 ± 292 mg/dl*min in WBd ($p<0.0001$). Compared to CBd the AUC for overall PPG was 12% lower in PBd and 19% lower in WBd ($p<0.0001$). The AUC for overall Insulin was 37% higher in PBd and 62% higher in WBd ($p<0.0001$). The AUC for overall intact GLP-1 increased more than in CBd, by 33% in PBd and by 70% in WBd ($p<0.0001$) Overall AUC for ghrelin was more suppressed in WBd by -11%, and by -5% in PBd vs CBd ($p<0.0001$). In contrast AUC for overall satiety was enhanced by 31% in PBd and more enhanced by 42% in WBd vs CBd ($p<0.0001$).

Conclusion: This study demonstrates that increasing protein content from at breakfast has a significant impact on overall postprandial gycaemia, HbA1c, weight loss and overall satiety. However, for the same protein content, whey protein vs other protein sources, yields additional benefits on reduction of overall postprandial glycaemia, HbA1c and for weight loss and enhancing satiety. Whey protein should be considered an important adjuvant in the management of type 2 diabetes.

Clinical Trial Registration Number: NCT01944449
Disclosure: **D. Jakubowicz:** None.

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A whey/guar "preload" improves postprandial glycaemia and HbA_{1c} in type 2 diabetes: a 12-week, single-blind, randomised and placebo controlled trial

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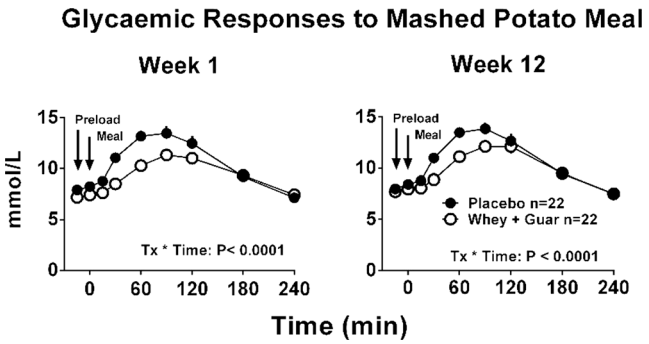
Background and aims: We have shown that whey "preloads", taken before meals for up to 4 weeks, slow gastric emptying and reduce postprandial glycaemia in type 2 diabetes (T2DM). Guar also slows carbohydrate absorption. We have evaluated the effects of 12 weeks' treatment with a whey/guar preload on gastric emptying, postprandial blood glucose, and overall glycaemic control (HbA1c), in T2DM.
Materials and methods: 47 patients with T2DM (26 male; age 64±7 years; body mass index 29.5±5.1 kg/m2; HbA1c 6.5±0.5%; 24 managed by diet alone and 23 by metformin) were randomised, in single-blind fashion, to receive 150ml flavoured shakes containing either 20g whey protein and 5g guar (90 kcal), or flavoured placebo (0 kcal), 15 min before two meals each day, for 12 weeks. No other specific dietary advice was given. During the first day (week 1) and last day (week 12) of treatment, patients attended the laboratory after an overnight fast and consumed the whey/guar or placebo preload shake 15 min before a mashed potato meal (368.5kcal: 61.4g carbohydrate, 7.4g protein and 8.9g fat) labelled with 13C-octanoic acid. Venous blood was sampled frequently for measurement of glucose, and the gastric 50% emptying time (T50) was calculated by quantifying breath 13C, over 240 min. HbA1c was measured on the first and last day of treatment. Data are mean ± SE.
Results: Gastric emptying was slower after the whey/guar preload compared with placebo at the beginning of treatment (T50: Whey/Guar 192.4 ± 9.2 min, Placebo 167.2 ± 7.2 min, $P<0.05$), although this effect was attenuated after 12 weeks (T50: Whey/Guar 177.2 ± 8.6 min, Placebo 163.7 ± 7.5min, $P=0.2414$). Postprandial blood glucose concentrations were lower after the whey/guar preload than placebo at both the beginning ($P<0.0001$) and end ($P<0.0001$) of treatment, without any attenuation of this effect at 12 weeks. During both week 1 ($r=-0.58$, $P=0.0001$) and week 12 ($r=-0.57$, $P=0.0001$) there was an inverse relationship between the blood glucose at 60min and gastric emptying (T50), so that when gastric emptying was more relatively rapid, the glycaemic response was greater. HbA1c decreased by 0.1±0.1% (1.0±0.6mmol/mol) with the whey/guar preload and increased by 0.2±0.1% (2.0±0.8mmol/mol) with placebo, with a significant difference between them ($P=0.005$). No meaningful changes in body weight were observed in either group (placebo: -0.4 ±0.3kg, whey/guar: -0.4±0.3kg).
Conclusion: In well controlled patients with type 2 diabetes, 12 weeks' treatment with a low dose whey/guar preload taken twice daily before meals reduces postprandial blood glucose, associated with slowing of gastric emptying, and leads to a modest improvement in HbA1c.



Clinical Trial Registration Number: ACTRN12614001131640
Supported by: NHMRC

Disclosure: **L.E. Mignone:** National Health and Research Council of Australia, Omniblend Innovation.

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Identification of a novel milk-protein derived bioactive for glycaemic management

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Background and aims: Milk proteins have been reported to have potential benefits for reducing risk of type 2 diabetes. However, what the active components of milk proteins are for glycaemic management has received little investigation in humans. The objectives of this study were to (1) investigate the effects of milk-derived bioactives on insulin secretion from pancreatic β-cells in vitro and in an ob/ob mouse model, and (2) ascertain the effects on postprandial glycaemia and possible underlying mechanisms in humans.
Materials and methods: Effects of a range of milk protein hydrolysates on β-cell insulin secretion from BRIN-BD11 cells, were first tested. Metabolic profiling and functional assays including intracellular calcium and plasma membrane potential (PMP) were further performed, following treatment of the cells with the most promising bioactive (identified as a casein hydrolysate). This bioactive was brought forward to be tested in an animal model and glucose tolerance tests (GTTs) were performed pre and post a 12 week intervention. The acute effect of the bioactive was subsequently tested in healthy human volunteers. Participants (n=62, mean ±SEM; age 53 ±0.9 years, BMI 31.3 ±0.6 kg/m2) were provided with a fixed breakfast containing 70g carbohydrate, along with a 10% (w/v) solution of either the casein hydrolysate or a sodium caseinate (intact control protein) in a randomised, crossover fashion. Blood samples were collected over 2 hours postprandially and analysed for a range of biomarkers. In a subsample (n=9), gastric emptying rate was assessed by paracetamol absorption test.
Results: The bioactive identified stimulated the highest insulin levels from BRIN-BD11 cells, with insulin secretion significantly increasing in the presence of 16.7mM glucose plus 1 mg/ml bioactive compared to 16.7mM glucose alone (25.65 ng/mg protein ±0.7 SEM vs 11.78 ng/mg protein ±1.2 SEM, $P<0.001$), and to a similar level as the positive control of 16.7mM glucose plus 10mM alanine (27.87 ng/mg protein ±1.1 SEM, $P=0.47$). Intracellular calcium and PMP of cells were not changed. Metabolomics analysis revealed increases in C18:1n9, C20:5n3 and MUFA, succinate and decreases in GABA and sn-glycero-3-phosphocholine ($P<0.05$ for all). In the ob/ob model, acute treatment with the bioactive improved glucose tolerance with a reduction in AUC during a GTT ($P<0.01$). In human subjects, postprandial insulin secretion was enhanced at 15min after acute ingestion of the casein hydrolysate in comparison to the intact protein (63.99 mU/L ±6.2 SEM vs 53.38mU/L ±4.8 SEM, $P<0.05$). This was mirrored by a reduction in blood glucose at the same time-point ($P<0.05$) and in postprandial delta glucose AUC ($P<0.01$). Non-esterified fatty acids were also reduced in response to the casein hydrolysate compared to the intact protein ($P<0.01$).
Conclusion: The milk protein-derived bioactive identified has a positive effect on insulin secretion from β-cells both in vitro and in humans. The bioactive promoted insulin secretion through a Ca2+-independent pathway and significantly altered the metabolic profile. Characterisation of the gastric emptying pattern will provide additional insight into potential mechanisms of action. Identification of this novel hydrolysate and underlying mechanisms provides an opportunity for future use as part of a functional food matrix for glycaemic management. Further work is underway to incorporate such a bioactive into food matrices and to examine longer term effects.
Supported by: CSC-UCD and FHI
Disclosure: **K. Horner:** None.

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Extra-virgin olive oil reduces postprandial glycaemia in type 1 diabetes by modulating GLP-1 levels and gastric emptying rate

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Background and aims: Extra-virgin olive oil (EVOO) attenuates the postprandial glycaemic response to a high glycaemic index (GI) meal compared with butter in patients with type 1 diabetes. We evaluated the possible mechanisms behind this effect.
Materials and methods: Eleven type 1 diabetes patients (6F and 5M) on insulin pump, age 41±9 y (M±SD), duration of diabetes 24±9 y, consumed in the metabolic ward in three successive weeks, according to a randomized cross-over design, three high-GI meals with identical carbohydrate composition (CHO 130 g, GI 66%) but differing for amount and quality of fat: 1) "Low-fat", total fat 10.6 g, 2) high-saturated fat ("Butter"), total fat 39.4 g, SFA 22.1 g, or 3) high-monounsaturated fat ("EVOO"), total fat 40.5 g, MUFA 27.9 g. Postprandial glycaemic response (0-6h) was evaluated by continuous glucose monitoring, gastric emptying rate (0-6h) by gastric ultrasound, and plasma active GLP-1 concentrations (0-3h) by ELISA. Pre-prandial insulin doses before the three meals (11.4±3.1 IU), based on the patients' individual insulin to glycaemic load ratio, were the same for each participant.
Results: Blood glucose 0-6h incremental AUC (mmol/Lx360 min) was significantly lower after EVOO (675±423) than after Butter (1321±603) and low-fat meals (1008±987) (p=0.034 by repeated measures analysis). Antrum gastric volume after EVOO meal ingestion was higher than after Butter meal at 1 hour (106[92,123] vs. 91[73,100] ml, median [IQ], p=0.041), while it was lower than Butter at 6 hours (42[35,50] vs. 49[38,70] ml, p=0.041), indicating a tendency to an early slower and a late faster gastric emptying with EVOO. Postprandial GLP-1 0-3h incremental AUC after EVOO was significantly higher (153[95,345]) than after Butter (76[8,199]) (pmol/Lx180 min, p=0.033).
Conclusion: This study suggests that EVOO attenuates postprandial glucose response to a high-GI meal in patients with type 1 diabetes by counteracting the rapid glucose absorption following higher-GI foods through the modulation of incretin secretion and gastric emptying rate.
Clinical Trial Registration Number: NCT02330939
Disclosure: **L. Bozzetto:** None.

17

Effects of a paleolithic diet with and without supervised exercise on liver fat and insulin sensitivity: a randomised controlled trial in individuals with type 2 diabetes

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Background and aims: Earlier studies have suggested that a Paleolithic diet has powerful metabolic effects in obesity and type 2 diabetes. Our hypothesis was that exercise training would improve the beneficial effects of a Paleolithic diet on liver fat, insulin sensitivity and insulin clearance.
Materials and methods: 26 patients with type 2 diabetes (age 60 ± 6 years) followed a Paleolithic diet for 12 weeks. The diet was based on consuming lean meat, fish, seafood, eggs, vegetables, fruits berries, and nuts. Cereals, dairy products, legumes, refined fats, refined sugars, and salt were excluded. The participants were randomized to either standard care exercise recommendations (PD) or 1-h supervised exercise sessions (aerobic exercise and resistance training) three times per week (PD-EX). Liver fat was measured with proton magnetic resonance spectroscopy. A hyperinsulinemic euglycemic clamp with continuous infusion of deuterated glucose and with 40 mU/m2/min insulin for three hours was performed at baseline and after 12 weeks.
Results: Body weight decreased by 7.1 kg (IQR −9.8, −5.6; P<0.001) in the PD group and by 7.0 kg (−9.7, −5.6; P<0.001) in the PD-EX group. Maximum oxygen uptake increased by 0.18 L/min (IQR 0.04, 0.37) in the PD-EX group and decreased by 0.06 L/min (IQR −0.13, −0.03) in the PD group (P<0.001 for the difference between groups). Liver fat decreased significantly more in the PD group (77%) versus the PD-EX group (48%; P<0.001 for difference between groups). Peripheral insulin sensitivity (rate of disappearance) increased by 57% in the PD group (P<0.05) and by 42% in the PD-EX group (P<0.01). Suppression of hepatic glucose production increased by 13% in the PD group and by 11% in the PD-EX group. Adipose insulin sensitivity (NEFA suppression during the clamp) increased in both groups by 3.4% (P<0.05 for PD; P<0.01 for PD-EX). Insulin clearance increased by 14% (P<0.05) in the PD group and by 1% in the PD-EX group.
Conclusion: A Paleolithic diet improved liver fat, peripheral insulin sensitivity, adipose tissue insulin sensitivity and insulin clearance. Exercise training reduced the effect on liver fat and insulin clearance despite an increase in cardiovascular fitness. Further analyses will reveal if the subjects in the PD group were more rigorous regarding the study diet.
Clinical Trial Registration Number: NCT01513798
Disclosure: **J. Otten:** None.

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Effect of overfeeding diets enriched with unsaturated or saturated fatty acids or sugars on de novo lipogenesis, lipolysis and liver fat in humans

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Background and aims: Lipolysis, *de novo* lipogenesis (DNL) and dietary fat (chylomicron remnants and spillover) are the sources contributing to increased liver fat content but there are no studies comparing effects of overfeeding different diets on these pathways and liver fat content in humans.
Materials and methods: To compare effects of overfeeding diets enriched with either unsaturated fat (UNSAT), saturated fat (SAT) or carbohydrates (CARB) on liver fat, hepatic DNL and insulin action on serum NEFA (antilipolysis), we recruited 38 subjects (age 48±2 years, BMI 31±1 kg/m2, weight 93±3 kg, liver fat 4.7±0.9%). Subjects were randomized to overeat 1000 extra calories/day either from UNSAT (79% mono- or polyunsaturated fatty acids, n=12), SAT (76% saturated fatty acids, n=14) or CARB (100% carbohydrate, n=12) diets for 3 weeks. Before and after overfeeding, hepatic DNL (palmitate enrichment in VLDL from ^2H_2O), NEFA during euglycemic hyperinsulinemia, and liver fat content (proton magnetic resonance spectroscopy) were measured. The fatty acid composition of VLDL-triglycerides was measured to assess the origin of fatty acids in intrahepatocellular triglycerides and compliance to the dietary regimes.
Results: The abundance of monounsaturated and polyunsaturated fatty acids in VLDL-triglycerides increased in the UNSAT group while saturated fatty acids increased significantly in the SAT and CARB groups. Body weight increased by 0.9±0.3, 1.4±0.3 and 1.4±0.5 kg in the UNSAT, SAT and CARB groups, respectively. Liver fat increased by 16% (from

4.5±1.4 to 5.5±1.4% ($p<0.02$)) in the UNSAT group, by 55% (from 4.9 ±1.8 to 7.6±2.3% ($p<0.001$)) in the SAT, and by 32% (from 4.3±1.5 to 5.7 ±1.5% ($p<0.02$)) in the CARB group. The increase was significantly greater in the SAT than the UNSAT ($p<0.02$) or CARB group ($p<0.05$). Fasting hepatic DNL increased in the CARB ($p<0.05$) but not other groups. NEFA during hyperinsulinemia were significantly ($p<0.01$) lower in the CARB group and in the UNSAT group after than before the diet ($p<0.05$) and remained unchanged in the SAT group.

Conclusion: Overfeeding a diet enriched either in mono- and polyunsaturated fat, saturated fat or carbohydrate increases liver fat, of which saturated fat seems most harmful. CARB diet increases liver fat via DNL while a SAT diet increases direct deposition of saturated fat and UNSAT unsaturated fat in the liver. NEFA availability remains relative higher during a SAT than during CARB or UNSAT diets.

Clinical Trial Registration Number: NCT02133144

Supported by: EMIF, EPoS, Sigrid Juselius,EVO, Novo Nordisk and British Heart Foundations

Disclosure: **P.K. Luukkonen:** None.

OP 04 The diabetic heart in men and mice

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A novel and practical screening tool for the detection of silent myocardial infarction in diabetes

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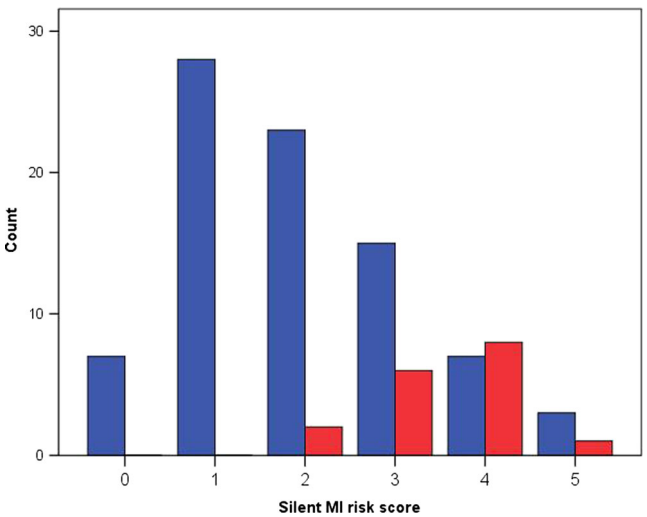
Background and aims: Silent myocardial infarction (MI) is a prevalent finding in patients with type 2 diabetes mellitus and is associated with significant mortality and morbidity. Late gadolinium enhancement (LGE) by cardiovascular magnetic resonance (CMR) is the most validated technique for detection of silent MI but is time consuming, costly and requires administration of intravenous contrast. We therefore planned to develop a population screening tool to identify those at highest risk of silent MI.

Materials and methods: 100 asymptomatic patients with type 2 diabetes underwent electrocardiogram (ECG), echocardiography, biomarker assessment and CMR at 3.0T including assessment of left ventricular ejection fraction and LGE. Global longitudinal strain (GLS) from 2 and 4 chamber cines was measured using feature tracking.

Results: 17/100 patients with no history of cardiovascular disease had silent MI defined by LGE in an infarct pattern on CMR. The current diagnostic test is the presence of Q waves on ECG. Only 4 out of 17 patients with silent MI had Q waves (sensitivity 24%, specificity 93%). Doppler echocardiography E/A ratio ≤ 0.79, GLS ≥ -18.4% and NT-proBNP > 29ng/L were all associated with silent MI. A combined risk score derived from these 4 factors and age had an area under the receiver operating characteristic (ROC) curve of 0.836 (0.749-0.902), $P<0.0001$ and had significantly better diagnostic accuracy that the presence of Q waves ($P<0.001$). A score of ≥3/5 had 88% sensitivity and 70% specificity for silent MI. The number of patients with silent MI according to their score is shown in the figure.

Conclusion: Using measures that can be derived in an outpatient clinic setting, we have developed a novel screening tool, for the detection of silent MI in type 2 diabetes. The screening tool had significantly superior diagnostic accuracy than current ECG criteria for the detection of silent MI asymptomatic patients. Figure. Number of patients with silent MI (red) and without silent MI (blue) according to their silent MI risk score.



Supported by: British Heart Foundation

Disclosure: **P.P. Swoboda:** None.

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Streptozotocin-induced diabetes mellitus increases the risk for atrial fibrillation

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Background and aims: Diabetes mellitus (DM) is an important risk factor for atrial fibrillation (AF) - the most common heart rhythm disorder. However, the mechanisms underlying this association are poorly understood. Altered cardiac metabolism, mitochondrial dysfunction, increased oxidative stress, inflammation, and fibrosis have all been described in atria of patients with DM, but their role in the pathogenesis of AF remains uncertain. An animal model would be needed to depict the relative importance of different proposed mechanisms. We tested whether streptozotocin (STZ) induced diabetes mellitus in mice recapitulate the atrial dysfunction observed in patients with DM.
Materials and methods: Multiple low-dose (50 mg/kg over 5 days) intra-peritoneal injections of streptozotocin (STZ) were used to induce DM in C57BL/6 mice. Vehicle (citrate buffer) injected mice were used as controls. After 12 weeks of DM, left ventricular (LV) systolic and diastolic function was characterized using echocardiography. Atrial electrophysiological properties and arrhythmia inducibility were assessed by ECG analysis and transoesophageal atrial pacing in anaesthetised mice. All animal procedures were conducted in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act of 1986.
Results: Mice with STZ-induced DM had significantly higher probability of AF induction compared to controls (18±3% vs. 8±2, p=0.03). AF induction was positively correlated with plasma glucose levels (R2=0.36, p=0.002). Mice in the STZ group also had longer PQ interval (45±1 ms vs. 38±1 ms in controls, p=0.001) and longer QRS interval (16.0±0.5 ms vs. 14.5±0.6 ms in controls, p=0.06), but showed no differences in the P wave duration, RR or QT interval. Furthermore, mice in the STZ group developed modest LV diastolic dysfunction (tissue Doppler E'/A' 1.02±0.05 vs. 1.20±0.06 in controls, p=0.04), but no LV systolic dysfunction (ejection fraction 83±4% vs. 83±3% in controls). Mice in the STZ group had lower LV mass (84±13 mg vs. 110±18 mg, p<0.001).
Conclusion: STZ induced diabetic mouse hearts are more susceptible towards pacing induced atrial arrhythmias. Diabetic hearts also have slowed atrial and ventricular electrical conduction. Furthermore, they have a modest LV diastolic dysfunction without any measureable LV systolic dysfunction. Characteristics of this novel mouse model of DM induced atrial remodelling are particularly suitable for studying different signalling pathways involved in the pathogenesis of AF; and can be readily used to screen for new potential therapeutic agents for diabetic cardiomyopathy.
Supported by: MRC, BHF
Disclosure: **K. Ziberna:** Grants; Medical Research Council (MRC), British Heart Foundation (BHF).

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Epicardial adipose tissue and cardiovascular disease in type 2 diabetes

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Background and aims: Epicardial adipose tissue (EAT) is the visceral fat of the heart. Observational studies indicate that patients with T2D have more and inflamed EAT compared to non-diabetic individuals, and EAT excretes biologically active substances that may provoke coronary atherosclerosis. Whether EAT promotes cardiovascular disease (CVD) in diabetic patients is currently unknown.The aim of this study is to evaluate the amount of EATand its association with the composite endpoint comprising incident CVD and all-cause mortality in patients with T2D, and to investigate the association between EAT and markers of low-grade inflammation and coronary artery calcium score (CAC).
Materials and methods: The study was a prospective study including 200 T2D patients without known coronary artery disease (CAD). EAT was measured from baseline echocardiography as the echo-free space at the free wall of the right ventricle in the parasternal long axis view averaged from two cardiac cycles. Baseline EAT was available for 194 patients. Coronary artery calcium score (CAC) and markers of low-grade inflammation (IL-1β, IL-6, IL-8, TNFα and hsCRP) were measured at baseline. Follow-up was performed after 6.1 years (5th to 95th percentile 2.9-7.1) with no lost to follow-up. Descriptive data is expressed as mean ±SD. EAT was investigated both as a continuous variable and split in quartiles (the highest vs. the three lowest). Time-to-event data were analysed by Kaplan-Meier survival plots and Cox regression models. Adjustment was performed for traditional risk factors of CVD (sex, age, LDL-cholesterol, smoking, HbA1C, and systolic blood pressure).
Results: 152 (76%) were men, with an age average of 59±9 years, and known diabetes duration of 13±7 years. Mean EAT was 3.3±1.7 mm. EAT in the highest quartile was 5.6±1.2 mm vs. 2.4±0.9 mm EAT in the three lower quartile groups. Median CAC (IQR) was 107 (3;547). During the follow-up period 66 patients had experienced a CVD event or died. In Cox regression analysis EAT was not associated with the composite endpoint (HR 1.1 (95% CI 0.9-1.2 p 0.18)). However, patients with high EAT had an increased risk of the composite endpoint when compared with patients with lower EAT (HR 1.8 (95% CI 1.0-3.2), p=0.047). After adjusting for traditional CVD risk factors, the association became insignificant (HR 1.5 (95% CI 0.8-2.7)).EAT was positively correlated to CAC (p=0.032) and TNFα (p=0.047), but not to other markers of low-grade inflammation (p≥0.31).
Conclusion: In a prospective study with 200 T2D patients without known CAD, we found that EAT correlated positively with CAC and TNFα. Elevated EAT was associated with our composite endpoint of CVD and all-cause mortality. However, the result attenuated after adjustment for traditional CVD risk factors. Our novel data suggest further research exploring the role of EAT in T2D and related cardiovascular complications.
Disclosure: **R.H. Christensen:** None.

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Platelets from poorly controlled type 2 diabetes subjects show an impaired ability to protect against the cardiac ischaemia/reperfusion injury

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Background and aims: In Type 2 Diabetes (T2D) platelet hyperreactivity is partially responsible of switch of the coagulation system towards a prothrombotic state. Ischemia and reperfusion (I/R) are implicated in a variety of clinical conditions including thrombolytic therapy for myocardial infarction. Reperfusion of previously ischemic tissue, while essential for the prevention of irreversible tissue injury, elicits inflammatory responses with increased production of reactive oxygen species (ROS) and soluble mediators leading to an impaired organ function. Aim of the study was to investigate the direct role of platelets to influence infarct size in a rat model of I/R after infusion of human platelets from T2D or healthy subjects (HS).
Materials and methods: Hearts from male Wistar rats were perfused in Langendorff mode with an oxygenated Krebs solution at constant flow (9-10 ml/min/g). After 20-min stabilization, hearts were divided in three

groups: a control group received 20-min of oxygenated buffer perfusion, a second and a third group received 20-min oxygenated buffer containing 30x10^6 /ml platelets from healthy subjects (n=6, 4M/2F, age: 49±3 years) and T2D patients (n=5, 4M/1F, age: 55±3 years, Hba1c: 14±1%), respectively. At the end of platelet-perfusion hearts underwent 30-min period of normo-thermic global ischemia followed by 60-min of reperfusion. Lactate dehydrogenase (LDH) release was measured by spectrophotometer analysis in buffer collected during reperfusion period. Infarct size was assessed via nitro-blu-tetrazolium staining. In platelet samples from each subject, we also evaluated: i) platelet aggregation to ADP (10 micromol/l), collagen (4 mg/L), arachidonic acid (AA) (0.5 mmol/L) (Born's method), ii) the activation of PI-3 kinase/Akt and MAPK/Erk-1/2 pathways (WB), iii) the intraplatelets ROS production, and iii) the expression of cyclooxygenase-1 (COX-1) (WB).

Results: Infarct size was 59±2% of risk area in control hearts and it was smaller in platelet-HS pretreated hearts (49±3% of risk area; $p<0.05$), but higher in platelet-T2D pretreated hearts (63±4% of risk area; $p<0.05$ vs platelet-HS). LDH release corroborated infarct size data. The perfusion pressure was not affected by the treatments. Interestingly, T2D-platelets, in comparison with HS-platelets, showed: i) higher trend to aggregate in response to AA (76±11 vs 51±3, $p<0.05$), ADP (78±14 vs 63±2, ns), and collagen (89±13 vs 65±2, ns), ii) higher fold increase on basal values of phosphorylated Akt and Erk-2 in response to collagen (2.9±0.2 vs 1.5±0.6 $p=0.07$ and 5.5±1.2 vs 15.6±3.3, $p<0.03$, respectively) and AA (2±0.3 vs 1.4±0.6, ns and 22±4.9 vs 5.4±1.5, $p<0.007$, iii) higher ROS production (Mean Fluorescence Intensity/min) stimulated by AA (73708±560 vs 35350±720, $p<0.0001$), iv) higher expression of COX-1 (arbitrary units: 26643±210 vs 13200±350, $p<0.0001$).

Conclusion: Platelets from poorly controlled T2D subjects not only lost their ability to protect against I/R injury but induced an increase of infarct size and marker of necrosis.These effects were associated with an increased tendency of platelets to aggregate in respose to the main physiological proaggregating agents, increased production of ROS and COX-1 expression. These findings suggest that, in T2D, platelet hyperreactivity could have predictor value on myocardial dysfunction after I/R injury.

Disclosure: **I. Russo:** None.

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The novel adipokine chitinase-3-like-protein 1 (CHI3L1) is up-regulated in fibrotic and calcified aortic valves of type 2 diabetic patients

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Background and aims: Type 2 diabetic patients (T2D) have an increased risk of developing cardiovascular disease, like atherosclerosis and calcific aortic valve disease (CAVD). Adipokine-induced pro-inflammatory signalling is described to be involved in the pathogenesis of atherosclerosis, while the role of adipokines in CAVD has been merely studied. Both diseases are linked to endothelial dysfunction, inflammation, followed by pro-fibrotic signalling and the accumulation of collagen. In CAVD, this results in thickened, fibrotic aortic leaflets with reduced biomechanical functionality. Therefore, the aim of this study was to characterize the expression of different adipokines, including activin A (INHBA), follistatin-like-1 (FSTL1) and CHI3L1, as well as a marker of fibrosis, collagen1A1 (COL1A1), and a marker for calcification, osteopontin (OPN), in human aortic valve tissue samples of non-diabetics (ND) and T2D in different disease stages of CAVD.

Materials and methods: Aortic valves were collected from patients with fibrotic (n=43) and calcific aortic tissue (n=40) undergoing aortic valve replacement. Morphologically unaltered aortic valves were gathered from hearts harvested at the time of cardiac transplantation (n=12).Via quantitative RT-PCR the gene expression of OPN, INHBA, FSTL1, CHI3L1 and COL1A1 was analyzed. Furthermore, we examined the regulation of the cytokines between ND (n=51) and T2D (n=44) with regard to the condition of the aortic valves, which led to the 6 different patient groups.

Results: OPN, a classical marker for osteogenic processes linked to aortic valve calcification, is significantly enhanced in fibrotic (9.95fold, $p<0.01$) and calcified aortic valves (18.27fold, $p<0.001$) compared to morphologically unaltered controls. The adipokine INHBA is not regulated between unaltered, fibrotic and calcified aortic valves. While FSTL1 is down-regulated ($p<0.001$) in calcified aortic valve tissue samples compared to fibrotic or unaltered controls, CHI3L1 is significantly up-regulated in fibrotic (2.62fold, $p<0.05$) and calcified aortic valves (2.84fold, $p<0.01$) compared to the controls. The diagnosis of type 2 diabetes had no influence on INHBA and FSTL1 mRNA expression within the three groups. In contrast, CHI3L1 mRNA expression is enhanced in aortic valve tissue of T2D compared to ND (ND 3.69 ± 0.74 vs. T2D 6.28 ± 0.83, $p<0.002$). Subdividing the groups of unaltered, fibrotic and calcified aortic valves in samples of ND and T2D revealed that CHI3L1 mRNA expression is especially enhanced in fibrotic aortic valves of T2D (2.41fold, $p<0.01$). Additionally, COL1A1 mRNA expression is significantly enhanced in fibrotic (9.66fold, $p<0.01$) and calcified aortic valves (19.65fold, $p<0.001$) compared to unaltered aortic valves. In line with the CHI3L1 mRNA expression, COL1A1 mRNA expression is especially enhanced in fibrotic valves of T2D (3.1fold, $p<0.01$).

Conclusion: The presented results indicate a significant link between enhanced mRNA expression of the adipokine CHI3L1 and the enhanced COL1A1 expression, most prominently in the fibrotic aortic valve tissue of T2D compared to ND. Thus, these presented data indicate a role of a novel adipokine in CAVD. Future studies will further clarify the effects and underlying mechanisms of CHI3L1 in the context of fibro-calcific remodelling of aortic valves and the progression of CAVD.

Clinical Trial Registration Number: ID2013091405

Disclosure: **J. Kistner:** None.

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Endothelial monocyte-activating polypeptide-II improves heart function and endothelium-dependent relaxation of aorta in type 1 diabetes mellitus

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Background and aims: Diabetes mellitus is accompanied by both heart and endothelium dysfunction and the development of diabetic cardiomyopathy. It acts as a risk factor for cardiovascular complications, such as coronary atherosclerosis and ischemic heart disease. We have recently shown that endothelial monocyte-activating polypeptide-II (EMAP-II) improves diastolic heart function in hypertension, decreased oxidative and nitrosative stress and increased the synthesis of NO. The aim of work was to investigate the effect of EMAP II on the heart function and endothelium-dependent relaxation of aortic smooth muscle in type -I Diabetes mellitus.

Materials and methods: Rats were divided into control and diabetic groups. Type-1 Diabetes mellitus was induced with a single intraperitoneally injection of streptozotocin (60 mg/kg). After 2 month animals with a random blood glucose level >15 mmol/l were considered to be diabetic and were included in the study. The functional cardiohemodynamic indicators registered via Pressure-Volume System.Endothelium-dependent relaxations investigate using aortic strips by add Acetylcholine (10-5 M/l) in aortic rings precontracted with noradrenaline. The recombinant endothelial monocyte activating polypeptide II - EMAP II (2,8 mkg/kg) was administered intravenously.

Results: It was shown that after EMAP II end-diastolic pressure in streptozotocin-induced diabetic rats was decreased by 25,4% ($P<0,05$), end-systolic pressure decreased by 13,8%. The end-diastolic myocardial stiffness decreased by 25,5% ($P<0,05$) in streptozotocin - induced diabetic rats. After EMAP II the arterial stiffness was decreased by 26,3%

(P<0,05), that indicates an improvement the ventriculo-arterial coupling. It has been shown that endothelium-dependent relaxation of aortic smooth muscle was decreased by 31,6% in streptozotocin - induced diabetic rats vs control group. Administration EMAP II results in improving and restoration of endothelium-dependent relaxation in streptozotocin-induced diabetic rats. Endothelium-dependent relaxation of aortic smooth muscle in streptozotocin - induced diabetic rats after EMAP II was increased by 1.7 times (average value was 85,22%±18,62%).

Conclusion: Thus, EMAP II has a positive effect on heart and vessels. EMAPII improves diastolic heart function, end-diastolic pressure decreased, end-diastolic myocardial stiffness and arterial stiffness reduced in streptozotocin - induced diabetic rats. EMAP II improves and restoration of endothelium-dependent relaxation in streptozotocin -induced diabetic rats.

Disclosure: **N. Dorofeyeva:** None.

OP 05 (Epi-)genetics of adipose tissue

25

Maternal and paternal pre-conceptional overweight results in different sex-specific transcriptome changes of blastocysts and spermatozoa sncRNAs

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Background and aims: Human epidemiological studies show that the offspring's risk to develop obesity in later life is strongly associated with maternal obesity. In a previous study we used a diet-induced obesity (DIO) model to show, that a peri-conceptional obesogenic exposure of female mice is sufficient to induce sex-specific abnormal health outcomes in offspring. In adult male offspring we found, overweight, insulin resistance, hyperleptinemia, hyperuricemia and hepatic steatosis were observed; all these changes were not present in female offspring. Instead, those showed impaired fasting glucose suggesting a prediabetic condition and a reduction in fat mass with adipocytes of smaller size than in males. In our current study we investigated if maternal or paternal pre-conceptional obesogenic exposure does already affect the transcriptome of the early embryo in utero, and if DIO changes the small RNA pattern of spermatozoa.

Materials and methods: One parent group received a high fat diet (HFD) and the other parent group obtained a control diet (CD), in a third control mating group both parents were fed with CD. At the age of 12 weeks the parents of the overweight group were mated with their counterparts of the lean parent group to generate the embryos. After mating blastocysts were collected at day 3.5 post coitum and a microarray experiment was performed. To address the underlying paternal mechanisms we investigated the alteration of the miRNAome in spermatozoa by next generation sequencing.

Results: Microarray analysis revealed different alterations of the gene expression profile for male and female blastocysts. Male embryos from an overweight parent, regardless of father or mother, had more upregulated genes than control embryos from lean parents. In contrast, 48 genes were downregulated in female blastocysts from obese fathers, whereas female blastocysts from overweight mothers showed only very few differentially expressed genes compared to female control blastocysts. The transcriptome changes induced by paternal or maternal periconceptional overweight in male and female blastocysts do not overlap. In contrast, within the same sex of blastocysts, the effects of maternal and paternal overweight on the embryonic transcriptome are partially overlapping. Next generation sequencing of small RNAs reveals several differentially expressed microRNAs and tRNA fragments in the spermatozoa of HFD fed males.

Conclusion: Our data indicate that overweight of parents during the periconceptional period may lead to sex-dependent transcriptome changes already in an early stage of development, the blastocyst. Additionally we found, that the spermatozoa of obese male mice have a different small RNA pattern, these RNAs may act as early regulators of the embryonic transcriptome. Finally our data indicate that DIO can inherited obesity by the mother and the father, but it mainly affects the male offspring.

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Disclosure: **K. Hedegger:** None.

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Family risk of type 2 diabetes is accompanied by a different epigenetic signature in pre-adipocytes from type 2 diabetic relatives

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Background and aims: Family history of Type 2 Diabetes (T2D) is accompanied by evidence of subcutaneous adipose tissue (scAT) dysfunction. Indeed, First Degree Relatives (FDRs) of T2D patients have a very high risk of developing T2D and also have considerably larger subcutaneous fat cells as a consequence of an impaired ability to recruit new adipose cells in response to calorie excess. Mechanisms driving this restricted adipogenesis are unclear but may depend on epigenetic effects. We have, therefore, explored global DNA methylation in adipocyte precursor cells from healthy FDRs and control individuals lacking a known family history of T2D.
Materials and methods: scAT Stromal Vascular Fraction cells (enriched in pre-adipocytes; SVFs) were isolated from fat biopsies obtained from 9 healthy and non-obese FDRs of T2D individuals and 12 carefully matched control subjects with no family history of T2D. Genome-wide DNA methylation profile was analysed in SVFs using methylated DNA immunoprecipitation sequencing (MeDIP-Seq). Advanced analyses were applied to identify differentially methylated regions (DMRs) between the two groups. DMRs were mapped to their corresponding genes, and tested for potential overlaps with biological pathways and gene network.
Results: The clinical characteristics of individuals with a genetic predisposition for T2D are shown in Table 1. MeDIP-Seq analysis provided non-biased DNA methylation maps covering almost the entire cell genome. 2,936 DMRs were detected with false discovery rate <5%. Most of the identified DMRs were located within gene bodies. The majority of the significant DMR (~90%) showed decreased DNA methylation in SVF from FDRs, suggesting that genetic predisposition for T2D is linked to global hypomethylation in scAT precursor cells. Moreover, DMRs mapped to 1,329 unique genes [differentially methylated genes (DMGs); false discovery rate <5%]. Of these, 88 genes had increased and 1,241 had decreased DNA methylation levels in SVF of FDRs. Intriguingly, most hypomethylated genes belonged to key adipocyte commitment and differentiation pathways such as Protein Kinase A (adjusted P = 0.003), Fibroblast growth factors (FGFs; adjusted P = 0.006), and the Wnt signaling pathways (adjusted P = 0.000005). DMGs also included genes with known function in adipocyte biology, such as TLE3 and FASN.
Conclusion: In FDRs, the epigenetic signature of subcutaneous fat precursor cells is characterized by global hypomethylation and differential methylation of genes linked to adipocyte commitment and metabolic functions, including genes of the Wnt family. Alterations of the methylation state of this gene network, and its inappropriate activation, may represent a mechanism responsible for the impaired adipose cell recruitment accompanying T2D predisposition in FDRs.

Measure	T2D FDR	Ctrl
Age, years	42.3 ± 8.7	39.4 ± 7.8
BMI, Kg/m^2	25.4 ± 1.5	24.5 ± 2.2
Fat percent, %	26.9 ± 7.3	24.3 ± 6.3
Cell size, µm	100.2 ± 5.2***	89.6 ± 6.0
GIR/bw, mg/min	7.9 ± 1.7**	11.3 ± 2.5
f-insulin, µU/mL	60.1 ± 22.7**	34.0 ± 13.3
fb-glucose, mmol/L	4.8 ± 0.4*	4.4 ± 0.4
OGTT p-glucose 2h, mmol/L	6.6 ± 1.7*	4.9 ± 1.2

Table 1. Comparison of lean individuals with or without a family history of T2D. Values are mean ± SD. Significances (by t-test) were only calculated between groups with heredity or not for type 2 diabetes.*p<0.05, **p<0.01 and ***p<0.001.

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Disclosure: **L. Parrillo:** Grants; LP is the recipient of the EFSD/Lilly Research Fellowship 2015.

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Epigenetic changes at PPARγ and Zfp423 promoters influence adipogenic commitment of NIH-3T3 cells

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Background and aims: Hypertrophic obesity is typically associated with insulin resistance and risk of developing type 2 diabetes. It is due to the inability to recruit and differentiate available precursor cells in the subcutaneous adipose tissue; a process requiring inactivation of the canonical WNT signaling pathway and activation of bone morphogenetic protein 4 (BMP4) to initiate their commitment. Emerging evidence suggests that the zinc-finger protein Zfp423 (key mediator of the BMP4 pathway) controls preadipocyte determination directly by regulating transcriptional activation of the peroxisome proliferator-activated receptor gamma (Pparγ) gene. Elucidation of molecular mechanisms regulating the expression of Zfp423 and its downstream target Pparγ could provide new insight into the reasons for the restricted adipogenesis in hypertrophic obesity. Genetic studies performed so far have not identified genetic variants of Zfp423 and Pparγ genes associated with hypertrophic obesity. This work aims at investigating the contribution of epigenetic mechanisms in the transcriptional regulation of these genes.
Materials and methods: 3T3-L1 and NIH-3T3 cells were used as fibroblasts committed and uncommitted to the adipocyte lineage, respectively. mRNA levels were measured by Real-time PCR, while methylation levels were analyzed by bisulfite sequencing. Chromatin structure was analyzed by Micrococcal nuclease protection assay, and DNA-methyltransferase were chemically inhibited by 5-azacytidine (AZA). Adipocyte differentiation rate was also evaluated by Oil Red O staining.
Results: 3T3-L1 cells show a significant increase of Zfp423 and Pparγ mRNA levels compared to NIH-3T3 cells (p<0.001). The DNA methylation at Zfp423 and Pparγ promoters is higher in NIH-3T3 compared to 3T3-L1 cells (NIH-3T3 vs 3T3-L1; Zfp423 % of CpG methylation: 90.2% vs 15.3%, p<0.001; Pparγ % of CpG methylation: 70% vs 30%, p<0.001). Nucleosome occupancy on both Zfp423 and Pparγ promoters is higher in NIH-3T3 compared to 3T3-L1 cells [NIH-3T3 vs 3T3-L1, Zfp423 % of nucleosome occupancy: Nucleosome (NUC) 1: 72.2% vs 51.5%, p<0.01; NUC2: 94.6% vs 46.4%, p<0.01; Pparγ % of nucleosome occupancy: NUC1: 62.0% vs 21.2%, p<0.01; NUC2: 45.1% vs 9.2%, p<0.01; NUC3: 53.1% vs 10.9%, p<0.01]. AZA treatment increases Zfp423 and Pparγ mRNA levels (Not treated vs AZA treated NIH-3T3; Zfp423 expression: 1 vs 1.87 REU, p<0.001; Pparγ expression: 1 vs 6.49 REU, p<0.001], reduces nucleosome occupancy on both Pparγ and Zfp423 promoters (Not treated vs AZA treated NIH-3T3; Zfp423 % of nucleosome occupancy: NUC1: 72.2% vs 65.1%, p<0.01; NUC2: 94.6% vs 46.9%, p<0.01; Pparγ % of nucleosome occupancy: NUC1: 62.0% vs 38.9%, p<0.01; NUC2: 45.1% vs 37.4%, p<0.01; NUC3: 53.1% vs 22.8%, p<0.01) and improves adipocyte differentiation of NIH-3T3.
Conclusion: Our results indicate that epigenetic events such as DNA methylation and chromatin remodeling influence the ability of precursor cells to differentiate into mature adipocytes by modulating Zfp423 and Pparγ genes expression. Further understanding of the molecular mechanisms regulating Zfp423 and Pparγ expression will help the identification of novel strategies aimed at preventing and treating the restricted adipogenesis typical of hypertrophic obesity.
Disclosure: **M. Longo:** None.

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Functional evaluation of the human fat distribution HOXC13 gene locus

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Background and aims: Human fat distribution shows strong associations with both insulin resistance and type 2 diabetes independent of total

fat mass. Genome-wide association (GWA) studies have identified several genetic loci associated with BMI-adjusted waist-to-hip ratio, but most of these loci have not been functionally evaluated. The *HOXC13* locus contains epigenetically regulated developmental genes, of which the HOX transcript antisense RNA (*HOTAIR*) gene maps right under the GWA peak and is also the most differentially expressed gene between abdominal and gluteofemoral fat tissue. *HOTAIR* is a long non-coding RNA under epigenetic control capable of modulating cell function by reprogramming chromatin states, but its regulation remains largely unexplored.

Materials and methods: Subjects were recruited through the Oxford Biobank/NIHR Bioresource in which participants have had a dual energy X-ray absorptiometry scan to monitor fat distribution. Paired adipose tissue biopsy samples were obtained from abdominal (ASAT) and gluteal (GSAT) subcutaneous tissue (n=204). A dense map of gene variants were genotyped across the region to assess haplotypes (n=7382). Two single nucleotide polymorphisms (SNPs) were haplotype-tagging (not in linkage disequilibrium) for minor alleles: rs76084431 and rs12427129. *HOTAIR* mRNA expression was measured by qPCR and expressed relative to suitable endogenous control transcripts. Associations for fat distribution were adjusted for BMI.

Results: *HOTAIR* expression was nearly undetectable in ASAT (0.007 [0.004; 0.019]), whereas it was higher, but variable in GSAT (0.910 [0.592; 1.429], P<0.001). Females had on average 50% lower GSAT *HOTAIR* expression than males (P<0.001). The minor allele haplotype-tagging SNPs showed significant but opposite expression quantitative trait loci (eQTLs); females heterozygous for rs76084431 had significantly lower (34%) GSAT *HOTAIR* expression compared to wild type (P=0.017), whereas heterozygous individuals for rs12427129 had 33% higher GSAT *HOTAIR* expression compared to wild type (P=0.018). The *HOTAIR* transcript-lowering variant (rs76084431) had a significant effect on body fat distribution. Females carrying the minor allele (TT+TC) showed significantly higher android-total fat ratio (beta: 0.036, P=0.013) and android-gynoid fat ratio (beta: 0.033, P=0.022) compared to wild type (CC).

Conclusion: In summary, we have identified two eQTLs with opposite directional effects on *HOTAIR* expression and a *HOTAIR* transcript-lowering variant shows strong associations with fat distribution. Current work involving bisulphite sequencing of *HOTAIR* regulatory regions to identify sites where these gene variants may interact with DNA methylation, as well as *HOTAIR* knockdown and overexpression in specific abdominal and gluteal adipocyte precursors is ongoing. Further functional studies will reveal effects on regional preadipocyte proliferation and differentiation. The eQTLs will enable epigenetic testing of regulatory regions by bisulphite sequencing and transcriptional testing to identify the genetic/epigenetic control of *HOTAIR*.

Disclosure: **F. Kuo:** None.

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Differences of SSPN methylation pattern in human subcutaneous and omental visceral adipose tissue

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Background and aims: Adipose tissue distribution plays a major within obesity risk development. Genome wide association studies (GWAS) for waist-to hip ratio (WHR) revealed numerous SNP markers including a variant near the *Sarcospan* (*SSPN*) gene locus. Nevertheless, based on the limited effect size on WHR variance and the rising evidence for epigenetic mechanisms playing an important role in obesity and related metabolic complications, we hypothesized that *SSPN* may be differentially methylated.

Materials and methods: We examined genome wide DNA promoter methylation along with changes in mRNA profiles in paired samples of human subcutaneous adipose tissue (SAT) and omental visceral adipose tissue (OVAT) from non-obese vs. obese individuals.

Results: In general, we identified several obesity-associated and adipose tissue depot-specific genes potentially playing a role in adipogenesis and differentiation, obesity, lipid metabolism and adipose tissue expandability (*ETV6, HAND2, HOXC6, PPARG, SORBS2, CD36, CLDN1*), which were successfully in silico replicated. In detail, results for *SSPN* indicate adipose tissue specific differences in non-obese subjects (P=0.017; NM_005086) as well as a trend of obesity specific differences in OVAT (P=0.08; and NM_005086). Further, we demonstrated significantly negative effects of DNA methylation (all $P \leq 0.03$, adj. for sex, age, lnBMI and T2D) and positive effects of expression (all $P<0.02$) on parameters of fat distribution such as waist and WHR in SAT among all subjects. In line with that, methylation levels of SSPN in SAT associate negatively with parameters of glucose homeostasis like fasting plasma insulin (P=0.001, ß=-2.8). Finally, an epigenome-wide association study (EWAS) for methylation and BMI (adj. sex, age) revealed the same probe (NM_005086) within the *SSPN* promoter region as top hit (P<1x10-5) in SAT.

Conclusion: In summary, our data hint at an epigenetic regulation of *SSPN* especially in human SAT which may further affect adipose tissue storage.

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Disclosure: **M. Keller:** None.

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Zinc finger 521 (ZNF521) is a key regulator of early commitment of human adipose precursor cells and adipogenesis

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Background and aims: Inability to recruit new adipose cells in the subcutaneous adipose tissue following weight gain leads to hypertrophic obesity, accumulation of fat in ectopic depots, insulin resistance and increased risk of type 2 diabetes (T2D). Furthermore, hypertrophic obesity is associated with a family history of T2D indicating that it is under genetic regulation. There is a ~10% annual turnover of the adipose cells but the early signals for recruitment and commitment of precursor cells into the adipose lineage are unclear. We have previously identified ZNF423 as a commitment factor regulated by bone morphogenetic protein (BMP)4 for its nuclear entry. In contrast, another Zinc Nuclear Factor, ZNF521, has been shown to be a suppressor of adipogenesis. We here characterized the role of ZNF521 for the early commitment and differentiation of human subcutaneous precursor cells and its relation to the development of hypertrophic obesity.

Materials and methods: Stromal cells from human subcutaneous adipose tissue were isolated with collagenase from healthy individuals with and without a family history of T2D (First Degree Relatives; FDR). ZNF521 was silenced in the precursor cells and adipocyte differentiation initiated. Gene expression were analysed with RT-PCR and protein levels with Western blots. Nuclear ZNF521 was detected with immunofluorescence.

Results: Following initiation of differentiation, ZNF521 declined rapidly but with large inter-individual differences (relative quantity in adipocytes, range 0.068 - 0.989, n=42). Ability to downregulate ZNF521 was negatively correlated with adipocyte differentiation measured as lipid accumulation and the differentiation markers peroxisome proliferator-activated receptor (PPAR)γ2, GLUT4 and free fatty acid receptor 4 (FFAR4/GPR120) (all $p<0.001$). Furthermore, ZNF521 expression in differentiated adipocytes correlated positively with both BMI and cell size of the donors ($p<0.001$ and $p=0.01$, respectively). Silencing ZNF521 induced a rapid induction of differentiation with increased early markers such as CCAAT/enhancer binding protein (C/EBP)β/δ and PPARγ2, likely mirroring commitment of the precursor cells. In addition, silencing ZNF521 induced BMP4, the early regulator of adipose precursor cell commitment, as well as activation of SMAD1/5/8. This induction of BMP4 was probably a consequence of an increase in the transcriptional co-activator with a PDZ domain (TAZ). In well-differentiated cells, ZNF521 was translocated from the nucleus and degraded.

Conclusion: ZNF521 is an early regulator of adipose precursor cell commitment and differentiation. It acts as a switch to prevent adipogenic commitment and, instead, favour the osteogenic linage. Our results show that silencing ZNF521 promotes mesenchymal precursor cells to enter adipogenic commitment following BMP4 induction. Thus, ZNF521 is an important early regulator of adipogenesis and may play a key role for the development of hypertrophic obesity and associated metabolic complications.

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Supported by: EFSD/Novo Nordisk award and Swedish Research Council

Disclosure: **B. Gustafson:** None.

OP 06 Diabetic foot screening, treatment and outcomes

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Combined retinal/neuropathy/renal screening service: an effective model for early detection of diabetic peripheral neuropathy

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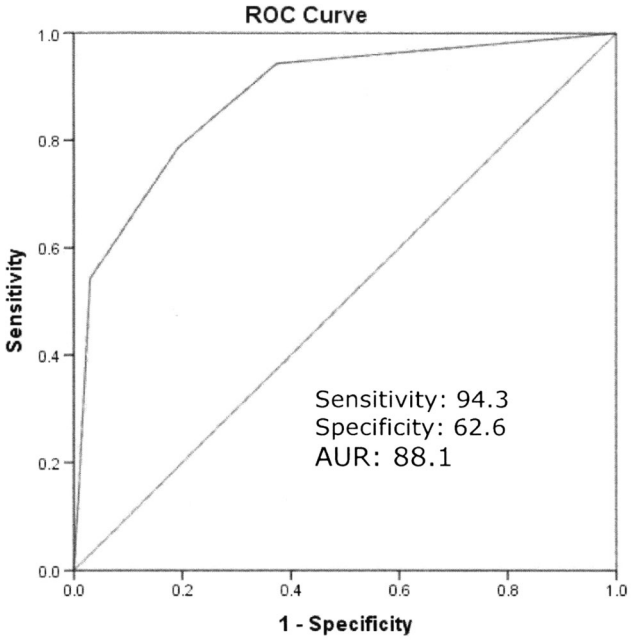
Background and aims: Diabetic foot ulceration and amputations are physically and emotionally devastating as well as being expensive. In the UK, high-uptake retinal-screening has resulted in retinopathy no longer being the commonest cause of working-age blindness. In contrast, diabetic distal symmetrical polyneuropathy (DPN) is diagnosed late using the 10g monofilament (10gMF), foot clinics are bursting with patients and amputations are increasing year-on-year. A more effective model of foot-screening is therefore required.

Materials and methods: Hospital and community-based, one-stop, combined eye/DPN/renal screening service has undergone a feasibility assessment. Patients attending retinal-screening have their feet assessed by a podiatrist whilst the instilled medriatic eye-drop is working. Assessments included: 1) gold-standard Toronto Clinical Neuropathy Score (TCNS, takes 15minutes), 2) the 10gMF and 3) two state-of-the art, validated, objective and quick measures of neuropathy: DPN-Check - a hand-held device that measures sural nerve conduction velocity and amplitude (3minutes) and SUDOSCAN that measures sudomotor function (3minutes).

Results: 180 consecutive diabetic patients, 20.5% of whom have never had their feet examined previously, have so far been evaluated. The prevalence of DPN using TCNS was 31%, massively underestimated by 10gMF (14%). The prevalence of DPN using DPN-check was 55% (91% sensitivity, 73% specificity), 40% using SUDOSCAN (79% sensitivity, 60.3% specificity) and 50.3% using abnormality in either (94% sensitivity, 63% specificity). Both devices co-related with TCNS ($p<0.001$). New diagnosis of painful-DPN was made in 12%. Participants rated the service very highly ($p<0.00$).

Conclusion: Combined eye, DPN and renal screening has high uptake, reduces clinic visits, leads to an early diagnosis of DPN, unmasks painful DPN, and is an effective model for the early diagnosis/management of DPN and foot complications.



Diagnostic performance of combined DPN-check and SUDOSCAN for TCSS-based DPN

Disclosure: **S. Tesfaye:** Honorarium; Astellas Pharma, Worwag Pharma, Miro. Lecture/other fees; Pfizer, Eli Lilly, Novo Nordisk, MSD.

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Impaired vibration perception thresholds at low frequencies are strong risk factors for diabetic foot ulcers in type 1 diabetic patients

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Background and aims: The aim was to evaluate multi-frequency vibrometry as a method to measure vibrotactile sense in the sole of the foot in subjects with type 1 diabetes and evaluate association between diabetic foot ulcers and vibration perception thresholds.
Materials and methods: Vibration perception thresholds were investigated at six frequencies (4, 8, 16, 32, 64 and 125 Hz) at two sites - first metatarsal (MTH1) and fifth metatarsal heads (MTH5) - in patients with type 1 diabetes (n=364). Thresholds for patients with a duration more than 10 years were compared with healthy, age- and gender-matched subjects (n=137). Foot ulcers were defined as at least Wagner grade 1 ulcer with a full thickness skin loss involving subcutaneous tissue. Twenty-four patients had a history of foot ulcers. There was a strong co-linearity between nearby frequencies. Therefore, we conducted a factor analysis and used the extracted factors in a logistic regression analysis to determine the risk factors for diabetic foot ulcer.
Results: Vibration perception thresholds measured at the MTH5 were significantly higher in subjects with type 1 diabetes with more than 10 years of duration compared with healthy subjects at all frequencies. Vibration perception thresholds measured at MTH1 were also significantly different, but only at 32, 64 and 125 Hz. The correlation between different frequencies was strongest between 4 and 8 Hz (correlation coefficient > 0.8) and was less when the difference between frequencies was larger (correlation coefficient < 0.5 between 4 Hz and 125 Hz). Patients with diabetic foot ulcers were older (60±13 vs. 46±16 yrs., p<0.001) and had longer duration of diabetes (36±15 vs. 22±15 yrs., p<0.0001), higher systolic (135±16 vs. 127±16 mmHg) and diastolic blood pressure (77±10 vs. 66±18 mmHg, p=0.009) than patients without history of foot ulcers. All measured vibration perception thresholds were higher in patients with than without foot ulcers. As a result of factor analysis with varimax rotation, seven different factors were extracted, "MTH1 low frequency", "MTH5 middle+MTH1 low-high frequency", "MTH5 low frequency"," duration and age", "smoking", "blood pressure" and "gender". Only factor three - "MTH5 low frequency" (4 and 8 Hz) - was associated with diabetic foot ulcers (OR 2.7 [1.6-4.7], p=0.0003).
Conclusion: Multi frequency vibrometry is an effective method for detecting neuropathy in the sole of the foot. Low frequencies, i.e. 4 and 8 Hz, measured at 5th metatarsal head, were associated with increased risk of diabetic foot ulcer, indicating that at least Merkel cell-neurite complex and Meissner's corpuscles were affected by diabetes.
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Disclosure: **E. Lindholm:** None.

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Inherited thrombotic disorders may influence the effect of autologous cell therapy of critical limb ischaemia and diabetic foot

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Background and aims: Some diabetic patients with no-option critical limb ischemia (CLI) treated by autologous cell therapy (ACT) using bone marrow-derived mononuclear cells do not show satisfactory improvement of limb ischemia after the procedure. Inherited thrombotic disorders could negatively influence the clinical outcome of ACT by microthrombosis. The insufficient effect of cell therapy may be caused by impaired cell function due to deteriorated perfusion on the cell injection site. The aim of our study was to analyze the risk factors for impaired response to ACT in patients with CLI and diabetic foot with respect to inherited thrombophilia.
Materials and methods: Seventy-two diabetic patients with CLI and diabetic foot from our foot clinic treated by ACT over 7 years were divided into responders (n = 57) and non-responders (n = 15). Non-responders were defined as an insufficient increase of transcutaneous oxygen pressure (TcPO2) by a maximum of 5 mm Hg at 3 months after ACT. Risk factors for the impaired response were assessed before cell therapy and divided into patient-related ones: e.g. age, sex, duration and treatment of diabetes, HbA1c, inherited thrombotic disorders, co-morbidities, product-related ones: number of leukocytes, lymphocytes, monocytes, platelets and CD34+ cells, and limb-related ones: e.g. severity of CLI (Rutherford classification), TEXAS classification, and infection - classified by PEDIS (up to grade 3) and WIfI (up to grade 2), presence of resistant microbes (MRSA, Pseudomonas, Klebsiella ESBL), osteomyelitis (confirmed by X-ray), and CRP.
Results: The main independent predictors for impaired response to cell therapy were heterozygote Leiden mutation (OR 10.5; 95% CI 1.72-64) and homozygote methylenetetrahydrofolate reductase (MTHFR 677) mutation (OR 3.36; 95% CI 1.0-14.3) in stepwise logistic regression. Univariate analysis revealed that lower mean value of protein C was significantly more prevalent in non-responders compared to responders (p = 0.041). No significant difference was found in the other tested factors.
Conclusion: Our study showed that the significant predictors for impaired response to autologous cell therapy in diabetic patients with CLI were inherited thrombotic disorders (Leiden mutation and MTHFR 677 mutation). These results may support future research into adequate antithrombotic therapy after cell therapy and, also, to prompt screening for inherited thrombophilia.
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Autologous mesenchymal stem cells in treatment of recalcitrant neuropathic diabetic foot ulcer: randomised controlled trial

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Background and aims: Study the effect of locally injected autologous bone marrow mesenchymal stem cells (BM MSCs) on ulcer healing in patients with resistant neuropathic diabetic foot ulcers.
Materials and methods: Twenty patients with resistant neuropathic diabetic foot ulcers were randomly assigned to conventional treatment and proper offloading modalities alone or with added MSCs injection. Aspiration of 40cc of Patients' own bone marrow under good aseptic technique. MSCs were characterized by adherence and trans differentiation. Cultured cells were subjected to microbiological and karyotyping testing. Cultured BM MSCs were injected in the edges of the wound at eight points in day 0 and day 7. Total injected cell number ranged from one million to 2 million cells. Cases were followed for 12 weeks for size of ulcer and any local reactions.
Results: In the group of MSCs ulcer size decreased by median 49.9%(9.09%,86.6%) after 6 weeks and reached median 68.24% (3.03%-100%) after 12 weeks while the conventionally treated group ulcer size reduction was median7.67% (-30%-35%) and median 5.27% (-133.33%- 25%) respectively (P value < 0.0001). Complete healing was achieved in one case in MSCs group. There were no systemic complications or local reactions to the stem cell therapy.
Conclusion: Local injection of autologous bone marrow derived mesenchymal stem cell is promising in healing of recalcitrant neuropathic diabetic foot ulcers. The procedure is safe and well tolerated by the patients. Optimum number of injected cells and frequency of injection is still to be determined.
Disclosure: **A. Albehairy:** None.

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MicroRNA-210 role for wound healing in diabetes

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Background and aims: Diabetic foot ulceration represents a major medical, social and economical problem. Specific therapeutically options are limited because the pathogenic mechanisms for delayed wound healing in diabetes are still unraveled. Even though prolonged exposure of the tissues to hyperglycemia is the primary causative factor for chronic complications of diabetes it has recently become increasingly evident that hypoxia plays an important role. Tissues' response to hypoxia is mediated by transcription factors called Hypoxia Inducible Factors (HIFs), which regulates various genes that adapt the cells to low oxygen concentration by modulating angiogenesis, cell proliferation, apoptosis, cell migration etc. In diabetes, however, the cellular response to hypoxia is impaired as a consequence of the repression of HIF-1. Reactivating the HIF signaling in diabetes is followed by improved wound healing rate despite chronic hyperglycemia. MicroRNA-210 (miR-210) is a robust target gene of HIF and mediates an important part of the hypoxic response by modulating cell cycle, mitochondria metabolism with direct effects on angiogenesis and cell survival. In this study, we aim to investigate the contribution of miR-210 for the HIF signature during wound healing aiming for a potential narrower specific therapeutic target.
Materials and methods: The effect of diabetes on miR-210 expression in skin and wounds was studied in two diabetic mouse models characterized by delayed wound healing: - db/db mice and Streptozotocin induced diabetic mice. The wound model consists of full-thickness wounds made on the dorsum of the animals. The wound area was determined every second day using a digital camera. The modulation of miR-210 by glucose and oxygen was investigated in human dermal fibroblasts (HDF) cultured in normal (5mM) and high (30mM) glucose concentrations exposed to normoxia (21% O2) or hypoxia (1% O2) for 24 hours. The expression of miR-210 was evaluated both in vitro and in vivo by qPCR The direct influence of miR-210 for wound healing in diabetes was studied in miR-210 knockout mice where diabetes was induced by Streptozotocin (50mg/kg i.p for 5 days). The wound model consists of full-thickness wounds made on the dorsum of the animals. The wound area was determined every second day using a digital camera.
Results: In concordance with the HIF repression in diabetes the expression of miR-210 in the skin of db/db mice was significantly reduced compared with the skin from the control mice ($p<0.01$, t-test; n=10). Moreover, miR-210 was induced by 1.7 folds in wounds compared with uninjured skin ($p<0.01$, t-test; n=10) but markedly reduced in the wounds of diabetic animals ($p<0.05$, n=10). The expression of miR-210 in vitro followed the HIF signaling regulation being increased (8 folds) in HDF cultured in hypoxia and normoglycemia ($p<0.01$, t-test; n=5) but repressed in the cells cultured in high glucose and hypoxia ($p<0.05$, n=5). In vivo the wound healing rate was delayed by diabetes and further modulated by the lack of miR-210 KO.
Conclusion: miR-210 is part of the HIF signature for wound healing both in diabetic and non diabetic conditions.
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Severity of foot pathology (IWGDF categories 2 and 3) shows the strongest association with mortality in diabetes

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Background and aims: Little is known on the association of foot lesions (amputations, ulcerations) and diabetic vascular complications vs. cardiovascular risk factors on mortality. Thus, we carried out a prospective 5-year study to examine the impact of established vascular complications and classical risk factors on mortality in diabetic patients.
Materials and methods: We included 244 patients attending a diabetes clinic during the preceding 5 years: of these, 53 (group A) had meanwhile died, and 191 (group B) are still alive. Cardiovascular risk factors (hypertension, triglycerides, HDLc, LDLc, fibrinogen, proteinuria, smoking), diabetes duration, macrovascular disease [coronary artery disease (CAD), cerebrovascular disease (CeVD) and peripheral arterial disease (PAD)] were assessed. Peripheral neuropathy diagnosed by the Neuropathy Disability Score (NDS), and retinopathy was diagnosed by funduscopy. Vibration perception threshold (VPT) and Neuropad time to colour change were studied as well. The International Working Group on the Diabetic Foot (IWGDF) risk categorisation was used to quantify severity of foot pathology.
Results: There were no differences between groups A and B in the following parameters: male gender [31(58.5%) vs. 94(49.2%), p=0.23], type 1 diabetes [6 (11.3%) vs. 24 (12.6%), p=0.80], HbA1c (8.9%±2.04 vs. 9.2 ±1.94%, p=0.17), triglycerides (1.9±1.51 vs. 1.93±1.7mmol/l, p=0.90),

HDLc (1.27±0.51 vs. 1.25±0.28mmol/l, p=0.80), LDLc (3.44±0.81 vs. 3.62± 0.89 mmol/l, p=0.18), smoking [7 (13.2%) vs. 36 (18.8%), p=0.34], diabetic retinopathy [34 (64.15%) vs. 105 (54.97%), p=0.29], proteinuria (385.2±609.9 vs. 443.9±1003, p=0.23), CeVD [4 (7.55%) vs. 10 (5.23%), p=0.52], CAD [5 (9.43%) vs. 16 (8.38%), p=0.81] and combined CeVD+ CHD [2 (3.77%) vs. 9 (4.71%), p=0.81]. Patients in group A exhibited significant differences in the following parameters: age at developing foot lesions (69.2±8.77 vs. 66.2±9.7years, p=0.036), DM duration (20.2 ±10.45 vs. 16.96±8.8, p=0.026), hypertension [42 (79.2%) vs. 117 (61.3%), p=0.015], fibrinogen (4.3±1.11 vs. 3.89±0.88, p=0.02), ankle reflexes (AR) score (3.42± 1.06 vs. 3.04±1.28, p=0.03), Neuropad response (13.8± 8.9 vs. 10.8± 7.2min., p=0.03), VPT (3.35±3.2 vs. 4.8 ±3.00 V, p=0.004), and IWGDF risk category (p=0.0002). However, in multivariable logistic regression analysis including risk factors significantly associated with mortality, it was only IWGDF category 2/3 that remained significantly associated with mortality (OR: 3.78, 95% CI: 1.72-8.28, p=0.001).

Conclusion: Severity of diabetic foot pathology was a stronger prognostic factor of mortality than cardiovascular risk factors. This finding underlines the importance of timely diagnosis and management.

Disclosure: **D.S. Tesic:** None.

OP 07 Novel insights into diabetic nephropathy

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Significant variation in rate of progression to end-stage renal disease between cohorts of patients with type 1 diabetes and proteinuria

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Background and aims: Renal decline, which leads to end-stage renal disease (ESRD) is clinically the most important phenotype of diabetic kidney disease. In this report we compare risks of ESRD and distributions of GFR slopes in four cohorts of patients with T1D and proteinuria, who were followed for a long period of time. We attempt to explain differences in distributions of slopes, risks of ESRD and deaths unrelated to ESRD between the cohorts using covariates associated with renal function decline and applying an analysis of competing risks between ESRD and mortality.

Materials and methods: We compare four cohorts of patients with type 1 diabetes and overt proteinuria with GFR at baseline ≥30 ml/min/1.73m^2: 368 patients from Steno Diabetes Center, Denmark, 232 recruited through INSERM, France, 432 from Joslin Clinic, USA and 486 participants of FinnDiane Study. They were followed for ESRD or death, with 2-3 annual GFR measurements. The study is a part of JDRF Diabetic Nephropathy Collaborative Research Initiative "Search for genes determining time to onset of ESRD in T1D patients with proteinuria".

Results: Age, duration of diabetes, HbA_{1c}, systolic blood pressure, baseline GFR and AER varied significantly between cohorts. The steepest slope of GFR was observed in Joslin Cohort, -5.2 ml/min/1.73m^2/year. The slope in FinnDiane and INSERM was -4.1 and -4.0, respectively and in Steno cohort it was -3.3. Adjusting for sex, age, HbA_{1c}, systolic blood pressure, antihypertensive treatment and smoking status did not materially change this pattern. The highest incidence of ESRD was observed in FinnDiane (40/1000 patient-years) and Joslin (37/1000 patient-years), while it was substantially lower in INSERM (26/1000 patient-years) and Steno cohorts (21/1000 patient-years). After adjusting for sex, age, HbA_{1c}, baseline GFR, systolic blood pressure, and smoking status Joslin cohort was characterized by the highest risk of ESRD, approximately 45% higher than in FinnDiane, while in INSERM and Steno the risk was 40% lower than in FinnDiane. Adjusting by the same covariates, the risk of mortality unrelated to ESRD was comparable in all cohorts. Competing risks of mortality and ESRD could not explain differences in cumulative incidence of ESRD between the four examined cohorts.

Conclusion: Slopes of GFR decline and risk of ESRD varied substantially between the four cohorts. These differences could not be explained by variation in patients characteristics related to the risk of kidney damage. The differences could not be attributed to competing risks between ESRD and mortality. Patient ascertainment procedures for each of the four cohorts do not seem to induce significant selection bias. This suggest that the clinical course of diabetic kidney disease varies between populations.

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Regression of albuminuria: causes and consequences in patients with type 1 diabetes

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Background and aims: Progression of diabetic nephropathy (DN) has been studied intensively, however, studies on regression are scarce. Therefore, the aim was to assess the regression of DN as well as its cardiovascular (CVD) consequences in patients with type 1 diabetes.
Materials and methods: All 3,642 patients were part of the prospective multicenter Finnish Diabetic Nephropathy (FinnDiane) study. At the baseline visit, three consecutive urine samples, as well as the prior history of albuminuria was available. Regression was defined as a change from a prior higher to a lower category of albuminuria at the baseline visit (in 2 out of 3 urine samples). According to the initial classification 2,729 had normal AER, 438 micro-, and 475 macroalbuminuria. Patients with ESRD were excluded. The main outcome measurements were incident first ever hard CVD endpoints (AMI, coronary bypass, stroke). CVD events were identified from the National Care Register for Health Care.
Results: According to the revised classification 102 (23.3%) initially microalbuminuric and 111 (23.4%) macroalbuminuric patients had regressed. In a logistic regression analysis, lower levels of total cholesterol [OR 0.69, (95%CI 0.51-0.94)], and prior onset of microalbuminuria (1.08, 1.03-1.13) were associated with regression of microalbuminuria. Factors associated with regression of macroalbuminuria were female sex (2.25, 1.15-4.40), lower systolic blood pressure (0.98, 0.96-0.99), higher eGFR (1.02, 1.01-1.03), and prior onset of macroalbuminuria (1.07, 1.02-1.12). The 15-year cumulative incidence (95%CI) and HR (95%CI) for incident CVD are shown below. The CVD risk increased with higher albuminuria category. Regression from micro- to normoalbuminuria resulted in a CVD risk no different from patients with normal AER at baseline. A similar phenomenon was seen for the regression of macroalbuminuria.
Conclusion: Progression of DN confers increased CVD risk. Notably, regression reduces the risk to the same level as for those that did not progress.

	15-year hard CVD events	Adjusted HR for incident CVD
normal AER	8.0% (6.7 - 9.3), n=163	1.00 (reference)
micro regression (normo)	13.2% (6.1 - 19.7), n=10	1.15 (0.61 - 2.19)
microalbuminuria	21.5% (17.4 - 25.4), n=58	2.28 (1.68 - 3.10)*
macro regression	28.7% (20.3 - 36.2), n=22	2.70 (1.73 - 4.24)
macroalbuminuria	40.1% (36.3 - 43.6), n=108	4.46 (3.46 - 5.77)*

HRs are adjusted for age, sex, and age at onset of diabetes. * P<0.05 vs. the preceding row.

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Disclosure: **F. Jansson:** None.

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Genome wide association study identifies novel loci associated with end stage renal disease in Chinese patients with type 2 diabetes

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Background and aims: Diabetic kidney disease is a major cause of morbidity and mortality in patients with diabetes. The genetic basis of diabetic kidney disease remains poorly understood. In this project, our overarching aim is to utilize a trans-omic approach to exmaine the genome, epigenome, and transcriptome of patients with diabetic kidney disease in order to unravel the genetic basis of this important complication.
Materials and methods: We performed a nested case-control study from the Hong Kong Diabetes Registry (HKDR), which includes more than 8,000 patients with type 2 diabetes (T2D) and prospective follow-up for development of complications and treatment failure. eGFR was calculated according to the Chinese Modification of Diet in Renal Disease (MDRD) equation. End stage renal disease (ESRD) was defined as renal transplantation, dialysis or peritoneal dialysis, or follow-up eGFR<15ml/min/1.73m2. Samples were genotyped using the Illumina Omni 2.5+ exome array and genotype data was imputed using minimac 3 (1000G phase 3v5). After standard quality control procedures pre- and post-imputation, 8.02million SNPs (MAF ≥ 0.01) were included in the final analysis. Association analysis was performed in EPACTS using the Firth Bias-corrected logistic likelihood ratio test, adjusted for age, gender, and principal components.
Results: After sample QC, we included 882 case subjects with T2D+ ESRD, and 2231 control subjects with T2D duration of >10 years but no diabetic kidney disease (DKD, eGFR<60ml/kg/m2) or cardiovascular disease (CVD) in the genome-wide association analysis. We identified 41 SNPs with suggestive association with end stage renal disease in Type 2 diabetes (p<10-5), with one of the strongest signal from a genotyped SNP on chromosome 14, OR 0.54 (95% CI 0.43-0.70, p=5.30x 10-7), with other top suggestive association signals from loci on chromosomes 1, 2, 3, 10, 16, 17, 18.
Conclusion: Our study has identified a number of novel regions associated with end stage renal disease in T2D. Additional genotyping and integrative analysis together with methylation data are currently in progress.
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Disclosure: **R.C.W. Ma:** None.

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Heritability of renal function decline measured by the rate of eGFR loss in type 1 diabetes

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Background and aims: End-stage renal disease (ESRD) is one of the most devastating complications of type 1 diabetes (T1D) and the cumulative incidence of ESRD has been reported to be 7.8% after 30 years of diabetes. Only a few genetic variants have been associated with the risk of ESRD in patients with T1D. The onset of ESRD can be predicted by the rate of renal function decline modeled by serial estimates of the glomerular filtration rate (eGFR), a measure of the intensity of the disease process leading to ESRD. Here we investigated the heritability of the rate of renal function decline measured by serial eGFR data utilizing genome wide single nucleotide polymorphism (SNP) data.
Materials and methods: We studied 1,703 patients with T1D recruited from the Finnish Diabetic Nephropathy (FinnDiane) study that were genotyped with the CoreExome BeadChip (Illumina). Patients were required to have insulin treatment initiated within 1 year from diagnosis of T1D, age at onset of T1D below 40, and a minimum follow-up time of 3 years of eGFR measurements. Patients with all eGFR measurements above 90 mL/min/1.73m^2 were excluded. The first eGFR measurement below 90 mL/min/1.73m^2 was set as baseline. The eGFR was calculated using the CKD-EPI formula using serum and plasma creatinine values retrieved from medical records. ESRD was considered present if the patient was on dialysis, had had a kidney transplantation, or an eGFR equal to 10 ml/min/1.73m^2 or less was observed. The rate of renal function

decline was estimated by fitting a line to the time series measurements of eGFR using linear regression. The slope of the fitted line was used to estimate the rate of renal function decline. The narrow sense heritability of the slope was estimated using the GCTA (Genome-wide Complex Trait Analysis) software.

Results: A total of 450 patients developed ESRD. The median number of eGFR determinations per patient was 15 (Q_1=6, Q_3=31) and the median follow-up time was 9.6 (Q_1=6.1, Q_3=13.1) years. The observed median decline of renal function was -1.15 (Q_1=-3.61, Q_3=-0.04) mL/min/1.73m^2/year. The fraction of the overall variability of the eGFR slope explained by additive genetic effects was h^2=0.42 (SE=0.16, P=0.003). When the heritability estimate was adjusted for sex, age and duration of T1D at first eGFR measurement, the heritability estimate was h^2=0.36 (SE=0.16, P=0.01).

Conclusion: Additive genetic effects are estimated to account for 36% of the variation in the rate of renal function decline. This suggests that there are still multiple genetic variants associated with the risk of ESRD that remain to be found.

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Disclosure: **E. Valo:** None.

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Identifying novel phenotype profiles and their genetic differences in patients with type 1 diabetes using machine learning approaches

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Background and aims: Many diabetic complications correlate with multiple biological markers and each other, and this interplay might limit traditional analyses studying one trait at a time. The aim was to create novel phenotype classes in patients with type 1 diabetes (T1D) considering multiple traits simultaneously by using a multi-layer self-organizing map (SOM), to evaluate their profile differences, and to test the presence of genetic effects.

Materials and methods: The study included 4,409 patients with T1D from the Finnish Diabetic Nephropathy (FinnDiane) Study. A total of 1,600 patients were used to train the SOMs, and rest as test data. We used 79 different cross-sectional measures of clinical variables or complications from two different time points to train 1,000 dual-layer SOMs. The optimal SOM mapping was selected, and its trained node prototypes were clustered using agglomerative hierarchical clustering. Multiple nested group assignments were defined by cutting the dendrogram from different resolution levels. The differences in the profiles between groups were evaluated by generalized linear regression, where the group assignments and the time point were used as explanatory variables (including their interaction). Finally, narrow-sense heritabilities between each pair of the created groups were evaluated (GCTA), with data from genome-wide genotyping (Illumina Human CoreExome BeadChip). Pairs of groups showing significant heritabilities were used as case-control phenotypes in genome-wide association study (GWAS) setting.

Results: The created groups demonstrated significant profile differences for most variables used to train the SOM, especially when the number of groups (k) was increased. In particular, modeling identified a group characterized by fast progression of phenotypes related to diabetic nephropathy (DN), occurring between the two time points. The most significant heritability between two of the groups was detected with k=4 (h^2=42%, $p=1.8\times10^{-4}$). The progression of DN, all-cause mortality and occurrence of incident CVD events were significantly higher in one of these groups compared to the other. The GWAS analyses between these two patient groups showed a single-nucleotide polymorphism (SNP) on chromosome 21 in the last intron of the NRIP1 gene (rs202095311, $p=3.81\times10^{-8}$) genome-wide significantly associated with the group assignment. In addition, the GWAS analyses highlighted multiple other SNPs with suggestive p-values ($p<1\times10^{-5}$) around regions with genes and pathways previously linked to diabetic complications, their risk factors, and/or related pathways and processes.

Conclusion: Multilayer SOM can capture the progressive nature of DN, and can create novel phenotypic profiles showing different progression rates of complications and divergent genetic background.

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Multiple novel regions for diabetic nephropathy revealed by linkage analysis with dense genetic marker set

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Background and aims: The diabetic nephropathy (DN) segregates in families with a sibling recurrence risk ratio of 2.3. Altogether, genetic factors are thought to explain approximately one third of the DN risk in patients with type 1 diabetes (T1D). Large case-control-based genome-wide association studies (GWAS) in recent years have yielded only few susceptibly loci that reach genome-wide suggestive P-values, and much of the predicted genetic risk of DN remains unexplained. These population-based studies excel in finding variants that occur commonly in the population that only slightly affect the disease risk. In contrast, genetic linkage studies offer superior statistical power in search of linkage peaks that contain medium-to-high-effect rare familial variants. We performed genetic linkage study for Finnish families with T1D, aiming to find novel variants and regions that segregate with the disease.

Materials and methods: Altogether 6,255 participants were recruited from the Finnish Diabetic Nephropathy Study (FinnDiane) cohort and genotyped with CoreExome BeadChip (Illumina), containing > 300,000 SNPs. We built pedigrees for these patients based on their pairwise genetic distances (Identity by descent, IBD) using PRIMUS software. A total of 339 pedigrees were formed of 729 patients. They consisted mostly of sibling pairs and -groups, parent- offspring pairs and nuclear families. Patients with micro/macroalbuminuria/ESRD were set as cases and patients with normal AER set as controls. All patients had prolonged duration of T1D (more than 10 years on cases and more than 15 years on controls). The linkage analysis was performed with the PSEUDOMARKER software.

Results: The linkage analysis resulted in 105 markers with genome-wide significant linkage values of LOD > 3.3. Of these markers, 86 loci localized on chromosome 6, several of them reaching LOD > 4. The two highest linkage values (LOD 5.4) were located at chromosome 6p22.2 (MHC class I) and at chromosome 6p21.3 (MHC class II). In order to confirm that the linkage peak on chromosome 6p did not originate due to the T1D HLA risk component, we performed another study, in which only patients with the common high T1D risk HLA DRB1*0401-DQA1*0301-DQB1*0302 and DRB1*0301-DQA1*0501-DQB1*0201 haplotypes were set as cases and controls. The highest LOD scores on chromosome 6p remained above 5 also after the stratification. Outside chromosome 6, there were linkage peaks on chromosomes 1, 3, 4, 11, 12, 17, 18 and 19 with genome-wide significant LOD scores. Based on their function, expression in kidneys and associated diseases, several plausible DN genes were located under, or close to these peaks. For example, the ARHGAP24 gene under the linkage peak at chromosome 4q22.1 (highest LOD 3.36) has been associated with Familial Idiopathic Steroid-Resistant Nephrotic Syndrome with Focal Segmental Hyalinosis (a rare familial kidney disease) but not with DN, whereas another peak at 1q43 with

LOD score 3.32 is localized between two genes, NID1 and GPR137B, that are both suggested to be related to kidney development and function.
Conclusion: We found several genome-wide significant linkage signals throughout genome. Several of these signals originated from regions that contain plausible genes involved in processes associated with DN or kidney function in general.
Supported by: Folkhälsan Research Center, Wilhelm and Else Stockmann Foundation, JDRF
Disclosure: **J. Haukka:** None.

OP 08 Can we make new beta cells?

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Differentiation of functional human insulin-producing stem cell derived beta cells from iPS cells

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Background and aims: Drug discovery in the islet and beta cell field is in need of an "unlimited" source of human, gene editable and functional islet cells to improve through-put and translability. We aimed to establish a differentiation protocol to allow induced pluripotent stem cells (iPS) to differentiate to mature beta-cells in vitro in an industrial setting suitable for use in screening for novel beta-cell drug targets.
Materials and methods: Two genetically different iPS cell lines were differentiated in spinner flasks applying a six weeks differentiation protocol developed at Harvard University. Quality controls were performed at each stage of differentiation. Sc-beta cells were characterized using flow cytometry, immunochemistry and functional assays. We also aimed to establish an sc-beta transplantation model to create a translatable in vivo disease models for testing of beta-cell regenerative and restorative drug candidates. Furthermore we used flow cytometry to sort dispersed cells after differentiation and to obtain an enriched population of sc-beta cells.
Results: We successfully produced functional sc-beta cells from iPSCs of two different genetic backgrounds. Markers for undifferentiated cells, e.g. Oct4, rapidly disappeared (from 95% to <3%) as markers of pancreatic progenitors and endocrine sc-beta cells, e.g. Pdx-1, Nkx6.1, insulin/c-peptide appeared. We obtained up to 53% Pdx positive cells, with 12% of cells being c-peptide and Nkx6.1 double positive. We then characterized sc-beta with different stimuli to induce insulin secretion. The sc-beta cells demonstrated significant responsiveness to KCl (+460%, $p<0.0001$) indicating proper production and processing of insulin. The cells also responded well to IBMX/Forskolin (+257%, $p<0.001$) indicating cAMP signalling to be intact. The glucose responsiveness was however weaker and gave only a maximal effect of 35% ($p<0.005$). After transplantation of differentiated clusters under the kidney capsule of NMRI nude mice, human c-peptide increased from 14±7 pM to 109+/- 27 pM between 2 and 10 weeks ($p<0.0005$), indicating that the cells were functional in vivo. Insulin positive cells represented ~10% of total cells in a cluster. We took advantage of the large amounts of zinc contained in pancreatic beta-cells to sort insulin positive cells with a zinc-specific probe, Zinpyr-1. We obtained an unprecedented level of enrichment in sc-beta cells, with up to 85% insulin positive cells after FACS cell sorting. This method will allow producing cell clusters with higher beta cell numbers and will represent a better cell model suitable for screening purposes or islet studies.
Conclusion: These cells represent a new opportunity for drug discovery in the islet and beta cell field,as they are of human origin, gene editable and functional. Besides opening new avenues for an improved understanding of human beta cell biology , they also provide a scalable platform for in vitro and in vivo drug discovery in diabetes.
Disclosure: **F. Chimienti:** None.

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Differentiation of human iPSCs into glucose responsive beta-like cells through programmable cell fate decisions by synthetic lineage control network

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Background and aims: The differentiation of human pluripotent stem cells into pancreatic β-like cells requires timely and well-controlled activation of critical TF that trigger cell fate decisions and guide development towards glucose responsive insulin secreting cells. While differentiation of human induced pluripotent stem cells (iPSC) into pancreatic progenitor cells can be reproducibly achieved with existing protocols further in-vitro differentiation into glucose-sensitive insulin secreting remains a difficult task.
Materials and methods: For programming of the differentiation process we designed a synthetic lineage-control network that combines vanillic acid (VA)-triggered gene switches for the transcription factors Ngn3 (OFF-ON-OFF; endocrine specification), followed by Pdx1 (ON-OFF-ON) with the concomitant induction of MafA (OFF-ON) for β-cell differentiation and maturation. This synthetic lineage-control network consists of a vanillic acid sensitive receptor (activated by medium and high VA) controlling the expression of a vanillic acid sensitive transcription factor (activated by medium VA but inhibited by high VA) that subsequently regulates the expression of Ngn3 and Pdx1shRNA. Therefore, the gene expression is turned only ON at medium vanillic acid levels. Additionally, the vanillic acid receptor signaling is directly rewired via a modified cyclic AMP signaling pathway to trigger the expression of Pdx1 and MafA at only high vanillic acid levels. Human iPSCs were first differentiated into pancreatic progenitor cells expressing Pdx1 and Nkx6.1 using established methods. Thereafter, an endocrine specification program was started with medium-VA induced expression of NGN3 for 4 days. This was followed by an induction of the β-cell differentiation and maturation program with high-VA induced expression of Pdx1 and MafA with concomitant silencing of Ngn3 for 7 days. All components of the network were transiently transfected without genomic integration.
Results: This synthetic lineage-control network was able to program human iPSCs from a 50-year-old donor into glucose-sensitive insulin-secreting beta-like cells. The glucose-stimulated insulin-release dynamics were comparable to human pancreatic islets. More than 75% of transfected cells differentiated into insulin positive cells while less than 10% were somatostatin or glucagon positive. In addition, glucose-responsive insulin secretion was preserved after longterm culture (4 weeks).
Conclusion: Such synthetic lineage-control networks that allow to program dynamic cell fate decisions at different stages of differentiation may complement induced pluripotent stem cell technology and provide new opportunities for controlled differentiation of human pluripotent stem cells into β-cells.
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Disclosure: **H. Zulewski:** None.

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CRISPR/Cas9-mediated transcriptional activation for maturation of human stem cell derived beta cells

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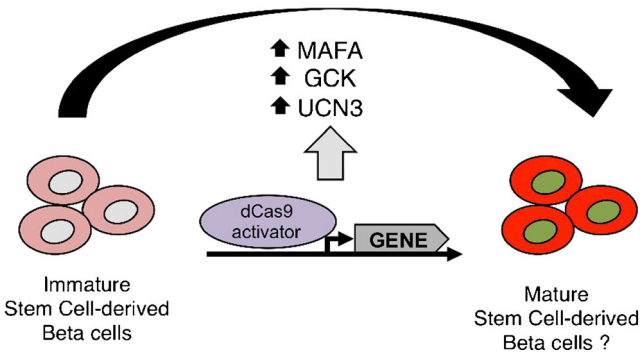
Background and aims: Human pluripotent stem cells (hPSC) can be efficiently differentiated into monohormonal pancreatic endocrine cells in islet-like aggregates. However, these cells are functionally immature. CRISPR-based systems enable genome editing and transcriptional control in hPSC. We have recently developed an inducible system to activate transcription of endogenous genes based on the use of the catalytically inactive Cas9 (dCas9) protein fused with artificial transcriptional activators. The dCas9 activator is targeted to the promoter area of the gene of interest using small guide RNA molecules (gRNAs). In order to induce the functional maturation of hPSC-derived beta cells in vitro we have generated hPSC-lines containing the CRISPR/dCas9 activator system to increase the transcription of the mature beta cell genes MAFA, GCK and UCN3.
Materials and methods: Healthy donor-derived hiPSC were differentiated to beta cells using an optimized version of previously published protocols. We generated sets of gRNAs targeting the promoters of MAFA, GCK and UCN3 and validated them. Stable transgenic hPSC lines containing inducible dCas9 activator construct with the correspondent set of gRNAs for the three target genes were generated.
Results: Differentiation of the hPSC resulted in the generation of large numbers of islet-like cell clusters that contain endocrine cells (10-20% single INS+; 15-30% single glucagon (GCG)+; 5-10% double INS+ GCG+ cells). In vitro static glucose stimulated insulin secretion (GSIS) showed that these cells are not responsive to glucose alone, but they do respond when primed with Forskolin (Stimulation index, SI=1,5). These cells also respond to closure of the K+ channels with Tolbutamide (SI=2,64), depolarization of the membrane with KCl (SI=4,66) or when other metabolic fuels, leucine + glutamine or pyruvate, are used. Expression levels of some beta cell markers like PDX1, NKX6.1 and MAFB are similar to adult human islet controls, while the levels of INS, and mature beta cell markers MAFA and GCK are significantly lower. After transplantation under the kidney capsule of immunodeficient NSG mice, the cells undergo functional maturation in vivo and start to exhibit glucose stimulated insulin secretion based on human C-peptide measurements. Induction of the dCas9-activation system in the transgenic hPSC lines leads to increase expression of MAFA, GCK or UCN3 in vitro. Characterization of the functional outcome is ongoing.
Conclusion: Our results suggest that the stem cell-derived beta cells are equipped with functional exocytotic machinery, but they are metabolically immature. The combination of CRISPR/Cas9 trancriptional activation systems with stem cell differentiation is a promising approach to dissect the importance of relevant genes in beta-cell maturation and to obtain functional beta cells in vitro.



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Disclosure: **D. Balboa:** None.

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Anterior-posterior patterning of definitive endoderm derived from human embryonic stem cells by BMP-, Wnt/beta-catenin-, and ATRA signalling

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Background and aims: The in vitro differentiation of pluripotent stem cells (PSCs) into the definitive endoderm (DE) is nowadays robustly performed. The next differentiation step requires anterior-posterior (A-P) patterning of the endoderm into the broad primitive gut tube domains foregut (SOX2+) and hindgut (CDX2+). Subsequently specific domains are patterned, which later give rise to the organ primordia. The activities of Wnt/FGFs and BMPs in the posterior half and all-trans-retinoic acid (ATRA), TGF-β-ligands, Wnt- and BMP-inhibitors in the anterior half of the endoderm sheet are thought to be responsible for A-P patterning process. However, it is currently unclear how these complex interactions can be translated into a differentiation protocol for PSCs.
Materials and methods: Two PSC lines (HES3, HUES8) were differentiated into DE-cells applying a single cell based protocol and then purified by MACS-sorting via the CXCR4 cell surface receptor to exclude residual pluripotent cells. Next, the effects of Wnt/beta-catenin-, TGF-β-, ATRA-, and FGF2-signaling were tested by various combinations of ligands and inhibitors. Differentiated cells were analyzed by RT-qPCR and immunofluorescence upon their lineage selection towards foregut or hindgut identity.
Results: The treatment of DE-cells with 5 μM CHIR-99021, 25 ng/ml BMP4, 100 nM ATRA and 100 ng/ml bFGF for 48 h resulted in a midgut/hindgut population positive for CDX2. These cells expressed a combination of HOXC5, HOXC6, and HOXB8 indicating their midgut/hindgut identity. If Wnt- and BMP4-signaling were chemically suppressed SOX2-positive cells could be detected that were double positive for FOXA2 and expressed the typical foregut marker genes. In contrast to previous reports activin A treatment did not yield in SOX2-positive foregut cells. The addition of ATRA could posteriorize the foregut population in a concentration-dependent manner towards the foregut/midgut boundary. Specifically 0.5/1/2 μM ATRA resulted in cells that highly expressed HNF6, HNF1B, and FOXA2 on the gene and protein level typical for foregut cells of the foregut/midgut boundary. The gene expression of MNX1, SHH, HOXC5, and HOXA3 was also detected whereas the anterior foregut endoderm markers TBX1 and HEX1 were suppressed. Duodenal PDX1-positive precursor cells were detected as first signs of pancreatic progeny, which then upon further differentiation, yielded in multipotent NKX6.1-positive progenitors.
Conclusion: In summary, the identity of the anterior/posterior axis of the endoderm derived from human PSCs is controlled by the interplay of BMP-, Wnt/beta-catenin-, and RA-signaling. Specifically BMP- and Wnt/beta-catenin inhibition was sufficient to induce a SOX2-positive foregut cell type whereas a strong activation of Wnt/beta-catenin-signaling promotes hindgut identity. TGF-β activation has no instructive or domain-establishing role. ATRA is able to posteriorize and dorsalize foregut cells in a concentration-dependent manner and suppresses anterior foregut identity. In particular the treatment of endoderm cells with 0.5-2 μM RA resulted in a cell population reminiscent of the PFE; a domain which gives rise to pancreas primordia during further human development.
Disclosure: **O. Naujok:** None.

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Neonatal beta cell but not acinar cell neogenesis evidenced by lineage tracing

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Background and aims: Stimulation of beta cell formation and beta cell transplantation offer hope as curative diabetes therapies. Yet, these are hindered by insufficient knowledge in the mechanisms of pancreas cell formation after birth. In adult mice, self-duplication is the main mechanism of beta cell formation. Though, the origin of different pancreas cells formed during the major pancreas expansion in the first month of life remains unclear and controversial. We wanted to uncover the source and quantity of the newly formed beta cells and to unravel cellular contributions during physiological neonatal pancreas growth.
Materials and methods: To specifically trace the fate of beta, duct, acinar and alpha cells in the first month of life, we used transgenic RIPCreER Rosa26LoxSTOPLoxYFP, Hnf1bCreER R26LacZ, ElastaseCreER R26YFP and Glucagon-rtTA TetO-Cre R26YFP mice, respectively. Pups received tamoxifen on the day of birth or continuous doxycycline from day 1 on and were followed during the next four weeks.
Results: In tamoxifen-treated RIP YFP mice, beta cells are labelled with a high efficiency and specificity. 82.2 ± 1.7% beta cells express YFP at postnatal day 7 (P7). Without tamoxifen, there is only limited 'leaky' expression. From week 1 to week 4, there is a 3- to 4-fold increase in beta cell mass. The islet size distribution shows a decrease in the fraction of small islets (1-5 beta cells) and an increase in the fraction of larger islets (11-50 beta cells and >50 beta cells). To quantify beta cell neogenesis, we assessed the beta cell labelling index. The percentage of labelled beta cells remains unchanged between week 1 and week 2, but starts to decrease from week 3 on and falls significantly to 70.8 ± 2.6% at week 4. The YFP+ fraction of beta cells are beta cells which were already present at birth or originate from self-duplication of beta cells. This decrease indicates that between 2 and 4 weeks of age new beta cells (about 16-17%) arise from non-beta cells (progenitor cells or stem cells). No further reduction in the beta cell labelling is observed from week 4 to week 6 (70.4 ± 0.9% YFP+ beta cells at P42) indicating that the period during which beta cell neogenesis occurs under physiological growth is restricted to the neonatal period between 2- and 4-weeks of age. Further, our lineage tracing experiments reveal that nor duct cells nor acinar cells nor alpha cells contribute to the neonatal beta cell neogenesis. And we show that physiological neonatal growth of exocrine acinar cells occurs primarily by self-duplication (and hypertrophy), as the labelling index of acinar cells in Ela YFP mice did not decrease during the first 4 weeks of life (35.2 ± 5.4% YFP+ acinar cells at P7). In line with this, duct cell-tracing in Hnf1b LacZ mice did not show significant increase in acinar labelling. Interestingly, we obtained indirect evidence that somatostatin-producing delta cells may be the origin of the newly formed neonatal beta cells as the percentage somatostatin/insulin double positive cells increases specifically at P21 and P28, coinciding with the timing of neonatal beta cell neogenesis, and declines again at P42.
Conclusion: We provide direct evidence (via genetic lineage tracing) which shows that new formation of beta cells from non-beta cells occurs in young mice and that self-duplication is the main mechanism for the increase in acinar cell number.
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Disclosure: **I. Houbracken:** Grants; FWO project and research fellowship, VUB price Ignace Vanderschueren.

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Pro-proliferative and anti-apoptotic action of exogenously introduced YAP in pancreatic beta cells

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Background and aims: Loss of functional pancreatic β-cell mass is a hallmark of both, type 1 and 2 diabetes (T1D/T2D). Identifying the pathways that promote β-cell proliferation and/or block β-cell apoptosis is a potential strategy for β-cell targeted diabetes therapy. The transcriptional co-activator Yes-associated protein (YAP), a major down-stream effector of the Hippo signaling pathway, is a key regulator of organ size and tissue homeostasis by modulating cell proliferation and apoptosis. YAP is not expressed in mature primary human and mouse β-cells. Given the pro-proliferative and anti-apoptotic role of YAP in other cell types, we aimed

to identify whether re-expression of a constitutively active form of YAP (aYAP) promotes β-cell proliferation in isolated human islets and whether such over-expression would also restore β-cell survival under diabetogenic conditions.
Materials and methods: Isolated human islets were infected with Ad-Luciferase (Ad-Luc)-control or Ad-aYAP, an adenoviral system to express aYAP with a serine 127 to alanine substitution (YAPS127A) that abolishes MST/LATS-mediated YAP inactivation and provides a constitutively active form of YAP. Immunostaining of proliferation markers (Ki67 & BrdU), glucose-stimulated insulin secretion (GSIS) and RT-PCR expression analysis of critical β-cell genes and reactive oxygen species (ROS)-associated genes were performed in isolated human islets and the rat β-cell line INS-1E. The effect of aYAP overexpression on apoptosis and signaling was assessed by Western blot analysis.
Results: Overexpression of aYAP remarkably induced β-cell proliferation in isolated human islets as shown by significantly increased Ki67- as well as BrdU-positive human β-cells. The transcription factor forkhead box M1 (FOXM1) was strongly upregulated upon aYAP-overexpression. FOXM1 inhibitor (Thiostrepton) fully blocked β-cell proliferation in the aYAP-infected human islets suggesting that increased FOXM1 is a crucial contributor of β-cell proliferation in YAP-reconstituted β-cells. While aYAP-overexpression neither affected glucose-stimulated insulin secretion (GSIS), nor key β-cell functional identity genes including endocrine hormones (INS and GCG), key β-cell transcription factors (Pdx1, NeuroD1, MafA, Nkx2.2, Nkx6.1 and Pax4) as well as critical genes involved in glucose sensing (GCK and Slc2a2), its overexpression protected β-cells from apoptosis triggered by multiple diabetic conditions. Small redox proteins thioredoxin-1 and thioredoxin-2 (Trx1/2) were upregulated by aYAP overexpression. Genetic and pharmacological disruption of the Trx system showed that YAP-dependent upregulation of Trx1/2 was required for the anti-apoptotic action of YAP in insulin producing β-cells.
Conclusion: Our data show the robust pro-proliferative and anti-apoptotic function of YAP in pancreatic β-cells with no influence on islet functionality. YAP reconstitution may represent a disease-modifying approach to restore a functional β-cell mass in diabetes.
Supported by: DFG
Disclosure: **S. Rafizadeh:** None.

OP 09 SGLT-2 inhibitors: metabolic effects

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Effect of empagliflozin (EMPA) on bone fractures in patients with type 2 diabetes (T2DM)

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Background and aims: Patients with T2DM have an increased risk of fractures compared with individuals without diabetes. Sodium glucose cotransporter 2 inhibition may alter the renal reabsorption of calcium and phosphate, potentially affecting bone metabolism. We assessed the effect of EMPA on bone fractures in patients with T2DM using pooled placebo-controlled trial data and data from a head-to-head study versus glimepiride (GLIM; EMPA-REG H2H-SU).
Materials and methods: Pooled safety data were analysed from patients who were randomised (1:1:1) to receive EMPA 10 mg, 25 mg or placebo (PBO) in 15 randomised, Phase I-III clinical trials (including the cardiovascular outcomes trial, EMPA-REG OUTCOME), plus 4 extension studies. In EMPA-REG H2H-SU, patients received EMPA 25 mg or GLIM as add-on to metformin for 104 weeks and could participate in a 2-year extension. Bone fracture adverse events (AEs) were evaluated through a search of investigator-reported AEs and analysed descriptively.
Results: In the pooled analysis, 4221, 4196 and 4203 patients received EMPA 10 mg, EMPA 25 mg and PBO, respectively, and median exposure was 698, 699 and 658 days in these groups, respectively. Bone fracture AEs were reported in 119 patients (2.8%), 105 patients (2.5%) and 123 patients (2.9%) in EMPA 10 mg, EMPA 25 mg and PBO groups, respectively. This corresponded to a rate of 1.55, 1.36 and 1.69/100 patient-years, respectively. In EMPA-REG H2H-SU, 765 and 780 patients received EMPA 25 mg and GLIM, respectively, and bone fracture AEs were reported in 31 patients (4.1%) and 33 patients (4.2%) in the EMPA 25 mg and GLIM groups, respectively. This corresponded to a rate of 1.28 and 1.40/100 patient-years, respectively. There were no changes from baseline in calcium or phosphate in any group in the pooled analysis or in EMPA-REG H2H-SU.
Conclusion: To conclude, in a pooled analysis of >12,000 patients with T2DM, EMPA did not increase the risk of bone fracture compared with PBO. In a 4-year head-to-head study, EMPA did not increase the risk of bone fracture compared with GLIM.
Clinical Trial Registration Number: NCT00885118, NCT00789035, NCT00558571, NCT00749190, NCT01011868, NCT01193218, NCT01210001, NCT01177813, NCT01159600, NCT01289990, NCT01131676, NCT01167881, NCT01164501, NCT01370005, NCT01306214, NCT01947855, NCT00881530
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **S. Kohler:** Employment/Consultancy; Boehringer Ingelheim.

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Effect of empagliflozin (EMPA) on diabetic ketoacidosis (DKA) in patients with type 2 diabetes (T2DM): pooled clinical trial data

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Background and aims: Sodium glucose cotransporter 2 inhibition leads to loss of glucose in the urine and decreased insulin-to-glucagon ratio, which may lead to a shift from glucose to lipid oxidation and increased

ketone bodies. We assessed the incidence of DKA with EMPA using pooled clinical trial data.
Materials and methods: Safety data were pooled from patients with T2DM treated with EMPA 10 mg or 25 mg in 18 randomised, Phase I-III clinical trials of 8 days' to 4 years' duration (including the cardiovascular outcomes trial, EMPA-REG OUTCOME), plus 6 extension studies. Based on adverse events (AEs) reported by the investigators, DKA was assessed through a search of 3 Medical Dictionary for Regulatory Activities (MedDRA) preferred terms (diabetic ketoacidosis; diabetic ketoacidotic hyperglycaemic coma; ketoacidosis) and analysed descriptively.
Results: Total exposure was 8368, 11017, and 10472 patient-years in the EMPA 10 mg, EMPA 25 mg and comparator groups, respectively. The proportion of patients with any DKA AE was low in all groups and comparable between groups. All patients with DKA AEs in EMPA groups recovered, except for 1 patient (EMPA 10 mg; post-treatment AE) scheduled for hospital discharge but lost to follow-up. Two patients discontinued treatment with EMPA due to DKA AEs.
Conclusion: To conclude, in an analysis of pooled data with >19000 patient-years' exposure to EMPA, the incidence of DKA was low and comparable between groups.

	Empagliflozin 10 mg (n=4558)		Empagliflozin 25 mg (n=5520)		Comparators (n=5599)	
	n (%)	Rate/100 patient-years	n (%)	Rate/100 patient-years	n (%)	Rate/100 patient-years
Any DKA adverse event	5 (0.1)	0.06	2 (<0.1)	0.02	5 (0.1)	0.05
Diabetic ketoacidosis	5 (0.1)	0.06	2 (<0.1)	0.02	4 (0.1)	0.04
Ketoacidosis	0	0	0	0	1 (<0.1)	0.01
Serious DKA adverse event*	5 (0.1)	0.06	1 (<0.1)	0.01	4 (0.1)	0.04
Data from patients treated with ≥1 dose of study drug. *Adverse events reported as serious adverse events by investigator.						

Clinical Trial Registration Number: NCT01924767, NCT00885118, NCT00789035, NCT00558571, NCT00749190, NCT01289990, NCT01316341, NCT01011868, NCT01193218, NCT01210001, NCT01177813, NCT01159600, NCT01131676, NCT01167881, NCT01164501, NCT01370005, NCT01306214, NCT01947855, NCT00881530
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **S. Lund:** Employment/Consultancy; Boehringer Ingelheim. Stock/Shareholding; Novo Nordisk.

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Metabolic mechanisms of increased plasma ketones with dapagliflozin

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Background and aims: Treatment of type 2 DM (T2DM) patients with sodium-glucose cotrasporter-2 inhibitors (SGLT2i) ameliorates glucotoxicity, increases insulin sensitivity, and improves beta-cell function. Development of ketoacidosis in T2DM subjects treated with SGLT2i has been reported, but the underlying mechanisms about how they may cause ketogenesis are poorly understood. The aim of the study was to examine the effect of reducing glucotoxicity with dapagliflozin (DAPA) on potential mechanisms leading to increased ketone production.
Materials and methods: 18 T2DM were randomized to DAPA (n=10), 10 mg/day, or placebo (n=8) for 2 weeks. Before and after 14 days of treatment, subjects received a euglycemic insulin (80mU/m2.min) clamp with tritiated glucose, indirect calorimetry (-30 to 0 and last 30 minutes of insulin clamp), and vastus lateralis muscle biopsy during the basal period prior to start of the insulin clamp.
Results: At baseline, placebo and DAPA groups were well matched for age (55.4 vs 51.9 years), BMI (32.6 vs. 30.9 kg/m2), HbA1c (8.7 vs. 8.5%), fasting plasma insulin (12 vs. 9 uU/ml), and fasting FFA (0.46 vs 0.50 mM). DAPA reduced FPG (167±13 to 128±6 mg/dl) and increased whole body insulin-stimulated glucose disposal (TGD) by 36% ($p<0.01$) versus placebo during the insulin clamp. Glucose oxidation (OX) during the insulin clamp decreased after dapagliflozin (1.36±0.16 to 0.62±0.17 mg/kg.min, $p<0.001$), while insulin-stimulated non-OX glucose disposal increased markedly (2.74±0.59 to 4.74±0.51 mg/kg.min, $p<0.001$ vs baseline and $p<0.01$ vs placebo). DAPA had no effect on fasting plasma FFA concentration, but the basal rate of lipid OX increased with DAPA (2.48±0.34 to 2.82±0.12 mg/kg.min, $p<0.05$ vs. baseline and vs. placebo), while it slightly decreased in placebo-treated subjects (2.06 ±0.15 to 1.89±0.10 mg/kg.min). The ratio of fasting plasma glucagon to fasting plasma insulin concentration increased from 14±5 to 35±11 ($p<0.01$ vs baseline and vs placebo) after DAPA treatment whereas there was no change after placebo. Fasting plasma ketone concentration increased from 0.05±0.01 to 0.2±0.01 mmol/L in DAPA treated subjects ($p<0.01$), while there was no change in the placebo group (0.09±0.02 mmol/L, $p<0.01$ vs. DAPA).
Conclusion: DAPA reduces plasma glucose levels and improves insulin sensitivity, but resulted in a shift in substrate oxidation from glucose to lipid in association with a marked increase in plasma glucagon/insulin ratio. These changes in substrate utilization and hormones provide a clear metabolic basis for increased ketone body production with DAPA. Care should be taken in clinical situations where this switch could result in ketosis, such as insulinopenic T2DM patients and following strenuous exercise in these patients, excessive alcohol intake, or severe medical/surgical stress.
Clinical Trial Registration Number: NCT01439854
Disclosure: **C. Solis-Herrera:** None.

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Impact of the SGLT-2-Inhibitor dapagliflozin on micro- and macrocirculation in type 2 diabetes

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Background and aims: Diabetes mellitus, primarily a metabolic disorder, must be considered also as a vascular disease. Early vascular changes are characterized by hyperperfusion (e.g. eye), vascular remodeling of small arteries and increased pulse wave reflection leading to increased (central) aortic pressure. We investigated the effects of the SGLT-2 inhibitor dapagliflozin on parameters of early micro- and macrovascular changes in patients with type-2 diabetes.
Materials and methods: In this prospective, double-blind, placebo-controlled, cross-over trial 59 patients (61±7.6 years) with type-2 diabetes were randomly assigned to dapagliflozin 10mg and placebo for 6 weeks. Retinal microvascular structure (wall-to-lumen ratio [WLR]) and retinal capillary flow [RCF]) were non-invasively assessed by scanning laser Doppler flowmetry. In addition, macrovascular parameters (central pulse pressure) were assessed by pulse wave analysis in addition to 24-h ambulatory blood pressure (ABP).
Results: Treatment with dapagliflozin for 6 weeks improved diabetic control (HbA1c, fasting and postprandial blood glucose, all $p<0.001$) compared to placebo. Compared to placebo treatment with dapagliflozin reduced numerically but not significantly both microvascular parameters (RCF and WLR). When compared to baseline, treatment with dapagliflozin reduced RCF (308±78 vs. 324±84 AU, $p=0.028$), indicative of a normalization of retinal hyperperfusion, and prevented vascular remodelling of retinal, which occurred in the placebo group (WLR: 0.356 ±0.1 vs. 0.391±0.1, $p=0.034$). Moreover, compared to placebo, treatment of dapagliflozin reduced systolic and diastolic 24-h ABP (126±11/75±8

vs. 129±12/77±7 mmHg, p=0.021/0.027), and central pulse pressure (40.9±11 vs. 43.9±12 mmHg, p=0.05).
Conclusion: Overall, our data indicate that treatment with the SGLT-2 inhibitor dapagliflozin exerts beneficial effects on vascular parameters of the micro- and macrocirculation, suggesting an improvement of cardiovascular prognosis.
Supported by: BMS AZ
Disclosure: **C. Ott:** None.

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Canagliflozin slows progression of renal function decline independent of glycaemic effects

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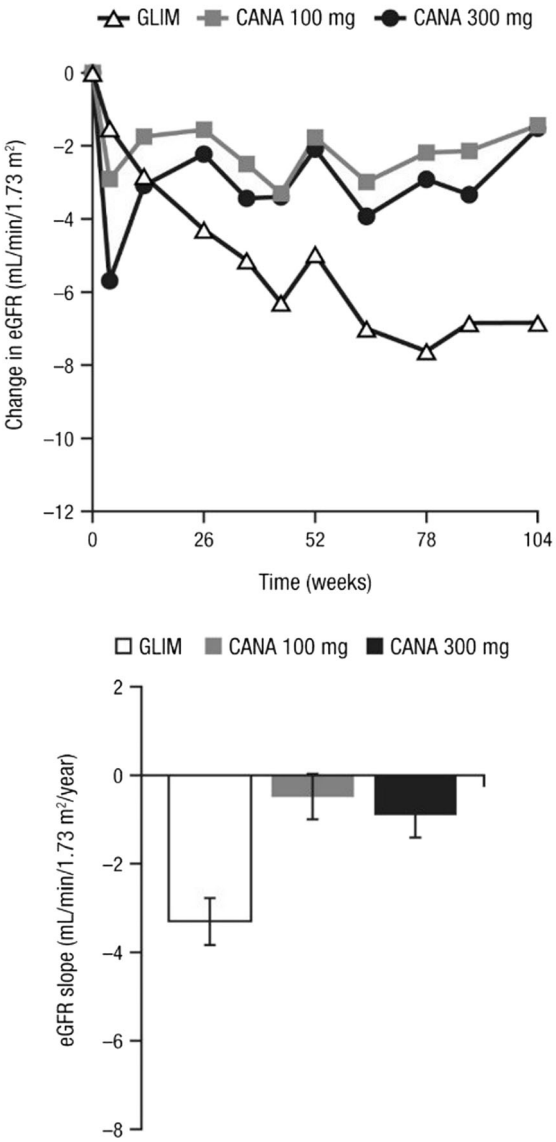
Background and aims: Sodium glucose co-transporter 2 (SGLT2) inhibition with canagliflozin (CANA) has been shown to decrease HbA1c, body weight (BW), blood pressure (BP), and albuminuria (UACR), implying that CANA may confer renoprotection. The aim of this analysis was to determine whether CANA decreases UACR and slows estimated glomerular filtration rate (eGFR) decline independent of its glycaemic effects.
Materials and methods: This was a post-hoc analysis of a Phase 3 clinical trial in 1450 patients with type 2 diabetes randomly assigned to CANA 100 or 300 mg or glimepiride (GLIM) 6 to 8 mg. Endpoints were change in eGFR and UACR over 2 years follow-up.
Results: Annual eGFR decline was 3.3 ml/min/1.73 m^2 (95% CI 2.8 to 3.8) with GLIM, 0.5 ml/min/1.73 m^2 (95% CI 0.0 to 1.0) with CANA 100 mg, and 0.9 ml/min/1.73 m^2 (95% CI 0.4 to 1.4) with CANA 300 mg (p <0.01 for each CANA group vs GLIM). Results were similar in patients with baseline UACR >3.39 mg/mmol (>30 mg/g). In this subgroup, relative to GLIM, UACR was lowered significantly more with CANA 100 mg (31.7%; 95% CI 8.6 to 48.9; p = 0.01) and CANA 300 mg (49.3%; 95% CI 31.9 to 62.2; p <0.001). Reductions in HbA1c with GLIM, CANA 100 and 300 mg were 0.81%, 0.82%, and 0.93% at 1 year, and 0.55%, 0.65%, and 0.74% at 2 years, respectively. Effects of CANA vs GLIM on eGFR and UACR were similar after adjustment for on-treatment differences in HbA1c, systolic BP, or BW.
Conclusion: CANA 100 or 300 mg/day slows the progression of renal disease compared to GLIM over 2 years. These beneficial effects of CANA appear unlikely to be due to differences in HbA1c, and suggest that CANA may confer renoprotection independent of its glycaemic effects.

Figure. eGFR decline and annual eGFR slope in the overall population.



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Disclosure: **H.J.L. Heerspink:** Employment/Consultancy; AbbVie, Astellas, AstraZeneca, Boehringer Ingelheim, Janssen, and ZS Pharma.

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Differential effects of dapagliflozin on cardiovascular risk factors at varying degrees of renal function

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Background and aims: Sodium glucose co-transporter 2 inhibition with dapagliflozin decreases HbA1c, body weight, blood pressure and albuminuria (urine albumin-to-creatinine ratio [UACR]). Dapagliflozin is also associated with small, predictable increases in haematocrit, likely related to osmotic diuresis. Prior studies suggest that the HbA1c lowering effects of dapagliflozin attenuate at lower estimated glomerular filtration rate (eGFR). However, effects on other cardiovascular risk factors at different eGFR levels are incompletely understood.

Materials and methods: This pooled analysis of 11 phase 3 clinical trials assessed changes in HbA1c, body weight, systolic blood pressure, haematocrit and UACR with placebo (N=2,178) or dapagliflozin 10 mg (N=2,226) over 24 weeks in patients with type 2 diabetes, according to baseline eGFR (eGFR ≥45 to <60; eGFR ≥60 to <90; eGFR ≥90 mL/min/1.73m^2).
Results: The HbA1c lowering effects of dapagliflozin were smaller at lower baseline eGFR levels (Table). However, the effects of dapagliflozin on body weight and systolic blood pressure were similar regardless of baseline eGFR. Moreover, among individuals with baseline UACR of ≥30 mg/g, the greatest reduction in UACR was observed in patients with an eGFR of ≥45 to <60 mL/min/1.73m^2, despite similar median values at baseline. Furthermore, dapagliflozin-induced volume contraction (as estimated by changes in haematocrit) was equal across eGFR subgroups, despite lower urinary glucose excretion in patients with low renal function. Adverse events occurred more frequently in the lowest eGFR subgroup; this was true for both dapagliflozin and placebo treated patients.
Conclusion: In conclusion, dapagliflozin was associated with favourable effects on a number of renal and cardiovascular risk factors, including reductions in body weight, systolic blood pressure and UACR, regardless of baseline eGFR. These effects, in conjunction with the finding of similar volume contraction independent of renal function, suggest that effects of dapagliflozin are partly mediated via non-glucosuria dependent mechanisms. Such a mechanism could potentially be an increase in sodium excretion. Collectively, these results suggest that dapagliflozin may confer renal and cardiovascular protection in subjects with type 2 diabetes and low eGFR.

Table: Dapagliflozin 10 mg induced placebo-corrected changes on cardiovascular risk factors at Week 24 by baseline eGFR.

	Difference vs Placebo (95% CI)		
	eGFR ≥45 to <60 mL/min/1.73m^2 (N=274 [placebo], 252 [DAPA])	eGFR ≥60 to <90 mL/min/1.73m^2 (N=1233 [placebo], 1251 [DAPA])	eGFR ≥90 mL/min/1.73m^2 (N=671 [placebo], 723 [DAPA])
HbA1c, mmol/mol*	−3.0 (−4.7, −1.2)	−5.1 (−5.9, −4.4)	−6.2 (−7.2, −5.1)
Body weight, kg*	−2.1 (−2.6, −1.5)	−1.8 (−2.0, −1.5)	−2.3 (−2.7, −2.0)
Systolic BP, mmHg*	−4.3 (−6.8, −1.8)	−2.6 (−3.6, −1.6)	−3.4 (−4.7, −2.1)
Haematocrit, %*	2.4 (2.0, 2.9)	2.6 (2.4, 2.8)	2.6 (2.3, 2.8)
UACR, %†‡	−38.3 (−54.4, −16.6)	−23.3 (−35.5, −8.7)	−16.1 (−32.3, 3.8)

*Data show mean differences versus placebo (95% CI) at Week 24; †Patients with baseline UACR ≥30 mg/g, data show % differences versus placebo (95% CI) at Week 24; ‡N (Placebo/DAPA) = 110/97. 316/322 and 186/179 for eGFR subgroup ≥45 to <60, ≥60 to <90 and ≥90 mL/min/1.73m^2, respectively. Values exclude data after rescue therapy. BP, blood pressure; DAPA, dapagliflozin; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio.

Clinical Trial Registration Number: NCT 00528372, NCT00528879, NCT00683878, NCT00859898, NCT00680745, NCT00673231, NCT00984867, NCT00855166, NCT01031680, NCT01042977, NCT00663260
Supported by: AstraZeneca
Disclosure: **C.D. Sjöström:** Employment/Consultancy; AstraZeneca. Stock/Shareholding; AstraZeneca.

OP 10 Predicting and preventing macrovascular disease

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Time to treatment intensification and its association with subsequent macrovascular outcomes among patients with type 2 diabetes

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Background and aims: Previous research has shown that earlier treatment intensification among patients with Type 2 diabetes mellitus (T2DM) and HbA1c levels ≥7% despite monotherapy is associated with shorter time to subsequent glycemic control, which could potentially result in better long-term outcomes. This study assessed the association between timing of treatment intensification and subsequent macrovascular outcomes among patients with T2DM in the UK.
Materials and methods: Patients aged 18-79 years diagnosed with T2DM who used metformin (met) or sulfonylurea (SU) for ≥3 months were selected using the UK Clinical Practice Research Datalink (1/2000 - 12/2014). The first record with HbA1c ≥7% after ≥3 months of met/SU monotherapy was the index event. Intensification was defined as initiating ≥1 non-insulin antidiabetic medication in addition to met/SU after index. The cohort was stratified into 4 groups based on time from index to intensification: <6 months, 6 to <12 months, 12 to <24 months, and 24 to <36 months. Multivariate cox proportional hazard models were used to compare the incidence of myocardial infarction (MI), congestive heart failure (CHF), stroke, and major adverse cardiovascular events (MACE; composite of MI, CHF, stroke, and cardiovascular deaths) after intensification between groups (<6 months as reference group). Models were adjusted for the following covariates, assessed during 6 months prior to index: age, gender, body mass index, HbA1c level, Charlson comorbidity index, duration of monotherapy, presence of nephropathy, and use of antihypertensive, statins, or antidepressants.
Results: Of the 40,036 patients included in the sample (mean age: 61 years, ~57% male), 17% intensified <6 months after index, 9% in 6 to <12 months, 13% in 12 to <24 months, and 8% in 24 to <36 months. The mean HbA1c at index was 8.9 for the <6 months group, 8.3 for 6 to <12 months group, 8.0 for 12 to <24 months group, and 7.9 for the 24 to <36 group. The rates of developing the various complications (per 1000 person-years) were lowest among the 6 to <12 months group and highest among the 24 to <36 months group (except MI, which was highest among the 12 to <24 months group) - MI: 5.0 vs. 5.7, CHF: 8.2 vs. 10.3, stroke: 5.0 vs. 6.7, MACE: 13.2 vs. 18.4. After adjusting for differences in patient characteristics, there was no statistically significant association between earlier intensification and incidence of macrovascular outcomes (Table 1).
Conclusion: The findings of this study suggest that early intensification alone is not associated with improved macrovascular outcomes in T2DM patients. Future research should evaluate the potential role of durability of response to intensification, in terms of glycemic control, as an explanatory variable.

Table 1: Likelihood of developing MI, CHF, stroke, and MACE among patients intensifying within 36 months after index, stratified by time to intensification

	Adjusted HR	(95% CI)
MI		
6 to <12 months	0.97	(0.70, 1.34)
12 to <24 months	1.09	(0.82, 1.44)
24 to <36 months	0.92	(0.65, 1.30)
CHF		
6 to <12 months	1.01	(0.78, 1.31)
12 to <24 months	1.04	(0.83, 1.30)
24 to <36 months	1.26	(0.98, 1.62)
Stroke		
6 to <12 months	0.79	(0.57, 1.09)
12 to <24 months	0.87	(0.66, 1.15)
24 to <36 months	1.14	(0.85, 1.54)
MACE		
6 to <12 months	0.83	(0.67, 1.03)
12 to <24 months	0.94	(0.79, 1.12)
24 to <36 months	1.15	(0.94, 1.40)

Notes:
MI = myocardial infarction, CHF = congestive heart failure, MACE = major adverse cardiovascular event; Intensifying <6 months was considered as the reference cohort for all comparisons.

Supported by: BMS, AZ
Disclosure: **J. Kim:** Employment/Consultancy; I am an employee and a stock/shareholder of AstraZeneca.

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Combination therapy with a SGLT2 inhibitor and a DPP-4 inhibitor suppresses macrophage foam cell formation in type 2 diabetic mice

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Background and aims: We recently reported that a SGLT2 inhibitor suppressed the acceleration of atherosclerosis in diabetic Apoe−/− mice and macrophage foam cell formation in diabetic db/db mice in glucose-dependent-manner. While, several previous studies including ours have revealed that a DPP-4i can exert anti-atherosclerotic effect in Apoe−/− mice in glucose-independent manner. Thus, it is expected that combination therapy of SGLT2i with DPP-4i synergistically suppresses atherosclerosis and foam cell formation. The aim of this experiment is to determine whether the combination therapy is superior to the monotherapy on macrophage foam cell formation, a critical process of atherosclerosis, in type 2 diabetic mice.
Materials and methods: Male db/db mice were given the SGLT2i (ipragliflozin, 1.0 mg/kg/day), the DPP-4i (alogliptin, 8.0 mg/kg/day), or these combination at these same respective dose with normal diet for 4-weeks. The peritoneal macrophages migrated by an intraperitoneal injection of thioglycolate were collected to evaluate foam cell formation measured by the incorporation of [3H]-oleate into cholesteryl-oleate stimulated by oxidized LDL. The gene expressions related to foam cell formation in the peritoneal macrophages were examined by real-time RT-PCR.
Results: The OGTTs revealed that ipragliflozin completely suppressed glucose intolerance, while alogliptin only slightly ameliorated this. The combination therapy did not further suppressed glucose tolerance compared with ipragliflozin alone. Similar changes of fasting glucose and HbA1c were observed in ipragliflozin, alogliptin, and the combination treatments. Macrophage foam cell formation was suppressed by ipragliflozin or alogliptin monotherapy to similar extent (33% and 30%), and further suppressed by the combination therapy (48%, $p < 0.05$ vs. monotherapies). The macrophage foam cell formation ex vivo was closely associated with HbA1c levels in each group (nontreated, ipragliflozin, alogliptin, and the combination group; $r = 0.76$, 0.69, 0.67, and 0.77, respectively, all, $p < 0.01$). The correlation curves encompassing foam cell formation and HbA1c levels were similar between nontreated group and ipragliflozin alone group. Alogliptin alone group or the combination group disproportionally further suppressed foam cell formation at corresponded HbA1c levels compared with nontreated and ipragliflozin treated groups. The gene expression of lectin-like ox-LDL receptor-1 was down-regulated by ipragliflozin or alogliptin to similar extent (24% and 29%, $p < 0.05$ vs. nontreated.), and further suppressed by the combination therapy (45%, $p < 0.05$ vs. monotherapies). Whereas those of ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette sub-family G member1 (ABCG1) were up-regulated by ipragliflozin or alogliptin to similar extent (ABCA1: 48% and 102%; ABCG1: 60% and 95%, respectively, $p < 0.05$ vs. monotherapies), and further increased by the combination (ABCA1: 141%; ABCG1: 165%, respectively, $p < 0.05$ vs. monotherapies).
Conclusion: The combination therapy of SGLT2i with DPP-4i more strongly suppresses macrophage foam cell formation than each monotherapy in mouse model of type 2 diabetes. These results suggest that scavenger receptors and cholesterol efflux are regulated by both glycemia and DPP-4 inhibition.
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Disclosure: **M. Terasaki:** None.

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Dapagliflozin, a SGLT-2 inhibitor, alleviates atherosclerosis in ApoE$^{-/-}$ mice by inhibiting NLRP3 inflammasome

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Background and aims: To observe the effects of dapagliflozin (a SGLT-2 inhibitor) on atherosclerosis of aorta in streptozotocin (STZ) induced diabetic ApoE$^{-/-}$ mice and the relevant mechanisms.
Materials and methods: The STZ induced diabetic ApoE$^{-/-}$ mice were fed with a high-fat diet to establish the experimental diabetic atherosclerosis mouse models. C57BL/6J mice served as control group and they were fed with a general diet. ApoE$^{-/-}$ mice were intragastrically administrated with dapagliflozin 1mg/kg.d or vehicle for 12w treatment, and thereafter their aortas were collected for oil red O staining to analyze the area of lesion and for hematoxylin-eosin (HE) staining to observe the pathologically morphological changes. The IL-1β, IL-18 and NLRP3 inflammasome in the serum were tested by ELISA, the macrophage infiltration in the lesion and the stability of lesion were detected by immunofluorescence, and the expression levels of NLRP3 inflammasome, IL-1β and IL-18 in aorta were analyzed by Western blot.
Results: Dapagliflozin decreased the blood glucose by 43% in diabetic ApoE$^{-/-}$ mice ($P<0.01$) and 28% in non-diabetic ApoE$^{-/-}$ mice ($P<0.05$), respectively; and it lowered FFA and TG in diabetic ApoE$^{-/-}$ mice ($P<0.05$). Oil red O staining results of aorta showed that dapagliflozin inhibited the formation of aortic atherosclerosis in diabetic ApoE$^{-/-}$ mice ($P<0.05$). The serum ELISA results demonstrated that dapagliflozin decreased the serum levels of IL-1β, IL-18 and NLRP3 in diabetic ApoE$^{-/-}$ mice ($P<0.05$), while it only reduced the serum IL-1β level in non-diabetic ApoE$^{-/-}$ mice ($P<0.05$). The immunofluorescence results of aortic root indicated that dapagliflozin reduced macrophage infiltration in the lesion, prevented the reduction of smooth muscle cells and increased the stability of lesion in diabetic mice. The Western blot results of abdominal aorta indicated that dapagliflozin suppressed the expression of NLRP3 inflammasome in abdominal aorta of diabetic ApoE$^{-/-}$ mice and decreased the production and release of mature IL-1β and IL-18.

Conclusion: Dapagliflozin can improve the formation of atherosclerotic lesion by lowering blood glucose and lipid, inhibit the macrophage infiltration of aorta and reduce the expression of NLRP3 inflammasome, thus hindering the production and release of IL-1β and IL-18 and relieving the inflammatory reaction in atherosclerosis.

Supported by: AstraZeneca China

Disclosure: **Z. Liang:** Grants; This study was supported by grants from AstraZeneca China.

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Performance of the validated ORIGIN cardiovascular biomarker panel in HOPE study participants with diabetes

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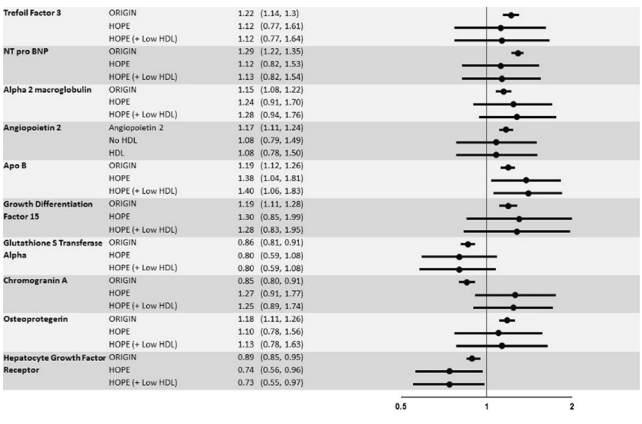
Background and aims: Novel biomarkers may identify people at high risk for cardiovascular outcomes.Stored serum from 8401 ORIGIN participants was assayed for 237 novel biomarkers that identified and validated 10 biomarkers that independently predicted the composite outcome of nonfatal myocardial infarction, nonfatal stroke or CV death after accounting for standard risk factors including sex, age group, prior CV event, albuminuria, smoking, established diabetes, LDL/HDL ratio, and hypertension.

Materials and methods: The performance of these biomarkers was assessed in a random sample of 350 HOPE participants with baseline diabetes and stored serum. To facilitate a case-cohort approach, all 77 participants who had a composite outcome during follow-up were included. A 1 ml sample was blindly analysed for the 10 biomarkers identified in the ORIGIN trial. The link between these biomarkers and the composite outcome was analyzed: a) after accounting for 7 of the 8 clinical risk factors noted above (because the 8th - LDL/HDL - was unavailable for HOPE participants); and b) after substituting a history of low HDL level for the 8th risk factor.

Results: All but chromogranin A (Figure) yielded hazards highly consistent with those in the ORIGIN trial and the biomarkers improved the C statistic for predicting the composite outcome from 0.66 to 0.74 ($p<0.001$).

Conclusion: Consistent performance of the ORIGIN biomarker panel in the HOPE dataset highlights its value.



Clinical Trial Registration Number: NCT00069784

Supported by: Sanofi-Aventis

Disclosure: **H.C. Gerstein:** Grants; Sanofi.

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Combined Nox1/4 inhibition with GKT137831 rescues established micro- and macrovascular diabetic complications

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Background and aims: Oxidative stress is a promising target in diabetes associated vasculopathies with inhibitors of NADPH oxidases (NOX), in particular isoforms 1 and 4, demonstrating attenuation of diabetic complications, specifically atherosclerosis and nephropathy. NOX inhibitors are now in early clinical development. Our studies have shown that NOX4 plays an injurious role in the kidney. With respect to atherosclerosis, our studies have shown that NOX1 exerts pro-atherosclerotic effects but that NOX4 may be vasculoprotective in longterm diabetes. Thus, its remains controversial as to which Nox isoform to target in diabetic complications. We have performed a late stage intervention protocol using the clinically most advanced compound, the NOX1/4 inhibitor GKT137831 which inhibits Nox isoforms 1 and 4. We aimed to determine if end-organ damage could be rescued when a Nox1/4 inhibitor is administered in the setting of established diabetic complications.

Materials and methods: GKT137831 was administered to STZ-induced diabetic ApoE-/- mice after 10 weeks of diabetes for a period of additional 10 weeks, at two doses, a low dose of 30mg/kg/day and a higher dose of 60mg/kg/day. At the end of the study animals were culled and used for analysis of aortic atherosclerosis and renal functional and structural parameters, using RT-PCR, ELISA and immunohistochemistry.

Results: Consistent with NOX4-/- mouse data, GKT137831 was renoprotective in a model of diabetic nephropathy at both the 30mg/kg/day and 60mg/kg/day dose, through suppression of pro-inflammatory (MCP-1) and pro-fibrotic (Collagen IV & Fibronectin) processes. Conversely, in diabetic atherosclerosis, where NOX1-/y and NOX4-/- mice has yielded qualitatively opposing results, the net effect of pharmacological NOX1/4 inhibition was protection, albeit to a lower extent, and only at the lower 30mg/kg/day dose. This was mediated through reductions in pro-inflammatory (TNFα & MCP-1) processes and reduced vascular macrophage accumulation.

Conclusion: Our findings demonstrate that targeting NOX in established diabetic complications is effective at rescuing further progression of disease. However, it should be appreciated that there appears to be tissue specific and dose dependent effects with respect to Nox isoform specific inhibition at the different sites of micro- versus macrovascular injury in diabetes.

Supported by: NHMRC, JDRF

Disclosure: **S.P. Gray:** None.

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Angiopoietin-like 2 is prognostic for all-cause death in patients with type 2 diabetes

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Background and aims: Angiopoietin-like 2 (Angptl2) plays an important role in insulin-resistance and atherosclerosis. Here we examined the prognostic value of Angptl2 for improving detah-risk stratification in patients with type 2 diabetes (T2DM).

Materials and methods: We followed consecutively recruited T2DM patients (SURDIAGENE) for all-cause death as a primary end-point, and the composite of cardiovascular (CV) death, myocardial infarction (MI), and stroke (Major CV Adverse Events; MACE) as a secondary end-point. For each patients, Angioptl2 level was evaluated at baseline.
Results: A total of 1353 T2DM patients (58% men) aged of 64±11 years were followed up for a median of 6.0 years. During follow-up, 367 patients died (11.4% person-year, PY, 95% IC (10,3-12,6) and 290 patients (9.4% PY, IC 95% IC (8.3-10.4)) had a MACE. Deaths and MACE were significantly more frequent in patients with high Angioptl2 concentrations (HR: 8,60 ; 95% IC (5.36-13.78); p<0.0001 and HR 7.15, 95% IC (4.19-12.18); p<0.0001 per log ng/ml respectively). When patients were divided into quartiles (Q) accordingly to baseline Angptl2 concentration, patients with Angptl2 levels ≥19.5 ng/ml (Q4) had a significantly increased risk of death and of MACE as compared to those with Angptl2<19.5 ng/ml (both p<0.0001) after adjustment for sex, age, and established CV risk factors. Using Angptl2, prediction of the risk of mortality as assessed by the integrated discrimination improvement (IDI) was significantly improved (pIDI =0.0001).
Conclusion: In T2DM, Angptl2 levels were independently associated with death and MACE. Therefore, measurement of Angptl2 is a promising candidate biomarker for improving all-cause-death risk stratification in T2DM patients.
Disclosure: **M. Fraty:** None.

OP 11 Novel approaches to unravel glucose homeostasis in humans

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Glucose-stimulated pancreatic blood flow in healthy subjects and in patients with type 1 diabetes using positron emission tomography

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Background and aims: Assessing the factors contributing to beta cell function in the development of type 1 diabetes (T1D) is of major importance. Animal experiments have demonstrated rapidly increasing pancreatic islet cell perfusion after intravenous glucose infusion. The islets of Langerhans, although minor in size, receive a remarkable proportion of the overall pancreatic blood supply, which emphasizes the importance of perfusion to the function of endocrine pancreas. Our aims were to quantitate glucose-stimulated islet blood flow in healthy adult human subjects and in patients with T1D and to investigate whether pancreatic blood flow is associated with insulin secretion.
Materials and methods: We have enrolled so far 7 healthy subjects (age 24.1 ± 2.6 years, BMI 23.9 ± 1.3 kg/m^2) and 5 subjects with T1D (age 19.8 ± 1.2 years, BMI 25.4 ± 4.5 kg/m^2). Patients with T1D had good or moderate glycaemic control and had no diabetic complications. Minimum duration of T1D was 5 years. Pancreatic blood flow was studied at baseline and at 1 and 12 minutes after an intravenous glucose tolerance test (IVGTT) using a combined positron emission tomography / magnetic resonance (PET/MR) scanner and 15O-labeled water as a tracer. One-tissue compartmental model was applied to measure the dynamics of pancreatic blood flow on regions of interests graphically defined on fused PET and MR images. Insulin secretion was calculated by C-peptide deconvolution.
Results: Pancreatic blood flow increased by a mean of 27% one minute after the IVGTT in healthy subjects (1.31 ± 0.23 ml/ml/min vs. 1.67 ± 0.35 ml/ml/min, p < 0.01) and approached the baseline level at 12 minutes after the IVGTT (1.57 ± 0.48 ml/ml/min). In patients with T1D, pancreatic blood flow at baseline tended to be 18% lower than in the healthy subjects (1.08 ± 0.18 ml/ml/min, p=0.11) and failed to increase significantly after the IVGTT (1.21 ± 0.20, p=0.37 and 1.26 ± 0.30, p=0.12 at 1 and 12 minutes, respectively). Mean incremental insulin secretion rate during the first 15 minutes of the IVGTT (ISR1) was 239 ± 59 pmol/min/m^2 in healthy subjects compared to no increase (1.4 pmol/min/m^2) in patients with type 1 diabetes. However, two patients still positive for GAD autoantibodies had minor insulin production and a mean ISR1 of 29 pmol/min/m^2. Their pancreatic blood flow increased from a mean of 0.93 ± 0.02 to 1.38 ± 0.06 ml/ml/min one minute after the IVGTT, as opposed to other patients with T1D with no significant response.
Conclusion: The study demonstrates that pancreatic blood flow is stimulated by hyperglycaemia in healthy subjects. This effect is blunted in subjects with T1D, but subjects showing increase in pancreatic flow had also higher residual insulin secretion response. The study suggests that pancreatic perfusion may be linked to insulin secretion.
Clinical Trial Registration Number: NCT02547337
Supported by: Academy of Finland
Disclosure: **J. Mäkinen:** None.

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Reduced skeletal muscle phosphocreatine concentrations in type 2 diabetic patients: quantitative image-based phosophorus-31 MR spectroscopy study

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Background and aims: Mitochondrial dysfunction and impaired [ATP]-production has been described in subjects with type 2 diabetes mellitus (T2DM). Phosphorus-31 magnetic resonance spectroscopy (31P-MRS) allows quantitation of substrate metabolism in skeletal muscle. Previous studies using 31P-MRS in T2DM individuals have reported results as relative concentrations of the 31P-MRS metabolite ratios, which could obscure simultaneous decreases in both phosphocreatine [PCr] and [ATP] and attenuate differences between T2DM and NGT. To our knowledge no previous human study has used an absolute concentration method utilizing in vivo techniques. The aims of this study were to develop a novel image-guided 31P-MRS method and to evaluate this method to quantitate intramyocellular phosphorus metabolites in the vastus lateralis muscle in T2DM and NGT subjects.
Materials and methods: 11 T2DM (age = 55 ± 3 years; BMI 31.0±1.5) and 12 NGT (47 + 4 years; BMI 27.8+1.1) subjects received an OGTT to provide a measure of insulin sensitivity (Matsuda Index) and beta cell function (InsAUC0-120/GluAUC0-120) × Matsuda Index. 31P-MRS was performed to measure the absolute concentrations (mM) of [PCr], inorganic phosphate [Pi], and [ATP].
Results: Matsuda insulin sensitivity index (1.61 vs 6.79, $p<0.01$) and beta cell function index (0.68 vs 9.84, $p<0.01$) were significantly reduced in T2DM compared to NGT. Fasting plasma glucose (145±10 vs 92±2, $p<0.05$), HbA1c (7.8 ± 0.4% vs 5.5 ± 0.1%, $p<0.05$) and fasting insulin (25 vs 8 μU/ml $p<0.05$) were higher in T2DM. T2DM subjects had lower absolute [PCr] (25.2 ± 1.0 mM) than NGT subjects (28.5 ± 1.0 mM, $p = 0.03$), while the [Pi] and [ATP] were not significantly different. In contrast, [PCr] values obtained using the traditional ratio method showed no significant difference between groups. Further, [PCr] correlated with HbA1c ($r = -0.43$, $p = 0.04$), beta cell function ($r = 0.49$, $p = 0.02$), and fasting plasma glucose ($r = -0.52$, $p = 0.01$). [PCr] and [γATP] were inversely correlated with age ($r = -0.45$, $p = 0.03$ and $r = -0.48$, $p = 0.02$).
Conclusion: A novel in vivo method for quantitation of absolute concentrations of phosphorus metabolites in skeletal muscle of T2DM and NGT subjects was developed and validated. Using this method we demonstrate that skeletal muscle [PCr] is reduced in T2DM. This difference is not appreciated when using the traditional ratio method, likely because it assumes a constant and uniform [ATP]. The strong correlation between [PCr] and HbA1c, FPG and beta cell function supports the concept that lower baseline skeletal muscle [PCr] is related to key determinants of glucose homeostasis.
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Disclosure: **V. Hamidi:** None.

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Skeletal muscle and liver, but not brain, account for impaired glucose utilisation in type 2 diabetes: whole-body PET/MR during hyperinsulinaemic euglycaemic clamp

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Background and aims: Type 2 diabetes (T2D) is characterized by impairment of beta cell function and insulin-mediated glucose turnover. To what extend glucose uptake is altered in different tissues is not fully clarified. Using 18FDG (fluoro-deoxyglucose) PET/MR scanning during a hyperinsulinemic euglycemic clamp, this pilot study in T2D and control subjects aimed to investigate: 1) simultaneous glucose uptake in peripheral organs and brain; 2) to what extent glucose uptake in specific organs correlate to whole-body insulin resistance; 3) whether glucose uptake in adipose tissue in vivo mirrors glucose uptake in adipocytes ex vivo.
Materials and methods: 7 healthy controls (Con) and 7 T2D subjects matched for age (64±3; 61 ±4), BMI (27.6±1.8 kg/m^2; 29.0±1.5 kg/m^2), and sex (4 males/group) were recruited. Subcutaneous (sc) abdominal adipose tissue biopsies were taken for ex vivo glucose uptake measurements and an OGTT was performed. 2 wks later, glucose uptake was measured in vivo using 18FDG PET/MR whole-body scanning during a hyperinsulinemic (56 mU/m^2/min) euglycemic clamp, allowing for simultaneous measurement of whole body insulin sensitivity and glucose uptake in several organs. This initial study focused on liver, thigh muscle, subcutaneous abdominal fat (SAT), and brain.
Results: T2D had significantly higher Hba1c and fasting insulin levels ($p<0.01$), and significantly lower M-values ($p<0.01$). In ex-vivo sc adipocytes, glucose uptake after stimulation with 1000 μU/mL of insulin was significantly lower in T2D ($p<0.05$). There was a significant correlation between the insulin-stiumlated SAT FDG uptake in vivo and sc adipocyte glucose uptake ex-vivo ($r=0.53$; $p<0.05$). Glucose uptake was lower in muscle ($p<0.01$) and liver ($p<0.01$) of T2D. There was a significant positive correlation between M-value and glucose uptake in SAT ($r=0.49$; $p<0.05$), muscle ($r=0.85$; $p<0.01$) and liver ($r=0.59$; $p<0.05$). In contrast, there was a significant inverse correlation between the M-value and brain glucose uptake ($r=-0.58$; $p<0.05$), and glucose uptake in the brain tended to be higher in T2D compared to Con ($p=0.062$).
Conclusion: Insulin-stimulated FDG uptake was decreased in liver and muscle tissue in T2D and this correlated to whole-body glucose utilization. In contrast, brain FDG uptake displayed an inverse correlation and was increased in T2D.Glucose uptake measured in the same subjects were positively correlated in sc adipocytes ex vivo and in SAT in vivo using FDG PET/MR, supporting the robustness of the methods. We propose that during hyperinsulinemia partitioning of glucose to the brain might be explained by insulin resistance in other organs, but the underlying mechanisms need to be explored further.

	Control	T2D
Glucose uptake subcutaneous adipocytes (Insulin 1000 uU/ml; % of basal)	257 ± 63	189 ± 20*
Hba1C (mmol/mol)	33.7 ± 0.3	54.6 ± 2.2**
OGTT Glucose (AUC) (mmol/L*min)	891 ± 93	1879 ± 174*
OGTT Insulin (AUC) (mU/L*min)	5518 ± 1318	2498 ± 486*
M-value (g/kg lean body mass/min)	10.7 ± 1.4	5.5 ± 1.1**
FDG uptake Muscle (umol FDG/100 g tissue min)	7.1 ± 0.6	3.3 ± 0.8**
FDG uptake Liver (umol FDG/100 g tissue min)	3.5 ± 0.4	1.9 ± 0.3**
FDG uptake SAT (umol FDG/100 g tissue min)	1.9 ± 0.3	1.5 ± 0.3
FDG uptake Brain (umol FDG/100 g tissue min)	8.5 ± 0.4	9.4 ± 0.4#
** P<0.01; * P<0.05; # p = 0.062		

Disclosure: **G.J. Boersma:** Grants; AstraZeneca AB. (to medical sciences UU).

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Load-dependent effects of small intestinal glucose on glycaemia, insulinaemia and incretin hormone release in obese subjects

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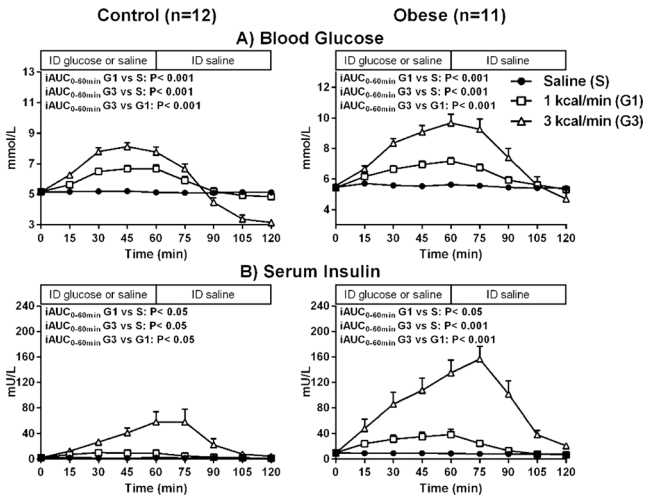
Background and aims: The mechanisms by which obesity predisposes to type 2 diabetes are incompletely understood. Studies relating to the glycaemic response to oral glucose, or meals in obesity have failed to account for gastric emptying, which is a major determinant of the glycaemic and incretin responses to carbohydrate. In health and type 2 diabetes we have shown that the relationship of glycaemia with the rate of duodenal glucose delivery is non-linear. We have now evaluated: (i) in both obese and healthy subjects, the effect of two intraduodenal glucose loads and (ii) in obese subjects, the comparative effects of oral and intraduodenal glucose, on glycaemic, insulinaemic and incretin hormone responses.
Materials and methods: 11 obese subjects (age 37.5±4.1 yr, BMI 35.7±1.4 kg/m2) and 12 healthy control subjects (age 34.7±4.0 yr, BMI 23.9±0.7 kg/m2) received, in randomised order, intraduodenal infusions of glucose at 1 or 3 kcal/min ('G1' and 'G3'), or saline ('S') for 60 min (t= 0-60 min), followed by intraduodenal saline (t= 60-120 min), after an overnight fast. In obese subjects, an oral glucose tolerance test was also performed. Blood glucose, serum insulin and plasma glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP) and glucagon were measured. Insulin resistance (HOMA2) was calculated. Data are mean ± SEM.
Results: 3 obese subjects had impaired, and 8 had normal, glucose tolerance. HOMA2 was greater (P<0.001) in the obese. In both groups, the iAUC0-60 min for glucose (Figure 1A) was greater with G3 compared with G1 (P<0.001 for both), and the iAUC0-120 min for glucose in response to G3 was greater (P<0.05) in the obese. Insulin responses (Figure 1B) to G1 and G3 were greater (P<0.001 for both) in the obese than controls. GLP-1 and GIP responses were greater (P<0.001 for both) in response to G3, without any difference between the groups. There was also no difference in the iAUC0-60 min for glucagon between the two groups. In the obese, glycaemic (R=0.80, P<0.01), insulinaemic (R=0.85, P<0.001) and GIP (R=0.80, P<0.01), but not GLP-1 or glucagon, responses to G3 and oral glucose were related.
Conclusion: The rate of duodenal glucose delivery is a major determinant of glycaemia, insulinaemia and incretin hormone release in non-diabetic obese subjects. Differences in glycaemic responses between obese and healthy subjects are more evident at higher rates of duodenal glucose delivery. The secretion of GLP-1 and GIP appears to be normal in obesity. The concept that strategies that slow gastric emptying may prevent progression to type 2 diabetes in obesity warrants exploration.



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Disclosure: **C.S. Marathe:** None.

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GIP contributes to post-meal hyperglucagonaemic response after bariatric surgery

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Background and aims: Incretin hormone glucose-dependent insulinotrophic polypeptide (GIP) has a bivalent role stimulating secretion of both insulin and glucagon. The present study was conducted to investigate the islet endocrine effects of GIP early after bariatric surgery and the contribution of GIP to the meal-induced hyperglucagonemic response seen after bariatric procedures.
Materials and methods: A total of ten obese patients with type 2 diabetes (two males, eight females; weight 114 [SD 18.9] kg; fasting plasma glucose 7.0 [0.4] mM; fasting plasma glucagon 40 [6.4] pM) were recruited to the study. On two separate days performed at fasting, patients received a constant GIP infusion at the rate of 4.0 and 2.0 pmol/kg/min for 0-15 and 15-60 minutes, respectively, and a 250-kcal meal solution for 90 minutes. During the experiments, blood was frequently sampled to measure plasma glucose, insulin, C-peptide, glucagon, GIP and glucagon-like peptide 1 (GLP-1). Insulin secretion rate (ISR) was estimated from deconvolution of C-peptide concentration. Bariatric procedures (five Roux-en-Y gastric bypasses and five vertical sleeve gastrectomies) were performed to patients and thereafter the experiments were repeated 74 [24] days after the surgery.
Results: After bariatric surgery, 14% excess weight loss was observed (98 [16.2] kg, P < 0.001 vs. pre-surgery) and fasting plasma glucose was normalized (5.8 [0.3] mM, P = 0.002 vs. pre-surgery). In contrast, fasting plasma glucagon was not changed from pre-surgery level (30 [6.5], P = 0.230). The total diabetic remission rate was 50%. GIP infusion resulted in a transient upregulation of ISR for five minutes from the start of the infusion and a constant increase in glucagon levels similarly both before and after surgery (P = 0.173 and 0.418 for time × group interactions, respectively). However, plasma glucose decreased to a nadir of 6.3 [1.0] mM only in patients before (P < 0.001 vs. baseline) but not after surgery (P = 0.358 vs. baseline). Post-meal responses of GIP, ISR and glucagon were markedly increased at post-surgery vs. pre-surgery (P < 0.001 for time × group interaction for all variables) whereas an increase in meal-stimulated plasma GLP-1 levels was observed only at post-surgery (P < 0.001 for time factor) but not at pre-surgery (P = 0.093 for time factor). During test meal, increase in plasma GIP was associated with corresponding increase in plasma glucagon after (r = 0.767, P = 0.016) but not before (r = -0.117, P = 0.765) surgery. Bypass and sleeve groups did not differ in ISR and glucagon responses during GIP infusion and test meal.
Conclusion: The effect of GIP on islet endocrine secretions is preserved in patients with type 2 diabetes both before and early after bariatric surgery, leading to a normalization of fasting hyperglycemia. We propose that the post-meal hyperglucagonemic response seen in patients who have undergone a bariatric procedure is connected to GIP hypersecretion, in conjunction with surgical manipulation of the gastrointestinal tract.
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Supported by: Academy of Finland, Finnish Cultural Foundation
Disclosure: **H. Honka:** None.

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Are incretin responses to oral glucose and mixed meal tests associated with glucose levels 7 years later?

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Background and aims: Reduced incretin effectivity is believed to contribute to impaired regulation of insulin secretion in type 2 diabetes. However, it is still unclear what comes first in the aetiology. Therefore, the aim of this study was to examine the association between incretin responses to oral glucose tolerance test (OGTT) and mixed meal test (MTT) and glucose levels 7 years later, in individuals who were non-diabetic at baseline.
Materials and methods: We used data from the Hoorn Meal Study; a population-based cohort study among 208 men and women (40-65y), who at baseline were subjected to a 75g-OGTT and a MMT (75g carbohydrates/ 50g fat/ 24g protein). During the OGTT and MMT, we measured GLP-1 and GIP levels from which we calculated the incremental area under the curve (iAUC). We assessed the association between fasting and iAUC incretin levels at baseline and fasting glucose levels after 7y, corrected for baseline glucose levels.
Results: We included 121 participants (age 61.0±6.7, 50% male) without diabetes at baseline. While the associations were non-linear, incretin responses were grouped in tertiles. We observed no significant associations for fasting GIP levels or GIP responses. In contrast, compared to the lowest tertile of fasting GLP-1 levels, those in the middle and highest tertiles had higher fasting glucose levels at follow- up, respectively 0.36 (95%CI, 0.1;0.6) mmol/l and 0.09 (-0.2;0.3) mmol/l, corrected for glucose at baseline. Additionally, compared to the lowest tertiles of the iAUC GLP-1 responses, the glucose levels at follow-up were respectively -0.28 (-0.5; -0.1) mmol/l and -0.37 (-0.7; -0.1) mmol/l for the OGTT, corrected for glucose at baseline. No significant association was observed for the MMT.
Conclusion: Within our population-based cohort, fasting GLP-1 and GLP-1 responses to OGTT were associated with altered fasting glucose levels after 7-year follow-up, suggesting that the reduced incretin response is associated to glucose deterioration.
Disclosure: **A.D.M. Koopman:** None.

OP 12 Health or disease?

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Glycaemic control and patient-reported outcome measures (PROMs) in type 1 diabetes

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Background and aims: Patient-reported outcome measures (PROMs) offer potential to improve the quality and outcomes of health care. One way to measure PROM is by using health-related quality of life questionnaires. The SF-36 is a widely used generic health-related quality instrument and measures health across eight dimensions of physical functioning (PF), role limitations due to physical problems (RP), bodily pain (BP), general health (GH), vitality (VT), social functioning (SF), role limitations due to emotional problems (RE) and mental health (MH). The dimension scores also form physical component summary (PCS) and mental component summary (MCS) scores. The aim of this study was to assess health-related quality of life at different levels of glycaemic control in type 1 diabetes (T1D) using the SF-36.
Materials and methods: The SF-36v2 questionnaire was sent to 2479 randomly selected individuals with T1D in Sweden registered in the National Diabetes Register (NDR). Background data on responders and non-responders were collected from NDR. SF-36v2 results are presented as norm based scores using the SF-36v2 2009 U.S. general population norms. Significance between groups by one- way ANOVA.
Results: 1373 individuals completed the questionnaire (response rate 55%). Responders were 50% male, mean age 49 years, diabetes duration 25 years, HbA1c 61.7±12.7 mmol/mol, 48% on lipid-lowering medication, 10% smokers, 66% had retinopathy and 26% on insulin pump therapy. Non-responders were younger (41 years), more often male (61%), fewer were on lipid-lowering medication (37%), HbA1c 62.2±15.1 mmol/mol, 15% smokers. In the overall cohort PCS was 50.22±9.42 and MCS 48.83±11.16. The overall scores did not differ between individuals using insulin pump therapy (n=356) or multiple daily injections (n=994), PCS 50.32±9.50 and 50.13±9.38 (p=0.74), MCS 48.86±10.41 and 48.87±11.56 (p=0.99), respectively. Figure 1 shows the results by group of glycaemic control, HbA1c below 52 (n=284), HbA1c 52-70 (n=806) and HbA1c above 70 mmol/mol (n=283).
Conclusion: This nation-wide study of individuals with T1D shows that poor glycaemic control is associated with lower health-related quality of life with lower scores of both physical and mental health and with the lowest scores for the general health and vitality dimensions. NDR is validating a new instrument which may facilitate further investigation of these findings.

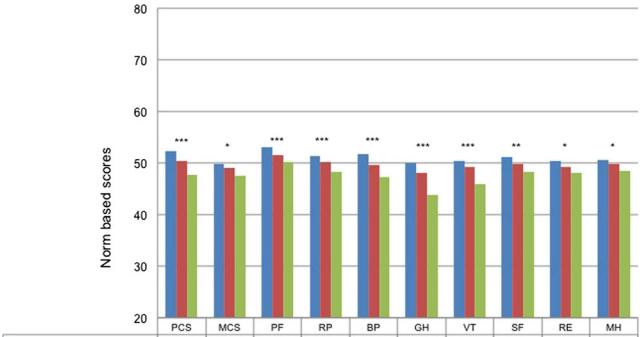

Figure 1. Norm based SF-36v2 scores at different levels of glycaemic control in individuals with type 1 diabetes. Significance between groups by one-way ANOVA * p<0.05, **p<0.01, ***p<0.001. PCS: Physical component summary, MCS: Mental component summary, PF: Physical function, RP: Role physical, BP: Bodily pain, GH: General health, VT: Vitality, SF: Social function, RE: Role emotional and MH: Mental health

Disclosure: **K. Eeg-Olofsson:** None.

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Doubled healthcare costs of type 2 diabetes mellitus during years 2006-2014: a nationwide cost-of-illness study in Sweden

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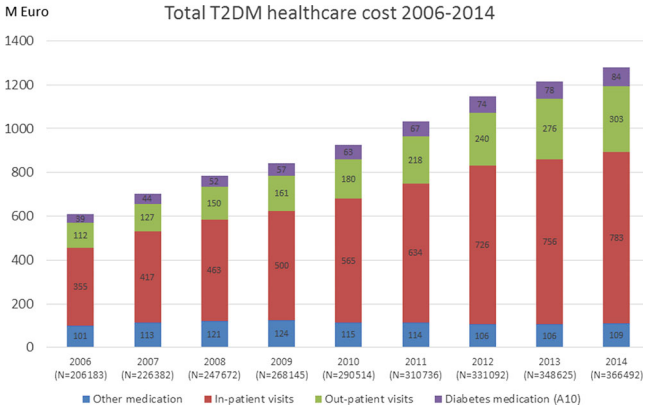
Background and aims: There is a lack of longitudinal studies estimating the total costs of type 2 diabetes (T2DM) in Sweden. The aim of this study was to examine changes in the total costs related to T2DM and potential reasons for changes in costs.
Materials and methods: All T2DM patients in Sweden who were dispensed any glucose-lowering drug (GLD) during years 2006—2014 were identified in the mandatory national Prescribed Drug Registry. Annual hospital admissions, discharges and hospital outpatient visits were extracted in the National Patient Register using ICD- and DRG codes for each occasion. A prevalence-based cost of illness-study was performed evaluating total costs of medication and healthcare consumption. The price level and exchange rate of 2014 (1 Euro= 9.10 SEK) was applied.
Results: The number of GLD-treated patients increased throughout the observation period (n= 206,183 in 2006 to n=366,492 in 2014). Patient mean age in 2014 was 67 years and 43% were female. The total cost for all T2DM patients in Sweden doubled over the period, from €608 million in 2006 to €1.279 billion in 2014, mainly due to the increased prevalence of patients. An increase in costs was also observed when estimating the total cost per patient, from €2,947 in 2006 to €3,490 in 2014 (+18%). The main driver in cost per patient was substantially increased costs for hospital care; from 77% of total costs in 2006 to 85% in 2014. Remaining costs were represented by medication costs, with costs for anti-diabetics (A10) accounting for 6% of total costs throughout the observation period. The same distribution occurred in costs per patient. The number of T2DM-related inpatient care visits increased from 45,559 in 2006 to 78,245 in 2014. The share of patients visiting inpatient care was 22% and 21% at observation start and end, respectively. However, the length of stay in inpatient care decreased from an annual average of 13.3 days per patient in 2006 to 11.6 days per patient in 2014. At the same time, cost for treating the most common comorbidities (i.e. heart failure, chest pain, myocardial infarction and cerebral infarction) increased over the period. The utilization of hospital outpatient care also increased over the period, from 105,653 visits in 2006 to 209,417 visits in 2014, showing an increase in patients visiting hospital outpatient care from 51% to 57%, respectively.
Conclusion: Total costs for T2DM in Sweden have increased substantially over the observation period 2006-2014, mainly due to increased costs for inpatient and hospital outpatient care. Antidiabetic drugs represented only a small portion of total costs, and this portion has remained stable during the recent years. Prevention of diabetes development and complications is of major importance for reducing future health care expenditures.



Supported by: AstraZeneca
Disclosure: **A. Kalkan:** Study sponsor: AstraZeneca.

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Direct medical costs of type 2 diabetes in Singapore (2011-2014) - a multiethnic Asians population with high prevalence of diabetes

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Background and aims: Singapore is a Southeast Asian country with three major ethnic groups: Chinese 74.2%, Malays 13.3% and Indians 9.1%. The prevalence of diabetes mellitus was 11.3% in 2010. In Singapore, information on economic burden of DM is scarce although there is a rising trend of diabetes prevalence. We aimed to assess the direct medical costs of Type 2DM in Singapore and identify factors associated with the costs.
Materials and methods: This was a prevalence-based cost-of-illness study which analysed the direct medical costs of T2DM. It involved 1390 adult patients with T2DM who attended the Diabetes Centre in a local hospital in 2011-2014. Data on demographics, clinical characteristics, complications and treatment were obtained from questionnaire and clinical measurements. Information on complications was further obtained from international classification codes for inpatient admissions using hospital administrative data. Annual medical costs for inpatient admissions, outpatient visits and emergency visits were assessed using hospital administrative data. Backward stepwise multiple linear regression was used to examine the relationship between annual direct medical costs and patient characteristics, adjusted for age, race and diabetes duration. Costs were expressed in year 2014 Singapore dollars. Consumer price index was used to estimate values older than 2014.
Results: The mean annual direct medical costs per patient rose by about 20% from S$2281 (€1418) in 2011 to S$2727 (€1695) in 2014. Inpatient costs and outpatient costs accounted for 40-47% and 47-53% of the mean annual direct medical costs respectively.

Investigations constituted the largest proportion of mean annual outpatient costs (33-37%), followed by medication and doctor visit (24-27% for both). Patients with neurological, eye, peripheral vascular disease and renal complications had 4.8, 3.2, 3.0 and 3.0 times higher direct medical costs than individuals free of any complications. Multiple linear regression revealed that higher haemoglobin A1c (HbA_{1c}), usage of insulin, usage of both insulin and oral hypoglycemic agent, neurological, renal and eye complications were independently associated with higher log-transformed direct annual medical costs, having adjusted for age, race and diabetes duration. For every unit increase in HbA_{1c}, there was 20% increase in direct medical costs. The direct medical cost was 379% and 74% higher for patients on insulin and combination of insulin and oral hypoglycemic than for those not on any medication. Patients with neurological, renal and eye-specific complications had 317%, 145% and 119% higher cost than those without the respective complications.

Conclusion: The keys to reducing direct medical costs appear to be optimizing metabolic control of diabetes, early screening and prevention of DM-related complications. The study also revealed the key drivers of the cost utilization (e.g. investigations). The findings provide useful reference for future economic modeling and intervention studies.

Supported by: Singapore National Medical Research Council Grant PPG/AH(KTPH)/2011

Disclosure: **S.K.M. Low:** None.

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Poor socio-economic status equals poor glycaemic control? Data from the Dutch Diabetes Pearl cohort

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Background and aims: Indicators of socio-economic status (SES), such as education and occupation, have been associated with glycemic control in people with type 2 diabetes (T2DM). Until now, studies predominantly focused on primary care populations. The aim of this study was to examine the association between SES variables and glycemic control in people with T2DM from primary to tertiary care in the Netherlands.

Materials and methods: The Dutch Diabetes Pearl is a large-scale cohort of people with T2DM from 2 primary care settings and 6 academic hospitals centers in the Netherlands. The SES factors included self-reported level of education and self-reported occupation, classified according to the ISCO-08 score. Glycemic control was measured as levels of HbA1c and fasting glucose. We used multivariate linear regression analyses to study the association between SES and glycemic control, and corrected for age, sex, BMI, recruitment center, diabetes medication use, and diabetes duration.

Results: In total, 6666 people with T2DM were included in our cohort, with 60% male, mean age of 62.3±10.3 years, and 39% in tertiary care. Preliminary analyses showed that compared to people with no education, HbA1c levels were lower for people with primary/secondary education (-2.4 mmol/mol (-4.1;-0.6)), vocational/pre-university (-3.5 mmol/mol (-5.3;-1.7)), and (professional) university education (-3.2 mmol/mol (-5.1;-1.3)). Also, compared to people with the lowest ISCO level (no job, housewives), HbA1c levels were lower for people with level of ISCO-1 (i.e. lunch ladies; -1.0 mmol/mol (-4.2;2.1)), ISCO-2 (i.e. secretaries; -1.7 mmol/mol (-4.6;1.3)), ISCO-3 (i.e. lab technicians; -2.2 mmol/mol (-5.2;0.8)), and ISCO-4 (i.e. professors; -2.4 mmol/mol (-5.4;0.6)). Similar associations were observed in subgroup analyses of people using insulin only, people with early onset of T2DM, and people with BMI >30. No statistically significant associations were observed between SES factors and fasting glucose.

Conclusion: This study showed that lower level of education was significantly associated with worse glycemic control in people with T2DM from primary to tertiary care in the Netherlands.

Disclosure: **A. Rutte:** None.

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The UK Civil Aviation Authority protocol to certify commercial pilots with insulin-treated diabetes: preliminary results of monitoring

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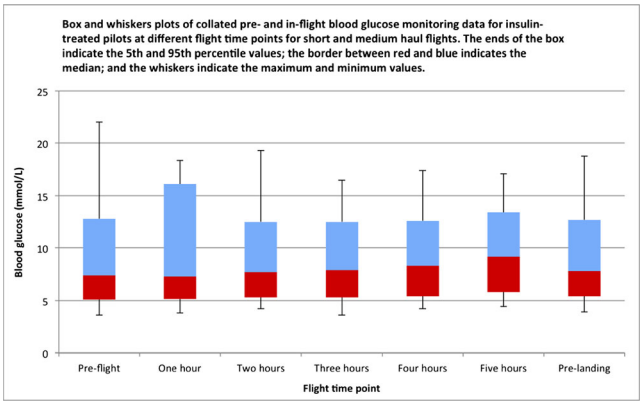
Background and aims: In 2012, the UK became the second country worldwide to issue insulin-treated individuals with Class 1 Medical Certificates for Commercial Pilot Licences (CPLs). The UK now has the largest cohort of insulin-treated pilots, and is leading the way in Europe and beyond to create and maintain employment and leisure opportunities for people with insulin-treated diabetes. A comprehensive protocol, developed by a panel of medical and aviation experts, governs the medical certification of insulin-treated pilots. Certified pilots are subject to strict requirements, directly overseen by the UK Civil Aviation Authority (CAA) medical department, including pre- and in-flight blood glucose monitoring. This study aimed to evaluate the early experience and safety of the UK programme.

Materials and methods: With the pilots' consent, the UK CAA-held medical files for all insulin-treated, Class 1-certified pilots were reviewed and data was collected. This included: age; date of issue of Class 1 Medical Certificate; diabetes type and duration; diabetes management regimen; comorbidities; diabetes complication monitoring; all available HbA1c values pre- and post-licence issue; and all flights undertaken with associated blood glucose monitoring values. Average pre- and post-licence HbA1c values were compared. Pre and in-flight blood glucose monitoring values were correlated against the CAA-specified "Green" (5-15mmol/l), "Amber" (4-5 and 15-20mmol/l), and "Red" (<4 or >20mmol/l) ranges.

Results: At the analysis date, 26 insulin-treated pilots had been issued with Class 1 medical certificates. All were male, with an average age of 40.7 years. The majority (84.6%) had type 1 diabetes, with an average diabetes duration of 8.1 years. Average follow up duration post-licence issue was 19.5months. The average pre-licence issue HbA1c was 53.1mmol/mol (95%CI 49.7- 56.5mmol/mol); the average of the most recent HbA1c was 54.8mmol/mol (95%CI 50.9-58.8mmol/mol). Paired t-test comparison between average pre- and post-licence issue HbA1c showed no significant change (p=0.25). 16 of the pilots had pre- and in-flight blood glucose monitoring data; cumulatively 8,897 blood glucose monitoring values had been recorded during 4,900 flight hours. For short and medium haul flights (<6hours), 95.8% of 7,829 blood glucose monitoring readings were within the 'green' range. For long haul flights (>6hours), 96.9% of 1,068 readings were within the 'green' range. A total of 19 (0.2%) readings were in the 'red' range. No pilot medical incapacitation due to low or high blood sugar has been reported.

Conclusion: A growing number of insulin-treated pilots have successfully applied for CPLs in the UK. To date, the CAA protocol has shown to work well in the cockpit, with no reported safety concerns, and without deterioration of diabetes control.



Disclosure: **J.L. Hine:** None.

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Overuse and underuse of acetyl-salicylic acid for primary prevention of cardiovascular disease in people with diabetes

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HealthPartners Institute, Minneapolis, USA.

Background and aims: Acetyl-salicylic acid use is recommended for primary prevention of atherosclerotic vascular disease (ASCVD) for people with and without diabetes when the ASCVD benefit outweighs the risk of gastrointestinal hemorrhage. In a primary care setting, the complexity and time required to assess acetyl-salicylic acid benefits and risks can result in inappropriate acetyl-salicylic acid use either through overuse or underuse. The objective of this analysis is to assess the appropriateness of acetyl-salicylic acid use for primary prevention in diabetes and other high ASCVD risk patients in a large primary care setting with good electronic health record acetyl-salicylic acid documentation.
Materials and methods: As part of an NIH-funded study to lower ASCVD risk, we successfully implemented electronic clinical decision support (CDS) algorithms to encourage appropriate acetyl-salicylic acid use. The algorithms recommended acetyl-salicylic acid if ASCVD risk scores were high and consistent with benefit greater than GI bleed risk using criteria from the United States Preventive Services Task Force; and acetyl-salicylic acid was not recommended if the ASCVD benefit was low or if major contraindications were identified (anticoagulant use or history of intracerebral hemorrhage). Providers were also alerted to the presence of other potential acetyl-salicylic acid risks including acetyl-salicylic acid allergy or intolerance, history of GI bleed risk conditions, and concomitant use of nonsteroidal antiflammatory drugs. Baseline study data was collected for whether acetyl-salicylic acid was algorithmically recommended for all patients at their first eligible primary care encounter in 20 clinics over 2012-2014. The analysis excluded patients with CHD and included 6065 adults with diabetes (mean age 55.6, mean 10-year ASCVD risk 27.9%) and 10,165 adults meeting pre-specified criteria for high ASCVD risk without diabetes (mean age 58.4, mean 10-year ASCVD risk 24.6%). Overuse and underuse was determined by comparing concordance with (a) acetyl-salicylic acid algorithm recommendations and (b) documented acetyl-salicylic acid use.
Results: For the targeted population with high CV risk, the CDS recommended acetyl-salicylic acid for 3,842 (63.3%) patients with diabetes and 7,552 (74.3%) without diabetes. Among patients with acetyl-salicylic acid recommended, acetyl-salicylic acid was underused in 761(19.8%) with diabetes and 5638 (74.4%) without diabetes. Among patients for whom the CDS did not recommend acetyl-salicylic acid, acetyl-salicylic acid was overused in 1322 (59.5%) with diabetes and 883 (33.8%) without diabetes.
Conclusion: In this large primary care setting, acetyl-salicylic acid was more likely to be overused than underused for patients with diabetes. Those with diabetes who were likely to benefit from acetyl-salicylic acid use had higher acetyl-salicylic acid use rates than similar high CV risk patients without diabetes. However, those with diabetes who were unlikely to benefit from acetyl-salicylic acid (risks greater than benefit) also had higher rates of acetyl-salicylic acid overuse compared to patients without diabetes. Strategies to ensure greater evidence-based use of acetyl-salicylic acid, such as providing electronic clinical decision support, may help providers more accurately assess individualized risks and benefits of acetyl-salicylic acid.
Clinical Trial Registration Number: NCT01420016
Supported by: NHLBI/R01HL102144
Disclosure: **A.L. Crain:** None.

OP 13 Pills or surgery for weight loss?

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Durable diabetes remission after bariatric surgery is associated with reduced incidence of microvascular events

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Background and aims: Bariatric surgery leads to diabetes remission and reduces the incidence of diabetes complications in many obese patients with type 2 diabetes (T2D). We have examined the importance of durable diabetes remission for the prevention of microvascular complications.
Materials and methods: The prospective, matched Swedish Obese Subjects (SOS) study examines long-term outcomes after bariatric surgery. Patients with baseline T2D (n=343) treated by bariatric surgery (banding, n=61; vertical banded gastroplasty, n = 227; gastric bypass, n = 55) were studied. Age was 48.7±5.9 years, BMI was 42.1±4.7, and median follow up time was 19 years. Microvascular events (retinopathy, nephropathy and neuropathy, whichever came first) were traced in nationwide registers. We considered a patient to have T2D if he or she reported the use of diabetes medication or if fasting blood glucose concentration was 110 mg per deciliter (6.1 mmol per liter) or higher.
Results: At the follow up examination 15 years after bariatric surgery, 30% of the patients were in diabetes remission. Patients who were in remission after 15-years had significantly lower incidence of microvascular events compared those who were not in diabetes remission (incidence rates of 8.0 and 26.0 per 1000 person-years, adjusted HR 0.19 [95% CI; 0.07-0.50], p=0.001.
Conclusion: We conclude that in patients treated by bariatric surgery who experienced long-lasting remission the risk of microvascular diabetes complications was reduced by over 80% compared to those that were not in remission at the 15-year follow up.
Clinical Trial Registration Number: NCT01479452
Supported by: NIH, Swedish Research council, NIDDKD
Disclosure: **M. Taube:** None.

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Combined actions of PYY and GLP-1 contribute to weight loss after Roux-en-Y gastric bypass

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Background and aims: Exaggerated secretion of the appetite-inhibiting gut-hormones, glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), may explain appetite reduction and the substantial weight loss seen after Roux-en-Y gastric bypass (RYGB), but causality has not been established. GLP-1 actions are effectively blocked by the specific GLP-1 receptor (GLP-1R) antagonist Exendin 9-39 (Ex-9), while PYY actions can be inhibited by administration of a dipeptidyl-peptidase 4 (DPP-4)-inhibitor preventing conversion of secreted PYY_{1-36} to the active version PYY_{3-36}. We hypothesized that both GLP-1 and PYY_{3-36} reduce appetite post-RYGB and investigated the individual and combined effects of Ex-9 and DPP-4 inhibition on *ad libitum* food intake.
Materials and methods: In a placebo-controlled, randomized, cross-over design, 12 patients with normal glucose tolerance (age: 35.4±7 (mean ±SEM) years, sex (f/m): 8/4, BMI: 33.5±6 kg/m^2) were studied 5±1 month after RYGB with standard mixed meal test (356 kcal, 53E% carbohydrate, 33E% fat, 14E% protein) followed 4 hours later by an *ad libitum* meal consisting of pasta bolognese (energy content 533kJ/100g, 53E %carbohydrate, 33E% fat, 14E% protein). On 4 separate experimental days, patients received: 1) placebo, 2) Ex-9 infusion to block the GLP-1R (900 pmol/kg/min), 3) the DPP-4 inhibitor sitagliptin (sita) to reduce formation of PYY_{3-36}, or 4) sita and Ex-9 (sita/Ex-9) to inhibit both PYY_{3-36} and GLP-1 simultaneously. Data were analyzed by use of linear mixed effects models.
Results: Combined administration of sita/Ex-9 increased *ad libitum* food intake ≈20% vs. placebo, whereas no effect was seen with sita or Ex9 alone (*Ad libitum* food intake: placebo: 245±22; Ex-9: 264±30, p=0.38; sita: 252±20, p=0.71; sita/Ex-9: 290±36 grams, p=0.04). Ex-9 markedly increased PYY_{3-36} concentrations compared with placebo conditions ($p<0.01$), whereas significantly decreased concentrations was seen during concurrent administration of sita/Ex-9 compared with Ex-9 alone ($p<0.01$) ($AUC_{PYY3-36}$: placebo: 1219±143, Ex-9: 2263±208, sita: 325 ±58, sita/Ex-9: 823±203 pmol ×L^{-1}×min). Intact GLP-1 increased during Ex-9 ($p<0.01$), sita ($p<0.05$) and even more during sita/Ex-9 ($p<0.01$) ($AUC_{intact\ GLP-1}$: placebo: 598±107, Ex9:1391±286, sita: 1759±332, sita/Ex-9: 4353±545 pmol×L^{-1}×min).
Conclusion: Combined blockade of actions from the two L-cell hormones GLP-1 and PYY increased *ad libitum* food intake by ≈20% in RYGB-operated patients, whereas neither GLP-1R blockage nor DPP-4 mediated lowering of PYY_{3-36}-formation affected food intake by itself. Notably, GLP-1R blockage alone markedly augmented concentrations of PYY_{3-36}, while DPP-4 inhibition alone greatly increased intact GLP-1. Thus concomitantly increased secretion of the other L-cell hormone might counteract effects on food intake and explain why Ex-9 or DPP-4 alone did not affect food intake. These results support that combined effects of GLP-1 and PYY_{3-36} play a role in decreased appetite and weight loss after RYGB and may implicate that enhancing L-cell hormone actions simultaneously can be an effective non-surgical treatment of obesity.
Clinical Trial Registration Number: NCT02336659
Supported by: University of Copenhagen
Disclosure: **M.S. Svane:** None.

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Safety- and efficacy-outcomes of the duodenal-jejunal bypass liner (DJBL) registry in obese patients with type 2 diabetes

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Background and aims: Obesity and type 2 diabetes mellitus are widespread and cost-intensive diseases. Current conservative concepts regularly fail to reach therapeutic targets. Even though bariatric surgery has proven to be effective, it is not suitable for all patients.
A less invasive approach for treatment of obesity and type 2 diabetes mellitus is represented by the temporary endoscopic duodenal-jejunal bypass liner (DJBL). This device consists of a self-expanding ring armed with barbs which is endoscopically anchored in the duodenal bulb, and an adapted impermeable Teflon liner covering 60 cm of the proximal jejunal mucosal resorption area, thereby mimicking Roux-en-Y gastric bypass. Up to now, small prospective studies have demonstrated promising improvements of diabetes control and loss of bodyweight. However, long-term safety and efficacy have not been investigated in a sufficiently large cohort of patients. Collecting these data is the aim of a newly established DJBL registry.
Materials and methods: Long term data were collected by approximately 30 centers. An electronic Case-Report-Form was designed for documentation of a pre-specified dataset. As of October 2015, 201 patients had been included in the registry and first preliminary efficacy and safety data

were obtained. Statistical analyses were performed by an independent statistician using t tests for continuous variables.

Results: The mean age at baseline was 50.9 years. 74 out of 201 patients were male, baseline BMI was 42.8 kg/m2. Baseline HbA1C was 8.36%, and mean blood pressure 139/81 mmHg. 20.7% of patients were treated with GLP-1-analogs and 95.8% received insulin (mean insulin dose: 90.8 IU/day). In 114 patients data were available at time of im- and explantation. A significant reduction was seen for weight, excess body weight, BMI, HbA1C, total cholesterol, LDL-cholesterol, and systolic blood pressure (-15 kg, -15.3 kg, -5.4 kg/m2, -1.47%, -28 mg/dl, -28 mg/dl and -6 mmHg, respectively). Mean insulin dose was reduced by 42.1 IU per day (all $p<0.05$). In addition a slight but not significant reduction was seen for diastolic blood pressure (3.3mmHg), triglycerides (41mg/dl). Moreover, a substantial proportion of patients who could reduce oral antihyperglycemic treatment (-33.7%), GLP-1-analogs (-56.5%), antihypertensive treatment (-14.6%), and lipid-lowering agents (-23.1%) was observed. In 31.8% of patients, side effects were documented: Abdominal pain was reported in 13.4%, nausea and vomiting in 8%, gastro-intestinal bleeding in 2%, and liver abscesses in 2%. Other side-effects were rare.

Conclusion: The DJBL registry for the first time provides substantial evidence on the value of the DJBL as a potentially new antidiabetic- and antiobesity-treatment in the largest cohort of diabetes patients investigated so far. Almost all patients are type 2 diabetic patients recieving high doses of insulin. Reductions in weight and HbA1C are beyond those previously published in clinical studies. Insulin doses were reduced by almost 50%. These data underline the importance of patient selection for DJBL treatment. However, side effects and long-term outcome of the DJBL need further investigation. We will present updated results and extended analyses supplemented by a prospective, 2:1-matched-pair, observational analysis of patients with diabetes on intensive antihyperglycemic treatment.

Supported by: GI Dynamics

Disclosure: **N. Sauer:** Grants; Establishment of the registry was supported by an unrestricted grant from GI Dynamics Inc., Lexington, MA, USA (GID).

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Coadministration of canagliflozin and phentermine for weight management in overweight and obese adults

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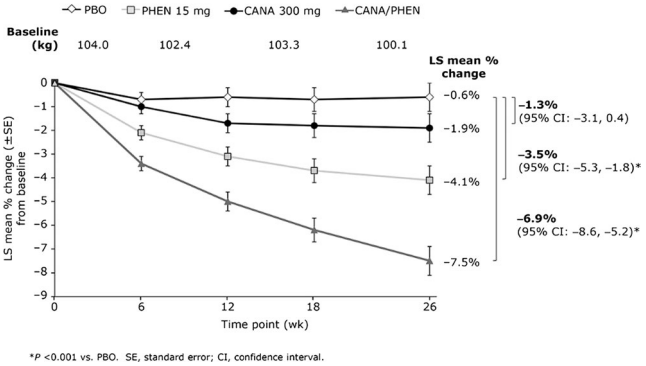
Background and aims: Canagliflozin (CANA) is an SGLT2 inhibitor approved for type 2 diabetes mellitus (T2DM) treatment that increases urinary glucose excretion and provides a calorie deficit, leading to weight loss that has plateaued over 26 weeks in T2DM studies. As CANA may cause increased calorie intake, adding phentermine (PHEN), an appetite suppressant, may facilitate further weight loss. This study evaluated the efficacy and safety of CANA combined with PHEN in overweight and obese adults without T2DM.

Materials and methods: This 4-arm, 26-week, Phase 2 study included adults without T2DM who had BMI 30 to <50 kg/m^2 or, if with hypertension and/or dyslipidaemia, BMI 27 to <50 kg/m^2. Patients (N = 334; mean weight, 102.9 kg; BMI, 37.3 kg/m^2) received CANA 300 mg + PHEN 15 mg (CANA/PHEN), PHEN 15 mg, CANA 300 mg, or placebo (PBO).

Results: At Week 26, weight loss was statistically superior with CANA/PHEN versus PBO (P <0.001; Figure). CANA/PHEN, PHEN 15 mg, CANA 300 mg, and PBO produced weight changes of -7.5%, -4.1%, -1.9%, and -0.6%, respectively. Significantly more patients achieved ≥5% weight loss with CANA/PHEN versus PBO (66.7% vs 17.5%; P <0.001). CANA/PHEN also resulted in a significant PBO-subtracted reduction in systolic BP (-4.2 mmHg; P = 0.015). CANA/PHEN was generally well tolerated, with no new or unexpected safety signals relative to CANA or PHEN. CANA/PHEN, PHEN 15 mg, CANA 300 mg, and PBO were associated with changes in heart rate of +3.5, +4.1, +0.7, and -0.7 bpm, respectively.

Conclusion: CANA/PHEN provided significantly greater weight loss versus PBO in overweight and obese adults, suggesting its potential use in chronic weight management.

Figure. Percent change in body weight over 26 weeks.



*P <0.001 vs. PBO. SE, standard error; CI, confidence interval.

Clinicla Trial Registration Number: NCT02243202

Supported by: Janssen Research & Development, LLC

Disclosure: **N. Erondu:** Employment/Consultancy; Janssen Research & Development, LLC.

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Comparable efficacy and safety of liraglutide 3.0 mg for weight management across baseline BMI subgroups: results from the SCALE obesity and prediabetes trial

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Background and aims: This 3-year trial investigated the effects of liraglutide 3.0 mg, as an adjunct to diet and exercise, in delaying the onset of type 2 diabetes mellitus (T2DM; primary endpoint) in adults with prediabetes and obesity (BMI ≥30 kg/m^2), or overweight (≥27 kg/m^2) with comorbidities of dyslipidaemia and/or hypertension. Here we evaluate glycaemic control endpoints and overall safety profile in 4 BMI subgroups post hoc.

Materials and methods: Individuals were randomised 2:1 to once-daily subcutaneous liraglutide 3.0 mg or placebo, with a 500 kcal/day deficit diet and 150 min/week physical activity. They were divided into the following 4 baseline BMI subgroups: 27-29.9 (n=62), 30-34.9 (n=624), 35-39.9 (n=737) and ≥40 kg/m^2 (n=831). Efficacy data are from an ANCOVA, with the last observation carried forward for missing values.

Results: Baseline characteristics of the 2254 randomised individuals were (mean±SD): age 47.5±11.7 years, 76.0% female, weight 107.6±21.6 kg, BMI 38.8±6.4 kg/m^2, HbA1C 5.7±0.3%. While on treatment, 26 individuals in the liraglutide group and 46 in the placebo group (3% vs 11%) developed T2DM over 3 years. No significant interactions between

treatment and baseline BMI subgroup were observed for body weight or glycaemic control endpoints (Table), indicating consistent treatment effects across the 4 BMI subgroups. Total adverse events and serious events, as well as gastrointestinal and hypoglycaemic events, had similar incidences across BMI subgroups. Low rates of confirmed pancreatitis and neoplasms were seen across the subgroups.

Conclusion: Continued treatment with liraglutide 3.0 mg as compared with placebo, both as an adjunct to diet and exercise, consistently improved glycaemic control endpoints and beta-cell function, and enhanced insulin sensitivity across BMI subgroups.

Changes in body weight and glycaemic control parameters

Mean changes from baseline at 160 weeks	BMI 27-29.9 Liraglutide / placebo N=39 / 23	BMI 30-34.9 Liraglutide / placebo N=415 / 912	BMI 35-39.9 Liraglutide / placebo N=480 / 243	BMI ≥40 Liraglutide / placebo N=538 / 280	P value
Body weight,% relative change	-5.7 / -1.8	-6.5 / -1.7	-6.2 / -1.8	-5.9 / -2.1	0.48
Body weight, kg	-4.5 / -1.4	-5.8 / -1.6	-6.4 / -1.8	-7.3 / -2.6	0.97
HbA_{1c}, % points	-0.29 / -0.28	-0.34 / -0.14	-0.36 / -0.13	-0.36 / -0.14	0.06
FPG, mmol/L	-0.51 / -0.03	-0.35 / 0.07	-0.33 / 0.00	-0.41 / 0.09	0.55
Fasting insulin, % rel. change	-17.8 / -8.2	-4.9 / 2.3	-0.7 / 4.0	-16.6 / 0.6	0.31
HOMA-B, % relative change	14.4 / 7.0	19.8 / -1.1	23.2 / 5.1	6.7 / -2.2	0.50
HOMA-IR, % relative change	-25.0 / 3.5	-10.4 / 4.3	-7.8 / 3.2	-22.3 / 2.2	0.59
2h OGTT PG AUC, h*mmol/L	-3.6 / -1.3	-2.6 / -0.06	-2.6 / -0.80	-2.3 / 0.43	0.97
% with normoglycaemia	67 / 36	67 / 34	70 / 40	63 / 33	0.92

P values in the end column are for interaction between treatment and BMI subgroup (expressed in kg/m^2). Data are estimated means.
HOMA-B: beta-cell function; HOMA-IR: insulin resistance; rel.: relative

Clinical Trial Registration Number: NCT01272219
Supported by: Novo Nordisk A/S
Disclosure: **S. Madsbad:** Grants; Novo Nordisk. Honorarium; Novartis Pharma, Novo Nordisk, Merck Sharpe & Dome, Sanofi-Aventis, Astra Zeneca, Johnson & Johnson, Boehringer-Ingelheim, E. Lilly, Intarcia Terapeutics, Bristol-Meyer Squibb. Lecture/other fees; Novo Nordisk, Merck Sharpe & Dome, Astra Zeneca, Sanofi-Aventis, Novartis Pharma, E Lilly, Bristol-Meyer Squibb, Boehringer-Ingelheim.

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Beneficial effects of canagliflozin on energy metabolism and visceral fat volume through a possible mechanism of fatty acid oxidation in an animal model of obese type 2 diabetes

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Background and aims: A sodium glucose co-transporter 2 (SGLT2) inhibitor such as canagliflozin (CANA) brings about the exacerbation of urinary glucose excretion and weight reduction due to caloric waste into urine. However, in clinical practice some patients treated with SGLT2 inhibitor shows weight reduction with no improvement of glycemic status, suggesting another mechanism for weight loss of this agent. Spontaneously Diabetic Torii (SDT) fatty rat exhibits hyperphagia, obesity associated with hyperglycemia. In the present study, we plan to investigate effects of CANA on body composition and energy metabolism including fat oxidation in this obese type 2 diabetic model.

Materials and methods: Six-week-old male SDT-fatty (Fa) rats and Sprague-Dawley (SD) rats fed control chow diet were treated by either CANA (10 mg/kg) or saline (vehicle) orally for 2 weeks. After 2 weeks, they were moved individually into acrylic metabolic chambers for the measurement of respiratory gas and the calculation of respiratory quotient (RQ). Then visceral and subcutaneous fat volumes were measured using in vivo micro-computed tomography. After the completion of anthropometric measurement, rats were sacrificed and samples of blood and liver tissue were collected. Blood glucose concentrations were measured with a handheld glucose meter immediately after blood sampling. From liver tissue samples, the expressions of fat oxidation-related enzymes were measured by RT-PCR.

Results: In Fa rats, marked hyperglycemia (28.3±0.5 mM, mean ±S.E.) was observed at 6 weeks old. By the administration of CANA, blood glucose level was reduced to 11.5±0.4mM after 2 weeks. Urine volume and sugar excretion were increased even after a single administration of CANA (40.7±0.2 ml, 3.1±0.3 g/day, respectively), and kept to be high until the end of experiments. Two weeks of CANA treatment brought about a significant reduction of visceral fat volume in Fa rats compared to vehicle-treated group (31 ±1 vs. 42±1%, $P<0.01$). An obvious diurnal rhythm of RQ was observed in CANA-treated Fa rats similar to that in SD rats, although RQ rhythm completely disappeared in vehicle-treated group (Figure). CANA treatment significantly ($P<0.05$) enhanced expressions of fat oxidation-related enzymes such as AMPK, CPT-1 and suppressed lipogenic enzyme such as ACC in the liver.

Conclusion: It was demonstrated that CANA directly affected and reduced visceral fat amount in an obese diabetic model, based on a lower RQ during light phase and its mobilization of enzymes associated with fat oxidation in the liver. It is thus plausible that CANA causes a potential shift of fuel resource into fat oxidation and a facilitation of it. A novel mechanism of CANA prompts the possibility that this new class of anti-diabetic agent could be a promising anti-obesity agent as well.

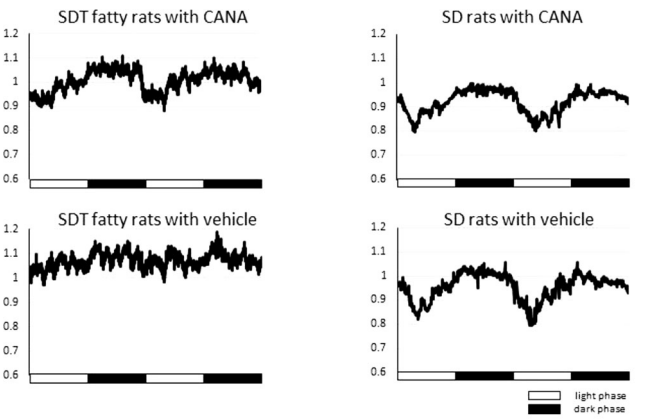

Figure. RQ in SDT fatty and SD rats treated by either CANA or vehicle for 2 weeks.

Supported by: Mitsubishi Tanabe Parma Co., Ltd.
Disclosure: **H. Mifune:** None.

OP 14 Hypoglycaemia: the low down

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Increased thalamic activation during hypoglycaemia in type 1 diabetes is associated with reduced adrenaline responses but preserved symptoms

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Background and aims: The effect of diabetes on counterregulatory responses to hypoglycaemia is well described however central correlates are less clearly understood. We sought to investigate the effect of hypoglycaemia on regional brain activation in healthy controls (HC) and subjects with type 1 diabetes (T1D) and good awareness of hypoglycaemia. Regional changes in cerebral perfusion were measured as a surrogate marker of brain activity, using arterial spin labelling (ASL) functional magnetic resonance imaging (fMRI).
Materials and methods: 15 controls, (age 40±11.3 years, 7 male) and 15 patients with T1D and intact hypoglycaemia awareness (age 39±13.5 years, HbA1C 7.6±1.0%, disease duration 24.0±12.8 years, Gold score ≤2, 6 male) underwent a hyperinsulinaemic (1.5 mU kg-1 min-1), two-step glucose clamp inducing sequential euglycaemia and hypoglycaemia in a 3 Tesla MRI scanner. Two scans were obtained at both euglycaemia (5 mmol/L) and hypoglycaemia (2.6 mmol/L). After each scan, adrenaline samples were taken and subjects quantified autonomic and neuroglycopenic symptoms on visual analogue scales. Each ASL scan provided a whole brain cerebral blood flow (CBF) map. Statistical parametric analysis of the images was performed and a 2x2 flexible factorial ANOVA used to examine the effect of diabetes. Post-hoc analyses of key regions of interest (ROI) were performed.
Results: During hypoglycaemia, autonomic and neuroglycopenic symptoms increased significantly in both groups (mean total symptom score HC; euglycaemia 10.1±3.7, hypoglycaemia 17.1±5.7 $p<0.0005$ and T1D; euglycaemia 9.4±3.5, hypoglycaemia 18.7±5.4 $p<0.0005$) and similarly (Δsymptom score HC vs T1D $p=0.25$). Preliminary analysis showed peak adrenaline responses were attenuated in T1D (HC n=6, 7.5±4.7nmol/L; T1D n=7, 3.0±1.5 $p=0.036$). Main effect of hypoglycaemia flexible factorial analysis across the whole brain showed no significant differences between the two groups' responses to hypoglycaemia. Mixed ANOVA ROI analyses demonstrated significant increases in regional (r)CBF in both groups in the thalamus ($p<0.0005$), anterior cingulate cortex ($p=0.001$), lateral orbitofrontal cortex (right $p<0.0005$, left $p=0.008$), right anterior insula ($p=0.006$), posterior cingulate ($p=0.002$), precuneus ($p=0.001$) dorsolateral prefrontal cortex (right $p<0.0005$, left $p=0.011$), and decreases in the left hippocampus ($p=0.004$). Interaction ROI analyses revealed significantly greater left thalamic rCBF in T1D ($p=0.028$).
Conclusion: These data demonstrate non-diabetic controls and T1D subjects with good hypoglycaemia awareness exhibit comparable cerebral and symptomatic responses to hypoglycaemia, despite a reduced adrenaline response in the T1D group. Activation of brain regions involved with interoception, perception of stressful stimuli and cortical function occurs in both groups, consistent with the similar symptom responses. Significantly greater left thalamic rCBF in T1D suggests that the thalamus, involved in alertness and relay of sensory stimuli, may have a role in modulating the perception of attenuated adrenaline responses, to preserve appropriate symptomatic awareness of hypoglycaemia in T1D subjects.
Supported by: Diabetes UK
Disclosure: **M. Nwokolo:** Grants; Diabetes UK.

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Cerebral blood flow response to hypoglycaemia is altered in patients with type 1 diabetes and impaired awareness of hypoglycaemia

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Background and aims: Impaired awareness of hypoglycemia (IAH) affects up to 30% of patients with type 1 diabetes (T1DM), which increases their risk of severe hypoglycemia. The mechanisms underlying the development of IAH remain to be resolved, but most certainly involves cerebral adaptations, including alterations in cerebral blood flow (CBF). The aim of the current study was to investigate the effect of hypoglycemia on both global and regional CBF in T1DM patients with IAH, as compared to patients with normal awareness (T1DM-NAH) and healthy controls (HC).
Materials and methods: Six T1DM-IAH subjects (3 males, age: 25±4 yrs, HbA1c: 7.5±0.3%), seven T1DM-NAH subjects (4 males, 26±2 yrs, 7.4±0.1%) and seven HC (3 males, 27±3 yrs) were enrolled. After an overnight fast, the subjects underwent a hyperinsulinemic euglycemic-hypoglycemic clamp, while lying in a 3T MR system. Global and regional changes in CBF were determined by pseudo-continuous arterial spin labeling (pCASL) MRI at three time points: just prior to initiating the glucose clamp (baseline), after 30 min of euglycemia and after 45 min of hypoglycemia. Global CBF was obtained by averaging the CBF in gray matter. The redistribution of regional CBF was evaluated by normalizing each CBF map to its global gray matter mean.
Results: Plasma glucose levels were 5.0±0.2 mmol/l and 2.8±0.1 mmol/l during the euglycemic and hypoglycemic phases, respectively. As expected, hypoglycemia generated typical symptoms in T1DM-NAH and HC, but not in T1DM-IAH. There was no change in global CBF between baseline and the end of the euglycemic phase in any of the groups. In response to hypoglycemia, global CBF increased in T1DM-IAH by 8±3% ($p<0.05$ versus euglycemia), tended to increase slightly in T1DM-NAH (5±2%, $p=0.08$), but did not change in HC (-2±2%, $p=0.70$). Furthermore, hypoglycemia altered the regional distribution of CBF. In HC and in T1DM-NAH, hypoglycemia increased regional CBF in the left and right thalamus ($p<0.05$). No such increase in thalamic CBF was present in T1DM-IAH. In T1DM-NAH, there was a further redistribution of regional CBF during hypoglycemia with a relative increase in the bi-lateral frontal lobes.
Conclusion: Hypoglycemia increased global CBF in patients with T1DM-IAH, but not in T1DM-NAH or HC, whereas the redistribution of CBF to the thalamus in the latter two groups was not observed in T1DM-IAH. We posit that the increase in global CBF may enhance nutrient supply to the brain, hence suppressing activation of brain areas involved in the autonomic response to hypoglycemia. This is in accordance with the blunted redistribution of CBF towards the thalamus in response to hypoglycemia in T1DM-IAH. Together these results suggest that changes in CBF may contribute to the development of IAH.
Clinical Trial Registration Number: NCT02146404
Supported by: Dutch Diabetes Research Foundation and EFSD
Disclosure: **E.C. Wiegers:** None.

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SWITCH 1: reduced risk of hypoglycaemia with insulin degludec vs insulin glargine U100 in patients with type 1 diabetes: a randomised, double-blind, crossover trial

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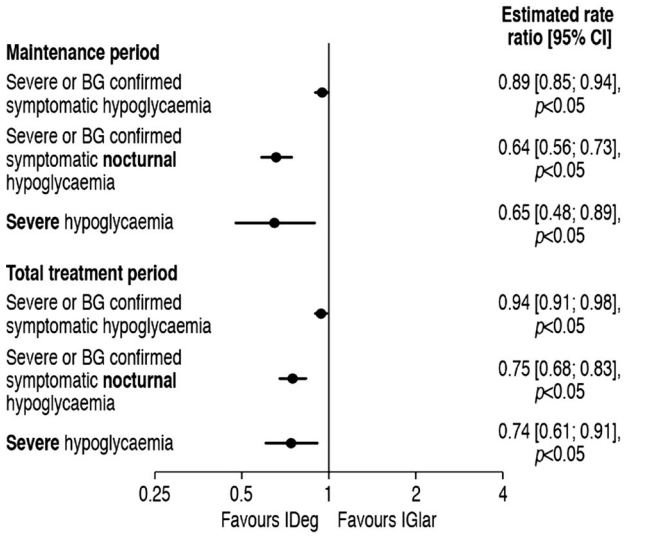
Background and aims: The SWITCH 1 trial aimed to compare the number of severe or blood glucose (BG)-confirmed (<3.1 mmol/L) symptomatic hypoglycaemic episodes in patients with type 1 diabetes (T1D), treated with insulin degludec U100 (IDeg) vs insulin glargine U100 (IGlar), both in combination with mealtime insulin aspart.
Materials and methods: In this 64-week, randomised, double-blind, treat-to-target crossover trial, 501 adults with T1D and ≥1 factor associated with increased risk of developing hypoglycaemia were randomised to once-daily IDeg or IGlar, both with mealtime insulin aspart for 32 weeks (comprising a 16-week titration period and a 16-week maintenance period), followed by crossover to IGlar or IDeg. The primary objective was to confirm non-inferiority in terms of the number of severe (requiring third-party aid, all externally adjudicated) or BG-confirmed (<3.1 mmol/L) symptomatic hypoglycaemic episodes during the maintenance periods.
Results: Treatment with IDeg vs IGlar resulted in an 11% significantly lower rate of severe or BG-confirmed symptomatic hypoglycaemia in the maintenance period; severe or BG-confirmed symptomatic nocturnal hypoglycaemia (00:01-05:59) was also significantly reduced by 36% for IDeg vs IGlar, and severe hypoglycaemia was significantly reduced by 35% with IDeg vs IGlar. Significant reductions for all three hypoglycaemia categories were also seen for the total treatment periods (Fig). In addition, IDeg was superior to IGlar regarding a lower proportion of patients experiencing severe hypoglycaemia during both the maintenance and total treatment periods (p=0.0016). The HbA_{1c} non-inferiority of IDeg vs IGlar was confirmed in both treatment periods (means, week 32: 6.95 vs 6.92%; week 64: 6.95 vs 6.97%). The rates of adverse events were similar for IDeg and IGlar.
Conclusion: In patients with T1D at increased risk of experiencing severe hypoglycaemia, IDeg was non-inferior in terms of HbA_{1c} reductions and significantly reduced the rates and proportions of severe hypoglycaemia and the rates of severe or BG-confirmed symptomatic overall and nocturnal hypoglycaemia vs IGlar.



BG, blood glucose (<3.1 mmol/L); CI, confidence interval; IDeg, insulin degludec U100; IGlar, insulin glargine U100.
P-values derived using a Poisson model with logarithm of the exposure time (100 years) as offset; estimates adjusted for treatment, period, sequence and dosing time as fixed effects and subject as a random effect.

Clinical Trial Registration Number: NCT02034513
Supported by: Novo Nordisk

Disclosure: **W. Lane:** Employment/Consultancy; Advisory panel: Novo Nordisk, Advisory panel: Insulet, Advisory panel: Thermalin, Consultant: Novo Nordisk, Consultant: Thermalin. Grants; Research support: Novo Nordisk, Research support: Eli Lilly. Lecture/other fees; Speakers' bureau: Novo Nordisk.

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SWITCH 2: reduced risk of hypoglycaemia with insulin degludec vs insulin glargine U100 in a type 2 diabetes population on basal insulin: a randomised, double-blind, crossover trial

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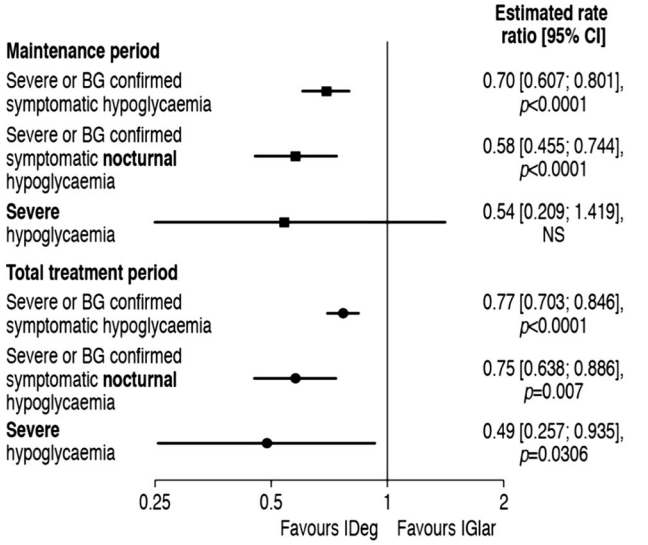
Background and aims: The aim of the SWITCH 2 trial was to compare the number of externally adjudicated severe or blood glucose (BG)-confirmed symptomatic hypoglycaemic episodes in patients with type 2 diabetes (T2D), treated with the long-acting basal insulin degludec U100 (IDeg) or with insulin glargine U100 (IGlar), both in combination with pre-trial oral antidiabetic drugs.
Materials and methods: In this 2x 32-week, randomised, double-blind, treat-to-target crossover trial, adults (n=721) with T2D were randomised 1:1 to once-daily IDeg/IGlar followed by a crossover to IGlar/IDeg. Each treatment period comprised a 16-week titration and a 16-week maintenance period. Patients included were previously treated with basal insulin with or without oral antidiabetic drugs excluding sulphonylurea/meglitinides, and had ≥1 factor associated with increased risk of developing hypoglycaemia. The primary endpoint was the number of severe (requiring third-party assistance and external adjudication) or BG-confirmed (<3.1 mmol/L) symptomatic hypoglycaemic events in the maintenance periods.
Results: Treatment with IDeg resulted in significantly lower rates of severe or BG-confirmed symptomatic hypoglycaemia and severe or BG-confirmed symptomatic nocturnal hypoglycaemia (occurring 00:01-05:59) vs IGlar in the maintenance and total treatment periods (Figure). The proportion of patients experiencing severe hypoglycaemia in the maintenance periods was 1.6% for IDeg vs 2.4% for IGlar (NS). The rate of severe hypoglycaemia was significantly lower with IDeg vs IGlar in the total treatment period (Figure). HbA_{1c} reductions with IDeg were non-inferior to IGlar. Adverse event rates were similar with IDeg and IGlar.
Conclusion: Compared with IGlar, IDeg resulted in a consistent reduction in hypoglycaemia in patients with T2D at an increased risk of developing hypoglycaemia.



BG, blood glucose (<3.1 mmol/L); CI, confidence interval; IDeg, insulin degludec U100; IGlar, insulin glargine U100; NS, not significant.
P-values derived using a Poisson model with logarithm of the exposure time (100 years) as offset; estimates adjusted for treatment, period, sequence and dosing time as fixed effects and subject as a random effect.

Clinical Trial Registration Number: NCT02030600
Supported by: Novo Nordisk
Disclosure: **C. Wysham:** Employment/Consultancy; Advisory panel: Astra Zeneca, Advisory panel: Boehringer Ingelheim, Advisory panel: Eli Lilly, Advisory panel: Sanofi, Advisory panel: Janssen, Consultant: Eli Lilly. Grants; Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Novo Nordisk, Sanofi. Lecture/other fees; Speakers' bureau: Astra Zeneca, Speakers' bureau: Boehringer Ingelheim, Speakers' bureau: Eli Lilly, Speakers' bureau: Janssen, Speakers' bureau: Novo Nordisk, Speakers' bureau: Sanofi.

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Hypoglycaemia is associated with increased risk of cardiovascular events: results from the EXAMINE trial

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Background and aims: Hypoglycemia is a known complication of some antidiabetic drugs (although not incretin-based therapies). The cardiovascular (CV) outcomes of patients experiencing hypoglycemia have not been well studied. We evaluated the consequence of reported hypoglycemia on the risk for subsequent major adverse CV events (MACE; CV death, nonfatal myocardial infarction or nonfatal stroke). Patients in the EXAMINE trial (N=5380) were at elevated risk for MACE due to baseline type 2 diabetes and acute coronary syndrome within the 15-90 days prior to study entry.

Materials and methods: EXAMINE patients were randomized to double-blind alogliptin or placebo in addition to standard antidiabetic treatment (adjusted throughout the trial). Most patients were men (68%), White or Asian (73%, 20% respectively) and the mean (SD) age was 61 (9.9) years. Metformin, sulfonylureas and insulin were commonly used at baseline (66%, 47% and 30% of patients, respectively).
Results: During the trial, 354 (6.6%) patients were reported to have hypoglycemia (6.7% with alogliptin and 6.5% with placebo); rates of serious hypoglycemia were low (0.7% with alogliptin and 0.6% with placebo). Using a Cox proportional hazards model adjusted for baseline covariates (age, sex, HbA1c, antidiabetic treatment) and study treatment, we found a significant increase in MACE among patients who developed serious hypoglycemia (12/34 [35.3%]) vs those who did not (609/5346 [11.4%]) (adj. HR: 2.42, 95% CI: 1.27-4.60; p=0.007). An increase in MACE was also found for patients with any hypoglycemia (64/354 [18.1%]) vs those without (557/5026 [11.1%]) (adj. HR: 1.38, 95% CI: 1.05-1.80; p=0.019).
Conclusion: Besides impairing cerebral function, hypoglycaemia may also have adverse effects on cardiovascular disease. Further research on the impact of treatment induced hypoglycemia on CV events is warranted.
Clinical Trial Registration Number: NCT00968708
Supported by: Takeda Development Center Americas
Disclosure: **S.R. Heller:** Employment/Consultancy; member of the EXAMINE steering Committee and has received personal fees from Takeda Development Center, Deerfield, IL. Honorarium; member of the EXAMINE steering Committee and has received personal fees from Takeda Development Center, Deerfield, IL.

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Differences in cognitions and behaviours between adults with type 1 diabetes with intact and impaired awareness of hypoglycaemia

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Background and aims: Impaired awareness of hypoglycaemia (IAH), seen in >25% of adults with type 1 diabetes (T1D), increases risk of severe hypoglycaemia (SH) 6-fold. IAH may be associated with unhelpful thinking styles, understanding which will help refine approaches to restore awareness. We compared hypoglycaemia-specific cognitions and behaviours of T1D adults with intact hypoglycaemia awareness (HA) with those with IAH.
Materials and methods: We recruited 148 T1D adults attending specialist clinics. Hypoglycaemia awareness was determined using Gold Score (≥4 = IAH). 117 had HA; 31 IAH. The IAH were supplemented with baseline data from 90 adults with IAH participating in the HypoCOMPaSS study. All completed the Attitudes to Awareness of Hypoglycaemia (A2A), a novel 19-item questionnaire defining beliefs around hypoglycaemia, rating items on a Likert scale, 0 (not true) to 3 (very true), and the Hypoglycaemia Fear Survey-II (HFS-II).

Results: Adults with IAH were older (mean±SD 46.7±13.0 vs 40.4 ±15.5 years, p=0.001), had longer T1D duration (29.0±12.7 vs 23.0 ±11.3 years, p<0.001) and were more likely to have had SH in the past year (41.5 vs 7.7%, p<0.001) vs those with HA. HbA1c did not differ (67.4 ±15.1 vs 70.8±19.1 mmol/mol, p=0.43). Those with IAH vs HA had higher A2A scores for beliefs that: they can function 'ok' with glucose <3mmol/l (median [IQR] score 1.0[0.0-2.0] vs 0.0[0.0-1.0], p<0.001); it is more important to avoid hyper- than hypo- glycaemia (1.0[0.0-2.0] vs 0.0[0.0-1.0], p=0.003); and optimal diabetes management is mainly about avoiding high blood glucose (2.0[1.0-3.0] vs 1.0[1.0-2.0], p=0.044). They had lower scores for the statement that they don't get worried about hypoglycaemia (1.0[0.0-1.0] vs 1.0[0.0-2.0], p=0.009) and for belief that they will not have SH in future (0.0[0.0-0.0] vs 0.0[0.0-1.0], p<0.001). People reporting SH in the past year scored higher for beliefs that: it is unnecessary to treat asymptomatic hypoglycaemia (0.0[0.0-2.0] vs 0.0[0.0], p=0.01); someone will always be around to help if I go low (0.0[0.0-1.0] vs 0.0[0.0-0.0], p=0.026); and they can function 'ok' with glucose <3mmol/l (1.0[0.0-2.0] vs 0.0[0.0-1.0], p=0.002). They scored lower on the belief that they will not have SH in future (0.0[0.0-0.0] vs 0.0[0.0-1.0], p=0.032) and were less likely not to worry about hypos (0.5[0.0-1.0] vs 1.0[0.0-2.0], p=0.032). The differences were driven by the HA group. The HFS-II Worry scale score did not differ between HA and IAH groups (p=0.64) but those with IAH had lower Behaviour scale scores (1.8[1.2-2.4] vs 2.1[1.8-2.4], p<0.001).

Conclusion: The A2A questionnaire identified differences in hypoglycaemia-related cognitions of adults with IAH compared to those with HA. Whilst people with IAH are concerned and recognise their increased risk for SH, they place excessive emphasis on avoiding hyperglycaemia and believe they can function normally during hypoglycaemia. Their lower behavioural HFS-II scores showing less action to avoid hypoglycaemia may be driven by these beliefs. The A2A will be helpful in identifying people at high risk of SH and in tailoring interventions to address cognitive barriers to future avoidance of severe hypoglycaemia.

Disclosure: **G. Margiotta:** None.

OP 15 Gestational diabetes from A to Z

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Gestational diabetes: What is the real level of risk? Data from the French population in 2012

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Background and aims: The risk of morbidities in infants born to mother with gestational diabetes (GD) is debated. We evaluated the risk of complications in case of GD in the French birth cohort in 2012 compared to the non-diabetic and the pregestationnal diabetic populations and according to maternal treatment.

Materials and methods: Data were obtained from the PMSI (French hospital discharge database) and the SNIIRAM (French national health insurance information system). All deliveries and terminations of pregnancy (TOP) after 22 weeks of gestation (WG) due to medical reasons were selected. Diabetes was identified by an algorithm based on the consumption of antidiabetics and hospitalization diagnoses before, during and after pregnancy. An identifier in the PMSI links mothers and infants, enabling analyses of associations between the mother's diabetes and outcomes.

Results: 796, 346 deliveries and TOP>22 WG were identified in the PMSI. Mother-infant chaining was obtained for 705, 198 deliveries. 57 629 (7.24%) GD including 16 108 treated by insulin, 1907 (0.24%) type 2 diabetes (T2D) and 1291 (0.16%) type 1 diabetes (T1D) were identified. Compared to the non-diabetic population, the risk in case of GD was increased for preterm birth < 37 WG (adjusted Odds Ratio from logistic model =1.2 [1.2-1.3]), caesarean section (1.4 [1.4-1.5]), preeclampsia and eclampsia (1.6 [1.5-1.7]), macrosomia (birth weight > 90th percentile) (1.8 [1.7-1.8]), perinatal asphyxia (1.2 [1.1-1.3]), respiratory distress syndrome (1.3 [1.2-1.3]), obstetrical trauma (1.3 [1.1-1.5]) and cardiac malformations (1.2 [1.1-1.3]). To take into account the immortal time bias, the analysis was also performed limited to deliveries after 28 WG and after 37 WG. The same outcomes were found associated with GD in addition to an increased risk for perinatal death among deliveries after 37 WG (1.3 [1.1-1.6]). The risks for adverse maternal and neonatal outcomes associated with GD were much lower than in case of T1D or T2D. Among deliveries after 28 weeks, compared to the non-diabetic population, the increased risk was higher in case of GD treated by insulin than treated by diet for prematurity (1.5 [1.4-1.6] vs 1.2 [1.2-1.3]), C section (1.7 [1.7-1.8] vs 1.3 [1.3-1.2]), macrosomia (2.1 [2.1-2.2] vs 1.6 [1.6-1.7]) and cardiac malformations (1.7 [1.4-2.0] vs 1.1[1.0-1.3]).

Conclusion: The risk of perinatal complications is slightly increased in case of GD compared to the non-diabetic population and is much lower than in T1D and T2D. GD treated by insulin was associated with more severe outcome than GD treated by diet alone. The increased risk for cardiac malformations observed in GD treated by insulin is probably related to the inclusion of unrecognized T2D.

Disclosure: **D. Mitanchez:** None.

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Seasonal pattern in the diagnosis of gestational diabetes mellitus in southern Sweden

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Background and aims: While seasonality in the onset of type-1 diabetes is well documented, less is known about the seasonality in the diagnosis of type-2 and gestational diabetes mellitus (GDM). Temperature-induced increase in post load glucose levels has been described, possibly related to increased arterialisation of venous blood at higher ambient temperature. The aim of the present study was to examine seasonal pattern in glucose tolerance and in the diagnosis of GDM.
Materials and methods: A number of 11 538 women underwent a universally applied 75-g OGTT in the 28th week of pregnancy during 2003-2005 in southern Sweden. The diagnostic criteria for GDM were a slight modification of those proposed by the WHO in 1999 based on the 2-h capillary plasma glucose concentration. OGTT results from the 3-year study period were grouped together into months and seasons. Chi-squared tests were used to test for differences in frequencies of GDM, and ANOVA to test for differences in mean glucose concentrations. Logistic regression analysis examined whether month or season were associated with the diagnosis of GDM and linear regression analysis was used to examine the corresponding associations with 2-h glucose levels. Information on mean monthly temperatures during the study period was obtained from the Swedish Meteorological and Hydrological Institute.
Results: A total of 487 women (4.2%) were diagnosed with GDM during the study period. The monthly frequency of GDM ranged from 2.9% in March to 5.8% in June. The seasonal frequency ranged from 3.3% in spring to 5.5% in summer. The differences were significant for both month ($p<0.01$) and season ($p<0.0001$). Mean 2-h glucose concentrations followed the same seasonal trend with a difference of 0.15 mmol/L between winter and summer ($p<0.0001$). Mean monthly temperature ranged from -0.6°C in the winter to 17.7°C in the summer. In a simple linear regression with 2-h plasma glucose as the dependent variable and mean monthly temperature as the predictor variable, the coefficient in the equation was 0.009, suggesting that the 2-h glucose level increased by 0.009 mmol/L for every degree increase in temperature. In regression analysis, adjusting for age, summer (June-August) was associated with increased 2-h glucose level ($p<0.001$) and increased frequency of GDM compared with the other seasons: OR (95% CI) 1.507 (1.241-1.829), $p<0.001$. These associations were no longer apparent when also adjusting for mean monthly temperature.
Conclusion: Our findings suggest seasonal variations in the 2-h glucose concentration of the OGTT and in the proportion of women diagnosed with GDM with a peak in the summer. A positive association with the ambient temperature was demonstrated. Further research is needed to explore the significance of these findings.

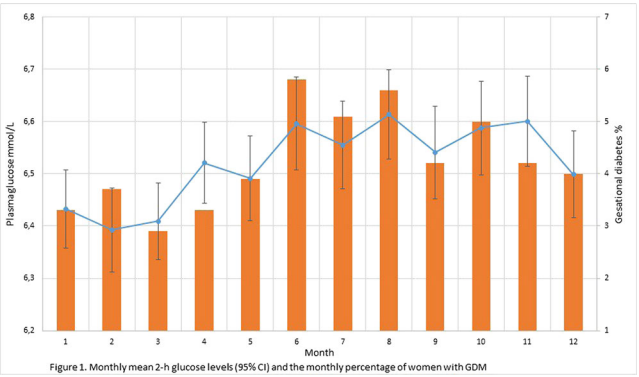

Figure 1. Monthly mean 2-h glucose levels (95% CI) and the monthly percentage of women with GDM

Supported by: Research Funds of Skåne University Hospital and Skåne County Council
Disclosure: **A. Katsarou:** None.

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Diet composition and gut microbiota composition of overweight pregnant women at risk of gestational diabetes are related to intestinal permeability

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Background and aims: Increased intestinal permeability may precede adverse metabolic conditions including diabetes. The extent to which gut microbiota composition and diet contribute to intestinal permeability during pregnancy is unknown. The aim was to investigate the impact of gut microbiota composition and intake of nutrients on the concentration of serum zonulin, a marker of intestinal permeability, in overweight pregnant women.
Materials and methods: This study included 100 overweight women in early pregnancy (mean 12.8, SD 2.5 weeks of gestation) participating in a clinical trial on the prevention of gestational diabetes. The mean (SD) prepregnancy BMI of the women was 30.3 (4.4) kg/m^2. Zonulin was determined from fasting serum samples using ELISA. The gut microbiota composition was analyzed from fecal samples using 16S rRNA sequencing. The dietary intake of nutrients was calculated from three-day-food diaries collected within a week prior to serum sample collection.
Results: Women were divided into low and high zonulin groups based on the median value of zonulin (46.4 ng/ml). The prepregnancy BMI did not differ between women in the low and high zonulin groups. The gut microbiota richness assessed by three indices was higher (p always <0.05) in the low zonulin group compared with the high zonulin group. The abundance of species *Faecalibacterium prausnitzii* was significantly ($p=0.04$) higher in the low zonulin group compared with the high zonulin group. Dietary absolute intake of n-3 polyunsaturated fatty acids was 20% ($p=0.03$) and that of fibre was 22% ($p=0.001$) higher in women having low zonulin concentration. In addition, the intake of a range of vitamins and minerals, including several B vitamins (p always <0.05), vitamin E ($p=0.01$) and vitamin K ($p=0.03$), magnesium ($p=0.008$) and calcium ($p=0.01$), were significantly higher in women having low zonulin concentration compared with those having high zonulin concentration.
Conclusion: The gut microbiota richness and composition and the intake of particular dietary nutrients are associated during early pregnancy with serum zonulin concentration, a marker of intestinal permeability. Modification of the gut microbiota and diet in overweight mothers may beneficially affect intestinal permeability and subsequently lead to improved metabolic health including prevention of gestational diabetes.
Clinical Trial Registration Number: NCT01922791
Supported by: Academy of Finland
Disclosure: **T. Rönnemaa:** Grants; Academy of Finland, Diabetes Research Foundation Finland.

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Global placental DNA methylation status and gestational diabetes

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Background and aims: Gestational diabetes mellitus (GDM), defined as glucose intolerance with a first recognition during pregnancy, is associated with adverse pregnancy outcomes. The placenta plays a key role in GDM and epigenetic alterations might contribute to the pathogenesis of GDM. The aim of this study was to examine whether differences in placental global DNA methylation are associated with GDM.
Materials and methods: Global DNA methylation was quantified by the current goldstandard method, LC-MS/MS. In total, 1030 placental samples were analyzed in this single-center birth cohort study.
Results: Mothers with GDM had a significantly higher degree of placental methylation (3.22±0.63% vs. 3.0±0.46%; p=0.013; ±SD). Bivariate logistic regression showed a highly significant correlation between placental methylation and the presence of GDM (p=0.0009). Quintile stratification according to the degree of placental methylation revealed that the frequency of GDM was evenly distributed in quintiles 1-4 (2.9-5.3%), whereas the frequency in the 5th quintile was significantly higher (10.7%; p=0.003). Bivariate logistic models adjusted for maternal age, BMI, ethnicity, recurrent miscarriages and familiar diabetes predisposition, clearly demonstrated an independent association between DNA methylation and GDM. Furthermore, an ANCOVA model considering known predictors of DNA methylation substantiated an independent association between GDM and placental DNA methylation.
Conclusion: This is the first study that employed a robust quantitative assessment of placental global DNA methylation in over a thousand placental samples. The study provides large scale evidence that placental global DNA hypermethylation is associated with an increased prevalence for GDM, independent of established risk factors.
Supported by: This study was partially funded by the DFG
Disclosure: **C. Reichetzeder:** Grants; DFG.

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miRNA signatures in maternal whole blood cells of women with gestational diabetes

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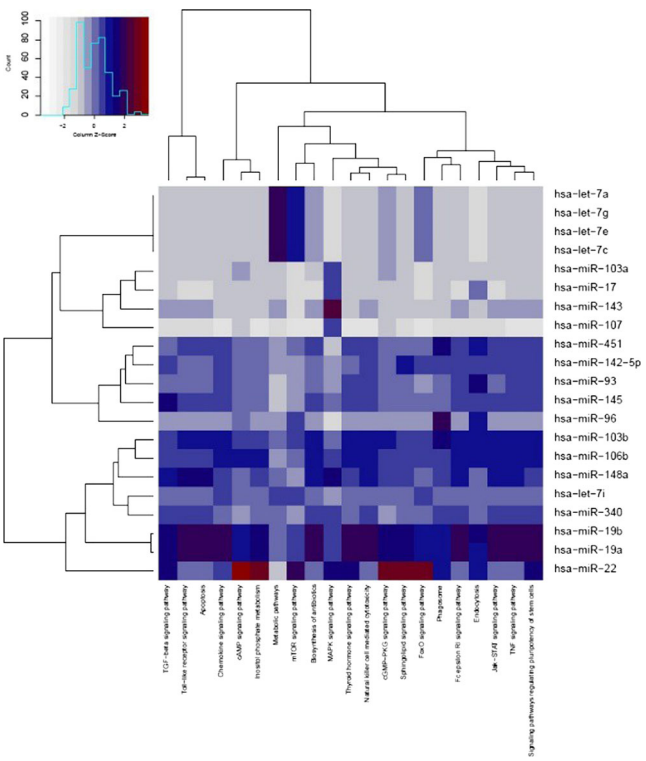
Background and aims: The number of pregnancies complicated by gestational diabetes (GDM) is increasing worldwide. So far, only a few studies have been conducted to profile the transcriptional changes that occur in peripheral blood mononuclear cells (PBMC) of patients with GDM. With support of the Tübingen PREG study, we are able to follow up GDM patients and their offspring up to ten years after birth. This allows us to determine whether these transcriptomic alterations are stable over time and can impact the offspring PBMC transcriptomic signatures.
Materials and methods: The PREG study has performed in accordance with the ethical guidelines laid down in the Helsinki Declaration. For the genome-wide miRNA and mRNA expression changes, eight NGT and eight GDM pregnant women were selected and matched based on their body mass index and age. Blood was collected during pregnancy (pregnancy week 24-32). After total RNA isolation from whole blood cells, genome-wide sncRNA and longRNA expression analysis was conducted. For genome wide long coding and non-coding RNA-Seq library preparation NuGen Ovation RNA-Seq system v2 ® was used. To investigate the expression of all small non-coding RNAs NEBNext® small RNA library Prep Set for Illumina technology was applied.
Results: Upon adjustment for pregnancy week and maternal weight gain during the first trimester, 21 mature miRNAs with differential expression (p-value<0.05 and FDR<0.0.5) due to GDM were found. Majority of these miRNAs are shown to be upregulated in GDM. The pathway analysis, showing cellular effects of the differentially expressed miRNAs, indicates strong effects of GDM on TGF-beta-, TNF-, toll-like receptor signaling pathways within whole blood cells (see graph). In a further step of the analysis, the significantly upregulated miRNAs were linked to the list of GDM-associated differentially expressed mRNAs, which are mostly downregulated. The network analysis, summarizing the effect of miRNAs on their putative target mRNAs, emphasizes a role of the miRNAs miR-19a and miR-19b.
Conclusion: Our results provide first evidence on miRNA-dependent reprogramming of whole blood cells in response (metabolic changes in) to GDM. Upregulated miRNAs indicate alterations in inflammatory, apoptotic and thyroid hormone signaling pathway. Additionally, the two major miRNAs miR-19a and miR19b were already shown to respond to diet-induced obesity and associate with metabolic disorders in other cells.



Disclosure: **L. Stirm:** None.

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Pregnancy outcomes of women with gestational diabetes based on the WHO 2013 and the NICE 2015 diagnostic criteria

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Background and aims: WHO has issued new recommendations for the diagnosis of gestational diabetes mellitus (GDM) in 2013. As the use of the new diagnostic criteria approximately doubles the prevalence of GDM, several professional bodies (including NICE) recommended the use of less stringent cut-off values to limit the prevalence of GDM. We aimed to compare pregnancy outcomes of women with untreated GDM (according to WHO 2013, WHO-GDM and NICE 2015, NICE-GDM) and women with normal glucose tolerance (NGT).
Materials and methods: During a universal screening program in a Western Hungarian region 4828 pregnant women had a 75 g OGTT with the determination 3 points glucose determinations in 4 years. Based on these OGTTs n=696 (14.4%) WHO-GDM and n=478 (9.9%) NICE-GDM cases were diagnosed. N=608 (12.6%) women were treated for GDM and thus were excluded from the present analysis.
Results: Untreated GDM women were older, had higher fasting , 60-minute , and 120-minute blood glucose, and blood pressure. No difference in marital status and education was found (all p>0.05). GDM women had higher body weight , while weight gain was similar in all groups (13.0-13.6 kg, p=0.610). WHO-GDM newborns had a higher birthweight (144, SE 31 g), NICE-GDM newborns had similar birthweigth (66, SE 43g) to controls. Hypertension during pregnancy was more frequent in the GDM groups (WHO-GDM OR 1.56, 95%CI 1.03-2.38, NICE-GDM 1.58 95%CI 0.90-2.78), as well as induced delivery(WHO-GDM OR 1.25, 95%CI 0.98-1.60, NICE-GDM 1.54 95%CI 1.11-2.13), forceps or vacuum use (WHO-GDM OR 1.29, 95%CI 1.01-1.64, NICE-GDM 1.35 95%CI 0.97-1.87), acute cesarian section (WHO-GDM OR 1.25, 95%CI 0.98-1.60, NICE-GDM 1.54 95%CI 1.11-2.13), and macrosomia (>4000g, WHO-GDM OR 1.95, 95%CI 1.39-2.72, NICE-GDM 1.16 95%CI 0.67-2.00). No difference in the risk of preeclampsia and malformations (p>0.40) was found. After adjustment for the mothers' weight at delivery, WHO-GDM women had similar outcomes to NGT women, while for the NICE-GDM group induced delivery and acute cesarian section remained more frequent.
Conclusion: Although the pregnancy outcomes of WHO-GDM women were worse compared to NGT women, most of the differences were explained by the higher body weight of these women. Some outcomes however remained worse for NICE-GDM women even after taking into account the weight difference between the NICE-GDM and NGT groups. Our data may suggest that treatment focused on weight gain during pregnancy might be enough the improve outcomes of these 'mild' GDM cases (WHO-GDM).
Disclosure: **A. Kun:** None.

OP 16 Browning: grow old along with me

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Transplantation of adipose tissue from mice overexpressing WISP2 increases FAHFAs and glucose tolerance in obese mice

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Background and aims: WISP2 is highly expressed in, and secreted by, mesenchymal precursor cells. It is an important early regulator of mesenchymal precursor cell commitment to the adipogenic lineage through dual effects; by forming a BMP4-regulated complex with the PPARγ transcriptional activator ZFP423 in the cytosol preventing its nuclear translocation and; as a secreted protein activating the canonical WNT pathway and inhibiting differentiation of the precursor cells. We have generated a transgenic (Tg) mouse overexpressing WISP2 under the aP2 promoter. The Tg mice have an improved metabolic phenotype with adipose tissue hyperplasia and growth, increased circulating levels of the novel fatty acids-hydroxy fatty acids (FAHFAs) and improved insulin sensitivity. To validate if Tg subcutaneous adipose tissue (sWAT) or brown adipose tissue (BAT) secrete factors which, in an endocrine fashion, improved insulin sensitivity, we transplanted sWAT or BAT to the abdominal cavity of obese recipient mice.
Materials and methods: 6 weeks old C57Bl/6N mice were fed high fat diet (HFD) for 10 weeks and at week 6 and 10 glucose tolerance tests (GTT) were performed. Sham operation (Sham) or transplantation of 0.8g sWAT and 0.1g BAT from sedentary (18 month) wildtype (wt) or Tg mice were performed at week 8. Conventional statistical methods were used to analyze results.
Results: There were no differences in weight among the groups (Sham, wt sWAT ,Tg WAT, wt BAT or Tg BAT) and all groups gained weight after transplantation (≈4.2 g p<0.05). Only the Sham group increased in fasting glucose (9.7 to 11.0 mmol/l p<0.05) and insulin (2.7 to 4.0 ηg/ml p<0.05) and also developed a reduced glucose tolerance (AUC p<0.05). Wt and Tg sWAT had no significant change in fasting insulin levels compared to Sham after transplantation. However, Tg sWAT had lower fasting glucose levels (p<0.05) and an improved glucose tolerance (AUC p<0.05), in spite of the same body weight. There was no difference between the wt and Tg BAT transplants in changing the glucose tolerance in the recipient mice. Serum levels of the novel FAHFA isoform palmitic acid-hydroxy stearic acids (PAHSAs), known to improve insulin action and insulin secretion, were also significantly increased in Tg compared to wt mice on HFD (p<0.05).
Conclusion: Transplantation of WISP2-secreting sWAT to obese recipient mice significantly improved glucose tolerance compared to wt sWAT. This effect was seen in spite of similar body weight and insulin levels, supporting an improved insulin sensitivity although only a small amount of adipose tissue was transplanted. Serum FAHFA/PAHSA levels were also increased in the recipient mice receiving Tg fat. These data support the concept of endocrine factors/lipids released by the adipose tissue which improve insulin sensitivity and support a role of the FAHFAs in mediating the improved insulin sensitivity seen in both the Tg mice and the recipient mice following transplantation. These results show that increased WISP2 expression in the adipose tissue is also associated with increased secretion of FAHFAs by the adipose cells. This makes WISP2 an interesting target for preventing obesity-related complications including insulin resistance and Type 2 diabetes.
Supported by: Edgar Sjölund Foundation, Wilhelm and Martina Lundgren's Foundation
Disclosure: **J.R. Grünberg:** None.

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Increased serum levels of BMP4 in mature mice protects from obesity by browning the subcutaneous fat

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Background and aims: Bone Morphogenetic Protein 4 (BMP4) is secreted by adipocytes and plays a key role for mesenchymal precursor cell commitment and differentiation into both white and oxidative beige adipocytes. This makes BMP4 a potential target for treatment of obesity and insulin resistance (IR). However, to be a potential therapeutic target it is important to examine if BMP4 also is able to increase browning of white adipose tissue (WAT) in adult mice. To examine the effect of BMP4, we injected adult, fully mature mice with adeno-associated viral BMP4- or control vectors targeting the liver and, thus, increasing circulating BMP4 levels.
Materials and methods: Six weeks old, male C57BL6/N mice were treated with control- or BMP4 vectors and fed control- or a high fat diet (CD or HFD) for 16 weeks (cohort 1). Additionally, even older (twelve weeks old) male C57BL6/N mice were treated with control- or BMP4 vectors and fed CD for nine weeks (cohort 2). Reported results are statistically significant. Statistical tests were performed using Students t-test or Mann-Whitney non-parametric U-test.
Results: The HFD BMP4 mice were protected from obesity (mean weight 36.7g and 44.3g for HFD BMP4 and HFD controls, respectively, $p<0.05$) and IR (25% reduced AUC during the insulin tolerance test (ITT), $p<0.01$), and the HFD BMP4 mice exhibited a marked browning of the subcutaneous (SubQ) WAT with increased UCP1 and PGC1a proteins. CD BMP4 mice had similar weight gain as controls in spite of increased food intake (mean intake 2.7g and 2.3g/day for CD BMP4 and CD controls, respectively, $p<0.05$). They also showed a marked browning of the SubQ WAT with increased UCP1 and mitochondrial OXPHOS proteins and also tended to be more insulin sensitive (15% reduced AUC during ITT, $p=0.074$). These results in fully mature mice suggest that BMP4 may induce trans-differentiation of white to beige adipocytes rather than increasing differentiation of beige preadipocytes. Detailed characterization of the stromal-vascular cells supported this concept. This was further supported by the findings in cohort 2 where mice were injected at 12 weeks of age with the BMP4 vectors when adipose tissue cellularity is fully developed. Also these mice had an increased food intake (mean intake 3.3g and 2.7g/day for CD BMP4 and CD controls, respectively, $p<0.001$), but, similar to cohort 1, no difference in weight gain. They showed increased energy expenditure (7% increased AUC of oxygen consumption, $p<0.05$) and electron microscopy of the SubQ WAT revealed increased numbers and size of mitochondria consistent with the increased PGC1α and other markers of mitogenesis.
Conclusion: Increased circulating levels of BMP4 in adult mice lead to browning of the SubQ WAT which prevents development of obesity and IR during HFD-feeding. Our results also provide evidence for BMP4-induced browning of the SubQ WAT and suggest a trans-differentiation of mature white adipocytes into more oxidative, beige adipocytes rather than increased recruitment of beige precursor cells. These results support a therapeutic potential of BMP4 and/or its signalling pathway in obesity.
Supported by: The WM Lundgren Foundation, the Swedish Research Council
Disclosure: **J.M. Hoffmann:** None.

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Differential effect of a GLP-1R/GCGR dual agonist versus a single GLP-1R agonist in the activation of BAT and browning in diet indiced obesity in mice

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Background and aims: Brown adipose tissue (BAT), a specialized fat that dissipates energy to produce heat, plays an important role in the regulation of energy balance. We have designed a pharmacological intervention protocol with a single GLP-1R or dual-acting GLP-1R/ GCGR agonist aimed to investigate their differential effects in preventing diet-induced obesity in mice by inducing the activation of BAT.
Materials and methods: 8 week-old male C57 Bl/6 mice were fed chow (group 1) or HFD for 10 weeks. HFD-fed mice were subsequently s.c. injected every 2 days with vehicle (group 2) or a GLP-1R agonist (10 nmol/kg; group 3) or G49, a GLP-1R/GCGR dual agonist (4.4 mg/kg; group 4), for 6 weeks. Parameters that assess obesity, glucose homeostasis, beta cell function and BAT activation were analyzed at 3 and 6 weeks of treatment.
Results: After 3 weeks of treatment, body weight loss was higher in mice injected with the G49 (-15.62%±4.57) vs HFD animals (+9.824%±4.14;$p<0.001$) and also vs GLP-1R agonist treated animals (3.88%±3.53: $p<0.001$). In parallel PET analysis revealed cold-induced activation of BAT in both drug treatment groups, but the effect was higher in G49 group (Mean activity 3232±668.4; $p<0.001$). These differential effects were maintained at 6 weeks of treatment (BW loss G49 animals -20.05%±0.937; PET of G49 animals 2090±756.9). At each time the adiposity (5.2%±2.081 vs 9.3%±1.39: $p<0.001$) and macrophage infiltration in eWAT were reduced and UCP1 immunostaining in both BAT and iWAT was increased ($p<0.05$). Moreover, adipocytes sizes were strongly reduced in G49 animals in iWAT (3725±2411;$p<0.01$) compared with GLP-1R agonist (10903±25.75;$p<0.01$) and also with HFD animals (10944±3250;$p<0.01$). Although, we also observed significant reduction in eWAT adipocytes sizes in G49 group (13671±4920) compared with HFD group (21695±5062;$p<0.05$) but not compared with GLP-1R agonist group. Also, the effect of G49 in inducing mRNA levels of markers of BAT activation and browning in were higher in mice treated with G49 ($p<0.001$). Moreover, we observed a strong decrease in insulin plasma levels (G49 0.6061±0.1991 vs HFD 2.075±1.10; $p<0.001$). This positive effects in glucose homeostasis corresponded with an improvement of islet morphology ($p<0.05$).
Conclusion: Our results strongly suggest a novel role of an oxyntomodulin-like dual acting GLP-1R/GCGR agonist in reducing obesity and improve insulin sensitivity by increasing energy expenditure due to its effects in BAT and pancreas phisiology.
Supported by: MEDIMMUNE INC. CIBERDEM.
Disclosure: **L. Ruis Cañas:** None.

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Knockout of thyrotropin receptor promotes energy expenditure by inducing the browning of white fat in mice

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Background and aims: Thyrotropin (TSH) is closely related to obesity. Many epidemiological studies found that serum TSH levels and BMI were positively correlated in euthyroid subjects and subclinical hypothyroidism patients. However, the molecular mechanism by which TSH affects obesity has not been fully elucidated. Obesity occurs when excess energy accumulates in white adipose tissue (WAT), whereas brown

adipose tissue (BAT), specialized for energy expenditure through thermogenesis potently counteracts obesity. Recent studies have showed that there are two types of brown adipocyte, interscapular BAT (iBAT) and interspersed brown-like adipocytes (also called 'beige' or 'brite' cells) within WAT. When obesity occurred, the development of beige cells was blocked. Here, we demonstrate that knockout of Tshr promotes energy expenditure and induces brown-like adipocytes in white adipose tissue.

Materials and methods: We used male Tshr knockout (Tshr-/-) mice treated with T4 to maintain normal thyroid function, and wild type littermates (Tshr+/+) as control group. Body weight was measured every week from the sixth week. Glucose and insulin tolerance tests were performed. In metabolic balance studies, mice were placed in metabolic cages to assess their food intake,O2 consumption, CO2 production and physical activity. After mice sacrifice, epididymal /inguinal white adipose tissue (eWAT/iWAT), BAT and blood were collected.Serum triglyceride(TG) and FT4 were measured respectively. The brown/beige markers in adipose tissues were assessed via qPCR, western blot and immunochemistry. Mice used in this study were aged between 8-12 weeks if not specially mentioned. All data are presented as the mean±s.e.m., and significant differences were analyzed by two-tailed Student t-tests.

Results: Our finding showed that the body weight of Tshr-/- mice was significantly lower than the control group (18.09±3.4 vs.23.85±1.25g; p=0.000; n=9), and so was the eWAT mass. Tshr-/- mice also showed decreased serum TG level and improved glucose tolerance.The data from metabolic cage showed that Tshr-/- mice had a higher metabolic rate (VO2: 5801±1703 vs.3898±972 ml/h/kg; p<0.0001; VCO2: 5796±1707 vs.3898±972 ml/h/kg; p<0.0001; n=7). For BAT, we found that the expression of BAT-specific marker Ucp-1 significantly decreased in Tshr-/- mice. For WAT, the relative expression of Ucp-1 and other BAT markers like Pgc1α, Cidea, Cox7a, Cox8b were significantly higher in Tshr-/- mice(p=0.012,0.013,0.025,0.005; n=6). Besides, beige markers Pat2 and P2rx5 also increased in Tshr-/- mice(p=0.020,0.046; n=6). Furthermore, the protein level of Ucp-1 and Pgc1α also increased in WAT of Tshr-/- mice. To explore the mechanism by which TSH affects the browning of the white fat, we detected some upstream regulatory genes and found that the relative expression of Prdm16 and Bmp7 increased in eWAT of Tshr-/- mice (p=0.046,n=6-8).

Conclusion: Therefore we conclude that knockout of thyrotropin receptor promotes energy expenditure by inducing the browning of white fat in mice.

Disclosure: **H. Wu:** None.

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Cannabinoid CB1 receptors in human brown adipose tissue during cold exposure

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Background and aims: The endocannabinoid system is important in energy metabolism and cannabinoid CB1 receptor (CB1R) antagonism leads to weight loss and increased energy expenditure. It is therefore a pharmaceutical target for treatment of obesity and dyslipidemia in humans. Animal studies have shown that CB1R expression is high in brown adipose tissue (BAT) and CB1R antagonism activates BAT metabolism. Recently it was shown in rodents that the CB1R radiotracer [^{18}F]FMPEP-d_2 (FMPEP) developed for brain studies can be used also for the measurement of CB1R density of BAT. The aim of this study was to investigate CB1R density in human BAT and brain using this tracer and PET in ambient and cold conditions. We hypothesized that CB1Rs are expressed in human BAT and FMPEP could be used as a new marker for human BAT imaging.

Materials and methods: We studied nine healthy lean male subjects with mean age of 32 years. Dynamic PET-CT scans of the neck area, abdomen and brain were done using FMPEP once in room temperature and on another day during controlled cold exposure. Activity of BAT was verified during cooling by measuring [^{18}F]FDG uptake using PET-MRI. Volume distribution of the dynamic images and standard uptake values (SUV) of FMPEP as well as glucose uptake values were measured in supraclavicular BAT, subcutaneous white adipose tissue (WAT), muscle tissue and the cortex of the brain.

Results: Cooling increased FMPEP uptake significantly as compared to that in room temperature both in BAT (SUV 5.8 ± 2.9 vs. 1.7 ± 1.9, P=0.003) and in brain cortex (SUV 1.5 ± 0.2 vs. 1.3 ± 0.2, P=0.003). In contrast, uptake of FMPEP in WAT and muscle tissue was low in room temperature and further decreased in cold conditions (WAT SUV 0.7 ± 0.2 vs. 0.4 ± 0.2 P=0.001, muscle SUV 0.6 ± 0.2 vs. 0.5 ± 0.2 P=0.03). Distribution volume values in BAT, derived from dynamic compartmental modeling, correlated with SUV in both warm and cold conditions (r=0.87, P=0.003 in warm, r=0.84, P=0.009 in cold) indicating that SUV is a suitable quantitative measurement for FMPEP binding in BAT. Under cold conditions, the uptake of FMPEP in BAT had a strong positive correlation with BAT glucose uptake (r=0.83, P=0.01). In room temperature, FMPEP uptake in BAT (SUV 1.7 ± 1.9) and WAT (SUV 0.7 ± 0.2, P=0.13) were overlapping.

Conclusion: In humans, using the CB1R tracer [^{18}F]FMPEP-d_2 and PET imaging we found that cannabinoid CB1 receptors are up-regulated during cold exposure in BAT, and this is correlated with the degree of functional BAT activity, measured as its glucose uptake. Cold exposure also increased FMPEP uptake in brain cortex. These results suggest that transient up-regulation of CB1R expression in metabolically active human BAT may be mediated via the central nervous system.

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Disclosure: **M. Lahesmaa:** None.

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Berberine promotes brown adipogenesis and energy expenditure

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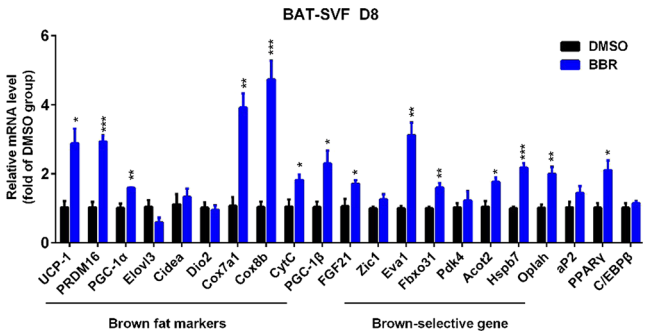
Background and aims: Activation and induction of brown fat is now recognized as an effective approach for treatment of obesity. The natural plant alkaloid Berberine (BBR) has been reported to defense against obesity. This study aims to clarify the impact of BBR on brown adipogenesis and its role in energy expenditure in vivo.

Materials and methods: BBR was i.p. (1.5 mg kg^{-1} per day) injected for 6 weeks in high-fat diet (HFD) fed mice (n=10). Body weight and body temperature were recorded daily. Oxygen consumption and heat were determined by metabolic cage study. 18F-PET/CT was performed to assess the glucose uptake of BAT. In vitro, BBR was added during the differentiation process of C3H10-T1/2 and stromal vascular fraction (SVF) from BAT. Gene and protein expression were detected by western blot and Q-PCR, lipid accumulation was determined by oil red staining, oxygen consumption rate (OCR) was also measured. ChIP assay, luciferase reporter assay, and loss of function study by shPRDM16 lentivirus were performed.

Results: The body weight of BBR group were markedly reduced compared to vehicle group after 6-weeks-treatment (37.25±0.70 vs 44.06 ±1.12 g, p<0.0001, n=6-8). The rectal temperature was increased (36.72

±0.12 vs 36.31±0.04°C, p<0.001; 37.46±0.11 vs 36.89±0.08°C, p<0.001, n=10 after 4 and 6 weeks treatment respectively). The oxygen consumption and energy expenditure of BBR group were also significantly upregulated (1489.8±39 vs 1315.6±15.3 ml/kg/h, p<0.0001; 6.86±0.18 vs 6.33 ±0.10 Kcal/kg/h, p<0.01, n=9, respectively). The mean and max SUV in BAT area was increased by BBR treatment (1.38±0.14 vs 0.86±0.10, p=0.02; 3.55±0.46 vs 2±0.39, p=0.04, n=4). BBR promoted lipid accumulation and enhanced both basal and uncoupled OCR (p<0.0001, n=4) in C3H10-T1/2 cells. BBR increased the protein level of brown fat markers such as UCP-1, PRDM16, PGC-1α and adipogenic markers like C/EBPα, C/EBPβ, PPARγ. Moreover, BBR dramatically increased brown fat markers and brown-selective genes expression in BAT-SVF cells. ChIP assay showed PRDM16 was recruited to UCP-1 enhancer by BBR treatment (3-fold, p<0.001). However, the upregulation of UCP-1 and PGC-1 protein by BBR was blunted after knocking down PRDM16. Luciferase assay suggested BBR upregulated UCP-1 transcription requiring the presence of PRDM16 (1.6-fold, p<0.01), and it also increased PRMD16 transcription in HEK293 cells (1.3-fold, p<0.01).
Conclusion: Our findings showed that BBR reduced obesity, increased BAT thermogenesis activity and energy expenditure in HFD mice. BBR promoted brown adipogenesis in C3H10-T1/2 and BAT-SVF cells. The upregulation of PRDM16 and UCP-1 by BBR treatment was dependent on PRDM16. These results indicated that BBR-induced brown adipogenesis may play a role in the anti-obese effect in vivo.



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OP 17 Type 2 diabetes and complications: the role of genes and environment

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Gene-obesogenic environment interactions in the UK Biobank study

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Background and aims: Susceptibility to obesity and type 2 diabetes in today's environment has a strong genetic component. However, little is known about how genetic variation interacts with the modern environment to predispose some individuals to obesity and type 2 diabetes whilst others remain slim. Previous gene-obesogenic environment studies have been limited by the need to perform meta-analyses of many heterogeneous studies. We aimed to use 120,000 individuals from the UK Biobank to test the hypothesis that high risk obesogenic environments accentuate genetic susceptibility to obesity and therefore increase type 2 diabetes risk.
Materials and methods: We used 120,000 individuals from the UK Biobank study to test the hypothesis that high risk obesogenic environments and behaviours accentuate genetic susceptibility to obesity. We used BMI as the outcome and genetics and self-reported estimates of the obesogenic environment as exposures. We used a 69-variant genetic risk score (GRS) for obesity as the genetic exposure and 9 self-reported measures, including TV watching, westernised diet and physical activity and a composite of these factors, as obesogenic environment/behaviour exposures. We tested the association of the genetic risk score with BMI in high and low environment groups and tested for interactions.
Results: The self-reported measures of the obesogenic environment and behaviour were all associated with BMI in the expected directions (all P<0.001). We found evidence of gene-environment interactions with self-reported TV-watching (Pinteraction=7x10-5), and self-reported physical activity (Pinteraction=5x10-6). For example, within individuals reporting watching ≥4 hours TV per day, carrying 10 additional BMI-raising alleles was associated with approximately 4.0kg extra weight in someone 1.73m tall. In contrast, within individuals reporting watching <4 hours TV per day, carrying 10 additional BMI-raising alleles was associated with approximately 3.1kg extra weight. Evidence of interaction using a composite measure of the obesogenic environment (Pinteraction=2x10-4) and permutations of the data based on randomly selecting groups of individuals of different BMIs, suggested that these differences were not specific to one aspect of the environment. The main limitations of our findings are that the environmental measures are complex mixes of environment and behaviour and are based on self-report.
Conclusion: Our findings suggest that the there is no particular aspect of the environment or behaviour that if altered would have a preferential benefit over others. It is premature to suggest public health measures should be targeted specifically at fried food reduction, fizzy drink consumption and diet in those genetically predisposed to obesity. Instead, public health measures aiming to alter all aspects of the obesogenic environment in small ways may have more impact in lowering the prevalence of obesity and type 2 diabetes than targeting a single or few aspects.
Supported by: J.T. is funded by a Diabetes Research and Wellness Foundation Fellowship.
Disclosure: **J. Tyrrell:** None.

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Dietary predictors of circulating urokinase plasminogen activator receptor

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Background and aims: Urokinase plasminogen activator receptor (uPAR) is a recently established biomarker of inflammation. Elevated levels of soluble uPAR have been associated with several diseases, including type 2 diabetes (T2D). While diet has been shown to associate with a wide range of inflammation biomarkers, less is known about the diet-uPAR association. The aim of this study was to identify individual dietary factors and dietary patterns that explain variation in circulating uPAR. Further, we examined if an uPAR-associated dietary pattern associates with incidence of T2D in a healthy population.
Materials and methods: The Malmö Diet and Cancer Study (MDCS) is a population-based prospective cohort from Sweden. In MDCS sub-cohort, fasting blood samples were collected at baseline (1991-1994) and analyzed using a high-throughput immunoassay by Olink Biosciences. In total 3,694 subjects, without cardio-metabolic diseases or related drug treatments at baseline, had complete information on uPAR and dietary intake. The main dietary, lifestyle, and anthropometric predictors of age- and sex-standardized uPAR levels were examined using a multivariable stepwise linear regression (SLR) with exclusion if P>0.05. Food and beverage intakes were adjusted for age, sex and total energy intake using the residual method. We extracted dietary patterns explaining variation in uPAR levels using partial least square (PLS) regression. The hazard ratios (HR) per quartile (Q) of a PLS-derived dietary pattern score were estimated using a sex-stratified Cox proportional hazards regression model adjusted for age, height, total energy intake, waist, smoking, leisure-time physical activity, and educational level.
Results: During a median follow-up of 21 years, 538 subjects developed T2D. High levels of uPAR associated with increased risk of T2D after adjustment for age, sex and smoking status (Q4 vs Q1: HR=1.49, 95% CI: 1.17-1.91). In SLR, the identified predictors (P<0.05) explained altogether 17.4% of the variation in uPAR. Age, HbA1c and systolic blood pressure associated positively (all P<0.0001), while non-smoking, HDL-C, and male sex associated inversely (all P<0.0001) with uPAR. Further, high intake of ready-to-eat powders (P=0.011) and sugar (P=0.015) associated positively, and high intakes of poultry (P=0.002), high-fiber bread (P=0.005), wine (P=0.021), and fruit and berries (P=0.017) associated inversely with uPAR. A PLS-derived dietary pattern explained in total 4.8% of the variation in uPAR and was characterized by high intakes of low-fiber bread, full-fat milk, and sugar, and low intakes of vegetables, fruit and berries, high-fiber bread, fermented milk and wine. After adjustment for potential confounders, subjects with higher PLS-pattern scores had a marginally increased incidence of T2D (Q4 vs Q1: HR=1.29, 95% CI: 0.99-1.67; P-trend =0.028); the result was strengthened in sensitivity analysis excluding potential energy misreporters and subjects with past food habit change (Q4 vs Q1: HR=1.48, 95% CI: 1.04-2.10; P-trend =0.026). The uPAR-T2D association and the PLS-score-T2D association were not significant after adjustment for HbA1c.
Conclusion: We identified some putative dietary predictors of uPAR and a dietary pattern that explained ~5% of the variation in uPAR. Further studies are needed to assess whether there is a direct effect of uPAR on T2D or if it is partly mediated by other disease risk markers, including HbA1c and other inflammatory markers.
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Disclosure: **I. Drake:** None.

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Low fructosamine levels and mortality: a 25-year follow-up of 215,011 Swedish non-diabetic subjects

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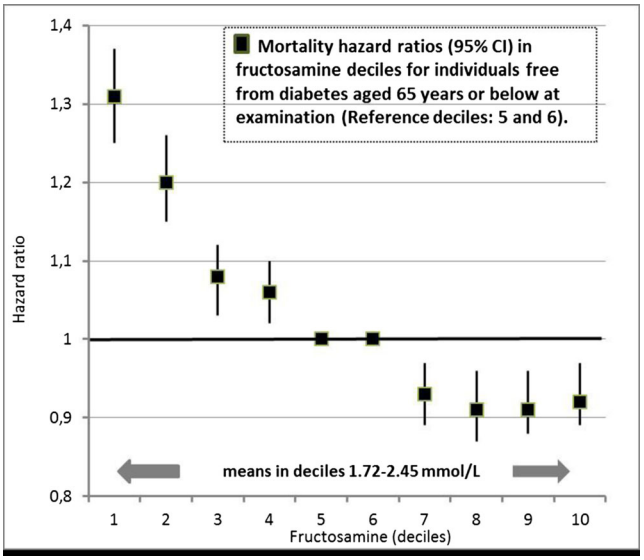
Background and aims: Both high and low levels of fasting blood glucose has been associated with an increased mortality among individuals without diabetes. This J-shaped association has also been shown for HbA1c in relation to all-cause and CVD mortality. Fructosamine reflects blood glucose levels over a 2-4 week period and is associated with increased mortality at high levels. In this study we aimed to evaluate the association of low fructosamine and mortality.
Materials and methods: A total of 215,011 subjects (48% women) undergoing routine occupational health screening or primary care health examination mainly in Stockholm, Sweden was included (AMORIS cohort). All had fructosamine, serum glucose, triglycerides, total cholesterol, albumin, haptoglobin, serum creatinine and uric acid respectively measured at the same examination. Cause specific mortality was obtained from the Swedish National Cause-of-Death Register by record linkage. Cox regression with repeated measurements was used to calculate hazard ratios in individuals ordered in deciles of fructosamine, vs. reference levels for all-cause (n=41,388 deaths) and diagnosis-specific mortality during up to 25 years of follow-up. We estimated time differences in survival by using Laplace regression for censored data.
Results: Increased all-cause mortality was observed for fructosamine below the median in particular for individuals up to 65 years of age. In this age group, the lowest decile of fructosamine had a sex, age, calendar year adjusted hazard ratio of 1.31 (95% CI: 1.25-1.37) . Increased mortality remained but was attenuated after further adjustment for all biomarkers (HR=1.20 (95% confidence interval 1.14-1.25)). The corresponding reductions of expected survival was 2.7 (95% CI: 2.2-2.3) and 1.7 (95% CI:1.2-2.1) years respectively. In sensitivity analyses we adjusted for smoking and BMI respectively and analysed the possibility of reverse causation due to cancer with only minor differences in hazard ratios compared to the main analyses.
Conclusion: Low to medium levels of fructosamine in individuals without diabetes was found to be associated with higher mortality in young and middle-aged subjects. This indicates a different type of association to mortality than that seen for fasting glucose and HbA1c possibly suggesting different mechanisms.



Disclosure: **H. Malmström:** None.

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Initiation of cardioprotective medication does not adversely affect health-related behaviours among people with recently diagnosed type 2 diabetes

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Background and aims: There is concern that cardioprotective medications (such as statins) detrimentally affect behaviour either directly, for example drug-related muscle pain limiting physical activity, or indirectly, via a process of false reassurance. However, few data are available from studies incorporating precise measures of behaviour before and after initiation of medication. We aimed to quantify associations between use of cardioprotective medications and changes in self-reported and objectively measured health behaviours over 5-years among individuals with recently diagnosed type 2 diabetes.
Materials and methods: We conducted a cohort analysis of individuals with either screen-detected or recently clinically diagnosed diabetes participating in the ADDITION-Cambridge and ADDITION-Plus studies. We used linear regression analyses to estimate associations between initiation of statins, anti-hypertensive or glucose-lowering medications between baseline and 1-year, and 1- and 5-year follow-up, and changes in physical activity (measured by EPAQ-2 questionnaire and objectively by a combined heart rate and movement sensor) and diet (measured by food frequency questionnaire and plasma vitamin C, a biomarker of fruit and vegetable intake) over the same time periods. Models were stratified by sex and adjusted for potential confounders including education, BMI and smoking status and baseline/1-year values of the relevant exposure and risk factor.
Results: In total 983 participants attended baseline and 1-year visits and 853 attended at 1- and 5-years. Mean (±SD)) age of the population was 60.5±7.4 years and 62% of participants were men. Among women, initiating statin therapy between baseline and 1-year was associated with a decrease in self-reported physical activity (-1.57 [95%CI -3.06, -0.07] MET.h/day) compared with women whose drug status did not change. In contrast, initiating statin therapy between 1- and 5-years was not associated with changes in self-reported or objectively measured physical activity. Among men, initiating statin therapy was associated with a 2.45 (0.35, 4.55) MET.h/day lower self-reported physical activity between 1- and 5-years but there was no association with objectively measured physical activity. In both men and women, there were no statistically significant associations between initiating use of anti-hypertensives and any of the behaviours measured over either time period. However, among women, initiating use of glucose-lowering medication between baseline and 1-year was associated with a decrease in polyunsaturated:saturated fat ratio (-0.09 [-0.15, -0.03]) and between 1- and 5-years was associated with a 1.88 (0.03, 3.73) MET.h/day lower self-reported physical activity.
Conclusion: In this large prospective analysis, we found no significant associations between initiation of cardioprotective medication and changes in objectively measured diet or physical activity. The few associations between initiation of medication and change in self-reported behaviours we identified differed by sex and were not clinically significant. Among people with diabetes there does not appear to be an important detrimental impact of medication use on diet and physical activity.
Supported by: MRC PhD Studentship
Disclosure: **M.J.E. Lamb:** None.

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Association between obesity and cognitive decline in people with type 2 diabetes may be mediated by inflammation

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Background and aims: A well established complication of type 2 diabetes is accelerated age-related cognitive decline and dementia. Obesity, identified as a major contributing factor in the development of type 2 diabetes, has also been identified as a risk factor for accelerated age-related cognitive decline. We aimed to determine whether obesity was associated with an increased risk of cognitive decline in older people with type 2 diabetes and to explore a possible mediating effect of inflammation.
Materials and methods: Body mass index and plasma inflammatory markers (interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα), c-reactive protein (CRP) and leptin) were measured in 1066 men and women aged 60-75 with type 2 diabetes participating in the prospective Edinburgh Type 2 Diabetes Study. Cognitive ability was assessed using tests of Logical Memory, Faces, from the Wechsler Memory Scale, the Digit Symbol Test, Letter Number Sequencing and Matrix reasoning from the Wechsler Adult Intelligence Scale, Trail Making Task (part B) and the Mini Mental State Exam. Scores from the 7 tests were used to obtain a general cognitive ability factor, 'g', via a principal components analysis, and cognitive decline over the subsequent 4 years was determined by adjusting follow-up test scores and 'g' for the respective baseline scores.
Results: In 831 subjects who attended for cognitive follow-up after 4 years, BMI was positively associated with cognitive decline as measured by several of the individual cognitive tests and by 'g' (standardised beta, β = -0.056, p = 0.006, 95% CI -0.017, -0.003). After adjustment for traditional cardiovascular risk factors the association between BMI and cognitive decline persisted (e.g. β for 'g' = -0.053, p = 0.010, 95% CI -0.017, -0.002). Raised BMI was associated with higher plasma inflammatory markers (IL-6, TNFα, CRP and leptin). Adjustment for these inflammatory markers markedly reduced the strength of the association between BMI and cognitive decline, which became non-significant (e.g. β for 'g' = -0.001, p = 0.783, 95% CI -0.011, 0.009).
Conclusion: Obesity was associated with increased cognitive decline in older adults with type 2 diabetes. This relationship may be at least in part mediated by systemic inflammation.
Supported by: MRC
Disclosure: **A.J. Sluiman:** None.

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All is not equal … or is it? Targeting uncontrolled cardiovascular risk factors in racial and ethnic populations to prevent major cardiovascular events

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Background and aims: Despite consistent improvement in cardiovascular (CV) risk factors (RF)over the past decade, uncontrolled, yet modifiable CV RFs remain significant contributors to major CVD. This study assessed the contribution of uncontrolled CV RFs to potentially preventable major CVD by race, ethnicity, and gender.

Materials and methods: Data were from 11 integrated health care systems in the United States for the years 2005-2011. Subjects included 760,971 adults (≥20 years) with diabetes enrolled for ≥ 6 months during the study period. Poor RF control was classified as LDL-c ≥ 100 mg/dL, HbA1c ≥ 7% (53mmol/mol), BP ≥ 140/90 mmHg, or smoker from electronic health records. Major CV events were from primary hospital discharge diagnoses for myocardial infarction, acute coronary syndrome, stroke, or heart failure. The four major race ethnicity groups were Hispanic and non-Hispanic White, Black, and Asian. Aggregate five-year incidence rates and average attributable fractions (AAF) were estimated using multivariable Poisson regression models for race/ethnicity and gender strata.

Results: At baseline, White, Black, Asian, and Hispanic subjects had a mean (SD) age of 61 (13), 57(13), 57(13), and 54(13) years; the percent female was 47.1%, 55.3%, 48.6%, and 49.7%; and a prior history of CVD was 39.3%, 32.4%, 24.2%, and 22.5%, respectively. Mean follow-up was 59 months. HbA1c and LDL-c were uncontrolled in 42-60% of subjects (Table). Five-year major CV event rates per 100 person-years for men and women were 85.8 and 66.6 for Whites, 100.9 and 83.9 for Blacks, 67.0 and 43.0 for Asians, and 83.0 and 59.3 for Hispanics. The percentages of CV events attributable to inadequate RF control for men and women were 13.3% and 18.0% for Whites, 15.4% and 21.3% for Blacks, 19.7% and 8.1% for Asians, and 18.3% and 12.7% for Hispanics, respectively. Within gender, AAF were different across race/ethnicity strata ($p<0.01$).

Conclusion: In this large cohort of adults with diabetes from 11 geographically dispersed U.S health systems, rates of major CV events differed by gender, race, and ethnicity as expected. Yet, excess major CV events due to suboptimal HbA1c, LDL-c, BP, and smoking levels were substantial within each subgroup. Improved CV risk factor control can prevent future CV events across all demographic groups.

Uncontrolled CV Risk Factors, Major CV Events, and Excess Major CV Events, 2005-2011

Race, Ethnicity, Gender	N	Uncontrolled CV Risk Factors (%)				5-yr Major CV Rates (per 100 person-years, age-adjusted)[a]	% Excess Major CV Events[b]
		HbA1c ≥ 7%	BP ≥ 140/90 mm Hg	LDL-c ≥ 100 mg/dL	Smoker		
Male							
White	204,765	44.7	20.2	43.0	11.3	85.8	13.3
Black	37,680	55.0	27.2	53.9	11.7	100.9	15.4
Asian	50,993	51.2	17.0	48.2	10.0	67.0	19.7
Hispanic	95,900	60.1	18.9	53.2	7.9	83.0	18.3
Female							
White	182,195	41.6	23.5	52.5	9.1	66.6	20.0
Black	46,645	50.1	29.7	58.9	9.0	83.9	21.3
Asian	48,162	48.7	19.7	54.0	3.5	43.0	9.2
Hispanic	94,631	54.8	18.9	58.3	4.5	59.3	12.7

a. Within gender categories, Black, Asian, and Hispanic rates are significantly different ($p<0.01$) compared to White.
b. Within gender categories, Black, Asian, and Hispanic percent preventable events are significantly different ($p<0.01$) compared to White.

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Disclosure: **J.R. Desai:** None.

OP 18 Altering the course of type 1 diabetes

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The incidence of pre-clinical type 1 diabetes and celiac disease in the general population from birth to 15 years of age

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Background and aims: Type 1 diabetes (T1D) and celiac disease (CD) are the most frequent autoimmune diseases of childhood. Importantly, 90% of the patients have no affected close relatives and often experience delay in care and severe complications, e.g., diabetic ketoacidosis. There are very limited data concerning incidence of pre-clinical T1D or CD in the general population that could inform optimal screening leading to timely diagnosis and clinical trials to prevent the diseases at a subclinical stage. Our goal was to provide age-specific estimates of the incidence of pre-T1D and subclinical CD in the U.S. general population.

Materials and methods: In 1993-2004, the Diabetes Autoimmunity Study in the Young (DAISY) genotyped HLA-DR-DQ of 31,766 children born in one hospital. They were representative of the general population of Denver, Colorado: non-Hispanic white (56%), Hispanic (30%), African American (7%), Asian American (2%) or biracial/other (5%). Of the 5693 children with the HLA genotypes: DR3/3, 3/x, 3/4, 4/4/, 4/x, (including only DR4,DQB1 03:02),1339 have been followed prospectively for four endpoints: persistent islet autoantibodies (IA) (to insulin, GAD, IA-2 and ZnT8), persistent transglutaminase autoantibodies (TGA), T1D and CD. Persistence was defined by presence of autoantibody on at least two consecutive examinations, at least 3 months apart. T1D was diagnosed using the WHO/ADA criteria; CD was diagnosed by intestinal biopsy or the presence or persistent TGA at 10x positivity cutoff.

Results: During follow-up from birth up to age 22 y, 101 of the children develop persistent IA (49 had a single IA that has not progressed to T1D and 52 developed multiple islet autoantibodies and/or T1D); 112 children developed persistent TGA, including 66 who were diagnosed with CD. The cumulative risk of these endpoints in the Denver general population was estimated by using genotype-specific risk weighting for the population frequencies of HLA genotypes (Table). By age 15 y, up to 2.7% Denver general population children experience a period of persistent islet autoantibodies and up to 5.1% may have persistent TGA. Clinical disease develops in about half of the cases; our previously published data indicate that progression from multiple IA to T1D takes more than 10 y in a 30% of the cases.

Conclusion: The incidence of pre-clinical T1D and CD in the general population is much higher than previously thought. Future general population screening programs will have to account for spontaneous remissions and slow progression to clinical disease in many screening-detected cases. Additional biomarkers may be needed to improve the predictive value of autoantibody-based screening.

Cumulative incidence (%) of persistent islet autoantibodies (IA), celiac autoantibodies (TGA) and celiac disease in the general population, by age (95% confidence interval)

Age, y	Single or multiple IA	Multiple IA or T1D	Persistent TGA	Celiac disease
5	1.1 (0.7-1.5)	0.5 (0.3-0.7)	2.4 (1.8-3.2)	1.7 (1.2-2.4)
10	2.1 (1.6-2.7)	1.0 (0.7-1.4)	4.3 (3.5-5.3)	3.1 (2.4-4.0)
15	2.7 (2.1-3.2)	1.4 (1.0-1.8)	5.1 (4.2-6.1)	3.3 (2.6-4.2)

Disclosure: **M. Rewers:** None.

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Zinc transporter 8 autoantibodies in children with HLA-conferred disease susceptibility and first-degree relatives affected by type 1 diabetes

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Background and aims: The TRIGR (Trial to Reduce IDDM in the Genetically at Risk) study is an international randomized, double-blind trial with the aim to assess whether weaning to an extensively hydrolyzed formula in infancy decreases the cumulative incidence of type 1 diabetes compared to standard cow's milk formula by the age of 10-14 years in children with an affected first-degree relative and HLA-conferred disease susceptibility. The first endpoint was positivity for multiple (≥2) autoantibodies by the age of 6 years. Beta-cell autoimmunity was studied by analyzing islet cell autoantibodies (ICA), insulin autoantibodies (IAA), and antibodies to glutamic acid decarboxylase (GADA) and islet antigen 2 (IA-2A). In previous analysis, the frequency of subjects testing positive for multiple autoantibodies by the age of 6 years was similar in the two groups. In the current analysis, autoantibodies to zinc transporter 8 (ZnT8A), the fourth biochemical autoantibody predictive for T1D, were assessed in the TRIGR study cohort.

Materials and methods: ZnT8A were analyzed in samples obtained at the age of 2, 3, 4, 6, 8, 10, and 12 years. If the child tested positive for ZnT8A in the 2-year sample, cord blood sample and samples taken at the age of 3-18 months were analyzed to exclude maternal antibodies and to define the seroconversion age. Similarly, samples obtained at the age of 5, 7, 9, and 11 years were analyzed, if needed. Altogether 10,956 samples from 2,077 subjects who had at least one serum sample available were analyzed with a specific radiobinding assay. The cut-off limit for ZnT8A positivity was 0.50 relative units (RU).

Results: ZnT8A positivity was observed in 675 of the 10,956 serum samples (6.2%) from 170 of the 2,077 children (8.2%). The mean age at seroconversion to ZnT8A positivity was 52 months (range 12-144 months). Altogether 333 children had a single biochemical autoantibody at seroconversion. IAA emerged as the first autoantibody in 156 (46.8%), GADA in 140 (42.0%), ZnT8A in 22 (6.6%), and IA-2A in 15 (4.5%) of these children. Among the 1,115 participants previously classified as autoantibody-negative based on the IAA, GADA, IA2-A, and ICA analyses, 15 (1.4%) tested positive for ZnT8A, thus changing their status from seronegative to seropositive. When ICA were included, analyzing ZnT8A changed the autoantibody status from previous single positivity to positivity for two autoantibodies in two cases. If the biochemical autoantibodies were exclusively taken into account, switch from single to multiple autoantibody positivity was observed in five children.

Conclusion: Although seroconversion to ZnT8A positivity peaks around the age of 5 years, ZnT8A may appear already by the age of one year. ZnT8A usually emerge together with one or more of the other diabetes-associated autoantibodies. ZnT8 autoantibodies contribute only modestly to multiple autoantibody positivity.

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Disclosure: **H. Siljander:** None.

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Differences in prevalence of ZNT8ab and other autoantibodies in children and adults at onset of type 1 diabetes

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Background and aims: Zinc transporter 8 (ZnT8) autoantigen is associated with type 1 diabetes (T1DM) pathogenesis. ZnT8 autoantibodies (ZnT8ab) are reported in the majority of T1DM patients prior to and at clinical diagnosis. Relationship between presence of ZnT8ab and severity of diabetes clinical manifestation is still ambiguous. This study aims to assess the prevalence of ZnT8ab and its correlation to other autoimmune markers and DKA presence in children and adults with T1DM onset.
Materials and methods: The study cohort comprised 367 patients, including 218 children (girls: 95; boys: 123;mean age: 9±4 years) and 149 adults (women: 78; men: 71; mean age: 35±11 years) Diabetes was diagnosed based on WHO criteria. To confirm autoimmune diabetes origin typical autoantibodies were tested (GAD-ab, IA2-ab, ZnT8ab). Diabetic ketoacidosis was defined as blood pH < 7.30. All statistical analyses were performed with „Statistica Software". All p-values was two-sided, and p-value < 0.05 was considered significant.
Results: On disease onset children were more frequently diagnosed with DKA (28,4 vs 10,7%, p=00002), had higher glycaemia (447±191 vs 339 ±145 mg/dl, p=0,0017), lower C-peptide level (0,4±0,2 vs 1,1±0,7 pmol/ml, p<0,0001) compared to adults. The presence of ZnT8ab (81,1 vs 34,9%, p<0.0001) and IA2ab (80,7 vs 41,6%, p<0,0001) had been reported more often in children, and the titter of IA2ab was higher among youth (401,8±871,9 vs 12,3±14,5 U/ml, p<0.00001). Adults had a higher titter of GADAab compared to children (446,8 ±734,6 vs 17,2±32,6 U/ml, p<0,000001). 78 patients were diagnosed with DKA (79.5% children). Individuals with DKA were younger (13 ± 12 vs 21 ± 15 years, p = 0.043), had reported more frequently the presence of ZnT8ab (79.4 vs 57.7%, p = 0.0017), and anti IA2ab (78.2 vs 61 2%, p = 0.0065), but the titters of IA2ab (70.3 ± 271.1 vs 191.2 ± 634.2 U / ml, p < 0.0001) and GADAab (76 ± 293.3 , vs 217 ± 547.9 U / ml , p < 0.00001) were lower compared to patients without DKA. There was no statistically significant difference in GADAab prevalence in patients with DKA compared to those without DKA.
Conclusion: Children and adults have a different autoantibodies pattern at the diabetes diagnosis. ZnT8ab and IA2ab are more often detected in children, while adults have frequently higher titter of GADAab. Similar pattern is observed among individuals with and without DKA. The presence of ZnT8ab is associated with acute onset of diabetes. Youth have a highest risk of developing ketoacidosis.
Disclosure: **A. Rogowicz-Frontczak:** None.

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Is there regulation of the autoimmune response in slow progressors to type 1 diabetes?

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Background and aims: Multiple islet autoimmunity increases risk of diabetes but not all individuals positive for two or more islet autoantibodies progress to disease within a decade. The SNAIL Study seeks to harmonise data from longitudinal studies to identify the characteristics of slow progression to type 1 diabetes.
Materials and methods: Samples from 131 individuals with multiple islet autoantibodies (IAA, GADA, IA-2A and ZnT8A) followed for more than 10 years without progression were available from five studies (Bart's-Oxford (BOX) - UK; BABYDIAB - Germany; DAISY and Pittsburgh Diabetes - USA; ABIS - Sweden). Individuals enrolled in BOX provided "Rapid Progressor" (diagnosed <age 5yrs) and "at diagnosis" samples. HLA Class I and II were analysed by PCR-SSP. Islet autoantibody profile was analysed by radioimmunoassay and/or ECL.
Results: Intermediate HLA-Class II risk was more frequent in Slow (60%) than Rapid Progressors (42%) with a reciprocal reduction in high risk genotypes (24% vs. 48%; pCorr=0.005); only one carried protective HLA DQ6. Slow Progressors carried fewer HLA-Class I B risk alleles (48%) than Rapid Progressors (86%; pCorr<0.001). Of 35 Slow Progressors with longitudinal data available, only 13 (37%) retained multiple autoantibodies after 10 years (p<0.001). A reduction in positivity for IAA and GADA was observed (p<0.001 and p=0.016 respectively) and in levels of IA-2A and ZnT8A even though autoantibody positive status had not changed (p<0.05 for all). In addition, Slow Progressors had lower levels of all IA-2A IgG (1-4) subclasses than individuals sampled close to diagnosis (p<0.05).
Conclusion: Multiple autoantibody positivity is not maintained in some Slow Progressors suggesting regulation of the autoimmune response. Continued immuno-phenotyping of these individuals is required to elucidate the mechanisms underlying a decreased humoral response and delayed progression.
Supported by: JDRF
Disclosure: **K.M. Gillespie:** None.

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In vitro TNFR2 agonism for correction of Treg activation defect in type 1 diabetes

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Background and aims: Tumor necrosis factor receptor 2 (TNFR2) is obligatory for induction, maintenance and expansion of activated regulatory T cells (aTregs), known to prevent or halt various forms of autoimmunity in animal models and humans. This study investigated the effect of TNFR2 agonism on aTreg expansion in patients with type 1 diabetes (T1D).
Materials and methods: Treg cell frequency in peripheral blood of T1D subjects (adults and children) and controls was assessed using flow cytometry and plotted against age and HbA1c. Treg expansion protocols were performed in culture using IL2, tumor necrosis factor (TNF) and TNFR2 agonistic antibodies.
Results: T1D patients had normal numbers of total Tregs, but had an increase in resting Tregs (rTregs) and a decrease in aTregs compared to controls (n= 55 T1D, n=45 controls, p=0.01). This Treg activation defect is lifelong (n=100 T1D, p<0.01). Lower numbers of aTregs were associated with having less residual C-peptide secretion from the pancreas (p=0.08) and poorer HbA1C control (p=0.03). TNFR2 antibody agonism corrected the T1D activation defect by triggering conversion of rTregs into aTregs (n=54 T1D, p<0.001). TNFR2 antibody agonism was superior to standard protocols of Treg expansion and superior to TNF in expanding the most potent subsets of Tregs. TNFR2 antibody expansion protocols exclusively expanded Treg cells but not CD4 T cells, thus creating homogenous populations of potent human Tregs in culture. In T1D, TNFR2 agonist-expanded Tregs were functionally potent by virtue of suppressing autologous cytotoxic T cells in a dose-dependent manner compared to controls.
Conclusion: Targeting the TNFR2 receptor for Treg expansion may be a means to correct the Treg activation defect in T1D.
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Disclosure: **D.L. Faustman:** Grants; Iacocca Foundation, American Autoimmune Related Disease Association. Other; Travel grant for Y.O.: Manpei Suzuki Diabetes Foundation.

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Proinsulin peptide immunotherapy in new-onset type 1 diabetes is well-tolerated and associated with reduced daily insulin usage

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Background and aims: In type 1 diabetes (T1D), antigen specific immunotherapy (ASI) strategies have been shown to be effective in disease prevention in pre-clinical settings. Such studies have demonstrated that ASI may be effective when autoantigen is delivered as a short peptide, representing a key target of the pathological T cells response that is characteristic of the disease. To date, however, it has proved challenging to translate these findings into clinical settings. Here, we report safety data on a phase 1b trial of the naturally processed and presented proinsulin peptide C19-A3 (PI C19-A3) in newly diagnosed T1D, as well as, preliminary metabolic and immune results. Aims: We aimed to examine the safety and tolerability of intradermal administration of (PI C19-A3) at a dose of 10μg in new-onset T1D. Secondary endpoints included metabolic and immune effects of (PI C19-A3).
Materials and methods: The trial was a multi-centre, randomized; double-blind, placebo-controlled design, using 10μg of PI C19-A3 administered intradermally at high frequency (HF) every 14 days, or low frequency (LF) every 28 days (a total of 12 doses and 6 doses, respectively) over 24 weeks with a subsequent follow-up phase of 24 weeks. Twenty-seven patients aged 18-45 with new-onset T1D (within a 100 days of diagnosis), HLA-DRB1*0401 genotype, antibody positivity (GAD, IA2 or ZnT8) and a stimulated C-peptide level >0.2 pmol/ml were recruited. To maintain blinding, all patients received 12 injections at fortnightly intervals. The placebo group (n = 8) received normal saline only; the LF group (n = 10) had alternate PI C19-A3 and normal saline administration; and the HF group (n = 9) had PI C19-A3 only.
Results: Administration of PI C19-A3 was safe and well tolerated. The only treatment emergent adverse event noted was transient erythema at the injection site. No systemic type 1 hypersensitivity or treatment related serious adverse events were reported. Within the power of the study, there was no significant change in C-peptide levels between the groups, but there was a trend to greater preservation in the treatment groups. No advantage was seen for 2 weekly over 4 weekly dosing. The mean changes in the daily insulin use were significantly lower in the HF group at 6, 9 and 12 months (p=0.03; p=0.04; p=0.01, respectively) and significantly lower in the LF group at 12 months (p=0.009) compared with the placebo group which showed a progressive rise. There was a trend for increased HbA1c levels in the placebo group, whereas change in the treatment groups was minimal or values declined.
Conclusion: PI C19-A3 peptide immunotherapy in the dosing regimen used was safe, well tolerated and free from any systemic hypersensitivity or serious adverse reactions. Treatment with PI C19-A3 was associated with reduced or stable daily insulin use, compared with apparent increase in the placebo group. Furthermore, the stable insulin use in either of the treated groups was not associated with poorer glycaemic control. This phase 1b trial paves the route for future phase 2 trials in new-onset T1D to examine effectiveness of PI C19-A3 on preservation of β cell function and amelioration of T cell autoimmunity.
Clinical Trial Registration Number: EudraCT Number: 2007-003759-35
Supported by: DVDC (Diabetes Vaccine Development Centre)
Disclosure: **M. Alhadj Ali:** None.

OP 19 Diabetes drugs of the future

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Early clinical development of the dual GLP-1/glucagon receptor agonist SAR425899 and establishment of a population PK/PD model

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Background and aims: GLP-1 agonists are well-established therapies offering robust glycemic control associated with weight loss in patients with T2DM. Clinical evaluation with a native dual GLP-1/glucagon receptor agonist, suggests a potential for superior weight loss over GLP-1. SAR425899 is a novel single peptide with dual activity (GLP-1 & glucagon receptor).
Materials and methods: Clinical development of SAR started with a single dose (SAD) study followed by a combined multiple dose study in healthy subjects (MAD-1) and T2DM patients (MAD-2, NCT02411825). Studies were placebo controlled, randomized and double blind. A pop PK/PD model using data from SAD, MAD-1 & MAD-2 was developed, focusing on predicting the effect of SAR on FPG & body weight.
Results: SAD: 32 subjects were randomized to receive 1 out of 5 SAR doses (10, 30, 50, 75 & 100 μg subcutaneously) or placebo. Drug exposure & Cmax increased dose proportionally, mean half-life was about of 11-17 h across doses. Glucose levels following a MMT were lowered by SAR at doses > 30 μg with a peak change at 75 μg (AUC0-24h: -18.14 (SD 6.79) mmol h/L). Adverse Events related to gastrointestinal disorders were reported most often. Heart rate increased at doses > 50 μg. MAD-1: 40 subjects were treated with 1 out of 5 dose regimens (25 - 50 - 75, 50 - 75 - 100, 50 - 100 - 150, 60 - 120 - 180, 50 - 100 - 200 μg SAR) over 21 days including two uptitration steps. FPG, PPG and BW were lowered at all doses with maximal weight loss at highest dose (-6.4% vs. 0.7% for placebo). Dose-dependent GI AEs were observed most frequently, increased heart rate and a few cases of mild plasma level elevations of lipase were noted for all dose groups. MAD-2: 36 overweight to obese subjects with T2DM were receiving 1 out of 2 dose regimens, 30 - 60 - 90 or 60 - 120 - 180 μg SAR, over 28 days with uptitration after day 7 and 14. FPG, PPG, HbA1c and BW lowering was higher in both treatment groups compared to the placebo group (FPG: -3.0 vs. -1.2 mmol/L; BW: -5.4 vs. -2.3 kg at high dose level). AEs were similar to those observed in MAD-1 while heart rate elevation was less pronounced. PK & PD: FPG & BW, were well described by the models and subject-specific parameters were fitted (PK: CL=0.37-0.72 l/h; V=6.5-12 l; Ka=0.12-0.23 1/h). Model predictions were confirming measured experimental data (goodness of fit: r2=0.91-0.95).
Conclusion: SAR efficiently lower glucose and BW while being safe and generally well tolerated except for occasional findings of GI side effects. The PK/PD models are predicting the individual profiles of FPG & BW and strengthen the glucose & BW lowering properties of SAR.
Clinical Trial Registration Number: NCT02411825
Supported by: Sanofi
Disclosure: **K. Lindauer:** Employment/Consultancy; Sanofi Aventis Deutschland GmbH. Stock/Shareholding; Sanofi. Other; Studies were funded by Sanofi.

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Pharmacological characterisation of MEDI0382, a glucagon/GLP-1 dual agonist for treatment of obesity and type 2 diabetes

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[1]Cardiovascular and Metabolic Disease, MedImmune, Cambridge, UK, [2]Cardiovascular and Metabolic Disease, MedImmune, Gaithersburg, USA, [3]Antibody Discovery and Protein Engineering, MedImmune, Cambridge, UK.

Background and aims: The endogenous peptide oxyntomodulin (OXM), a glucagon-like peptide-1 (GLP-1)/glucagon dual agonist peptide, decreases body weight in obese subjects by reducing appetite and increasing energy expenditure. MEDI0382 is a synthetic GLP-1/glucagon dual agonist peptide modified for extended half-life, characterized by an optimum balance of agonist activity at the GLP-1 and glucagon receptors, designed to achieve superior weight loss effect and glycaemic control. Here we report on the pharmacological characterization of MEDI0382 in vitro and in vivo in wild type (wt) and GLP-1 receptor knock out (GLP-1R KO) mice.

Materials and methods: Intracellular accumulation of the second messenger cyclic AMP was measured in GLP-1 and glucagon receptor over-expressing cell lines and physiologically relevant cell lines from different species endogenously expressing these receptors. Functional assays include glucose-dependent insulin secretion (GDIS) in pancreatic beta cell line (INS-1) and hepatic glucose output in primary hepatocytes. In vivo, MEDI0382 was acutely administered s.c. in wt and GLP-1R KO mice and its effect on i.p. glucose tolerance test (GTT) and food intake were compared to liraglutide. Glucose levels were also measured after mice were pre-treated with somatostatin.

Results: In vitro data show that dual pharmacology of MEDI0382 at GLP-1 and glucagon receptors is preserved across multiple species, with its activity being slightly biased towards GLP-1 receptor and weaker at the glucagon receptor (glucagon receptor/GLP-1 receptor relative potency ratio = 0.2 as measured by cyclic AMP accumulation in presence of physiological levels of plasma albumin). Importantly, MEDI0382 exhibits similar potency as GLP-1 in the GDIS assay (1068±273 pM vs 1506±447 pM) and greater potency than OXM in the hepatic glucose output (83±28 pM vs 1954±1139 pM). In wt mice, a single subcutaneous administration of MEDI0382 or liraglutide (3 nmol/kg) decreased glucose excursion following an i.p. glucose challenge (AUC reduced by 52.8% and 26.1% respectively). MEDI0382 (30 and 100 nmol/kg) or liraglutide (30 nmol/kg) also reduced food intake up to 24 hours by 17.5%, 26.9% and 33.7% respectively. The same treatments in GLP-1R KO mice did not alter glucose disposal and food intake, suggesting that the acute metabolic effect is solely driven by the GLP-1 activity of MEDI0382. Glucose levels measured after pre-treatment with somatostatin were increased by MEDI0382 in GLP-1R KO mice, but this effect was absent in wt mice, corroborating the balanced dual GLP-1/glucagon agonist activity of MEDI0382, while the glucagon analogue IUB288 increased glucose levels in both models.

Conclusion: The in vitro pharmacological profile of MEDI0382 indicates that its activity is biased towards the GLP-1 receptor over glucagon receptor. The relative potency at each receptor translates between transfected and endogenous receptor systems and across species. MEDI0382 acute effects on food intake and glucose disposal are mediated solely by the GLP-1 component activity. Chronic administration of MEDI0382 will be performed to determine the beneficial metabolic effects mediated by the glucagon activity residing in the peptide.

Disclosure: **A. Rossi:** None.

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YH25724, a novel long-acting GLP-1/FGF21 dual agonist provides potent and sustained glycaemic control, body weight loss and lipid profile improvement in animal models

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Background and aims: Targeting multiple metabolic pathways provides better therapeutic potential compared to molecules targeting a single pathway, because metabolic disorders are triggered by various complicated factors. Glucagon-like peptide-1 (GLP-1) receptor agonists have been developed and approved for the treatment of type 2 diabetes given their benefit from the glucose-dependent insulinotropic effects of GLP-1. Fibroblast growth factor 21 (FGF21) is a promising drug candidate for the treatment of metabolic diseases, which enhances insulin sensitivity. Thus, a complementary and synergistic therapeutic potential may be anticipated with a dual agonist capable of delivering both GLP-1 and FGF21 agonism. However, dual agonist should be rationally designed to provide balanced activity and to reduce the GLP-1 related side-effects. YH25724 is a novel long-acting dual agonist, which is an immunoglobulin Fc-fused protein comprising GLP-1 variant and FGF21 variant. Here we report the characterization of a novel long-acting GLP-1/FGF21 dual agonist, YH25724.

Materials and methods: In vitro activation of GLP-1 receptor was measured by cAMP formation in CHO cells stably expressing the human GLP-1 receptor. In vitro activation of the FGF receptor and βKlotho complex was measured by ERK phosphorylation in HEK293 cells stably expressing human βKlotho. Pharmacokinetic characterization of YH25724 was performed in mice and monkeys. Pharmacodynamic effects of YH25724 were investigated in db/db mice and low-dose streptozotocin-treated mice fed with high-fat diet (HFD/STZ) by measuring blood glucose levels and body weight change. The effects on body weight loss and lipid profiles by YH25724 were investigated in diet-induced obese (DIO) mice. Immunogenicity of YH25724 was assessed using ex vivo T cell activation assays.

Results: YH25724 exhibited balanced in vitro activity for GLP-1 and FGF21. GLP-1 activity of YH25724 was deliberately attenuated in order to reduce side effects, while FGF21 activity was maintained to exert beneficial metabolic outcomes. Based on PK data in mice and monkeys, YH25724 has an optimal PK profile for once-weekly dosing in human. After single subcutaneous injection in db/db mice and HFD/STZ mice, YH25724 elicited rapid, potent, sustained and dose-dependent glucose-lowering and profound weight loss compared to either single agonism (GLP-1 or FGF21 agonist) or dulaglutide. Following repeat dosing in DIO mice, YH25724 produced a synergistic effect with more potent and sustained body weight loss compared to dulaglutide or single agonism. In the adipose tissue of DIO mice, YH25724 induced UCP-1 and PGC1α expression, indicating that these effects may be due to increased energy expenditure. YH25724 treatment also improved serum lipid profiles with reduction of hepatic triglyceride contents. Furthermore, ex vivo T cell activation assay results indicate that YH25724 has a low potential risk for clinical immunogenicity.

Conclusion: Given these findings, YH25724, a novel long-acting, well-balanced GLP-1/FGF21 dual agonist, has a complementary and synergistic mechanism of insulin secretion and insulin sensitivity, which is well translated into a potent and sustained glycemic control with the additional benefits of body weight loss and lipid profile improvement, indicating that YH25724 may be the ideal therapeutic for the treatment of type 2 diabetes.

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Disclosure: **H.N. Hong:** Employment/Consultancy; Employee of Yuhan Co.

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CRISPR/Cas-9 engineered pancreatic beta cell lines can delineate the pharmacology of novel dual GIPR/GLP-1R agonists

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Background and aims: Determining the relative activity of dual agonists at their target receptors is challenging but essential for the development of therapeutics. An innovative strategy for diabetes treatment uses dual-agonists to target both glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) receptors to enhance insulin secretion. Our aim was to develop novel dual-agonists and evaluate their activity in physiologically relevant β-cell lines.

Materials and methods: Clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9 endonucleases were used to ablate GIP or GLP-1 receptors from the INS-1 832/3 pancreatic β-cell line. Clonal cell lines confirmed as functional knock-outs in cyclic AMP Homogeneous Time-Resolved Fluorescence (HTRF) accumulation assay and glucose stimulated insulin secretin (GSIS) assays were used to profile dual agonists before progression to an acute i.p. GTT study. Wild-type (Glp1r+/+) and Glp1r knockout (Glp1r−/−) littermates on a C57BL/6 background were fasted for 6 h before receiving vehicle or dual agonist s.c. 2 hour prior to i.p. 2g/kg glucose challenge. 24 hours prior to the glucose challenge mice received a s.c. dose of GIP receptor antagonist (Gipg013) or isotype control antibody (NIP228). Blood samples were collected from tail vein for glucose measurements. Statistical significance was assessed using a one way ANOVA comparing vehicle to compound with a Dunnetts post-hoc test (*$p<0.05$).

Results: Cyclic AMP production following GIP or GLP-1 receptor activation was abolished in corresponding CRISPR/Cas9 knock-out INS-1 cell lines. GSIS was also attenuated. Profiling dual agonist molecules in knock-out cell lines allowed for clarification as biased towards the GIP or the GLP-1 receptor, or with relatively balanced potency. Dual agonist bias was confirmed in wild-type INS-1 cells using GLP-1 or GIP receptor selective antagonists exendin (9-39) and Gipg013 respectively. Dual agonists with balanced dual activity in in vitro assays were progressed to i.p. GTT in wild-type and GLP-1 receptor knock-out mice. Dual agonists reduced glucose excursion compared to vehicle control in wild-type mice demonstrating an improved glucose disposal. In wild-type mice pre-treated with Gipg013, a modest reduction in glucose excursion due to dual agonist was still apparent despite GIP receptor blockade. In GLP-1 receptor knock-out mice, dual agonists still improved glucose tolerance. In GLP-1 receptor knock-out mice pre-treated with Gipg013, the effects of dual agonists were completely attenuated, suggesting glucose excursion occurs via activation of both GIP and GLP-1 receptors.

Conclusion: CRISPR/Cas9 engineered knock-out cell lines can be used to profile pharmacological contributions of dual agonist molecules in native expression systems where signalling pathways overlap. This has allowed for the development of novel scaffolds for dual GIP/GLP1 agonists. Preliminary data for a balanced dual agonist in an acute i.p. GTT study shows activity at both GIP and GLP-1 receptors as predicted. Activity at just one receptor compensates for the absence of signal through the other receptor, suggesting balanced dual agonism is achievable in vivo.

Disclosure: **J. Naylor:** None.

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PXL770, a novel direct AMPK activator, improves metabolic disorders in a diet-induced mice model of obesity and diabetes

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Background and aims: PXL770 directly activates adenosine monophosphate-activated protein kinase (AMPK), an enzyme that regulates cellular energy metabolism by inhibiting energy-consuming pathways and stimulating energy-producing pathways. Accordingly, PXL770 could play an important role in the management of diabetes and hyperlipidemia. The aim of this study was to assess the effect of PXL770 on body weight, food intake and energy metabolism, in a mouse model of obesity and type 2 diabetes.

Materials and methods: 5-week old C57BL/6J mice were fed during 10 weeks with a normal chow diet (NC) or a high-fat diet (HFD). HFD mice were then orally treated for 5 weeks with PXL770 75mg/kg bid or a vehicle. A pair-fed group, fed with the same amount of food than this consumed in the PXL770 75 mg/kg bid group, received oral administrations of vehicle during the same period of time. Body weight, food intake, respiratory exchanges (indirect calorimetry), glycemia, oral glucose tolerance and fat mass were assessed during the study.

Results: PXL770 75mg/kg bid induces a rapid, marked and transient caloric intake decrease and body weight loss, during the first 4 days of treatment. The pair-fed group experiences the same body weight loss during this period. From day 4 till the end of the treatment, the PXL770 group experienced a steady body weight gain, whereas a body weight gain increase was observed in the pair-fed-group despite the same caloric intake as in the PXL770 group. This suggests that the long-lasting effect of PXL770 on body weight loss is independent of its effect on caloric intake. After 3 weeks of PXL770 treatment, an increase in fat oxidation (+21%, $p<0.01$ vs vehicle) and a decrease in CHO oxidation (-29%, $p<0.01$ vs vehicle) occur in comparison to both the vehicle and the pair-fed groups. In parallel, a trend in total energy expenditure increase was observed with PXL770 vs pair-fed group. Additionally, PXL770 treatment induces a significant decrease in 3h-fasted glycemia by contrast to the pair-fed animals that remained hyperglycemic throughout the experiment. PXL770 after 4 weeks significantly improves glucose tolerance in HFD mice (-32% vs pair-fed group, $p<0.001$). This improvement may be due to a caloric intake-independent metabolic effect, since no benefit on glucose tolerance was observed in the pair-fed animals, whereas they ingested the same calorie amount as the PXL770-group. Consistently with the results obtained on body weight, the HFD and the pair-fed groups show a significantly increased adiposity when compared with NC-fed group. PXL770 reduces the fat mass in relation with the fat oxidation increase. The weight of two white adipose tissues (epididymal and perirenal) was decreased by 53% ($p<0.0001$) for both adipose tissue pads compared to the pair-fed group.

Conclusion: These results show that PXL770, in a model of diet-induced obesity and diabetes, induces a weight loss by increasing energy expenditure and fat oxidation, leading to a decrease in fat mass. These effects may contribute to the improvement of basal glycemia and glucose tolerance by PXL770 treatment. These results confirm the therapeutic potential of PXL770 for metabolic disorders, such as type 2 diabetes.

Disclosure: **S. Bolze:** Employment/Consultancy; employment.

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Transient effect of glucokinase activator on beta cell proliferation

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Background and aims: Glucokinase activators (GKAs) stimulate both insulin secretion and glucose utilization in the liver and have antidiabetic efficacy in rodents. We have proposed that GKAs could improve glucose metabolism and exert an effect on beta cell proliferation as well as beta cell function. However, the results of recent clinical trials have shown that some GKAs led to improvements in glycemic control that were not sustained. Moreover, other reports showed that genetic activation of glucokinase in beta cells, which initially triggered proliferation, led to beta cell death. Therefore, a better understanding of the effect of glucokinase activation on beta cell survival is needed to resolve the problem identified in clinical trials. In the present study, we investigated the effect of GKA on beta cell proliferation and changes in related gene expressions.
Materials and methods: C57bl/6J mice were fed standard chow until 8 weeks of age, and then given free access to either standard chow or standard chow containing 0.04% GKA. After 1, 3 and 7 days, we compared beta cell proliferation and mass in the two groups. The expression levels of genes involved in beta cell proliferation and function, oxidative stress and apoptosis were examined in isolated islets from mice before and after 3 and 7 days of GKA treatment.
Results: Although there were no significant differences in body weight between mice with or without GKA, the blood glucose decreased shortly after GKA and the fed blood glucose level of mice with GKA was significantly lower than that of mice without GKA until 7 days. Immunohistochemical analysis revealed a significant increase in the BrdU incorporation rate in mice with GKA compared with mice without GKA after 3 days, although there were no differences between the two groups after 1 or 7 days (after 1 day, 0.62 ± 0.13% without GKA, 0.48 ± 0.10% with GKA; after 3 days, 0.68 ± 0.14% without GKA, 1.84 ± 0.39% with GKA, $P < 0.05$; after 7 days, 0.44 ± 0.17% without GKA, 0.61 ± 0.14% with GKA). There were no differences in beta cell mass between the two groups after 1, 3 or 7 days. Real-time quantitative PCR showed that Ki67, Irs2, Glut2 and Cyclin D1 mRNA levels were significantly increased in the isolated islets from mice after 3 days of GKA treatment compared with those before treatment. Moreover, the levels of expression of genes for the reduced-form nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex such as p22phox and p47phox were coordinately elevated in islets isolated from mice after 3 and 7 days of GKA treatment in comparison with those before treatment. Similarly, caspase3 mRNA level was significantly increased in the isolated islets from mice after 3 days of GKA treatment compared with those before treatment and tended to be increased after 7 days of GKA treatment.
Conclusion: The effect of glucokinase activator on beta cell proliferation was transient. In pancreatic beta cells, augmentation of glucose signaling by glucokinase activation could lead to an initial boost of beta cell proliferation and later fueling of a vicious cycle of glucotoxicity that precipitates beta cell failure.
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Disclosure: **A. Nakamura:** None.

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Large scale exome array meta-analyses identify multiple novel common, low frequency and rare coding variants associated with glycaemic traits

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Background and aims: Genetic risk loci for complex diseases and traits, including type 2 diabetes and glycaemic traits, frequently contain several genetically linked single nucleotide variants (SNVs) that can alter the coding sequence or expression of one or more genes. Since the effects of coding variants on the protein sequence can be exactly predicted, identification of trait-associated protein-coding SNVs may help to efficiently identify transcripts that are directly involved in expression of the trait. Our aim was to explore the role of coding SNVs in glycaemic traits.
Materials and methods: We investigated the association of 241,320 common (minor allele frequency (MAF)>5%), low frequency (MAF 1-5%) and rare (MAF<1%) exome array variants with fasting glucose (FG), fasting insulin (FI), 2-hour glucose (2hGlu) and HbA1c levels in up to 144,060 non-diabetic individuals of European (85%), African-American (6%), South Asian (5%), East Asian (2%) and Hispanic (2%) ancestry from up to 66 studies. We combined single variant results from linear mixed models by fixed effect meta-analyses.
Results: Forty-three loci showed significant ($P<2.07\times10^{-7}$ for coding and $P<5\times10^{-8}$ for non-coding variants) associations with FG, including 12 novel FG-associated loci, 50 loci (19 novel) associated with HbA1c, 16 (4 novel) with FI and 14 (5 novel) with 2hGlu. Among the lead SNVs at novel loci, 21 were missense variants, including two low-frequency and four rare SNVs. These included rare variants in ANKH and MAP3K15 associated with FG (ANKH: R187Q, MAF=0.4%, β= -0.19 mmol l^{-1}, $P=5.7\times10^{-10}$; MAP3K15: G838S, MAF=0.4%, β=-0.18 mmol l^{-1}, $P=1.4\times10^{-11}$). The rare exonic ANKH variant also maps to the vicinity of a putative antisense lncRNA expressed in the adjacent intron in pancreatic beta cells, an alternative mechanism through which the variant, or a variant in LD with it, might influence FG levels. A common missense SNV in OBSL1 was associated with FI (E1273D, MAF=27.1%, β=0.02 log-pmol l^{-1}, $P=8.5\times10^{-10}$) while two common missense SNVs in SWAP70 (Q447E, MAF=38.3%, β=-0.01%, $P=1.2x10^{-21}$) and MLXIPL (A265V, MAF=11.7%, β =0.01%, $P=6.2\times10^{-9}$) were associated with HbA1c. OBSL1 is implicated in 3-M syndrome, one of the commonest primordial growth disorders which is associated with IGF1 resistance. SWAP70 has recently been implicated in coronary artery disease while MLXIPL maps to a known lipid-associated locus. A low-frequency missense variant in DVL2, a locus previously implicated in T2D in East Asian individuals, was associated with 2hGlu in individuals of European and African ancestry (T535I, MAF=2.1%, β=0.20 mmol l^{-1}, $P=4.1\times10^{-11}$).
Conclusion: We identified novel coding variants across the allele frequency spectrum associating with glycaemic traits, further unravelling the genetic architecture and biology underlying these traits.
Disclosure: **S.M. Willems:** None.

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Genetically driven hyperglycaemia increases risk of coronary artery disease separately from type 2 diabetes

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Background and aims: Observational evidence suggests that individuals with type 2 diabetes (T2D) have higher coronary heart disease (CHD) risk. However, the putative role of hyperglycaemia per se on CHD risk independently of the risk conferred by T2D as a whole remains unclear. The incomplete overlap existing between genetic associations with fasting glucose (FG) and T2D provides an avenue to disentangle this relationship. We sought to test the hypothesis that genetically raised FG increases CHD risk independently of the risk conferred by T2D.

Materials and methods: We conducted a Mendelian randomization instrumental analysis using summary-level statistics from the largest meta-analyses of genome-wide association studies (GWAS) for FG (n = 133 010 non-diabetic participants) and CHD (n = 63 746 cases / 130 681 controls). We excluded FG-increasing variants that were also associated with T2D (P < .05) from the biggest GWAS for T2D (n = 34 840 cases / 114 981 controls). We excluded genetic variants with pleiotropic effects on other CHD risk factors (blood lipids, blood pressure and body mass index) using summary level results sourced from the largest GWAS meta-analyses conducted to date for each trait. The main outcome was the estimation of the risk of CHD. Instrument validation was conducted using individual data from the Framingham Heart Study (n = 5113). All datasets included participants that were predominantly of European descent.

Results: We identified 12 independent FG-raising genetic variants. A polygenic instrument comprising all FG-raising variants increased CHD risk (odds ratio [OR], 1.43; 95% confidence interval [95% CI], 1.14-1.68 per 1 mmol/L increase in FG, P < .01). The association was preserved after excluding variants for heterogeneity and pleiotropic effects on other CHD risk factors (OR, 1.33; 95% CI, 1.02-1.73; P= .03, per 1 mmol/L increase in FG). We confirmed that genetically isolated FG did not increase T2D risk (OR, 1.05; 95% CI, 0.90-1.22; P = .54). In the validation study, the 12 FG-raising genetic instrument explained 5.03% of the proportion of variance in FG.

Conclusion: Genetic predisposition to hyperglycaemia raises CHD risk independently of T2D and other CHD risk factors. These findings suggest that modulating glycaemia may provide cardiovascular benefit.

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Characteristics of patients with low and high HbA_{1c} variability in type 2 diabetes

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Background and aims: HbA1c variability is reported to be associated with increased risk of vascular events and mortality in patients with type 2 diabetes (T2D). Short-term studies have been conducted to determine the factors associated with increased glucose variability. However, no studies have been carried out to determine the factors associated with increased HbA1c variability over an extended time period. This study set out to identify factors that were associated with HbA1c variability over a 4-year period in a cohort of over 10,000 patients with T2D.

Materials and methods: We conducted a retrospective observational study on a Scottish (Tayside) population of T2D patients (n=10,131). HbA1c readings were collected from the Scottish Care Information- Diabetes Collaboration (SCI-DC) database between 1/1/2010 and 1/1/2014. Exclusion criteria: less than 4 HbA1c readings over the 4-year time period; T2D diagnosed after 1/1/2010; leaving the Tayside area before 1/1/2014 and death before 1/1/2014. The SD and CV of the HbA1c readings were calculated. A high variability group was defined as the top quartile of the distribution; and low variability as the bottom quartile. A logistic regression analysis was then undertaken including the following baseline predictors: sex, age, duration of diabetes (<2.5 years, 2.5-7 years and >7 years), treatment (diet, mono and dual therapy, triple and insulin therapy), HDL-cholesterol (<1 mmol/l, 1.0-1.3 mmol/l and >1.3 mmol/l) and BMI (<25 kg/m^2, 25-35 kg/m^2 and >35 kg/m^2). The analyses were stratified by mean HbA1c for the period into two groups: low HbA1c (<58 mmol/mol) and high HbA1c (≥58 mmol/mol).

Results: Similar results were observed in both variability metrics with SD giving a superior fit in the logistic regression model; results are therefore presented for the SD. Treatment, independent of other factors including duration of diabetes, had a big impact on the risk of being highly variable with those on triple or insulin therapy having nearly 10-fold increased risk of being highly variable in the low HbA1c group (OR 9.86, p<0.001 compared to diet treatment) and a 3-fold increased risk in the high HbA1c group (OR 2.94, p=0.01). Whereas those treated with mono or dual therapy were only 3 times more likely to be highly variable in the low HbA1c group (OR 3.22, p<0.001). In addition increased HbA1c variability was seen in men compared to women (low HbA1c group OR 1.49 p<0.001; high HbA1c group OR 1.81 p=0.018); with younger age (low HbA1c group OR 0.98 per year p<0.001; high HbA1c group OR 0.97 per year p=0.012); and increasing BMI in the low HbA1c group (OR 2.38, p<0.001). Relative to an HDL <1.0 mmol/l, an increased HDL was protective, with those with an HDL >1.3 mmol/l more likely to have low HbA1c variability in the low HbA1c group (OR 0.55, p<0.001) and the high HbA1c group (OR 0.40, p=0.007).

Conclusion: For poorly or well controlled patients, based upon their mean HbA1c, the variability of the HbA1c around this mean is

consistently increased with younger age, greater obesity, and lower HDL-cholesterol. It is striking, although not surprising, that the HbA1c variability in well controlled insulin treated patients is far more than in patients treated with diet, or mono and dual therapy. Further work is required to establish if HbA1c variability per se is causally associated with adverse outcomes or whether the reported associations reflect our findings that the group who are most variable are the group with the highest cardiovascular risk factors.

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Disclosure: **J.D. Noyes:** None.

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Prediabetes is associated with white matter atrophy: the MAASTRICHT study

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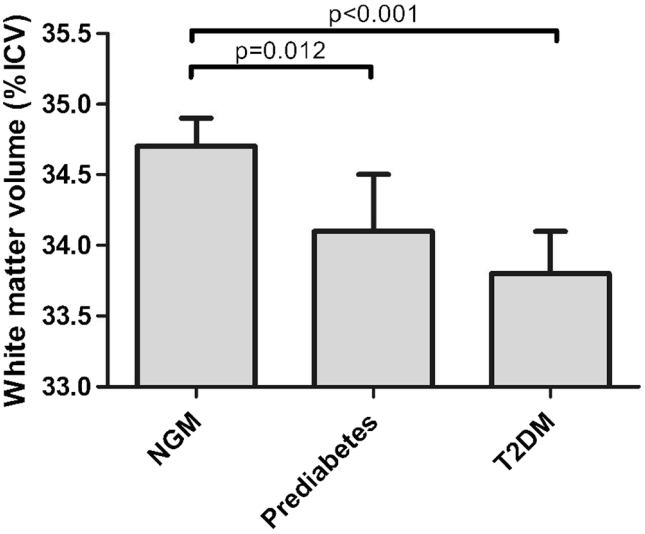
Background and aims: Type 2 diabetes mellitus (T2DM) is associated with brain atrophy and cerebral small vessel disease (CSVD), both of which are believed to be microvascular in origin. Prediabetes has been associated with microvascular dysfunction as well, however information on the relation between prediabetes and brain atrophy and CSVD is scarce. The aim of the present study was to evaluate whether prediabetes and T2DM are both associated with brain atrophy and white matter hyperintensities (WMH), a proxy of CSVD, in a population aged 40-75 years.

Materials and methods: We used cross-sectional data from The Maastricht Study, a population-based cohort study with an oversampling of T2DM (n for the present analysis=933, mean age 59.8 ±8.3, 45.8% female, 557 normal glucose metabolism (NGM), 146 prediabetes, and 230 T2DM). We determined white matter hyperintensities, white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) volumes, relative to intracranial volume (ICV), using automated segmentation of 3 Tesla MR T1, T2 and FLAIR weighted images. NGM, prediabetes and T2DM were determined by use of an oral glucose tolerance test and glucose lowering medication use, according to the WHO 2006 criteria. We used linear regression analyses to assess the association between glucose metabolism status and tissue volumes, and adjusted for age, sex, BMI, systolic blood pressure, total-to-HDL cholesterol ratio, and triglyceride levels. Results are reported as standardized regression coefficients (stβ) with 95% CI.

Results: Prediabetes and T2DM were associated with a smaller WM volume after full adjustment. The stβ of WM volume was -0.084[-0.010;-0.001], p=0.012 and -0.166 [-0.013;-0.005], p<0.001. T2DM was associated with a smaller GM volume (stβ=-0.095[-0.010;-0.002], p=0.008), while prediabetes was not (stβ=0.023[-0.003;0.006], p=0.458). T2DM was associated with a larger CSF volume (0.136 [0.005;0.013], p<0.001), while prediabetes was not (0.040[-0.001;0.007], p=0.160). T2DM was associated with a larger WMH volume (stβ 0.123 [0.084-0.358], p=0.002), while prediabetes was not (stβ 0.013 [-0.115;0.169], p=0.709).

Conclusion: Prediabetes is associated with white matter atrophy, while T2DM is associated with brain atrophy as indicated by both white and grey matter atrophy and a larger CSF volume. T2DM is associated with a larger WMH volume, while prediabetes is not. These data may indicate that, in a middle-aged population, changes in the cerebral white matter occur before onset of T2DM.



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Primary care management of type 2 diabetes mellitus in Denmark, Norway and Sweden: a long term observational study

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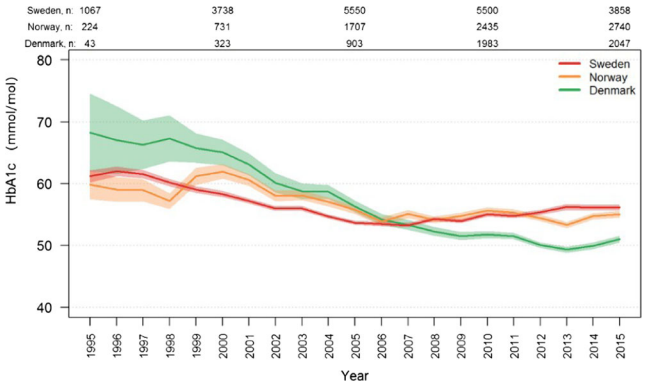
Background and aims: Despite similar health care infrastructure and recommendations for treatment goals of type 2 diabetes (T2DM), country specific differences in management of T2DM patients might be reflected in differences in blood glucose and cardiovascular risk factor control. The aim was to describe and compare management of T2DM in primary care using electronic medical records (EMR) data from Denmark (DK), Norway (NO) and Sweden (SWE).

Materials and methods: EMR data were extracted from 60 primary care clinics in DK, NO and SWE comprising all patients having a diabetes diagnosis and/or prescription of any glucose lowering drug during 1995-2015. Patients with T1DM and gestational diabetes were excluded. Patient characteristics, laboratory- and prescribed drug data were analysed annually from 1995 to 2015, and in more detail for the years 2005 and 2015, respectively.

Results: A total of 41,918 T2DM patients were identified in DK (6443), NO (6818) and SWE (28,657). From 1995 to 2005 HbA1c showed a considerable improvement and has since 2005 stabilized except for in DK where a continuous improvement was observed (Figure). Similar trends were also observed for systolic blood pressure and LDL-cholesterol. In 2015, mean ages of the diabetes populations were 64.5, 64.3 and 67.3 years in Denmark, Norway and Sweden,

respectively; remaining stable from 2005. No gender differences were seen (males 55.3, 54.9 and 56.1%, respectively). Mean HbA1c was 52.1, 57.1 and 58.4 mmol/mol, systolic blood pressure (SBP) 136.0, 137.8 and 135.8 mmHg, and low-density lipoprotein (LDL) 2.3, 2.8 and 2.8 mmol/l in the respective countries. Mean (95% CI) changes from 2005 to 2015 were: HbA1c: -9.9 (-11.8 to -8.0), -1.7 (-3.3 to -0.2) and +4.3% (3.2 to 5.3); SBP -4.4 (-6.6 to -2.3), -3.9 (-5.8 to -2.0) and -7.6 (-8.2 to -7.1) mmHg; and LDL -0.46 (-0.54 to -0.38), -0.31 (-0.39 to -0.23) and -0.08 (-0.12 to -0.03) mmol/l for DK, NO and SWE, respectively.

Conclusion: Treatment of type 2 diabetes seems to have improved considerably in all three countries during the last 20 years regarding control of blood glucose, blood pressure and cholesterol. However, during the recent 10 years HbA1c has remained relatively unchanged in Norway and Sweden, whereas in Denmark HbA1c continuously improved. This may indicate a more proactive disease management in Denmark, and also point out a potential areas of improvement in the management of type 2 diabetes patients particularly in Norway and Sweden.



Supported by: AstraZeneca
Disclosure: **K.I. Birkeland:** None.

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Trends in drug therapy for type 2 diabetes in Denmark from 1996 to 2014

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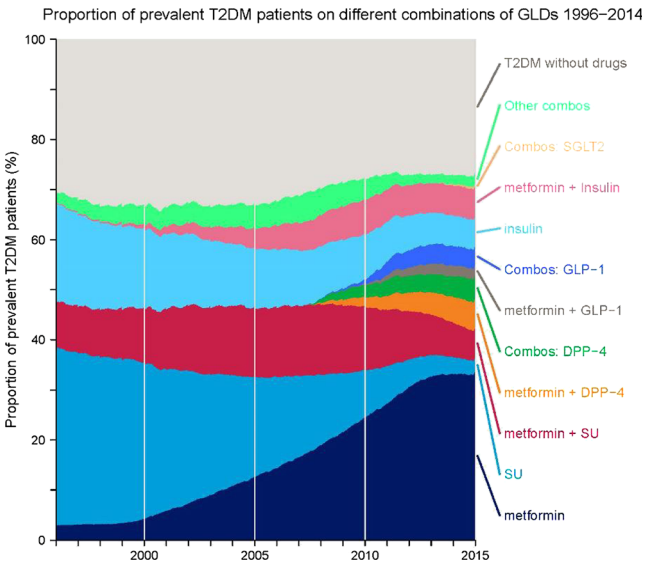
Background and aims: Danish guidelines for treatment of type 2 diabetes (T2DM) follow international guidelines, with the most recent update in 2014. The availability of new classes of glucose-lowering drugs (GLD) has had an impact on the glucose-lowering treatment practices of T2DM during the last few years. With access to the national prescription register for the entire Danish population, the aim was to map all GLD use (including combination therapy) by Danish T2DM patients during 19 years.

Materials and methods: Danish residents with a diabetes diagnosis in the National Patient Register (NPR) or the Danish Adult Diabetes Database (DADD) (excluding individuals diagnosed with type 1 diabetes); at least one GLD filled prescription in 1995-2014; or registered in the Danish National Diabetes Register (DNDR) were included. Exclusion criteria were a filled prescription for a GLD, or appearance in the NPR, DADD or DNDR before 30 years of age. Drug exposure was calculated from the first fill of a prescription, and an algorithm including information on daily prescribed dose was applied to estimate duration of drug exposure.

Results: The study included 412,290 T2DM patients covering a total of 3.0 million person-years between 1996 and 2014, from 80,735 individuals in January 1996 to 246,579 by end of 2014. Patients (53.3% men) with median age 63.0 years IQR [52.6; 72.7] at study entry were followed for a median of 6.0 years IQR [2.9; 10.9]. At study entry, 47.0% were either retired or outside the workforce; 40.1% had lower secondary education as highest educational level; 53.1% were married, while 30.8% were either widowed or divorced. By end of 2014, 180,572 (73.2%) T2DM patients were exposed to one or more GLD, leaving 26.8% (n= 66,007) of the patients on no GLD. Total exposure to one or more GLD was 2.1 million person-years. Monotherapy with metformin accounted for 27.5% of person-years exposed during the observation period, and other monotherapies accounted for 47.8% [Figure]. The most commonly used combination therapies in 2014 were metformin + insulin (6.1% of total GLD exposure), metformin + sulfonylureas (SU) (6.3%) and metformin + DPP-4 inhibitors (5.5%). From 1996 to 2014, the proportion of T2DM patients on monotherapy with SU decreased from 51.0% to 3.7% of the T2DM patients on GLD. Exposure to therapy including DPP-4, GLP-1 or SGLT2 covered 7.1% of total exposure to therapy during the observation period. Of the 180,572 patients exposed to GLD by end of 2014, 14.3% used DPP-4; 8.1% used GLP-1; and 0.7% used SGLT2.

Conclusion: During 19 years' observation period, consistently one in four Danish T2DM patients was untreated with GLD. Use of insulin has decreased and use of SU has declined markedly. Use of newer drugs like DPP-4 inhibitors and GLP-1 analogues have increased since the introduction on the Danish market in 2007.



Supported by: AstraZeneca
Disclosure: **M. Linnemann Jensen:** Honorarium; AstraZeneca. Stock/Shareholding; Novo Nordisk A/S.

OP 21 Retinopathy screening: Can we do better?

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Automated diabetic retinopathy screening: large-scale study on consecutive patient visits in a primary care setting

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Background and aims: Diabetic retinopathy (DR) is the leading cause of new-onset blindness among working-age adults in the western world. Vision loss due to DR is preventable by early diagnosis and intervention, and with the large and growing diabetic population, fully-automated DR screening is becoming essential. EyeArt is a computerized cloud-based DR screening system that automatically analyzes multiple color fundus images captured during a patient visit/encounter and provides a "refer" or "no refer" screening recommendation for patients. A "refer" recommendation is provided when the images indicate (i) moderate non-proliferative DR (NPDR) or higher on the International Clinical Diabetic Retinopathy (ICDR) severity scale and/or (ii) presence of surrogate markers for clinically significant macular edema (CSME) defined to be hard exudates within one disc of the macula. This study evaluates the safety and efficacy of EyeArt version 2.0 for automated DR screening on a large set of 78,685 consecutive patient visit/encounters obtained from the EyePACS DR telescreening program covering over 300 primary care clinics across the United States. We report the screening sensitivity, specificity, and area under the receiver operating characteristic curve (AUROC) measures.
Materials and methods: 78,685 consecutive patient encounters (totaling 627,490 images captured between January 2014 and May 2015) were obtained from the EyePACS database without any patient identification data, each with 1-38 images including external eye images. The DR severity on the ICDR scale and indication of surrogate markers for CSME provided by EyePACS graders was the clinical reference standard. Prevalence of encounters with moderate NPDR or higher or with surrogate markers for CSME was 20.1% and prevalence of encounters with potentially treatable DR (severe NPDR or proliferative DR) was 5.3%. EyeArt analyzed these images and produced a "refer" or "no refer" screening recommendation for each patient. Encounters with fewer than two gradable retinal images were automatically flagged as non-screenable, given a "refer" recommendation, and included in the performance analysis.
Results: EyeArt's screening sensitivity was 91.7% (95%CI: 91.3%-92.1%) and specificity was 91.5% (95%CI: 91.2%-91.7%). This corresponds to 19,728 "refer" recommendations (including 545 encounters flagged by EyeArt as non-screenable) and 1309 false negatives out of which 95.3% had moderate NPDR and did not meet the general treatment criteria. The AUROC was 0.968 (95%CI: 0.967-0.970). The sensitivity for referring potentially treatable DR was 98.5% i.e. less than 0.08% of the 78,685 cases had potentially treatable DR not detected by EyeArt.
Conclusion: EyeArt v2.0 achieves high sensitivity for detecting both referable DR and for potentially treatable DR at a high specificity making it both safe and effective for automated DR screening.
Supported by: NIH EB013585
Disclosure: **K. Solanki:** Employment/Consultancy; Eyenuk, Inc. Grants; NIH EB013585.

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Validation of automated diabetic retinopathy screening with the IDx-DR device in the Hoorn Diabetes Care System

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Background and aims: Grading of retinal images is often performed by graders, under supervision of an ophthalmologist. The subjectivity of this grading method and the high workload ask for an automated grading method. The aim of this study is to determine the accuracy of an automated retinopathy screening device in people with type 2 diabetes managed in primary care.
Materials and methods: 1,371 Persons with type 2 diabetes treated by the Hoorn Diabetes Care System, were included in the current study. Image quality analysis and exam grading were provided by the IDx-DR automated screening system and three retinal specialists (gold standard). After the independent expert reviews were complete, the experts came together for a consensus meeting to discuss cases without initial agreement and discussed these cases until they achieved consensus. Diagnostic accuracy of the IDx-DR device was compared to three retinal specialists using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Area under the receiver operating characteristic curve (AUC) was calculated. Results are presented for referable diabetic retinopathy (RDR) which was defined as EURODIAB≥2 and/or macular edema, and vision-threatening retinopathy (VT) defined as EURODIAB ≥3 and/or macular edema. Inter-rater reliability of the three retinal experts was estimated calculating sensitivity and specificity.
Results: Of the 1,371 persons included in this study 1,138 persons (83.0%) had images of sufficient quality according to the three experts compared to 938 persons (68.4%) according to the IDx-DR device, leaving 898 persons (65.5%) available for analysis. According to the gold standard, RDR was diagnosed in 22 persons (2.4%). The number of persons diagnosed with VT was 14 (1.6%). The sensitivity, specificity, PPV and NPV of IDx-DR for detection of RDR were 91%, 85%, 12%, 99% respectively. AUC was 0.94 (95% CI: 0.88 to 0.993). Using VT as the outcome measure, sensitivity, specificity, PPV and NPV of IDx-DR were 64%, 96%, 16%, 99%. The AUC was 0.91 (95% CI: 0.83 to 0.98). Graders' sensitivity and specificity were 72% and 99% respectively.
Conclusion: The automated grading method using IDx-DR for detection of referable retinopathy is a valid method and can be used in primary care. The high number of retinal images of insufficient quality may be due to under-utilization of the immediate quality feedback feature incorporated into the automated detection software and of the opportunity to dilate.

Table. Classification of diagnosis according to IDx-DR compared to the gold standard using EURODIAB grading

	Gold standard			
IDx-DR	Negative	Moderate DR	Vision-threatening DR	**Total**
Negative	732	1	1	734
Moderate DR	101	3	4	108
Vision-threatening DR	43	4	9	56
Total	876	8	14	898

DR=diabetic retinopathy

Disclosure: **A.A.W. van der Heijden:** Employment/Consultancy; cosultancy.

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A randomised controlled trial on the impact of financial incentives on attendance at diabetic eye screening in London

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Background and aims: The effectiveness of the UK national diabetic eye screening programme depends on levels of uptake, and currently has an attendance rate of 81%. Simple and cost-effective methods are needed to increase levels of screening uptake. Financial incentives can promote healthy behaviours, and there is evidence that incentives are more effective at promoting infrequent behaviours (e.g. vaccinations) compared to frequent behaviours (e.g. exercise). Therefore, incentives may be an effective way to increase diabetic retinopathy screening uptake. The primary outcome of this study was to determine whether financial based incentives were effective in increasing attendance at diabetic eye screening and secondly to study the impact of two different incentive schemes, based on principles from behavioural economics, on diabetic eye screening uptake.
Materials and methods: Patients aged 16 or over, who had not attended after invite to a diabetic eye screening appointment for two years or more, were included in a three arm, randomised controlled trial. Eligible participants were randomised to three conditions: 1. Control condition (usual invitation letter) 2. Fixed incentive condition (usual invitation letter, including a voucher for £10 if they attend their appointment) 3. Probabilistic incentive condition (invitation letter, including a voucher for a 1 in 100 chance of winning £1000 if they attend their appointment). The primary end point was attendance at the diabetic eye screening appointment.
Results: 1051 participants were included in the study (control N=435, fixed group N=312, probabilistic group N=304, according to 1.4:1:1 randomisation ratio for maximum statistical efficiency). 7.82% of participants from the control group, 5.45% of participants from the fixed incentive group, and 3.29% of participants from the lottery incentive group attended their appointment. When combining both incentive groups, participants who received an incentive were significantly less likely to attend their appointment than those in the control group (Risk Ratio=0.56; 0.34, 0.92). Those in the probabilistic incentive group were significantly less likely to attend than those in the control group (RR=0.42; 95% CI 0.21, 0.84). There was no significant difference between attendance rates in the fixed incentive or the probabilistic incentive group (RR=1.66; 95% CI 0.77, 3.56). Subgroup analyses by demographic factors showed no significant associations between attendance and gender, those 65 years and under, those in the lowest three index of multiple deprivation deciles, or distance from screening location.
Conclusion: Those receiving financial incentives and particularly those in the lottery condition were significantly less likely to attend screening than those who received the standard invitation letter. It is possible that being offered the incentive reinforces the view of patients that screening is painful or unpleasant, and therefore makes them less likely to attend. The findings suggest that incentives should not be used to promote screening in similar populations at risk of diabetic retinopathy, and potentially also in other screening programmes.
Clinical Trial Registration Number: ISRCTN14896403
Supported by: NIHR, Health Services and Delivery Research Programme
Disclosure: **G. Judah:** None.

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Retinal arteriolar and venular diameter are associated with diabetic retinopathy in people with type 2 diabetes

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Background and aims: Retinal vessels offer a direct and non-invasive measurement of the human microcirculation. Quantitative assessment of the retinal vascular parameters is a potentially precise and reliable method of investigating retinal traits which have been linked with macro- and microvascular diabetic complications such as cardiovascular disease (CVD) and diabetic retinopathy (DR). In the general population, several studies have consistently documented a relation between narrower arterioles and hypertension and other retinal traits such as tortuosity have been associated with ischaemic stroke. The purpose of this study was to determine the association of retinal vascular traits with DR in people with type 2 diabetes.
Materials and methods: We analysed 948 men and women aged 60 to 75 years with type 2 diabetes participating in the Edinburgh Type 2 Diabetes Study (ET2DS). DR cases were characterised according to Early Treatment Diabetic Retinopathy Study (ETDRS) classification. Retinal traits were measured from fundus photographs. The eye (right or left) with the fundus photograph of better quality was analysed for each subject using semi-automated image software (VAMPIRE 3.1). This software generates the arteriole-to-venule ratio (AVR), the central retinal arteriole equivalent (CRAE), central retinal venule equivalent (CRVE) and arteriolar and venular tortuosity. We used logistic regression analysis to assess the association of the retinal traits with DR, adjusted for covariates (age, sex, duration of type 2 diabetes, glycosylated haemoglobin (HbA1c) and smoking status).
Results: We included 615 individuals with no DR and 289 individuals with mild to severe DR. A total of 44 subjects were excluded due to incomplete data. As expected, we found an association of DR with male sex (OR 0.71, 95% CI 0.52-0.97, p=0.035), HbA1c (OR 1.31, 95% CI 1.15-1.50, p<0.001]) and duration of diabetes (OR 1.04, 95% CI 1.02-1.06, p<0.001). DR was also associated with CRAE and CRVE, even after adjustment for the other co-variates (OR 1.04, 95% CI 1.00-1.08, p= 0.03) and (OR 1.04, 95% CI 1.00-1.07, p=0.01), respectively. No significant associations were found for the other retinal traits.
Conclusion: CRAE and CVRE may provide useful early markers of the risk of DR. This will be investigated further in the prospective phase of the ET2DS, when the baseline retinal traits measured in this project will be analysed in relation to the development of DR over the subsequent 10 years.
Disclosure: **E. Sandoval:** None.

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Loss of visual acuity due to diabetic macular oedema arrives with hastened annual decline in renal function

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Background and aims: Diabetic nephropathy and retinopathy are long-term microvascular complications affecting the quality of life and functionality of an individual before ample loss of organ function. Diabetic macular oedema (DME) and associated increased central retinal thickness (CRT) is an indicator of progressive pathogenesis and amplified permeability and leakage of plasma constituents. However, related clinical symptoms do not necessarily correlate with less consistently assessable diagnosis of loss of visual acuity (VA) in subjects with long-term diabetes,

especially towards the lower range of the VA scale. We hypothesised that the impaired retinal vascular functionality correlates, or at least coincides, with the presence or risk of other microvascular complications such as renal impairment. We explored the factors characterising the relationship between renal function (reduced eGFR, proteinuria) and impaired retinal function (reduced VA or increased CRT) in patients with DME.

Materials and methods: We pooled baseline data from 5 DME studies which enrolled patients with VA <85 letters (corresponding to <20/40), and applied a linear regression model and multivariable analysis including baseline factors, VA, urine dipstick results and optical coherence tomography-defined CRT (with 400 microns as cut-off value for categorical analysis).

Results: Our cohort (n=1355, 59% men) comprised patients with mean (±SD) HbA1c 7.8±3.65%, diabetes and DME duration of 14.1±9.50 and 1.8±2.54 years, respectively; age, 62.9±9.48 years; BMI, 28.4±5.36 kg/m^2; and best corrected VA 64.5±11.22 letters (best eye 70.7±11.34; worse eye 58.3±12.97) and mean CRT of 431.98±137.04 microns. The mean blood pressure (BP) was 138.0±14.50/78.4±9.49 mmHg, sitting pulse 75.9±10.99 bpm and eGFR 81.5±25.04 mL/min (MDRD). 48.8% had an additional microvascular manifestation beyond DME. Most patients (98%) received glucose-lowering therapies, 28% beta blockers and 65% RAA blockers. Out of baseline factors only VA, BMI and age were significantly associated with eGFR (r^2= 0.17, p<0.001 for the overall model). The modelled mean (±SEM) eGFR declined annually with age at a rate of −0.87±0.07 mL/min/year (95% CI −1.00, −0.72, p<0.0001) and with BMI −0.28±0.12 mL/min/BMI unit (95% CI −0.52, −0.03, p=0.0231). One letter drop (mean VA loss for both eyes) corresponded to a reduction of eGFR by 0.22±0.06 mL/min (95% CI 0.11, 0.34, p=0.0002). The association was similar for the worse and best eyes (r^2=0.170, p=0.008; r^2=0.191, p=0.0009, respectively) but not affected by sex, HbA1c, pulse or sBP, regardless of the tested eye. Baseline CRT correlated only moderately with VA (r^2=0.107) and accordingly there was no association between CRT and eGFR (adjusted for baseline factors, p=0.234). Presence or absence of protein in urine (dipstick) occurred as frequently in subjects with elevated CRT (greater than 400 microns) vs. those with more normal CRT values.

Conclusion: Our exploratory analysis in patients with DME suggests that concomitant retinal and renal microvascular complications are associated. VA but also eGFR appears to describe a more continuous, fluctuating and partially reversible condition. Impairment of VA secondary to DME should prompt assessment of the renal function, while absence of proteinuria does not predict lack of progression of the underlying retinal microvascular complications.

Supported by: Novartis

Disclosure: **F. Ziemssen:** Employment/Consultancy; Alimera, Allergan, Bayer Healthcare, Novartis. Lecture/other fees; Alcon, Alimera, Allergan, Bayer Healthcare, Heidelberg Engineering, Novartis. Other; Allergan, Novartis.

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Retinal microperimetry: a new tool for identifying type 2 diabetic patients at risk of developing Alzheimer´s disease

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Background and aims: There is an emerging association between type 2 diabetes (T2D) and Alzheimer's disease (AD) which has been attributed to common signaling pathways. However, at present there are no markers to identify T2D patients at risk of developing AD. As the retina shares many anatomical and physiological features with the brain, including embryological origin, it has been suggested that it may provide an easily accessible and non-invasive way for examining the pathology in the brain. The multifocal electroretinogram (mfERG) is considered the gold standard procedure in the evaluation of the retinal function, but due to its complexity is reserved only to research studies. The retinal microperimetry is a simple, non-invasive examination that in some aspects could be considered a surrogate of the mfERG and can be easily used in the daily clinical practice. On this basis, the aim of this study is to identify T2D patients at risk of developing AD based on the assessment of retinal neurodegeneration by means of retinal microperimetry.

Materials and methods: We have evaluated 94 consecutive T2D patients attended at the Memory Unit of our centre (40 patients affected of AD, 35 patients with mild cognitive impairment (MCI) and 19 patients with subjective memory complaints (SMCs). All patients were functionally literate, without severe auditory and visual abnormalities. Neuropsychological, neurological and psychiatric evaluations, as well as a biochemical analysis were performed. Retinal function was assessed by standard microperimetry (MAIA microperimeter 3rd generation). Microperimietry, measures retinal sensitivity as the minimum light intensity that patients can perceive when spots of light stimulate specific areas of the retina. The standard MAIA test covers a 10° diameter area with 37 measurement points. The stimuli size is Goldmann III, background luminance is 4 asb and maximum luminance is 1000 asb, with a 36 decibels (dB) dynamic range. Brain neurodegenerative and functional changes were evaluated by MRI and 18-FDG- SPECT respectively.

Results: There was a significant difference in the retinal sensitivity between the three groups: 17,79±6,71 in AD, 21,58±4,35 in MCI and 23,10±3,03 in SMCs (ANOVA, p< 0,01). The time of performance was significantly higher in the AD and MCI groups than in the SMCs: 3,1±1,21 min in AD, 2,31±0,80 min in MCI and 1,68±0,36 min in SMCs, (ANOVA, p<0.05). A direct correlation was observed between retinal sensitivity and the whole brain and cortex volumes, as well as 18-FDG cerebral uptake.

Conclusion: The assessment of the retinal neurodegeneration by using a non-invasive functional test (MAIA microperimetry) identified T2D patients with cognitive impairment. This test might be a useful tool for screening cognitive impairment in diabetic patients.

Supported by: EFSD supported by Novartis

Disclosure: **A. Ciudin:** Grants; EFSD/Lilly Mental Health and Diabetes Programme 2013.

OP 22 The challenge of angry lipids

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Specific phospholipids are differently associated to human visceral and non-visceral adipose tissue depending on the inflammatory state in the NUGAT Twin study

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Background and aims: The aim of the study was to correlate lipidomic and secretory data as well as the amount of visceral and non-visceral adipose tissue of human subjects during a high-fat diet to assign the function of specific lipid metabolites.

Materials and methods: In the NUGAT-Study 46 healthy mono- and dizygotic twin-pairs first were standardized for their nutritional behavior by a carbohydrate-rich low-fat diet for 6 weeks, immediately followed by an isocaloric high-fat diet for 1 week and additional 5 weeks. At each CID periumbilical fat biopsies were taken for determination of gene expression. Plasma was measured for lipid metabolites and cytokines (ELISA). The amount of visceral (VAT) and non-visceral (nVAT) adipose tissue was determined by MRI.

Results: Phospholipid (PL) species like phosphatidylcholines (PCs), phosphatidylethanolamines (PEs) and phosphatidylinositols (PIs) were mainly correlated with the amount of VAT during the 3 CIDs (r=-0.166 to -0.429, p=0.035 to 1.9x10-8), while their lysoforms (LPLs) were mainly correlated with the amount of nVAT (r=-0.301 to -0.568, p=1x10-4 to 3.9x10-15). In people with CRP≥1 most of the LPLs decreased significantly (LPE18:1 -0.13±0.05, p=0.021; LPC16:0 -9.76±-4.71, p=0.017), while their concentrations didn't change in people with CRP<1. In people with CRP≥1, the majority of LPLs was correlated with VAT instead of nVAT. For PL species those effects appeared largely vice versa. In people with CRP≥1 most of the PL concentrations remained constant while they increased in people with CRP<1 (PC33:1 0.60±0.56, p=0.038; PC36:2 39.29±10.08, p=7.56x10-7).

Conclusion: Our data demonstrate that high-fat diet induced inflammation might either be triggered or lead to a specific pattern of phospholipid and lysophopholipid metabolism in association with their release and/or deposition in VAT and nVAT. Low concentrations of LPLs appear to be associated to higher inflammatory states, while higher concentrations of PLs are paralleled by lower inflammation.

Clinical Trial Registration Number: NCT01631123
Supported by: BMBF

Disclosure: **M.A. Osterhoff:** None.

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Serum ceramides are linked to low microbiome richness and diabetes risk in overweight and obese subjects

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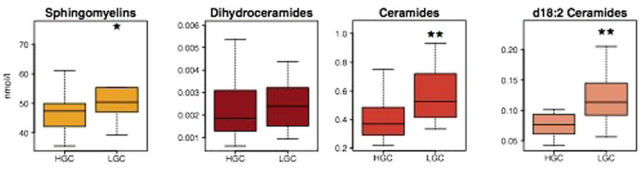
Background and aims: Low gut microbiome richess, i.e. low vs. high metagenomic gene count (LGC vs. HGC), is associated with dyslipidemia and insulin resistance; yet these lipid perturbations are poorly understood. Ceramides (Cer), which can directly induce insulin resistance, and sphingomyelins (SM) are important components of the human lipidome, therefore we tested whether these circulating sphingolipids were correlated with LGC and increased diabetes risk.

Materials and methods: 49 participants (41 women, 8 men) had previously undergone extensive clinical phenotyping. Serum samples were analyzed by HPLC-MS/MS to quantify 45 individual Cer and SM. Employing quantitative shotgun metagenomics, participants were characterized as LGC/HGC, and bacterial species abundances were quantified as metagenomic species (MGS). Data were analyzed using non-parametric statistics, and when appropriate, p-values were adjusted for the false discovery rate (fdr).

Results: Confirming previous reports, Cer were inversely associated with fasting (HOMA-S) and OGTT-derived (Matsuda Index) measures of insulin sensitivity (r=-0.31 and -0.32, p≤0.05). Cer were also associated with fasting glucose (r=0.39, p=0.006) and the OGTT-derived insulin-secretion-sensitivity-index-2 (r=-0.4, p=0.01), suggesting lower beta cell function. Establishing a link with gut dysbiosis, SM and Cer were elevated in LGC subjects by 13% (p=0.03) and 30% (p=0.007), respectively (see figure). Longer acyl-chain Cer, including Cer(d18:1-26:1) and Cer(d18:1-24:1), were the most elevated molecular lipids. 44 MGS were decreased in LGC relative to HGC (fdr<10%). Numerous Cer but fewer SM were strongly associated with the depletion of 9 MGS in particular (fdr<10%), which consisted of firmicutes and a methanogen. Shotgun serum metabolomics and analysis of the functional and metabolic capacities of the metagenome provide further insights into the mechanistic link between altered gut microbiota and elevated host Cer.

Conclusion: Our data establish a new link between gut microbiota richness, Cer, and diabetes risk in overweight/obese humans. If confirmed, restoration of specific bacteria or pathways that regulate host Cer may provide therapeutic targets for preventing the development of type 2 diabetes.



HGC = High gene count, LGC = Low gene count, * and ** for P<0.05 and <0.01 by the Wilcoxon rank-sum test.

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Supported by: ANR-MICRO-Obes, Fondation Coeur et Arteres, H2020-EPoS

Disclosure: **B.D. Kayser:** Grants; Agence Nationale de la Recherche, Association Fondation Coeur et Artères, Horizon 2020 (EPoS).

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Regulation of lipolysis and adipose tissue signalling during acute endotoxin-induced inflammation: a human randomised crossover trial

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Background and aims: Lipolysis is accelerated during the acute phase of inflammation, a process being regulated by pro-inflammatory cytokines, stress-hormones, and insulin. The intracellular mechanisms remain elusive and we therefore measured pro- and anti-lipolytic signaling pathways in adipocytes after in vivo endotoxin exposure.

Materials and methods: Eight healthy, lean, male subjects were investigated using a randomized cross over trial with two interventions: i) bolus injection of saline (Placebo) and ii) bolus injection of lipopolysaccharide endotoxin (LPS). A 3H-palmitate tracer was used to measure palmitate rate of appearance (Ra-palmitate) and indirect calorimetry was performed to measure energy expenditures and lipid oxidation rates. A subcutaneous abdominal fat biopsy was obtained during both interventions and subjected to western blotting and qPCR quantifications.
Results: LPS caused a mean increase in serum free fatty acids (FFA) concentrations of 90% (CI-95%: 37-142, p=0.005), a median increase in Ra-palmitate of 117% (CI-95%: 77-166, p<0.001), a mean increase in lipid oxidation of 49% (CI-95%: 1-96, p=0.047), and a median increase in energy expenditure of 28% (CI-95%: 16-42, p=0.001) compared with Placebo. These effects were associated with increased phosphorylation of hormone sensitive lipase (pHSL) at ser650 in adipose tissue (p=0.03), a trend towards elevated pHSL at ser552 (p=0.09) and cAMP-dependent protein kinase A (PKA) phosphorylation of perilipin 1 (PLIN1) (p=0.09). Phosphatase and tensin homolog (PTEN) also tended to increase (p=0.08) while phosphorylation of Akt at Thr308 tended to decrease (p=0.09) during LPS compared with Placebo. There was no difference between protein or mRNA expression of ATGL, G0S2, and CGI-58.
Conclusion: LPS stimulated lipolysis in adipose tissue is associated with increased pHSL and signs of increased PLIN1 phosphorylation combined with a trend toward decreased insulin signaling. The combination of these mechanisms appear to be the driving forces behind the increased lipolysis observed in the early stages of acute inflammation.
Clinical Trial Registration Number: NCT01705782
Supported by: Aarhus University, Danish Council Strategic Research, Lundbeck foundation
Disclosure: **N. Rittig:** None.

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Intrahepatic lipid content is an independent predictor of circulating CD36

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Background and aims: The increased release of free fatty acids (FFAs) from insulin-resistant adipose tissue is the first essential step for excessive fat accumulation within hepatocytes. Indeed, circulating FFAs are the major source of hepatic lipids in patients with in non-alcoholic fatty liver disease (NAFLD), suggesting that the rate of influx of FFAs to the hepatocytes is crucial for the development of steatosis. Membrane-bound CD36 plays an important role in facilitating uptake of FFAs in hepatocytes, adipocytes and other cell types. CD36 is detectable in plasma and circulating CD36 (sCD36) levels may be increased in NAFLD patients, however details of sCD36 in NAFLD are yet unclear. We hypothesized that sCD36 would be positively associated with intrahepatic lipid content (IHL) rather than the level of obesity and the body composition in a large NAFLD cohort.
Materials and methods: We investigated 111 patients with NAFLD and 33 normal or overweight controls. IHL was measured by MR-spectroscopy; and subgroups of participants had a dual-energy X-ray absorptiometry (n = 101), magnetic resonance imaging (n = 94, subcutaneous and visceral adipose tissue, SAT and VAT) and liver biopsy (n = 28 NAFLD patients, of which 17 had a repeat biopsy after 6 months) performed. Plasma sCD36 was measured by an in-house ELISA kit. Hepatic CD36 expression was assessed by real-time PCR.
Results: NAFLD patients had elevated sCD36 levels compared to both normal weight and overweight controls (0.68 (0.12 − 2.27) versus 0.48 (0.12 − 0.73) and 0.42 (0.1 − 1.18), $p < 0.01$). The sCD36 level correlated with the IHL content (Spearman's rho = 0.30, $p < 0.01$). Further, plasma sCD36 correlated with ALT levels (Pearson $\rho = 0.30$); HOMA-insulin resistance ($\rho = 0.24$); HDL ($\rho = -0.32$); and plasma triglyceride ($\rho = 0.44$, all $p < 0.01$). sCD36 and BMI were weakly correlated ($\rho = 0.17$, $p = 0.04$), yet, we found no significant correlations between sCD36 and other measures of fat distribution. In contrast, we found a negative relation to VAT (rho = −0.21, $p < 0.05$). In a multiple regression analysis, IHL was an independent predictor of sCD36 ($p < 0.02$). sCD36 was significantly correlated with hepatic CD36 mRNA expression in the 44 available specimens ($\rho = 0.42$, $p < 0.01$).
Conclusion: Circulating sCD36 levels were elevated in NAFLD patients and increased with the amount of intrahepatic lipid, insulin resistance and plasma triglyceride levels. Further, IHL was an independent predictor of sCD36. We report only a weak positive correlation between sCD36 and BMI and a negative correlation between sCD36 and VAT. sCD36 correlated with hepatic CD36 expression. This suggests that the excess sCD36 in NAFLD is derived from the liver and supports that CD36 is involved in NAFLD development.
Clinical Trial Registration Number: NCT01464801, NCT01412645, NCT01446276
Disclosure: **S. Heebøll:** None.

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Obesity paradox: increased adiposity plays beneficial role in PCSK9-dependent uptake via VLDLR by visceral adipose tissue during sepsis

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Background and aims: Obesity is a major risk factor contributing to mortality worldwide, but in certain conditions obesity may be a beneficial factor. Evidence indicates that obese sepsis patients have lower mortality and similar functional outcomes as normal weight patients. This so-called “obesity paradox” suggests that excess adipose tissue may cause the body to respond differently to acute infection together with the immune response, leading to sepsis. Whether there is a true relationship between increased adiposity and sepsis-induced outcome is unknown. Our group showed that reduced function of PCSK9 also improves outcome of sepsis. We hypothesized that PCSK9 inhibits bacterial lipopolysaccharide (LPS) clearance in sepsis through visceral adipose tissue (VAT).
Materials and methods: To mimic early stages of sepsis PCSK9-/-, Ldlr-/-, Vldlr-/-, and WT C57BL/6J control mice were injected into a tail vein with FITC-conjugated LPS (5mg/kg). Blood, liver and visceral fat tissue were collected at 1 and 6 hours after injection, and fluorescence measured using confocal microscopy and plate reader. Flow cytometry was used to access LPS clearance from blood plasma. Immunobloting was used to assess gene expression. Human recombinant PCSK9 (50 ug/animal) or saline were injected into the tail vein of Ldlr-/- mice, followed by FITC-LPS injection after 30 min for 1 hour.
Results: PCSK9-/- mice injected with FITC-conjugated LPS had 2.5 ±0.3-fold (n=8) increase in LPS plasma clearance 6 hours after injection, which was accompanied by 2.0±0.5-fold (n=8) increase in LPS uptake by VAT compared to the WT. PCSK9 is a well-known negative regulator of LDL clearance via downregulation of LDLR. LPS can form complexes with LDL in the bloodstream, thus we suggested LDLR-dependent mechanism of LPS uptake by adipose tissue. Ldlr-/- mice had 2.3±0.4-fold (n=8) decrease in plasma LPS clearance 6 hours post injection. However indicated inhibition of plasma clearance was entirely explained by decreased hepatic uptake of LPS, whereas no significant effect on LPS uptake by VAT was observed. This suggested alternative route of LPS uptake in adipocytes. Vldlr-/- mice had 3.3±0.6-fold (n=10) decrease in VAT uptake of LPS 6 hours post injection, with no significant change in hepatic

uptake, suggesting that VLDLR is a main mechanism of LPS uptake in VAT. Using 3T3-L1 adipocytes treated with recombinant PCSK9 (3 μg/mL) we showed decrease in VLDLR levels (1.5±0.1 fold). Finally, Ldlr-/- mice injected with recombinant PCSK9 prior to LPS injection, had 2.0±0.2 (n=5) fold decrease in VAT, but not hepatic LPS uptake.

Conclusion: We conclude that PCSK9 can affect outcome of sepsis via regulation of novel VLDLR-dependent clearance of LPS by VAT. This mechanism explains protective effect of obesity during sepsis.

Supported by: CIHR

Disclosure: **E. Topchiy:** None.

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Angiopoietin like protein 4 (ANGPTL4): a marker of alteration in lipid metabolism, insulin resistance and ectopic fat accumulation

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Background and aims: Alteration in lipid metabolism is a benchmark in the development of insulin resistance, type 2 diabetes and related cardiometabolic diseases. Angiopoietin like proteins (ANGPTL) are a family of secreted glycoproteins present in tissues (liver, heart, muscle, adipose tissue), macrophages and blood with pleiotropic effects on vascular cells, stem cell biology, and lipid metabolism. Studies in animals and cell lines have shown that ANGPTL4 is involved in the regulation of lipoprotein lipase (LPL). In plasma, ANGPTL4 inhibits VLDL- and chylomicron-triglyceride (TG) hydrolysis while in adipose tissue stimulates lipolysis. However, the relationship between this secreted glycoprotein and lipid metabolism in vivo in humans has not been studied. Since ANGPTL4 is secreted in plasma, we wanted to explore if circulating ANGPTL4 might be related with lipid dysfunction, ectopic fat accumulation and insulin resistance (IR).

Materials and methods: We studied 54 non diabetic subjects (45 with biopsy proven NAFLD and 9 without NAFLD; age and BMI (41±1.4 ; 27.5± 0.6). In all subjects we measured fasting hepatic glucose production (EGP) and lipolysis (Ra glycerol) by stable isotope tracer infusions, abdominal and hepatic fat by MRI, free fatty acid (FFA) concentration and composition (by GCMS), indexes of IR (Adipo-IR FFA x Insulin ; Hep-IR= EGP X Insulin) and of De Novo Lipogenesis index (DNL=16:0/18:2) that we correlated with plasma levels of ANGPTL4. Liver histology was scored according to Kleiner.

Results: Circulating ANGPTL4 levels were increased with hepatic fat (R=0.41, p=0.0064) and upper subcutaneous fat (R=0.44, p=0.0045) but not visceral fat. Overall, ANGPTL4 correlated positively with plasma concentrations of FFA (R=0.49, p=0.0003), TG (R=0.30, p=0.03), DNL index (R=0.45, p=0.0009), peripheral lipolysis (R=0.28, p=0.04), Hep-IR (R=0.30, p=0.03) and Adipo-IR (R=0.52 p<0.0001). ANGPTL4 was strongly associated with plasma MCP-1 (R= 0.52, p=0.0001) that is a marker of macrophage related inflammation. Moreover ANGPTL4 was significantly increased with the degree of hepatic fibrosis (in Fib3-4 +24% in vs Fib0-2; +60% vs CT) and hepatic inflammation (by 30% vs CT). Multiple regression analysis showed that ANGPTL4 levels were associated with the degree of fibrosis independently of BMI, AT-IR and MCP-1.

Conclusion: Increased plasma levels of ANGPTL4 are markers of alterations of both hepatic and adipose tissue lipid metabolism, whole body and hepatic inflammation and liver damage.

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Disclosure: **A. Gastaldelli:** None.

OP 23 Tracking insulin resistance in different tissues

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Benefit of hepatic denervation on glucose disposal: a long term efficacy study in a canine model of insulin resistance

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Background and aims: Chronic consumption of a high fat high fructose diet (HFFD) induces hepatic insulin resistance, abnormal glucose tolerance, and impaired β cell function in the dog. Enhancement of hepatic glucose uptake by portal glucose delivery is lost in this model, but since the latter appears to depend in part on the withdrawal of hepatic sympathetic input, we hypothesized that hepatic denervation would improve glucose tolerance in HFFD animals.

Materials and methods: Dogs were fed a HFFD for 4 months. Animals were randomized to either a surgical hepatic sympathetic denervation (HDN, n=5) or a sham surgery (SHAM, n=4) after one month of HFFD. Glucose tolerance was assessed before surgery (HDN-0 and SHAM-0) and at 4, 8 and 12 weeks after (HDN-4, -8 and -12 and SHAM-4 , -8 and -12). We performed an oral glucose tolerance test (OGTT) with a gavage of 0.9 g of Polycose / kg and measured glucose, insulin, glucagon and C-peptide at regular intervals during the 3 hours following gavage. Data are expressed as the area under the curve over 120min compared to the fasting value (Δ-AUC) and were compared to a group of untreated chow-fed dogs (CTR, n=8).

Results: There were no clinical issues attributable to the treatment of any of the animals. Likewise, there were no histologic or hematologic abnormalities noted. The HFFD-SHAM dogs gained 3.3 kg over the course of the study, while the HFFD-HDN animals gained 3.2 kg (P = 0.81). Analysis of liver norepinephrine (NE) content at the end of the study revealed over 98% denervation overall for the liver (individual lobes values <15 ng of NE / g tissue) compared to the SHAM group where values ranged from 316 to 658 ng of NE / g tissue. The HFFD diet induced an abnormal glucose excursion before surgery in both groups as indicated by the α AUC of glucose over the first 120 min of the OGTT : 3215±352, 7926±1115 and 6333±1847 mg/120min/dL respectively for CTR, HDN-0 and SHAM-0, values being significantly different from CTR (P=0.02 for HDN-0 and 0.04 for SHAM-0). After hepatic denervation in the HDN cohort, the defect observed was reduced by 57% (HDN-4), 76% (HDN-8) and 65% (HDN-12). Comparatively, the α AUC for plasma glucose in the sham-denervated HFFD dogs did not improve over time. The excess hyperglycemia seen in response to the OGTT after HFFD (before surgery) resulted in a greater increase in plasma insulin over that seen in the chow fed controls (+21% and +52% for HDN-0 and SHAM-0 respectively, NS). That increase was similar in the sham cohort after surgery (+23%, +37% and +60% for SHAM-4, -8 and -12, NS). Surprisingly, although the glucose excursion was improved after HDN, the insulin levels were the same or exceeded those seen prior to HDN (+18%, +27% +49% compared to CTR for HDN-4, -8 and -12, no significant difference).The C-peptide data confirmed these observations. Neither HFFD nor HDN had any effect on the fall in plasma glucagon.

Conclusion: The hyperglycemic defect induced by HFFD was in part attenuated by hepatic sympathetic denervation and that effect persisted for the 3 months of the study. Insulin secretion was maintained after denervation, even though there was a lower glucose excursion, suggesting that insulin beta cell function was enhanced by HDN.

Disclosure: **G. Kraft:** Other; Contract : Metavention.

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Hyperglycaemia-induced endoplasmic reticulum stress decreases skeletal muscle insulin sensitivity

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Background and aims: Experimental hyperglycemia induces insulin resistance, while the normalization of hyperglycemia can restore insulin sensitivity. Therefore, it is likely that hyperglycemia directly impairs insulin sensitivity and glucose metabolism; however, the mechanisms are unclear. Since hyperglycemia is associated with endoplasmic reticulum (ER) stress and since skeletal muscle ER stress is associated with insulin sensitivity, we aimed to test the hypothesis that hyperglycemia reduces skeletal muscle insulin sensitivity via an ER stress-related mechanism.
Materials and methods: We performed a clinical study whereby experimental hyperglycemia was established in ten healthy subjects (age 56 ±3years, BMI 31.3±1.2 kg/m^2; mean±SE) for 24 hours and changes in insulin sensitivity and muscle ER stress markers were determined. We compared these changes to the same variables measured in ten age- and BMI-matched type 2 diabetic patients (age 56±3 years, BMI 30.0±1.2 kg/m^2, HbA1c 6.84±0.21%) as a clinical model of chronic hyperglycemia. Furthermore, we evaluated insulin-stimulated glucose uptake in C2C12 muscle cells exposed to normo- (5mM) vs. hyperglycemic (25mM) conditions in the presence/absence of the chemical ER stress inhibitor tauroursodeoxycholate (TUDCA).
Results: Inflammation (plasma interleukin [IL]-6; muscle tyrosine-185 phosphorylation of c-Jun N-terminal kinase [p-JNK]) and ER stress (muscle serine-51 phosphorylation of eukaryotic translation initiation factor 2 alpha [p-eIF2α]) were greater in subjects with type 2 diabetes, who had lower insulin sensitivity than healthy subjects (4.69±0.73 vs. 8.12±1.44 x10^3 μmol/kg/min/pM.mM; P=0.05). Hyperglycemia reduced insulin sensitivity (5.08±1.08 vs. 8.12±1.44 x10^3 μmol/kg/min/pM.mM; P=0.01) in the presence of elevated markers of inflammation (plasma IL-6; muscle p-JNK) and ER stress (p-eIF-2α) in healthy subjects, but without impairment in canonical insulin signaling or plasma membrane glucose transporter type (GLUT)-4. In C2C12 muscle cells, TUDCA-induced ER stress inhibition prevented hyperglycemia-induced IRS-1 Ser307 phosphorylation (TUDCA+25mM glucose vs. 25mM glucose: 1.73±0.28 vs. 6.07±0.33 x10^{-5} a.u.; P=0.02) as well as hyperglycemia-induced reductions in GLUT4 protein (1.08±0.14 vs. 0.29±0.04 a.u.; P=0.001) and insulin-stimulated glucose uptake (2341±348 vs. 1256 ±282 x10^{-3} F/μg protein/min; P=0.04).
Conclusion: These findings indicate that hyperglycemia per se reduces insulin sensitivity in skeletal muscle via an ER stress-related mechanism. As such, reducing ER stress may provide a novel strategy to improve glucose metabolism in states of chronic hyperglycemia.
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Supported by: EFSD supported by AstraZeneca
Disclosure: **T.P.J. Solomon:** Grants; EFSD.

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Lack of inducible nitric oxide synthase prevents high fat diet-induced adipose tissue fibrosis in mice

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Background and aims: Fibrosis limits adipose tissue (AT) expandability and promotes obesity-associated insulin resistance. Activation of the inducible nitric oxide synthase (iNOS) has long been considered to be involved in the pathogenesis of inflammation and fibrosis. Recent studies have suggested that DNA damage is linked to adipose tissue inflammation and systemic insulin resistance, and that the p53 signaling pathway can repress PGC-1α expression. In particular, NO has been shown to increase p53 accumulation. This study was undertaken to examine the possible role of iNOS activation in AT macrophages in the genesis of AT fibrosis.
Materials and methods: Seven-week-old wild-type (WT) mice and iNOS-/- mice were given HFD for 16 weeks. Histologic analysis and the expression of inflammation- and fibrosis-related genes and mitochondrial biogenesis factors, and mRNA and protein levels of hypoxia-inducible factor (HIF)-1α in epididymal AT were determined. RAW264.7 cells or bone marrow derived macrophages (BMDMs) from WT and iNOS-/- mice were treated with 10 ng/mL of lipopolysaccharide (LPS), and the conditioned media were transferred to 3T3-L1 preadipocytes or primary preadipocytes. To examine the role of iNOS activation, macrophages were cultured in media containing LPS with 30 μM of S-methylisothiourea (SMT), an iNOS-selective inhibitor. In another set of experiments, 3T3-L1 preadipocytes were treated with the NO donors, sodium nitroprusside or Deta-NONOate. Protein levels of HIF-1α and phosphorylated p53 protein levels, and the mRNA expression of profibrogenic genes and PGC-1α were measured in preadipocytes.
Results: HFD-induced glucose intolerance and insulin resistance were attenuated in iNOS-/- mice, and iNOS-/- mice showed significantly less inflammation and fibrotic changes in the eWAT. Both the mRNA and protein levels of HIF-1α in AT were significantly increased in mice fed the HFD for 16 weeks. In HFD-fed iNOS-/- mice, the HIF-1α transcript levels were similar to WT, but the protein levels were significantly reduced, along with an increased expression of mitochondrial biogenesis factors. NO produced by activated macrophages increased HIF-1α protein levels in cultured preadipocytes and reduced PGC-1α expression. siRNA directed against p53 reversed NO-mediated changes in HIF-1α protein and phosphorylated p53 protein levels, and PGC-1α mRNA levels in preadipocytes. The phosphorylated p53 level was also significantly elevated in the AT of wild-type but not iNOS-/- mice on a HFD. Rosiglitazone treatment of preadipocytes reversed NO-mediated inhibition of mitochondrial respiration and decreased HIF-1α protein expression levels. NO also inhibited preadipocyte differentiation into mature adipocytes and increased the frequency of cells with a profibrotic phenotype in preadipocytes
Conclusion: Our data show that NO produced by activated macrophages induces p53-dependent PGC-1α suppression and mitochondrial dysfunction in preadipocytes and that this is responsible for the increased HIF-1α protein stabilization, defective adipocyte differentiation, and increased fibrogenesis These findings suggest that iNOS expression in macrophages plays a pivotal role in determining the preadipocyte fate. Interactions between macrophage iNOS and preadipocytes may represent a novel target for restoring healthy AT function.
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Disclosure: **J. Jang:** None.

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Hepatic hypomethylation and increased secretion of PDGFA links chronic hyperinsulinaemia to liver disease in obese subjects with diabetes

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Background and aims: Insulin resistance is a key feature of obesity and of T2D, but its contribution to non-alcoholic fatty liver diseases (NAFLD) and its complications including, fibrosis and cancer is unknown. We investigated the liver methylome and transcriptome in European women undergoing bariatric surgery.
Materials and methods: Liver samples were collected from European women undergoing bariatric surgery (96 T2D cases and 96 normoglycemic controls matched for age and body mass index).

Methylome and transcriptome were assessed using Infinium HumanMethylation450 BeadChip arrays (Illumina) and HumanHT-12 v4.0 Whole-Genome DASL HT Assays (Illumina), respectively. Immortalized Human Hepatocytes were used for hepatocyte model. Gene expression and protein content was monitored by quantitative real-time RT-PCR and Western Blotting experiments, respectively. Secretion of PDGFA was measured by ELISA.

Results: When we compared the methylome of T2D cases versus controls, we identified only one genome-wide significant differentially methylated CpG site in PDGFA which encodes a marker of both fibrosis and cancer. The average DNA methylation at this site was 41.3% in T2D cases and 60.3% in normoglycemic controls ($p=2.5\times10\text{-}8$). Hypomethylation at this site was associated with elevated PDGFA gene expression, hyperinsulinemia, systemic insulin resistance and non-alcoholic steatohepatitis (NASH). Moreover, increased PDGFA expression and decreased methylation in T2D cases were associated with liver fibrosis. This association was replicated in other European liver samples. Mendelian randomization confirmed a direct effect of fasting serum insulin levels on PDGFA hypomethylation. In Immortalized Human Hepatocytes (IHH), chronic hyperinsulinemia hampered insulin signaling and caused a reduction of methylation at this PDGFA site and an increase in PDGFA mRNA and protein levels. Exposure of IHH cells to chronic hyperinsulinemia further increased PDGFA secretion. IHH culture with blocking PDGFA antibodies improved insulin signaling induced by hyperinsulinemia, suggesting an autocrine and negative role of PDGFA in hepatocyte insulin signaling

Conclusion: These findings provide novel biological mechanism linking chronic hyperinsulinemia to hepatic insulin resistance and liver complications of obesity and T2D. The data may open new avenues to treat NAFLD associated complications in T2D.

Supported by: ERC advanced grant

Disclosure: **A. Abderrahmani:** None.

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A novel role of fibroblast growth factor 21 in activating pancreatic islet autophagy in type 2 diabetes mellitus

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Background and aims: Autophagy is an intracellular degradation pathway, which is promoted when cells are prepared to remove damaging cytoplasmic components upon oxidative stress or protein aggregate accumulation. Emerging studies point to the potential action of pancreatic islet autophagy in controlling glucose and lipid metabolism, as well as the progression to type 2 diabetes mellitus (T2DM). Recent researches have shown the beneficial roles of fibroblast growth factor 21 (FGF21) in regulating insulin sensitivity and glucose homeostasis; however, there have been no reports to further elucidate the interaction between FGF21 and pancreatic islet autophagy. This study aimed to investigate into the physiological role and the signaling pathways involved in FGF21-stimulated autophagy in pancreatic islets.

Materials and methods: c57/BL6J mice were fed a standard diet or a 60% high-fat diet (HFD) for 12 weeks. Blood glucose level and body weight were measured. Intraperitoneal glucose tolerance tests were performed to assess glucose tolerance between these two groups. Pancreatic islets were isolated from normal c57/BL6J and global FGF21 knockout (KO) mice by intraductal injection of collagenase P. For ex vivo study, isolated islets were cultured and exposed to different concentrations of glucose (normal: 5.6mM; high: 28mM), palmitic acid (normal: 0mM; high: 0.5mM) with/without FGF21 (100nM) or compound C (20μM, AMPK inhibitor) for various time course. Real-time quantitative PCR and Western blotting were performed for mRNA and protein expression levels of targeted genes, respectively.

Results: HFD-treated mice showed increases in fasting plasma glucose (9.02±0.53 vs 12.78±0.70, $p<0.001$), body weight (28.75±0.77 vs 44.87 ±1.50, $p<0.001$) and impaired glucose tolerance (1484±120.0 vs 2251 ±194.5, $p<0.01$). Interestingly, islet protein expression of FGF21 was induced after HFD treatment (1.00±0.17 vs 1.58±0.12, $p<0.05$). Meanwhile, protein expression levels of FGF21 (1.00±0.06 vs 1.64 ±0.04, $p<0.001$) and LC3-II (marker of autophagy; 1.00±0.05 vs 2.32 ±0.39, $p<0.05$) were induced in mouse islets treated with high concentrations of palmitic acid and glucose, while phosphorylation of AMPK was reduced (1.00±0.19 vs 0.31±0.12, $p<0.05$), compared with the respective controls. In addition, induction of LC3-II protein expression was reduced in islets isolated from FGF21 global KO mice (1.00±0.12 vs 0.71±0.08, $p<0.05$). Furthermore, exogenous administration of FGF21 diminished phosphorylation of AMPK (1.00±0.26 vs 0.64±0.10, $p<0.05$), and stimulated protein expression of LC3-II (1.00±0.09 vs 1.85±0.16, $p<0.001$). Consistently, compound C significantly induced islet autophagy, as revealed by increased protein expression of LC3-II (1.00±0.05 VS 1.86 ±0.18, $P<0.01$). Meanwhile, the effect of FGF21 on activating islet autophagy was blunted by exposure to high concentrations of glucose and palmitic acid, of which this effect might be due to suppressed mRNA expression of islet beta-klotho (1.00±0.14 VS 0.50±0.08, $P<0.05$), a co-factor of FGF21, and FGF receptor 1 (1.00±0.07 VS 0.32±0.07, $p<0.001$).

Conclusion: These data indicate that glucolipotoxicity-induced FGF21 activation mediates islet autophagy, which is dependent on AMPK inhibition. Our findings provide a scientific basis for FGF21 activation or the use of FGF21 analogues, as being therapeutic target for obesity-related T2DM.

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Disclosure: **T.W. Cheng:** Grants; Hong Kong Research Grants Council (Ref. # CUHK 14107415).

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Decreased circulating bone morphogenetic protein (BMP)-9 levels in patients with type 2 diabetes is a signature of insulin resistance

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Background and aims: Bone morphogenetic protein 9 (BMP-9) has been demonstrated to improve glucose homeostasis in diabetic mice. However, no report has demonstrated any relationships between circulating BMP-9 levels and insulin resistance (IR) or T2DM in humans. The aim of the objective of the study was to (1) determine circulating BMP9 levels in patients with T2DM and control subjects, (2) examine the relationship of BMP9 and conventional marker of insulin resistence, and (3) examine BMP9 changes with interventions modulating insulin resistence.

Materials and methods: One hudrded and fifty eight subjects with T2DM and 121 healthy controls were recruited for a series of cross-sectional and interrventional studies. The oral glucose test (OGTT) and the euglycemic- hyperinsuliemic clamps (EHCs) were performed to assess glucose tolerance and insulin sensitivity. Circulating BMP9 was measured with ELISA kit. Plasma glucose and HbA1c were measured by the glucose oxidase method and HPLC, respectively. Plasma insulin was detected by radioimmunoassay. Plasma TC, LDL, HDL, and TG were analyzed enzymatically using an autoanalyzer. The percentage of body fat (FAT%) was determind by bioelectrical impedance. The area under the curve for glucose (AUC glucose) and insulin (AUC insulin) during the OGTT was calculated gemetrically using the trapezoidal rule. The ROC curves of BMP9 was analyzed to investigate the predictive of BMP9 for insulin resistance. Acute insulin resistence which was induced by lipid infusion for 2 h in 20 healthy volunteers, a 4-hour EHC was performed. Muscle and fat biopsies were obtained for real-time RT-PCR and Western blot analysis. The effect of a GLP-1 receptor agonist (PEX168) on circulating BMP-9 was investigated in a 24-week treatment trial.

Results: Circulating BMP-9 levels were lower in patients with nT2DM than that in normal subjects. Circulating BMP-9 were inversely associated with TG, TC, LDL, HDL, HbA1c, FBG, 2-h OGTT, FAT%, FIns , 2hIns and HOMA-IR. Multivariate regression analyses showed that FFA (r=0.376; P<0.001) and AUGglucose (r= 0.432; P<0.001) were independently related factors influencing BMP-9 levels. Both hyperinsulinemia and lipid infusion decreased circulating BMP-9 levels. BMP-9 mRNA expression in T2DM patients was markedly decreased by 70.6% in muscle and 74.7% in adipose tissue compared with that in healthy subjects (both P < 0.01). BMP-9 protein expressions in both muscle and fat were also markedly decreased in T2DM patients compared with control subjects (by ~ 2.55-fold for muscle and ~ 2.43-fold for fat; both P <0.01). The ROC curve analyses revealed that the best cutoff value for circulating BMP-9 to predict T2DM was 56.2 ng/L (sensitivity 60.3% and specificity 67.3%), and to predict IR was 44.7ng/L (sensitivity70.3% and specificity 53.9). Circulating BMP-9 levels did not change as a result of PEX168 treatment, but were lowered by a placebo.
Conclusion: Circulating BMP-9 is reduced in T2DM and is associated with insulin resistence, making it a potential biomarker of insulin resistance in humans.
Clinical Trial Registration Number: ChiCTR-OCS-13003185
Supported by: NSFC (81500666)
Disclosure: **J. Chen:** None.

OP 24 Making beta cells work better

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Glycine and insulin autocrine signalling in human pancreatic beta cell

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Background and aims: Insulin is secreted from pancreatic islet β-cells and is regulated by nutrients, circulating hormones, and neurotransmitters. Glycine is an inhibitory neurotransmitter and recent metabolic studies identify glycine as a potential biomarker of type 2 diabetes (T2D) risk. A strong correlation exists between plasma glycine concentrations and insulin sensitivity, glucose disposal, and obesity. The mechanism for glycine's action in diabetes is unknown.
Materials and methods: Human islets were isolated in the Alberta Diabetes Institute Islet Core and the Clinical Islet Laboratory at the University of Alberta from donor organs. Electrical recordings and calcium imaging was performed on dispersed human islets from healthy donors and donors with T2D. For studying glycine secretion from single β-cells, (auto-) synapses were created by transfecting cells with plasmids encoding the mouse glycine receptor α1 subunit.
Results: Glycine receptors were identified on both α and β cells in humans and quantitative immunofluorescence determined that glycine receptor expression is decreased in T2D islets (from 480±35 average pixel intensity [PI] in non-diabetic donors to 309±24 averge PI in T2D donors). Application of 0.3mM glycine was found to produce 27±6pA/pF current in healthy human β cells whereas it was significantly lower in T2D β cells (16±2pA/pF). Downstream of the receptors, application of 0.3mM glycine resulted in depolarization of β cells (16±6mV), elevation of intracellular calcium (0.06±0.01 arbitrary units), and stimulation of insulin secretion. Interestingly, glycine receptor activity was increased by exogenous insulin (10uM by 56.0±0.2% and 100nM insulin in a similar manner) in a PI3 kinase sensitive manner. However, this effect was completely lacking in T2D β cells. β cells were observed to secrete glycine from vesicles and within the islet the clearance of extracellular glycine is mediated by glycine transporters that we have identified in human islets both by immunofluorescence and functionally by replacement of extracellular Na+ with Li+ which demonstrated a doubling of endogenous glycine signalling (from 18±6 to 42±6 ms).
Conclusion: Here we have evidence that glycine acts directly on glycine receptors in human pancreatic islets to stimulate insulin secretion. Pancreatic β-cells are directly involved with the clearance, storage, and release of glycine to produce a positive feed-forward effect on insulin secretion. Furthermore insulin autocrine feedback in human β-cells further amplifies the glycine response. Finally, individuals with T2D demonstrate a reduced glycine receptors expression and insulin is unable to promote an enhanced glycine response.
Supported by: ADI
Disclosure: **R. Yan-Do:** None.

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Biased agonism alters GLP-1 receptor trafficking and glucose homeostasis

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Background and aims: Exendin-4 (Ex4) is a glucagon-like peptide-1 (GLP-1) mimetic used to treat type 2 diabetes. Binding of Ex4 to the

GLP-1 receptor (GLP-1R) rapidly results in G-protein activation, β-arrestin recruitment, and receptor internalisation into the endocytic pathway. Each of these events has been implicated in signalling to insulin secretion in beta cells, but their precise roles remain incompletely understood. We therefore sought to develop peptide analogues of Ex4 with differing biases between G-protein activation and β-arrestin recruitment, which might 1) facilitate further understanding of GLP-1R trafficking and signalling in beta cells, and 2) be useful therapeutically.

Materials and methods: The effect of mutating specific residues within Ex4 on GLP-1R-induced cAMP production and β-arrestin2 recruitment was measured in GLP-1R-expressing Chinese hamster ovarian cells. Agonist-induced GLP-1R trafficking was investigated by confocal and FACS analysis in mouse insulinoma MIN6B1 cells stably expressing SNAP-tagged GLP-1R, labelled with membrane-permeable or impermeable, cleavable, SNAP-tag fluorescent probes, and by electron microscopy with a membrane-impermeable biotinylated SNAP-tag probe followed by gold-conjugated streptavidin. Insulinotropism of biased agonists was investigated in rat insulinoma INS-1 (832/3) cells and human islets. In vivo glucose lowering was measured after a single intraperitoneal injection of agonist in hyperglycaemic high fat fed C57BL/6J mice.

Results: Substitution of L-His to D-His at position one of Ex4 generated an agonist ("ex4-dHis1") with 5-fold bias for cAMP production over β-arrestin2 recruitment (ΔlogRA 0.72, 95% CI 0.47-0.96, n=6). Ex4-dHis1 100nM induced significantly less surface SNAP-GLP-1R loss than Ex4 100nM after overnight incubation (residual surface receptor 87% vs. 61%, percentage of vehicle control, $p<0.01$, n=3-4), most likely as a result of a reduced rate of internalisation (37% vs. 96% after 1h agonist exposure) as recycling rates were similar (9% vs. 11% over 30 min). Ex4-dHis1 induced greater insulin secretion than Ex4 in INS-1 (832/3) cells (insulin stimulation index [ISI] 1.84 vs. 1.32, $p<0.01$, n=6) and in human islets (ISI 1.67 vs. 1.34, $p<0.05$, n=3). Finally, glucose lowering in mice over 24h was greater with 100 ug/kg of Ex4-dHis1 compared to same dose of Ex4 (glucose AUC 101.9 vs. 132.2 mM.hr, $p<0.05$, n=6-7).

Conclusion: A single amino acid switch to the N-terminal of Ex4 induced significant agonist bias to favour G-protein signalling over β-arrestin recruitment, resulting in marked differences in GLP-1R trafficking and improved biological activity, both in vitro and in vivo. These findings suggest 1) a key role for β-arrestins in stimulating GLP-1R internalisation, and 2) GLP-1R-induced signalling to insulin secretion originates primarily at the plasma membrane rather than the endosome. Further experiments to define the role of β-arrestins in GLP-1R desensitisation and trafficking are underway.

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Disclosure: **B. Jones:** None.

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Role of circular RNAs in the control of beta cell functions

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Background and aims: There is already strong evidence, that different types of non-coding RNAs, including microRNAs and long non-coding RNAs, are key players in the regulation of β-cell functions and in the development of diabetes. However, the role of the newly discovered class of circular RNAs (circRNAs) remains unknown.

Materials and methods: We used microarrays and RNA-sequencing approaches to identify circRNAs present in samples of insulin-secreting cell lines and pancreatic islets. We amplified by real-time PCR and sequenced several of them to verify their expression and then analysed their role in the regulation of insulin secretion, cell proliferation and apoptosis by specifically modifying their expression level. All data are expressed as the mean ± SD and were analysed either by two-tailed Student's t-test or by one-way ANOVA.

Results: We identified thousands of circRNAs expressed in the mouse and rat β-cell lines MIN6B1 and INS832/13, respectively, as well as in human, rat and mouse islets. One of these transcripts, ciRS-7, is particularly interesting because it is selectively expressed in brain and islets, and possesses more than 60 binding sites for miR-7, one of the most abundant microRNAs expressed in β-cells. miR-7 has been proposed to inhibit proliferation of β-cells by decreasing the level of several components of the mTOR signaling pathway. In agreement with these observations, we found that the overexpression of human ciRS-7 mimics the effect of anti-miR-7 and increases proliferation of MIN6 cells by 60% (N4, $p = 0.02$). Furthermore, we identified the circular isoform of HIPK3 exon 2 (circHIPK3) as one of the most abundant circRNAs expressed in mouse and human islets. We were able to inhibit circHIPK3 expression in MIN6 cells by 80% (N7, $p < 0.0001$) using a siRNA directed against the circularised splice junction. CircHIPK3 knockdown resulted in a decrease in glucose-stimulated insulin secretion by 38% (N7, $p = 0.02$) and in a reduction of 54% of the insulin content (N7, $p = 0.01$). In addition, circHIPK3 was found to be necessary for β-cell survival, as its inhibition significantly increased apoptosis of MIN6 cells by 87% (N4, $p = 0.045$). To elucidate the mechanism of action of this particular circRNA, we analysed by microarray the global impact of circHIPK3 silencing on gene expression. In line with the observed phenotype, pathway analysis of the down-regulated genes revealed an enrichment for genes involved in insulin secretion and PI3K/AKT signaling. The changes of some of the key genes were then confirmed by quantitative PCR: AKT1 was decreased by 54% (N3, $p = 0.004$), MTPN by 50% (N3, $p = 0.037$), SLC2A2 by 65% (N3, $p = 0.01$) and eIF2B1 by 65% (N3, $p = 0.001$).

Conclusion: Our data indicate that circRNAs are important regulators of β-cell functions and suggest that alterations in the expression of these newly discovered class of RNAs may potentially contribute to the development of diabetes.

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Disclosure: **L. Stoll:** None.

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Activation of the alternative pathway of complement, represented by factor Bb, is longitudinally associated with beta cell conservation in type 2 diabetes: the CODAM study

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Background and aims: Complement factor D (FD, also known as adipsin) is the rate-limiting protease in activation of the alternative pathway (AP) of complement, and was recently reported to be inversely related to β-cell failure in type 2 diabetes (T2DM) patients. These data were obtained in a cross-sectional case-control setting (Controls: T2DM, Cases: T2DM with β-cell failure defined as insulin treatment) with matching for only age, sex and obesity. Based on mouse studies, the underlying β-cell preserving mechanism was proposed to be related to local generation of C3a in the β-cell. We now investigated the longitudinal associations of FD and the AP activation factors C3a and Bb with T2DM and insulin-treated T2DM (insT2DM) over a 7 yr period.

Materials and methods: The Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) is a prospective cohort (baseline n=574, age 60±7 yr, 61% men; 7 yr follow-up n=495). At baseline, n=129 had impaired glucose metabolism and n=146 had T2DM (based on oral glucose tolerance test). Complement factors FD, C3a and Bb were measured at baseline. First, generalized estimating equations (GEE) were done with T2DM (n=988 observations, max 2 observations per participant) and with insT2DM as outcomes, respectively, with adjustments for age, sex, follow-up time, obesity, kidney function, smoking, physical activity, caloric intake, and lipid and/or blood pressure-lowering medication. GEE with insT2DM as outcome was additionally adjusted for oral glucose

medication and duration of diabetes. Statistically significant associations in GEE were subsequently evaluated using prospective logistic regression, using only incident cases.
Results: Higher baseline concentrations of FD, C3a and Bb were consistently but weakly associated with slightly less prevalent and incident T2DM, over the 7 yr period. Odds ratios (ORs) were 0.95 [95% CI: 0.75; 1.20] for FD (in SD), 0.90 [0.75; 1.08] for C3a (in SD), and 0.87 [0.72; 1.06] for Bb (in SD). These associations were not statistically significant. Therefore logistic regression for incident T2DM was not done. Particularly for the activated factors C3a and Bb, the longitudinal associations were stronger when insT2DM was the outcome. ORs were 0.92 [0.58; 1.47] for FD (in SD), 0.62 [0.27; 1.41] for C3a (in SD), and 0.31 [0.15; 0.62] for Bb (in SD). The significant association between higher baseline Bb and less insT2DM was then evaluated in prospective logistic regression (n=440, 25 incident cases of insulin treatment). Individuals with 1 SD higher baseline Bb were 64% less likely to start insulin therapy in the 7 yr follow-up period (OR was 0.61 [0.37; 0.98], adjusted for age and sex). In the fully adjusted model, this association did not remain significant 0.73 [0.36; 1.50], which could be due to the low number of cases
Conclusion: Activation of the AP of complement may be related to less diabetes progression towards insulin therapy in human T2DM patients. In contrast, it did not seem to protect against T2DM in itself. This suggests a potentially protective effect of AP activation in β-cell preservation. The observed inverse association of AP activation with insT2DM may be due to local AP activation in the β-cell or pancreas and/or to systemic AP activation, and was most pronounced for factor Bb.
Supported by: NHS2010B194
Disclosure: **M.M.J. van Greevenbroek:** None.

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Expression of DPP-4 in beta cells of type 2 diabetic subjects and the effects of its inhibition

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Background and aims: It has been suggested that the incretin system, including regulated GLP-1 secretion and locally expressed DPP-4, is present in pancreatic islets. Here we comprehensively evaluated the expression and role of DPP-4 in islets from 17 human non-diabetic (ND) and 16 type 2 diabetic (T2D) individuals, as well as in human insulin-producing EndoC-βH1 cells.
Materials and methods: Morphological (including confocal microscopy), ultrastructural (electron microscopy, EM), functional (glucose-stimulated insulin secretion), survival (EM and nuclear dyes) and molecular (RNAseq and qPCR) studies were performed under several different conditions.
Results: DPP-4 was found in human pancreatic islet cells, co-localizing mainly with glucagon and, although to a less extent, also with insulin. In addition, DPP-4 was expressed in EndoC-βH1 cells. Both the proportion of DPP-4 positive alpha and beta cells and DPP-4 gene expression were significantly lower in T2D than in ND islets. A DPP-4 inhibitor protected primary ND human beta cells and EndoC-βH1 cells against cytokine-induced toxicity. Finally, DPP-4 inhibition augmented glucose-stimulated insulin secretion, reduced apoptosis and improved ultrastructure in T2D beta cells, as compared to controls.
Conclusion: These results demonstrate the presence of DPP-4 in human beta cells, with reduced expression in T2D islets, and show that DPP-4 inhibition has beneficial effects on human ND and T2D beta cells at least in part independently from GLP1. This suggests that DPP-4 directly affects beta cell pathophysiology besides its role in incretin effects.
Supported by: T2DSystems (grant agreement No 667191)
Disclosure: **M. Bugliani:** None.

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Hyperactivation of mTORC1 in human type 2 diabetic human pancreatic islets

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Background and aims: Mechanistic target of rapamycin (mTOR) is a master regulator of diverse cellular functions such as metabolism, proliferation and survival through the formation of at least two functionally distinct complexes, mTOR complex-1 (mTORC1) and mTOR complex-2 (mTORC2). mTORC1 is a key determinant of nutritional status at the cellular and organismic level. While mTORC1 mediates β-cell growth and expansion, its hyperactivation has been observed in pancreatic islets from animal models of type 2 diabetes and leads to β-cell loss. We sought to determine whether such mTORC1 activation also occurs in human type 2 diabetic or metabolically stressed human islets and whether mTORC1 blockade can restore β-cell function of type 2 diabetic islets.
Materials and methods: Human islets isolated from non-diabetic controls and individuals with type 2 diabetes as well as treated human islets and INS-1E cells with increased glucose concentrations (22.2 mM) were analyzed for mTORC1/2 activities by Western blot analysis of phosphorylation of mTORC1 down-stream targets S6K1 at Thr 389 (pS6K), the direct S6K substrate ribosomal protein S6 at Ser 235/236 (pS6) and 4E-BP1 at Thr 37/46 (p4E-BP1) as well as mTORC2 down-stream targets AKT at Ser 473 (pAKT) and NDRG1 at Thr 346 (pNDRG1). mTORC1/2 complexes integrity was assessed by immunoprecipitation of mTOR and subsequent Western blot analysis of mTOR associated proteins raptor (representing mTORC1 activity) and rictor (representing mTORC2 activity).
Results: While mTORC1 activity, shown by phosphorylation of S6K, S6 and 4E-BP1, was markedly increased, mTORC2 activity, shown by phosphorylation of AKT and NDRG1 was reduced in human islets and INS-1E cells exposed to increased glucose concentrations as well as in type 2 diabetic human islets. Consistently, under such glucotoxic conditions in metabolically stressed human islets, raptor co-precipitated with mTOR (raptor-mTOR complex) was increased whereas rictor co-precipitated with mTOR (rictor-mTOR complex) was drastically reduced suggesting reciprocal functional regulation of different mTOR complexes.
Conclusion: Our results show that elevated mTORC1 activation is a pathogenic hallmark of type 2 diabetic islets contributing to impaired β-cell function and survival in the presence of metabolic stress. Its inhibition may provide a new strategy to restore β-cell survival and function in diabetes.
Supported by: DFG
Disclosure: **K. Gorrepati:** None.

OP 25 GLP-1 RA: the longer, the better?

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Improved glycaemic control and weight loss with once weekly dulaglutide versus placebo, both added to titrated daily insulin glargine in type 2 diabetes patients (AWARD-9)

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Background and aims: This was a 28-week (wk), randomised, double-blind study that compared once weekly injection of dulaglutide (DU) 1.5 mg to placebo (PL), both added to titrated once daily insulin glargine (±metformin) in patients with type 2 diabetes and inadequate glycaemic control (HbA1c ≥7% [53 mmol/mol] and ≤10.5% [91 mmol/mol]).
Materials and methods: Patients (N = 300; mean baseline characteristics: age 60.4 y; HbA1c 8.4% [68 mmol]; BMI 32.7 kg/m^2; glargine dose 39 U [0.42 U/kg]) were randomised (1:1) to DU 1.5 mg, or PL; glargine was titrated to fasting plasma glucose target (71 to 99 mg/dl [3.9 to 5.5 mmol/l]). The primary objective was change from baseline in HbA1c at wk 28 tested for superiority.
Results: At wk 28 (Table), DU 1.5 mg resulted in significantly greater reductions than PL in HbA1c and fasting serum glucose (P<0.001, both). Body weight decreased with DU 1.5 mg and increased with PL (P<0.001); hypoglycaemia rate (≤70 mg/dl [3.9 mmol/l] and/or symptoms) was 7.69 and 8.56 events/pt/y for DU 1.5 mg and PL, respectively (P=0.488); severe hypoglycaemia events were (n): DU 1.5 mg (1), PL (0). A statistically greater increase (P<0.001) in glargine dose was observed with PL compared to DU 1.5 mg. Nausea and diarrhoea were more common with DU 1.5 mg (12.0%, 11.3%) vs PL (1.3%, 4.0%).
Conclusion: Once weekly DU 1.5 mg compared to PL, both added on to titrated daily glargine, resulted in better glycaemic control and weight loss without significantly increasing the risk of hypoglycaemia.

Primary Endpoint (28 wk, ITT)	DU 1.5 mg (N=150)	PL (N=150)	Treatment Difference (SE)[a]
HbA_{1c} change (%), LS Mean (SE)	-1.44 (0.09)	-0.67 (0.09)	-0.77 (0.10)[#]
HbA_{1c} change (mmol/mol), LS Mean (SE)	-15.74 (0.98)	-7.32 (0.98)	-8.42 (1.09)[#]
% of pt with HbA_{1c} <7% (53 mmol/mol)	66.7	33.3	-33.4[#]
FSG change (mmol/l), LS Mean (SE)	-2.48 (0.23)	-1.55 (0.23)	-0.93 (0.26)[#]
Weight change (kg), LS Mean (SE)	-1.91 (0.30)	0.50 (0.30)	-2.41 (0.39)[#]
Glargine dose change (U), LS Mean (SE)	12.75 (2.27)	25.94 (2.30)	-13.19 (3.21)[#]

[a] Least squares mean difference for DU 1.5 mg vs PL
Abbreviations: DU = dulaglutide; FSG = fasting serum glucose; ITT = intent-to-treat; LS = least squares; PL = placebo; pt = patient
[#] 2-sided p 0.001 of DU vs PL

Clinical Trial Registration Number: NCT02152371
Disclosure: **P. Pozzilli:** Grants; Eli Lilly and Company. Lecture/other fees; Eli Lilly and Company.

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Efficacy and safety by duration of diabetes with once weekly dulaglutide in the AWARD programme

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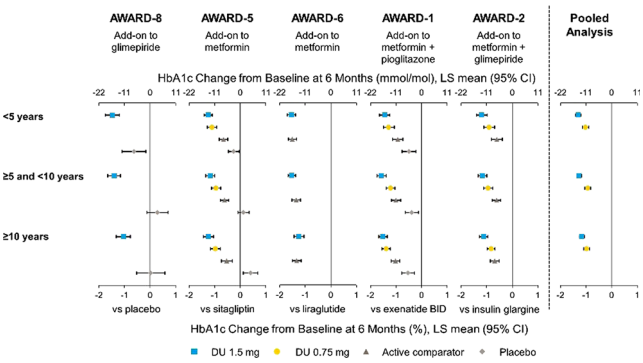
Background and aims: Dulaglutide (DU), a once weekly GLP-1 receptor agonist, was studied in the AWARD clinical trial programme in adult patients (pts) with T2D and demonstrated significant HbA1c reduction and potential for weight loss.
Materials and methods: To evaluate the efficacy and safety of DU 1.5 mg and DU 0.75 mg in T2D pts by duration of diabetes (DoD) <5 years, ≥5 and <10 years, and ≥10 years, we conducted a post-hoc analysis on the completed studies AWARD-1, 2, 5, 6 and 8 at 6 months. AWARD-3 and 4 were not included in the analysis because, due to the population studied, small numbers of pts had DoD ≥10 years in AWARD-3 and DoD <5 years in AWARD-4.
Results: Across the 5 studies, the proportions of pts with DU treatment were similar among DoD subgroups. The ranges of HbA1c reductions with DoD <5 years, ≥5 and <10 years, and ≥10 years, respectively, were: DU 1.5 mg: -1.19 to -1.54% [-13 to -17 mmol/mol] , -1.16 to -1.56% [-13 to -17 mmol/mol], and -1.04 to -1.50% [-11 to -16 mmol/mol]; DU 0.75 mg: -0.90 to -1.28% [-10 to -14 mmol/mol], -0.94 to -1.21% [-10 to -13 mmol/mol], and -0.82 to -1.38% [-9 to -15 mmol/mol]. The HbA1c changes were similar from the pooled analysis with DoD <5 years, ≥5 and <10 years, and ≥10 years: DU 1.5 mg: -1.31% [-14 mmol/mol], -1.27% [-14 mmol/mol], and -1.17% [-13 mmol/mol]; DU 0.75 mg: -1.02% [-11 mmol/mol], -0.93% [-10 mmol/mol], and -0.98% [-11 mmol/mol], respectively (Figure). The effects on weight were similar among DoD subgroups with both DU doses, respectively. DU treatments were well tolerated among DoD subgroups.
Conclusion: Irrespective of DoD, pts treated with DU demonstrate similar HbA1c reduction, weight change, and acceptable safety profile.



Clinical Trial Registration Number: NCT01064687, NCT01075282, NCT00734474, NCT01624259, NCT01769378
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Disclosure: **B. Gallwitz:** Employment/Consultancy; Amgen, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Lilly, MSD, Novo Nordisk, Sanofi. Lecture/other fees; Abbott, AstraZeneca, Boehringer Ingelheim, Bristol Myers Squibb, Lilly, MSD, Novartis, Novo Nordisk, Sanofi.

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Efficacy and safety of once-weekly semaglutide vs exenatide ER after 56 weeks in subjects with type 2 diabetes (SUSTAIN 3)

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Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). This study evaluated the efficacy, safety and tolerability of once-weekly s.c. semaglutide vs exenatide extended-release (ER) in subjects with T2D inadequately controlled on 1-2 oral antidiabetic drugs (OADs: MET, SU, TZDs).
Materials and methods: In this phase 3, open-label study, 813 adults with T2D (HbA_{1c} 7-10.5%) were randomised 1:1 to once-weekly semaglutide 1.0 mg or once-weekly exenatide ER 2.0 mg for 56 weeks. The primary endpoint was change in HbA_{1c} from baseline to Week 56. Secondary efficacy endpoints included change in body weight (BW), blood pressure and other glycaemic parameters.
Results: Baseline characteristics were similar in both arms; overall mean age 56.6 years, duration of T2D 9.2 years. Mean HbA_{1c} (overall baseline mean 8.3%) was reduced by 1.5% with semaglutide and 0.9% with exenatide ER (estimated treatment difference vs exenatide ER [ETD] -0.62%; p<0.0001). HbA_{1c} <7% was achieved by 67% of semaglutide-treated subjects vs 40% with exenatide ER; 47% and 22% achieved HbA_{1c} ≤6.5%, respectively. Mean BW (overall baseline mean 95.8 kg) was reduced by 5.6 kg with semaglutide and 1.9 kg with exenatide ER (ETD -3.73 kg; p<0.0001). Improvements in other secondary endpoints, including fasting plasma glucose and self-measured plasma glucose, also occurred (Table). Adverse event (AE) rates were comparable between groups: 75.0% and 76.3% of subjects reported AEs with semaglutide and exenatide ER, respectively. Serious AEs were reported by 9.4% of subjects receiving semaglutide and 5.9% of subjects receiving exenatide ER (spread over multiple system organ classes). Two fatal events were reported in the semaglutide arm (both advanced stage neoplasms considered unrelated to treatment). The proportion of subjects discontinuing treatment due to AEs was 9.4% for semaglutide and 7.2% for exenatide ER. The most frequent AEs were gastrointestinal (GI), which were mainly mild or moderate in severity. GI AEs were reported by 41.8% and 33.3% of subjects receiving semaglutide and exenatide ER, respectively. Injection site reactions were reported by 1.2% of subjects receiving semaglutide and 22.0% of subjects receiving exenatide ER.
Conclusion: Semaglutide (s.c. once weekly) 1.0 mg was superior to exenatide ER 2.0 mg in improving glycaemic control and reducing BW in subjects with T2D inadequately controlled on 1-2 OADs. Semaglutide was well tolerated, with a safety profile similar to that of other GLP-1 receptor agonists.

Table. Key primary and secondary outcomes from the SUSTAIN 3 study

	Overall baseline, mean	Change at Week 56		ETD (95% CI)
		Semaglutide 1.0 mg n=404*	Exenatide ER 2.0 mg n=405*	
HbA_{1c}, %	8.3	−1.5	−0.9	−0.62 (−0.79; −0.44)[a]
Body weight, kg	95.8	−5.6	−1.9	−3.73 (−4.53; −2.93)[a]
Fasting plasma glucose, mmol/L	10.5	−2.8	−2.0	−0.83 (−1.20; −0.46)[a]
7-point SMPG: mean, mmol/L	10.9	−2.2	−1.5	−0.73 (−1.02; −0.44)[a]
Post-prandial increment of 7-point SMPG, mmol/L	2.2	−0.6	−0.3	−0.24 (−0.44; −0.04)[b]
Systolic blood pressure, mmHg	133.5	−4.6	−2.2	−2.36 (−4.28; −0.44)[c]
Pulse rate, beats/min	75.1	2.1	1.1	1.03 (−0.19; 2.25)[d]
Overall treatment satisfaction (DTSQs)	27.3	5.0	4.0	1.02 (0.28; 1.76)[e]

CI, confidence interval; DTSQs, Diabetes Treatment Satisfaction Questionnaire Status Version; ETD, estimated treatment difference; SMPG, self-measured plasma glucose.

*Overall, 813 subjects were randomised; 809 subjects were exposed to treatment; [a]p<0.0001; [b]p=0.0189; [c]p=0.0162; [d]p=0.0973; [e]p=0.0068.

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Supported by: Novo Nordisk A/S
Disclosure: **A. Ahmann:** Employment/Consultancy; Sanofi, Lilly, Novo Nordisk. Grants; Novo Nordisk, Sanofi, Medtronic.

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Efficacy and safety of once-weekly semaglutide vs once-daily insulin glargine in insulin-naïve subjects with type 2 diabetes (SUSTAIN 4)

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Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). This trial evaluated the efficacy, safety and tolerability of subcutaneous semaglutide vs insulin glargine (IGlar) in insulin-naïve subjects with T2D.
Materials and methods: In this phase 3a, randomised, open-label study, 1082 adults with T2D (HbA_{1c} 7-10%) received semaglutide 0.5 mg (n=362) or 1.0 mg (n=360) once weekly or IGlar (n=360; starting dose 10 IU) once daily for 30 weeks, added to stable metformin +/- sulphonylurea (SU). In both semaglutide groups, 51% of subjects were on metformin + SU vs 52% with IGlar. Investigators were instructed to titrate IGlar to a pre-breakfast self-monitored plasma glucose (SMPG) target of 4.0-5.5 mmol/L. The primary endpoint was change in HbA_{1c} from baseline to Week 30.
Results: Mean HbA_{1c} (baseline 8.2%) was reduced with semaglutide 0.5 and 1.0 mg by 1.2% and 1.6%, respectively, vs 0.8% with IGlar (estimated treatment difference vs IGlar [ETD] -0.38% and -0.81%; p<0.0001 for both). Mean IGlar dose at Week 30 was 29.2 IU/day. HbA_{1c} <7% was achieved by 57.5% and 73.3% of 0.5 and 1.0 mg semaglutide-treated subjects vs 38.1% with IGlar. HbA_{1c} ≤6.5% was achieved by 37.3%, 54.2% and 17.5% of subjects, respectively. Mean fasting plasma glucose (baseline 9.7 mmol/L) was reduced with semaglutide 0.5 and 1.0 mg by 2.1 and 2.7 mmol/L vs 2.1 mmol/L with IGlar (ETD 0.07 mmol/L [p=0.7]

and -0.61 mmol/L [p=0.0002]). Mean 8-point SMPG (baseline 10.9 mmol/L) was reduced by 2.4, 2.9 and 2.4 mmol/L, respectively (ETD -0.07 mmol/L [p=0.6] and -0.58 mmol/L [p<0.0001]). Mean body weight (BW; baseline 93.4 kg) decreased with semaglutide 0.5 and 1.0 mg by 3.5 and 5.2 kg vs a 1.2 kg increase with IGlar (ETD -4.62 kg and -6.34 kg; p<0.0001 for both). Overall treatment satisfaction (Diabetes Treatment Satisfaction Questionnaire; baseline 26.9) improved by 4.9, 5.4 and 4.0 points, respectively (ETD 0.87 [p=0.025] and 1.38 [p=0.0005]). The proportions of subjects reporting adverse events (AEs) were 69.9%, 73.3% and 65.3% with semaglutide 0.5, 1.0 mg and IGlar, respectively; 6.1%, 4.7% and 5.0% reported serious AEs. Fatal AEs were reported in 4 semaglutide subjects (1 pancreatic cancer, 3 cardiovascular) and 2 IGlar subjects (both cardiovascular). Discontinuation due to AEs occurred in 5.5%, 7.5% and 1.1% of patients, respectively. The majority of discontinuations with semaglutide were due to gastrointestinal (GI) AEs; mild, transient GI AEs were the most common AEs with semaglutide. The proportions of subjects reporting GI AEs were: 21.3%, 22.2% and 3.6% for nausea; 16.3%, 19.2% and 4.4% for diarrhoea; and 6.6%, 10.3% and 3.1% for vomiting. Severe/blood glucose-confirmed hypoglycaemia was reported by 4.4%, 5.6% and 10.6% of subjects, respectively.

Conclusion: Semaglutide (0.5 and 1.0 mg s.c. once weekly) provided superior glycaemic control and BW reduction vs IGlar in patients with T2D treated with metformin +/- SU. Semaglutide was well tolerated, with a safety profile similar to that of other GLP-1 RAs.

Clinical Trial Registration Number: NCT02128932
Supported by: Novo Nordisk A/S

Disclosure: **J.H. DeVries:** Employment/Consultancy; Eli Lilly, Johnson & Johnson, MSD, Roche Diagnostics, Novo Nordisk. Lecture/other fees; BD, Dexcom, Eli Lilly, Novo Nordisk, Roche Diagnostics, Senseonics. Non-financial support; Abbott, Dexcom, Medtronic, Novo Nordisk, Senseonics.

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Dose-dependent glucose lowering and body weight reductions with the novel oral formulation of semaglutide in patients with early type 2 diabetes

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Background and aims: Glucagon-like peptide-1 (GLP-1) receptor agonists are available for the treatment of type 2 diabetes (T2D), but injectable formulations can be a barrier to acceptance and adherence for some patients. A novel oral formulation of the GLP-1 analogue, semaglutide, is in development. The aim of this study was to explore the dose-response relationship of oral semaglutide vs placebo (PBO) on glycaemic control in patients with T2D.

Materials and methods: Adults with T2D on diet/exercise ± metformin (n=632; HbA_{1c}, 7.0-9.5%) were randomised to double-blind oral semaglutide (2.5, 5, 10, 20 or 40 mg once-daily) or PBO; or open-label subcutaneous (s.c.) semaglutide (1.0 mg once-weekly). A mixed model for repeated measurements was used to analyse the primary endpoint, change in HbA_{1c} from baseline to week 26, and other continuous endpoints. Two other arms were included to evaluate dose escalations (efficacy data not shown).

Results: Baseline characteristics were comparable across treatment arms (mean HbA_{1c}, 7.9%; duration of diabetes, 6 years; body weight [BW] 92.3 kg; BMI, 32 kg/m^2); 63% were male and 85% on metformin. Mean HbA_{1c} decreased dose-dependently with oral semaglutide (2.5 mg, 0.7%; 5 mg, 1.2%; 10 mg, 1.5%; 20 mg, 1.7%; 40 mg, 1.9%) vs 1.9% for s.c. semaglutide and 0.3% for PBO, and was statistically significant vs PBO (p=0.007 for 2.5 mg, <0.0001 for other doses). Reductions in fasting plasma glucose were greater with oral (1.0-2.8 mmol/L; p=0.008 for 2.5 mg, <0.0001 for other doses) and s.c. (3.1 mmol/L) semaglutide vs PBO (0.1 mmol/L). The proportion of patients achieving HbA_{1c} <7% was higher for oral (44-90%; dose-dependent) and s.c. (93%) semaglutide vs PBO (28%). Reductions in BW were greater with oral (2.1-6.9 kg) and s.c. (6.4 kg) semaglutide vs PBO (1.2 kg); with oral semaglutide they were dose-dependent and statistically significant vs PBO for doses ≥10 mg (p<0.0001). The proportion of patients achieving ≥5% weight loss was higher for oral (21-71%) and s.c. (66%) semaglutide vs PBO (13%). Change in mean heart rate ranged from -1.7 to +3.0 beats/minute (bpm) with oral semaglutide, vs +2.6 bpm with s.c. semaglutide and -4.0 bpm with PBO. Adverse events (AEs) were reported by 63-86% patients on oral semaglutide (trend for dose-dependency) vs 81% on s.c. semaglutide and 68% on PBO. Mild/moderate gastrointestinal (GI) AEs were more common for oral semaglutide (dose-dependent) and s.c. semaglutide than for PBO. The proportions of patients with GI events with oral and s.c. semaglutide and placebo were, respectively: nausea 13-37%, 32%, 1%; vomiting 6-24%, 9%, 4%; diarrhoea 7-23%, 15%, 10%. Discontinuation due to AEs was more frequent with oral and s.c. semaglutide vs PBO, appeared dose-dependent and was mostly due to GI events. Pancreatitis was confirmed in 3 patients (1 with s.c. and 2 with oral semaglutide [20 and 40 mg]); none were serious AEs and all were mild/moderate in severity. Lipase levels (ratio to baseline) increased with oral (1.09-1.55) and s.c. (1.36) semaglutide vs PBO (0.99).

Conclusion: Once-daily oral semaglutide improved glycaemic control and BW in T2D. Oral semaglutide had no new safety or tolerability findings compared with once-weekly injectable semaglutide.

Clinical Trial Registration Nubmer: NCT01923181
Supported by: Novo Nordisk A/S

Disclosure: **M. Davies:** Employment/Consultancy; Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca and Janssen. Grants; Novo Nordisk, Sanofi-Aventis and Lilly. Honorarium; Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca and Janssen. Lecture/other fees; Novo Nordisk, Sanofi-Aventis, Lilly, Merck Sharp & Dohme, Boehringer Ingelheim, AstraZeneca, Janssen, Mitsubishi Tanabe Pharma Corporation and Takeda Pharmaceuticals International Inc.

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Efficacy and tolerability of ITCA 650 versus sitagliptin in uncontrolled type 2 diabetes patients on metformin monotherapy: results of the FREEDOM-2 study

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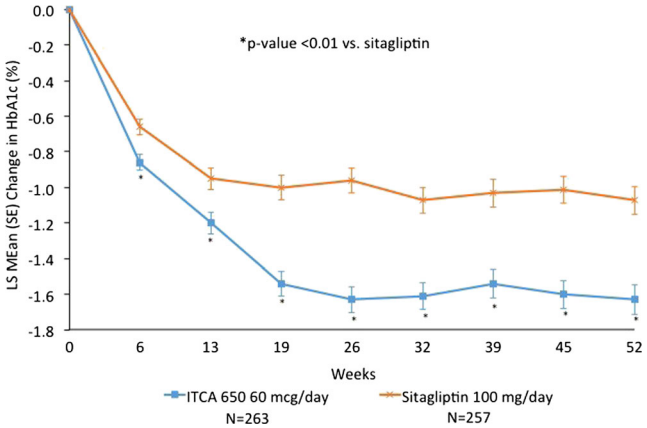
Background and aims: ITCA 650 can deliver continuous s. c. exenatide for up to 12 months from a sub-dermal placement of a small match-stick sized osmotic mini-pump. The objective of this 52-week, randomized, double-blind, double-dummy, active-controlled study was to compare ITCA 650 to sitagliptin for reducing HbA1c in patients with type 2 diabetes (T2DM).

Materials and methods: FREEDOM-2 randomized 535 T2DM patients on metformin ≥1500 mg/d with HbA1c ≥7.5 to ≤10.5% to ITCA 650 60 mcg/day or sitagliptin 100 mg (SITA) for 52 weeks. Change in HbA1c, composite of HbA1c plus weight reduction, change in body weight, and % achieving HbA1c <7% at week 52 were tested.

Results: Mean baseline characteristics were similar: 55 yrs; HbA1c 8.6%; BMI 32.6 kg/m2, T2DM duration 8.3 years. At Week 52, least square (LS) mean reduction in HbA1c from baseline was -1.5% with ITCA 650 vs -0.8% with SITA (LS mean difference: -0.71, p0.5% and body weight ≥2 kg were achieved by 61% with ITCA 650 and 28% with SITA (OR = 3.6, p<0.001). Body weight was reduced -4.0 kg with ITCA 650 vs -

1.3 kg with SITA (p<0.001). HbA1c <7% was achieved in 61% with ITCA 650 vs 42% with SITA (OR = 3, p<0.001). Rescue therapy was required in 15% with ITCA 650 and 35% with SITA. ITCA 650 had more gastrointestinal (GI) adverse events that were mostly transient. Minor hypoglycemia occurred in 4.2% with ITCA 650 vs 1.9% with SITA.
Conclusion: Continuous s. c. delivery of exenatide with ITCA 650 was statistically superior to SITA with sustained reductions in HbA1c and body weight over 52 weeks, with a major proportion achieving HbA1c <7% and the composite HbA1c/body weight loss endpoint.



Clinical Trial Registration Number: NCT01455870
Supported by: Intarcia Therapeutics, Inc.
Disclosure: **M.A. Baron:** Employment/Consultancy; Intarcia Therapeutics.

OP 26 Starving the fatty liver

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Hepatic fat content decreases with 3 weeks of intermittent fasting in obese patients with and without type 2 diabetes

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Background and aims: Two weeks of intermittent fasting increases insulin sensitivity in young healthy males. It was hypothesized that this was due to oscillations in energy stores induced by repeated periods of 24 hr. fasting. Intrahepatic fat accumulation is associated with liver insulin resistance and a decreased ability to suppress gluconeogenesis. We hypothesized that intermittent fasting with maintained body weight in obese patients with insulin resistance would increase whole body insulin sensitivity and decrease intrahepatic fat content.
Materials and methods: Male patients with T2DM (n=8) and obese controls (CON; n=8) (BMI: 32.2 ± 1.0 and 32.5 ± 0.8 kg/m2; age: 59 ± 2 and 53 ± 2 yrs., respectively) underwent 3 weeks of intermittent fasting (complete fasting every other day) corresponding to 10 days of caloric abstinence. Body weight was maintained by consumption of double amounts of food on non-fasting days. Insulin sensitivity (M-value by euglycemic, hyperinsulinaemic clamps (80mU/min/m2)) hepatic fat content (by Proton Magnetic Resonance Spectroscopy) and body composition (by Dual Energy X-ray Absorptiometry) was determined before and after the intervention. Twenty four hour blood glucose was measured for one week before and throughout the entire intervention using Continuous Glucose Monitoring (CGM) to assess changes in glucose homeostasis and to ensure adherence. For statistical comparison of results before and after the intervention, paired t-test and one way ANOVA for repeated measures was applied.
Results: Intrahepatic fat content decreased (P=0.021) with intermittent fasting (T2DM: 9.9 ± 2.6 to 7.9 ± 2.7%; CON: 8.9 ± 2.2 to 6.7 ± 1.8%), but whole body insulin sensitivity was not changed (M-value in T2DM and CON was 5.2 ± 0.7 and 6.3 ± 0.4 before and 4.8 ± 0.7 and 6.5 ± 0.5 mg/kg/min after the intervention, respectively). Fasting plasma glucose concentrations [Glucose] did not change (T2DM: 9.3 ± 0.8 to 9.3 ± 0.9 mmol/l; CON: 5.8 ± 0.2 to 6.0 ± 0.2 mmol/l). HbA1c was unchanged (T2DM: 52.5 ± 2.7 to 52.6 ± 3.3 mmol/mol; CON: 37.3 ± 1.0 to 37.1 ± 1.0 mmol/mol). Compliance to the intervention was good as judged by only a small decrease in BMI after the intermittent fasting (T2DM: -0.4±0.3; CON: -0.1±0.1 kg/m2 (P=<0.001)), unchanged body fat % (T2DM: 35.8 ± 1.5 to 35.5 ± 1.5%; CON: 34.7 ± 2.1 to 34.4 ± 2.3%) and by stable [Glucose] on fasting days and expected excursions on non-fasting days (CGM data not shown).
Conclusion: 3 weeks of intermittent fasting with maintained body weight elicits a marked decrease in hepatic fat content. Whole body insulin sensitivity did not change with intermittent fasting in this study. We speculate that frequent oscillations in hepatic and muscle glycogen stores support a mechanistic explanation for wholesome effects on metabolism in insulin resistant individuals.
Clinical Trial Registration Number: NCT02420054
Supported by: the Danish Council of Independent Research
Disclosure: **A. Ingersen:** None.

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Chronic intranasal insulin does not affect hepatic lipid content in healthy male subjects: a randomised, double-blind, placebo-controlled trial

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Background and aims: Hepatic steatosis is common in obesity and the insulin resistant state and represents the hepatic manifestation of the metabolic syndrome. Non-alcoholic liver disease (NAFLD) results from a net retention of lipids in the liver, thus a pivotal mechanism for the liver to prevent steatosis is to increase the secretion of triglycerides (TG). Rodent TG flux studies from our lab demonstrate that brain insulin signaling increases liver TG secretion, which results in a reduction in hepatic TG content. Similarly, acute intranasal insulin administration, a method to preferentially deliver neuropeptides to the CNS, has been shown by others to reduce hepatic lipid content in healthy volunteers as assessed by 1H-magnetic resonance spectroscopy (1H-MRS).
Materials and methods: Based on these findings we tested in this feasibility study, designed as a randomized, double-blind, placebo-controlled trial (RDBPC), whether chronic intranasal insulin (40 IU of recombinant human insulin; 4x daily) administered over 28 days reduces hepatic TG content in a sustained manner in healthy male subjects (n=20) with a BMI at the upper normal limit (BMI = 25 kg/m2 for both groups). Hepatic lipid content and intramyocellular lipids in the soleus muscle were assessed non-invasively using 1H-MRS.
Results: The baseline hepatic lipid content was 4.15 ± 2.5% water signal for the intranasal insulin group and 2.05 ± 0.8% water signal (p=0.43) for the placebo group (insulin dilution buffer). Intranasal insulin treated subjects showed no significant % change to baseline in hepatic lipid content compared to controls (2.2 ± 11% vs. -5 ± 16%; p=0.7). Intramyocellular lipid content in the soleus muscle was also unchanged after chronic intranasal insulin treatment (12 ± 14% vs. -0.1 ± 8%; p=0.48).
Conclusion: To our knowledge this is the first RDBPC trial using intranasal insulin delivery in humans. Here we demonstrate that despite the recently reported acute effects of brain insulin on hepatic TG content, chronic insulin delivery to the CNS using an intranasal route of administration does not reduce steatosis in healthy male subjects. In conclusion, while the brain remains a promising drug-target to modulate hepatic TG content, future efforts in developing an effective NAFLD therapy should concentrate on improved intranasal insulin formulations that cross the blood brain barrier more effectively and target certain brain regions more specifically.
Clinical Trial Registration Number: NCT02164032
Disclosure: **T. Scherer:** None.

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The interplay of fatty liver with fatty pancreas accenuates local islet inflammation in humans

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Background and aims: The role of ectopic fat accumulation for diabetes, and the influence of perivascular fat on cardiovascular and kidney diseases gained increasing attention the last years. However, the origin, the amount and the role of pancreatic fat for beta-cell (dys)function is not clarified at all. Previously we found in 200 subjects that increased pancreatic fat content (up to 9% of the gland) was associated with impaired insulin secretion in patients with IGT. Aim of this ex vivo study was to characterize pancreatic adipose tissue in humans. Since we have evidence that the fatty liver negatively impacts fat depots of several other organs, e.g. the kidney, we analyzed the inflammatory potential of pancreatic preadipocytes versus differentiated adipocytes under the influence of neighboring islets and the hepatokine fetuin-A.
Materials and methods: In 30 human pancreatic resections immune cell infiltration was determined by CD68 immunostaining. Human pancreatic preadipocytes (PPAC) were isolated from 9 patients, expanded in vitro and differentiated into pancreatic adipocytes (PAC). To mimic the crosstalk between the fatty liver and the "fatty pancreas", PPAC and PAC were cocultured with isolated human islets for 24 h in the presence of 600 μg/ml human fetuin-A and 60 μmol/L palmitate. Cellular mRNA levels and released cytokines, adhesion molecules, angiogenic and pro-inflammatory factors in PPAC vs. PAC were quantified by real-time PCR analysis, ELISA or Luminex, respectively.
Results: Immunohistochemistry revealed that PAC, displaying oil droplets and adiponectin, infiltrated the exocrine tissue. Fat cells were in tight contact with microvessels and many CD68+ monocytes/macrophages were found between fat cells. CD68+ staining was increased in islets neighbouring adipocytes. In vitro, both PAC and PPAC expressed and secreted IL-6, IL-8, MCP-1, ICAM-1, HGF and VEGF. Fetuin-A and palmitate differentially stimulated IL-6, IL-8 and MCP-1 release from PAC and PPAC. Coculture with islets further increased IL-6 and MCP-1 secretion in PPAC but not in PAC. In addition, the angiogenic factor HGF was strongly decreased by fetuin-A in both PPAC and PAC while VEGF was increased. Of note, human islets produce and release high amounts of VEGF.
Conclusion: The secretome of PAC and PPAC which is stimulated by the diabetogenic milieu (fetuin-A/palmitate) and modified by the islets contributes to immune cell infiltration, local inflammation and angiogenesis. These observations suggest that pancreatic fat represents a pathogenic factor accelerating diabetes progression.
Supported by: German Federal Ministry of Education and Research
Disclosure: **D.I. Siegel-Axel:** None.

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Exposure to hypoxia markedly ameliorates hyperglycaemia and hepatic steatosis without concomitant insulin sensitisation in obese mice

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Background and aims: The prevalence of obesity and type 2 diabetes is reduced in mountain dwellers, which may in part be due to lower partial oxygen pressure at high altitude. Although at least some preceding studies suggested that adaptation to hypoxia could have beneficial effects on metabolic traits, it has never been dissected, whether the improvement went beyond the indirect consequences of the well-known appetite-reducing effect of hypoxia.
Materials and methods: Male C57BL/6J mice received high fat diet (60%) to induce obesity and derangements of glucose and lipid metabolism. At the same time they were maintained in a hypoxic environment comparable to approximately 5.500m above sea level for three months (10% O2 in normobaric breathing air). To account for the reduction in calorie consumption under these conditions (-22%), the control group was fed restrictedly so that food intake, body weight, and body composition (lean/fat mass) were as in the hypoxia-treated mice. In a second experiment, mice were pre-fattened for three months with high fat diet, before subjected to the same hypoxia protocol. All metabolic tests and measurements were performed 24h after removal from hypoxia in order to portray

durable metabolic adaptations to hypoxia rather than acute responses to oxygen deficiency.
Results: Hypoxia caused major metabolic improvement indirectly via weight loss (data not shown in the abstract), but also retained distinct efficacy when comparison was made to weight-matched controls. Benefits obtained at weight neutrality included marked ameliorations of hyperglycaemia (mg/dl: control, 148±5, vs hypoxia, 113±4, p<0.001), glucose intolerance (AUC, min*g/dl: control, 33.7±1.4, vs hypoxia, 27.6±0.8, p<0.001) and hepatic steatosis (liver triglycerides, mg/g: control, 41.6±3.9, vs hypoxia, 24.4±4.0, p=0.008). The improvement was even more impressive in pre-fattened mice with more severe obesity and hyperglycaemia (basal glucose, mg/dl: control, 165±8, vs hypoxia, 104±4, p<0.001; AUC, min*g/dl: control, 43.7±1.2, vs hypoxia, 29.6 ±1.0, p<0.001). Further examination suggested that hypoxia did not act via "classic" mechanisms of metabolic improvement, since no changes were observed in plasma insulin (nmol/l: control, 0.25±0.03, vs hypoxia, 0.24±0.04, ns), plasma adipokines (leptin, μg/l: control, 19.4±2.6, vs hypoxia, 20.8±3.6, ns; adiponectin, mg/l: control, 8.3±0.5, vs hypoxia, 8.8±0.3, ns), plasma lipids (triglycerides, mg/l: control, 0.74±0.03, vs hypoxia, 0.85±0.09, ns; free fatty acids, mmol/l: control, 0.74±0.01, vs hypoxia, 0.74±0.02, ns), and insulin sensitivity (glycaemia 30min after insulin injection in an insulin tolerance test, mg/dl: control, 84±7, vs hypoxia, 87±5, ns).
Conclusion: The present study shows for the first time that hypoxia can profoundly protect from obesity-induced hyperglycaemia and liver steatosis independently of changes in calorie consumption and body weight. Absence of concomitant changes in insulin, lipids, adipokines or insulin sensitivity hints at the involvement of a yet undiscovered mechanism, via which the body can counter obesity-associated metabolic derangements. Hence, unravelling of the mechanisms addressed by hypoxia bares a unique chance to find novel approaches for prevention and therapeutic intervention in common metabolic disease.
Disclosure: **C. Fürnsinn:** None.

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Involvement of PRDX6 in the pathogenesis of obesity and nonalcoholic fatty liver disease

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Background and aims: Obesity and fatty liver disease are chronic disorders associated with an excess in reactive oxygen species (ROS) production. Increasing evidences suggest that the antioxidant enzyme peroxiredoxin 6 (PRDX6) is involved in atherosclerosis pathogenesis and type 2 diabetes mellitus; however the role of this protein in obesity condition and fatty liver disease is still unclear.
Materials and methods: We compared wild-type (WT) and PRDX6 knockout(-/-) mice following a 24-week high-fat-diet (HFD). Metabolic parameters, such as carbon dioxide production (VCO2), oxygen consumption (VO2) and respiratory exchange ratio (RER) were determined using metabolic cages. Intraperitoneal insulin tolerance test (IPITT) and intraperitoneal glucose tolerance test (IPGTT) were used to evaluate insulin and glucose tolerance, respectively. In liver and pancreas tissue sections we performed histological analysis. Gene expression of enzymes involved in lipid and glucose metabolism were analyzed by real time polymerase chain reaction (RT-PCR).
Results: When underwent to HFD, PRDX6 -/- mice showed weight gain as well as higher food and drink intake as compared to controls (p<0.05). In addition, we observed a VO2 consumption and VCO2 production decrease in PRDX6 -/- mice and a RER increase compared to WT mice (p<0.05), indicating a less fat oxidation in mice lacking of this enzyme. PRDX6-/- mice developed a significant glucose intolerance compared to control animals and this condition was correlated with a reduced insulin secretion in response to glucose. This metabolic phenotype typical of PRDX6-/- mice was accompanied by a lower number and/or size reduction of Langerhans islet. In PRDX6-/- mice, β-cells insulin secretion failure was not able to suppress adipose tissue lipolysis and this was leading in a higher free fatty acids (FFAs) serum concentration. As consequence, in liver, PRDX6-/- mice exhibited an increased VLDL assembly, secretion and activation of gluconeogenic pathway with more glucose release compared to WT mice. In addition, as consequences of this hepatic lipid overload, in PRDX6-/- mice a marked activation of kupferr cells, macrophages and hepatocytes cells and the upregulation of soluble pro-inflammatory cytokines, played a pivotal role in the induction of NASH.
Conclusion: In conclusion, our data provides evidence that PRDX6 have a protective role in the development of nonalcoholic fatty liver disease and obesity-related metabolic disorder. Therefore, by modulating pathways associated to this antioxidant enzyme may be useful to develop novel therapeutic strategy against metabolic diseases.
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Disclosure: **R. Arriga:** None.

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Adipose tissue macrophages induce hepatic macrophage accumulation during the development of non-alcoholic steatohepatitis

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Background and aims: Obesity is a key risk factor for type 2 diabetes. This increased risk has been mainly attributed to the expansion of visceral adipose tissue (vAT) associated with increased pro-inflammatory mediators. An important driver of vAT inflammation is the accumulation of adipose tissue macrophages (ATMs). A causal link between ATMs and insulin resistance has been reported. Moreover, a relationship between hepatic inflammation and insulin resistance has been described. In the current study, we aim to study a role of ATMs in hepatic inflammation during the development of non-alcoholic steatohepatitis (NASH).
Materials and methods: To specifically investigate the effects of ATMs on hepatic inflammation, vAT was transplanted to acceptor Ldlr-/- mice resulting in the following three groups: lean transplanted acceptors (LTA), obese transplanted acceptors (OTA) and ATM depleted (using clodronate liposomes) obese transplanted acceptors (DOTA). All acceptor mice received high cholesterol diet for 4 weeks before and for 8 weeks after transplantation to induce NASH. In a second experiment, the same vAT transplantations were executed with lean acceptor animals and these mice were sacrificed 2 weeks post AT transplantation. We assessed hepatic inflammation with the use of flow cytometry, gene expression analysis, histology, ELISA and plasma lipid assays.
Results: Donor vAT from high fat diet fed mice displayed increased inflammatory CD11c+ macrophages, which was reversed by clodronate liposomes without affecting the inflammatory cytokine profile. LTA, OTA and DOTA mice all had comparable plasma cholesterol and circulating neutrophil levels. Plasma triglyceride levels were slightly higher in OTA mice, which was reversed in DOTA mice suggesting an effect of ATMs on plasma triglycerides. No difference in hepatic steatosis was observed. Interestingly, hepatic macrophage content was increased in OTA mice when compared to LTA mice and this was reversed in DOTA mice. These data indicate that ATMs directly induce hepatic macrophage accumulation in the liver. A second experiment was performed to investigate the initiating effects of ATMs on hepatic inflammation in lean acceptors. OTA mice had higher circulating neutrophil levels compared to LTA mice and this was not observed in DOTA mice. Consistent with lower circulating neutrophil levels, DOTA mice had reduced hepatic gene expression of neutrophil markers (CD11b and myeloperoxidase) compared to OTA mice while macrophage markers were unchanged. Flow cytometry data

and immunohistochemistry confirmed the reduction of hepatic neutrophils in DOTA mice. Donor AT derived from obese mice had higher expression levels of macrophage colony-stimulating factor when compared to lean or ATM depleted mice, suggesting that ATMs may increase recruitment of neutrophils from the bone marrow. Indeed, flow cytometry data displayed less granulocyte-macrophage progenitor cells present in bone marrow from DOTA mice compared to OTA mice.

Conclusion: ATMs induce hepatic macrophage accumulation in established NASH, possibly as a consequence of hepatic neutrophil infiltration in early NASH development. Therefore, the link between ATMs and insulin resistance might be mediated, at least in part, by hepatic inflammation.

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Disclosure: **M. Bijnen:** Grants; NHS 2013T143.

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Enhanced dermal nerve regeneration despite pronounced nerve fibre loss in painful and painless diabetic polyneuropathy

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Background and aims: The determinants and mechanisms contributing to the development of diabetic sensorimotor polyneuropathy (DSPN) as a painful or painless clinical entity remain unclear. We examined the degree of cutaneous nerve fibre loss and regeneration in patients with newly diagnosed diabetes mellitus (N-DM) and those with painful DSPN (DSPN+p) or painless DSPN (DSPN-p). DSPN was diagnosed using modified Toronto Consensus (2011) criteria.

Materials and methods: Skin biopsies from the distal lateral calf were obtained from 36 patients with DSPN-p, 25 patients with DSPN+p (DSPN-p/DSPN+p: age [mean±SD]: 69.9±8.2/67.8±8.6 years; BMI: 28.8±4.1/30.6±4.8 kg/m^2; diabetes duration: 16.5±13.8/21.0±14.2 years; HbA1c: 7.4±1.0/7.8±1.2%), 21 patients with N-DM (duration: 0.6±0.3 years), and 21 control subjects (diabetes/controls: age: 42.2±11.2/40.0 ±15.0 years; BMI: 27.2±5.0/27.8±8.2 kg/m^2; HbA1c: 6.2±1.0/5.2 ±0.3%). Double immunofluorescence staining for Protein Gene Product (PGP)9.5 (pan-neuronal marker) and growth-associated protein (GAP)-43 (nerve regeneration marker) was applied to quantify intraepidermal nerve fibre density (IENFD) and dermal nerve fibre length (DNFL).

Results: After adjustment for age and sex, patients with DSPN+p showed reduced IENFD compared to those with DSPN-p (GAP-43: 2.18±2.71 vs 3.88±3.23 fibres/mm; P=0.043; PGP9.5: 2.27±2.90 vs 4.10±3.46 fibres/mm; P=0.041), while DNFL did not differ between the groups (GAP-43: 4.03±2.44 vs 4.97±1.89 μm/mm^2; P=0.160; PGP9.5: 3.70±2.53 vs 4.71 ±2.05 μm/mm^2; P=0.149). Both DSPN groups showed markedly diminished IENFD and DNFL when compared to the control and N-DM groups (GAP-43: IENFD: 8.41±2.57 and 7.32±3.46 fibres/mm; DNFL: 6.91 ±2.24 and 6.88±1.88 μm/mm^2; PGP9.5: IENFD: 9.22±2.97 and 7.94 ±3.85 fibres/mm; DNFL: 7.26±2.28 and 7.21±1.92 μm/mm^2) (P<0.05 vs DSPN-p and DSPN+p). Mean dermal GAP/PGP ratio was higher in patients with DSPN (1.20±0.38) than controls (0.95±0.06) and those with N-DM (0.95±0.05) (P<0.05), while no difference was noted between the groups with DSPN-p and DSPN+p. Linear regression analyses showed a distinct inverse association between the GAP/PGP ratio and IENFD (β=-0.496; P<0.001).

Conclusion: Intraepidermal nerve fibre loss is more pronounced in painful DSPN than in painless DSPN, while dermal nerve regeneration is enhanced in both entities. Thus, susceptibility to nerve repair in DSPN is preserved despite progressive fibre loss, albeit independent of neuropathic pain.

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Disclosure: **G. Bönhof:** None.

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Painful polyneuropathy is common but largely undiagnosed in subjects with and without diabetes participating in a nationwide educational initiative (PROTECT Study)

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Background and aims: Painful distal sensory polyneuropathy (DSPN) is associated with considerable morbidity and an increased risk of mortality, but neuropathy screening is underutilised in primary care practice. We conducted a nationwide educational initiative to determine the prevalence and risk factors of diagnosed and previously undiagnosed painful and painless polyneuropathy.
Materials and methods: Among 1,589 individuals participating in the initiative, 643 had no diabetes by history (ND) (age [mean±SD]: 67.7 ±11.8 years, 39% male), 113 had type 1 diabetes (age: 59.4±15.6 years, 47% male), and 833 had type 2 diabetes (age: 69.7±9.7 years, 51% male). DSPN was assessed by history and foot examination including pressure (10 g monofilament), temperature (tip therm instrument), and vibration (tuning fork) perception and was classified as possible, probable, and severe if 1 of 3, 2 of 3, and 3 of 3 tests were abnormal. Painful DSPN was defined as the presence of DSPN with pain and/or burning at rest in the feet, while painless DSPN was defined as the presence of paraesthesias, numbness, or absence of symptoms. Foot pulses, HbA1c (point-of-care testing), and symptom questionnaires were determined in subsets of participants.
Results: DSPN was detected in 49.3% (95% CI: 46.0-52.6) of ND, 43.5% (35.4-51.9) of type 1, and 52.9% (50.0-55.9) of type 2 diabetes subjects. The percentages of subjects with painful DSPN among those with DSPN were 66.7% (60.1-72.8) in ND, 61.5% (43.6-77.4) in type 1, and 61.8% (56.2-67.2) in type 2 diabetes subjects. Among participants with painful polyneuropathy, the latter was reported as previously undiagnosed by 75.8% (67.5-82.9) of ND, 28.5% (16.6-64.5) of type 1, and 60.2% (52.5-67.4) of type 2 diabetes participants. These rates were around 20% higher in subjects with painless DSPN. Apart from age, painful DSPN was associated with higher BMI in participants with type 2 diabetes (r=0.242; P=0.001). Among ND participants, 30.1% (26.0-34.5) had HbA1c values of 5.7-6.4%, while 4.1% (2.5-6.4) showed HbA1c levels ≥6.5%. Painful DSPN was associated with HbA1c in type 2 diabetes subjects (r=0.121; P=0.040) and in ND individuals who had HbA1c levels ≥6.5% (r=0.837; P<0.001).
Conclusion: Almost half of subjects with and without diabetes participating in an educational initiative had DSPN which was painful but previously undiagnosed in almost two thirds each. Since the risk of diabetes was increased in one third of participants without known diabetes, effective strategies to reveal both undetected diabetes and neuropathy should be implemented.
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Disclosure: **D. Ziegler:** Lecture/other fees; Wörwag Pharma.

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The relationship between vitamin D and thalamic neurochemistry in painful diabetic neuropathy

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Background and aims: We have previously demonstrated lower vitamin D levels in painful compared to painless diabetic peripheral neuropathy (DPN). However, the mechanistic basis for this remains unclear. Recent mouse studies have reported an association between vitamin D deficiency and prominent changes in behavior and brain neurochemistry. Thalamic Glutamate / glutamine (Glx) was also found to be lower in DPN compared to non- neuropathic diabetic subjects. The aim of this study was therefore to examine any potential relationship between vitamin D levels, neuropathic pain status and thalamic brain neurochemistry.
Materials and methods: Forty-four patients with type 2 diabetes (T2DM) (14 Painful-DPN, 15 Painless-DPN and 15 No-DPN) and 15 non-diabetic healthy volunteers (HV) were examined by detailed clinical and neurophysiological assessment to determine their neuropathy composite score [NIS(LL)+7 and Douleur Neuropathique 4 score(DN4)]. 25(OH)-Vitamin D was measured between May-September and all subjects had seasonal sunlight exposure and daily activity measured. Single-voxel proton Magnetic Resonance Spectroscopy (H-MRS) was used at 3T to yield thalamic Glx resonance information relative to that of water in each subject (MEGAPRESS; echo time=68ms).
Results: There was no significant difference in age between the study groups (painless-DPN, 59(SD10); painful-DPN, 60(7); no-DPN 57(7) and HV 55(10) years; ANOVA p=0.39). Subjects with painful-DPN (35.8nmol/l) had the lowest vitamin D levels (painful DPN, 35.8(17.1); painless-DPN, 56.1(28.7); No-DPN, 46.8(19.2) and HV, 58.5(27.8); ANOVA p=0.039) and Glx levels 1.27(0.23), 1.37(0.14), 1.34(0.21), 1.45(0.36); ANOVA p=0.28). There was a significant correlation between vitamin D and Glx in patients with painful-DPN only (r=0.69, p=0.03).
Conclusion: We have demonstrated a significant correlation between vitamin D levels and thalamic Glx in patients with painful DPN. This is the first study to demonstrate an association between vitamin D deficiency and neurochemical changes in the brains of patients with painful DPN.
Supported by: Wellcome Trust
Disclosure: **I. Wilkinson:** None.

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External muscle stimulation differentiates circulating haematopoietic stem cells in diabetes patients

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Background and aims: External electric muscle stimulation (EMS) has been shown to reduce pain resulting from diabetic neuropathy (DN), a vascular complication of diabetes. It was hypothesized that EMS-treatment did so by affecting circulating CD34+ hematopoietic stem cells (HSC), which can participate in vessel repair by differentiating into endothelial progenitor cells (EPC).
Materials and methods: 28 DN patients and 6 healthy sham-controls were treated for three 60-minutes lasting sessions within 10 days. 24 patients were evaluated for neuropathic symptoms using the Diabetic Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) tools. The frequency of HSC and their expression of surface proteins required for vessel wall binding, homing and markers of differentiation were identified by flow cytometry.
Results: Median symptom severity was reduced from 8 to 6 (p=0.001) as measured by NSS and from 5.5 to 5 (p=0.027) as measured by NDS. The one hour treatment also caused an immediate decrease in HSC frequency in the blood in participants with DN (a median decrease of -20%, p<0.001). HSC remaining in blood of 9 EMS-treated DN patients showed significant increases of surface molecules required for binding to vessel walls (JAM-A and CD31), homing toward hypoxic tissue (CXCR4) and differentiation into endothelial cells (CD31). The proportion of HSC expressing VEGFR2, defining the cells as EPC, was increased by a median of 36% (p=0.011). Increased frequency of HCS expressing VEGFR2 was also observed in the 6 healthy sham-controls (34% increase, p=0.028) after EMS treatment, but not after sham-treatment.
Conclusion: EMS-treatment alleviated symptoms of DN, increased differentiation of HCS and caused a reduction of HSCs in circulation. We hypothesize that EMS-induced differentiation of HSC promotes tissue regeneration which contributes to the improved symptoms of painful DN. To our knowledge, this is the first protocol described for in vivo differentiation of HSC in diabetes patients.

	Baseline		median change	p	n
	Median	IQR			
Age (years)	**70.5**	15			
Female / Male	**12 / 16**				
Diabetes Type 1 / 2	**4 / 24**				
Body-Mass-Index (kg/m^2)	**31.9**	7.1			
HbA1c (%; mmol/mol)	**7.1; 54**	1.5; 16.3			
Frequency HSC of Lymphocytes (%)	**0.051**	0.029	-20%	**0.001**	27
Frequency of HSC expressing VEGFR2 (%)	**7.5**	12.3	36%	**0.011**	9
Frequency of HSC expressing JAMA (%)	**5.57**	11.26	37%	**0.021**	9
Frequency of HSC expressing CD31 (%)	**4.58**	11.15	25%	**0.021**	9
Frequency of HSC expressing CXCR4 (%)	**15.2**	15.0	15%	**0.028**	9

Median change: *Median of individual changes after 60 minutes of EMS treatment*

Disclosure: **I. Spanidis:** None.

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Disruption of white matter integrity in sensory and motor pathways in diabetic peripheral neuropathy: evidence from diffusion tensor imaging

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Background and aims: There is now emerging evidence that diabetic peripheral neuropathy (DPN) is associated with both structural change and functional reorganization of the somatosensory cortex (SSC). We have previously demonstrated spinal cord atrophy and a reduction in grey matter volume in the primary SSC in DSPN but the integrity of the white matter tracks between these two anatomical areas has not been studied. In this study we have applied Magnetic Resonance (MR) Diffusion Tensor Imaging (DTI) to estimate cerebral water diffusion directionality which can be used to infer intracranial axonal tract architecture.
Materials and methods: 11 type 1 diabetes patients (8 males, mean age 54.6+13.8 years) with DPN and 13 healthy volunteers (7 males, age 49.3+15.0 years) without diabetes underwent detailed clinical and neurophysiological assessments (NIS-LL+7 - confirming DPN). All subjects had DTI on MR at 3T (Achieva, Philips Healthcare) using a spin-echo, EPI-based 32-direction technique. Group mean fractional anisotropy maps were generated and compared.
Results: We found significantly lower fractional anisotropy in the primary SSC (MNI coordinates: -32,-30,46), supramarginal gyrus (-28,-40,39) and corticospinal tract (-24,-17,36) in DSPN compared to the healthy volunteer group ($p < 0.05$). There were no regions where the fractional anisotropy was higher in the DPN compared to healthy volunteer group.
Conclusion: This study for the first time has shown alterations in white matter water diffusion fractional anisotropy in patients with DPN. This finding implies disruption of white matter tract integrity of the sensory and motor pathways in the brain in DPN. Changes in the degree of white matter structure, as indexed by a decline in fractional anisotropy, might provide a bio-mechanical understanding of CNS involvement in DPN. Larger studies are now required with including a diabetic control group without DPN.
Supported by: Juvenile Diabetes Research Foundation
Disclosre: **D. Selvarajah:** None.

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Neuropathy in patients with type 2 diabetes does not contribute to the development of microvascular dysfunction

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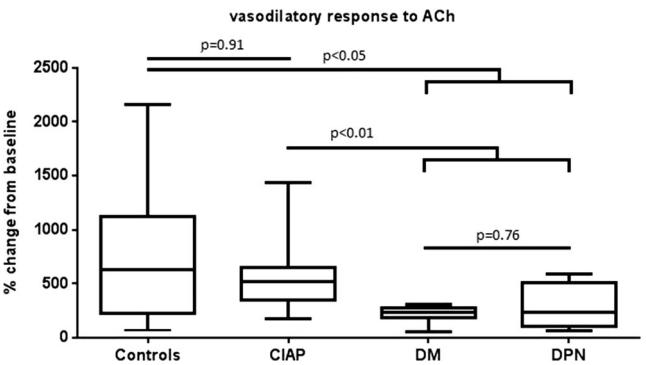
Background and aims: In type 2 diabetes (T2DM) more severe microvascular endothelial dysfunction can be demonstrated in patients with an accompanying neuropathy. A bidirectional causal relationship between microvascular endothelial dysfunction and neuropathy has been proposed, but most studies addressing this hypothesis are hampered due to confounding by diabetes-related factors influencing both microvascular and neural function. This study aims to investigate whether the relationship between neuropathy and microvascular endothelial dysfunction is independent of diabetes-related factors. For this purpose, microvascular function of T2DM patients with and without neuropathy was compared to microvascular function of patients with chronic idiopathic axonal polyneuropathy (CIAP), a polyneuropathy clinically comparable to diabetic neuropathy but not due to diabetes and of unknown aetiology.
Materials and methods: Cross-sectional information was collected from four groups; 16 healthy controls, 16 patients with CIAP, 11 T2DM patients without neuropathy (DM) and 15 T2DM patients with neuropathy (DPN). Neuropathy severity was assessed with skin biopsy and nerve conduction studies for small and large fibre involvement respectively. Microvascular endothelium-dependent and -independent vasodilatation was measured by laser Doppler fluxmetry in combination with iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP). Data were log-transformed and analysed by one-way ANOVA and post-hoc Fisher's LSD test.
Results: The characteristics showed no relevant differences in age. We observed differences in BMI (controls (23.9 ± 2.3 kg/m^2); CIAP (26.5 ± 3.2 kg/m^2); DM (28.2 ± 2.2 kg/m^2); DPN (28.2 ± 3.5 kg/m^2)) and HbA1c (controls (36.0 ± 2.0 mmol/mol); CIAP (37.1 ± 3.5 mmol/mol); DM (54.1 ± 11.8 mmol/mol) DPN (62.5 ± 18.2 mmol/mol)). Diabetes duration was equal in the two diabetic groups. Compared to healthy controls, CIAP and DPN patients demonstrated a similar decrease in intra-epidermal nerve fibre density and a similar reduction in sural nerve sensory nerve action potential. The vasodilatory response to ACh was similar in CIAP patients (630.8[69.6 - 2155.1]%), but lower in the diabetic patients with (238.3[64.9 - 805.3]%) and without neuropathy (233.6[53.0 - 305.2]%) compared to healthy controls (687[110.5 - 2155.1]%). No significant difference was found between the diabetics with vs without a neuropathy. The vasodilator response to SNP did not show significant differences among the groups (p=0.090).
Conclusion: In this study, endothelium-dependent vasodilatation was reduced in T2DM patients regardless of the presence of neuropathy. In addition, endothelium-dependent and -independent vasodilatation was normal in CIAP patients. These data suggest that neuropathy does not contribute to impaired microvascular endothelium-dependent vasodilatation in type 2 diabetes.



Supported by: Grants from STW and Biogen
Disclosure: **A.L. Emanuel:** Grants; Stichting Technologische Wetenschappen (STW) grant 10730, Biogen Netherlands B.V.

OP 28 Novel mechanisms determining beta cell dysfunction

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E2F1 controls pancreatic beta cell function and identity

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Background and aims: Dysfunction of pancreatic β cells, associated with a decrease in their number are responsible for diabetes development. In this regard, cell cycle regulators play key roles in the control of cell proliferation and cell fate. We previously reported the important role of the CDK4-pRb-E2F1 pathway, a key component of the cell cycle machinery, on glucose homeostasis, post-natal β-cell proliferation, mass and function. However, the molecular link between E2F1 and the control of endocrine cell fate or differentiation remains unknown.
Materials and methods: To identify the potential role of E2F1 in pancreatic β-cell differentiation, we used E2f1 +/+ and -/- mice and studied the proportion of α and β cell in these genetic backgrounds. To further understand the potential role of E2F1 in maintaining β-cell identity, we used shRNA technology in MIN6 cells, transient transfection experiments, chromatin immunoprecipitation assays and E2F1 inhibitors in vitro and in vivo. Moreover to confirm the cell-autonomous function of E2f1 in β cells, we have generated a beta-cell specific inactivation of the E2f1 gene in mice. We further used human islets to demonstrate the key role of maintaining E2F1 activity for β-cell identity.
Results: We show here that E2f1 deficiency induced a β-to-α-like cell conversion, suggesting that E2f1 could be directly involved in this process. Knocking-down E2f1 in the β-cell line Min6 induced the α-cell specific transcription factor Arx at the mRNA and protein levels. We also demonstrated that E2F1, in concert with pRb, repressed Arx in β cells at the promoter level, through an epigenetic mechanism involving CpG island methylation. These results were confirmed in a E2f1 beta cell specific knock-out mouse model where the mice present glucose intolerance associated with a decrease of insulin secretion in response to glucose. Finally, inhibiting E2F1 activity in human islets induced a β-to-α-like cell conversion, suggesting an important role for E2F1 in human islets.
Conclusion: Our data demonstrate that E2f1 could be a constitutive repressor of the α-cell specific transcription factor Arx, crucial to maintain the cellular identity of pancreatic β cells. The underlying repressive mechanisms are still under investigations but could involve a demethylation process.
Supported by: ARD, Lille 2 university, Labex, SFD, conseil régional Nord Pas de Calais
Disclosure: **X. Gromada:** None.

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The elevated expression of the ER-stress induced miR-29a in individuals with type 1 diabetes mellitus

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Background and aims: MicroRNAs are 19-25 noncoding RNA molecules which function as post-transcriptional regulators and are recognized as playing a crucial role in obesity, insulin secretion and action. Metabolically stressed human β-cells display markers of endoplasmic reticulum (ER)-stress and apoptosis. Obesity, hyperglycaemia and insulin resistance associated with a chronic inflammatory state induce such metabolic ER-stress. The expression of two specific microRNAs; miR-29a and miR-376a have been identified as being induced during ER-stress by our group. The aim of this study was to determine the expression of the ER-stress induced miR-29a and miR-376a in human subjects with type 1 and type 2 diabetes mellitus (T1DM and T2DM).
Materials and methods: 35 individuals with T1DM and 30 individuals with T2DM participated in the current study. The mean duration of diabetes in the cohort studied was 13 years. Participants were phenotyped, including performing a 75g oral glucose tolerance test with insulin and C-peptide at baseline and 30 minute intervals to clarify insulin secretory response. Absolute levels of serum miR-29a and 376a were determined using real time PCR. We also correlated expression levels with clinically relevant indices including AUC insulin, oral glucose insulin sensitivity (OGIS) and triglyceride levels. All data is presented as median ±SEM. Correlation analysis was performed using Spearman correlation coefficient.
Results: The expression of miR-29a was higher in the T1DM cohort than in the T2DM cohort (448000 [9183000-2 x 10^6] vs. 240500 [58425-485750], p=0.01). In contrast, miR-376a expression levels were not significantly higher in the T1DM cohort than in the T2DM cohort (7.9 x 10^6 [369000- 7.6 x 10^8] vs. 6.9 x 10^7 [8.8x 10^6 - 2.4 x 10^8], p=0.3). In the T1DM and the T2DM there was no significant correlation observed between miR-29a or miR-376a and markers of insulin resistance including BMI, OGIS, AUC insulin, LDL, total cholesterol, HDL , triglyceride level or CRP. There was a significant correlation between miR-29a and diastolic blood pressure in the T2DM cohort (rho=–0.4, p=0.01).
Conclusion: For the first time, we demonstrate the higher expression of the ER-stress induced miR-29a in the serum of subjects with T1DM when compared to T2DM. ER-stress is implicated in the β-cell failure associated with T2DM. However, the role of ER-stress in the propagation of T1DM remains undefined. In the β-cells of a NOD mouse model, the over expression of miR-29a promotes apoptosis by decreasing levels of the anti-apoptotic protein Mcl-1. Our findings may suggest that there is ongoing ER-stress in a T1DM cohort despite a long duration of diabetes.
Supported by: Health Professional Fellowship awarded from the Health Research Board to SB
Disclosure: **S. Bacon:** None.

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Hyperlipidaemia-induced microRNA-155 expression in pancreatic islet cells maintains glucose homeostasis by targeting MafB

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Background and aims: High fat diet-induced hyperlipidemia is associated with low-grade endotoxemia, which increases insulin secretion due to IL-6-induced glucagon-like peptide-1 (GLP-1) production. Because lipopolysaccharide (LPS) and oxidatively modified LDL upregulate microRNA-155 (miR-155) in macrophages, we aimed to study the role of miR-155 in glucose homeostasis during hyperlipidemia.
Materials and methods: Fasting glucose and LPS blood levels were determined in Mir155+/+Ldlr-/- and Mir155-/-Ldlr-/- mice fed a normal chow or a high fat diet. Immunostaining of insulin and glucagon was performed in murine islets. MiR-155 expression was quantified in laser microdissected murine islets by quantitative real-time PCR (qPCR). IL-6, GLP-1, glucagon, and insulin protein levels were determined by Luminex assay or ELISA. Gene expression in islets was studied by microarray analysis. The miRNA-induced silencing complex (RISC) was immunoprecipitated from MIN6 cells overexpressing a tagged GW182 protein after treatment with miR-155 mimics and the enrichment of genes was quantified by qPCR. MIN6 cells were transfected with miR-155 inhibitors and a luciferase reporter vector containing the Il6 promoter with or without mutations in the putative V-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB) binding sites. MIN6 cells were treated

with antisense oligonucleotides that inhibit the interaction between miR-155 and MafB or control oligonucleotides.
Results: LPS levels in serum and miR-155 expression in islets was increased in Ldlr-/- mice fed a high fat diet. In Mir155-/-Ldlr-/- mice, glucose and plasma glucagon levels were increased, whereas plasma insulin and GLP-1 levels were reduced compared with Mir155+/+Ldlr-/- mice. The α-to-β-cell ratio and the glucagon protein level were higher, whereas the insulin and GLP-1 protein content was reduced in islets from Mir155-/-Ldlr-/-mice. Insulin, GLP-1, and IL-6 signaling pathways were inhibited and several putative miR-155 targets, such as MafB, Sema5a and Stmn2, were upregulated in islets from Mir155-/-Ldlr-/- mice. Overexpression of miR-155 in MIN6 cells primarily enriched MafB mRNA in the RISC. MiR-155 suppressed glucagon expression, and upregulated proprotein convertase subtilisin/kexin type 1 (Pcsk1), insulin and GLP-1 expression in MIN6 cells. This effect of miR-155 was mediated by the targeting of MafB and the diminished MafB-mediated repression of IL-6.
Conclusion: Our data indicate that upregulation of miR-155 expression in islets during high fat diet-induced endotoxemia improves glucose homeostasis by targeting MafB, which results in increased IL-6-mediated GLP-1 production. Thus, increasing miR-155 levels in β-cell may be a promising therapeutic strategy to improve insulin secretion in patients with diabetes.
Supported by: German Federal Ministry of Education and Research
Disclosure: **M. Zhu:** None.

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Metabolic characterisation of beta cell specific IDE knock-out mice
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Background and aims: Insulin Degrading Enzyme (IDE) is a ubiquitous protein with a broad spectrum of activity against proteins such as insulin, glucagon and beta-amyloid. There are genetic associations, in human and rodents, between IDE polymorphisms and the risk to suffer type 2 diabetes mellitus (DM2). However, the physiological role of IDE on the regulation of glucose homeostasis and its potential therapeutic benefit remains largely unknown. Interestingly, there is a controversy on IDE role due to contradictory results obtained in the metabolic characterization of total IDE KO mice. In order to clarify if IDE is a possible target for DM2 and its specific role in pancreatic beta-cell function we have generated beta-cell specific IDE null mice (β-IDE-KO).
Materials and methods: We have characterized glucose and insulin metabolism in β-IDE-KO and control mice fed a standard diet (2 and 6 months old) or a high fat diet (6 months old). Body weight, blood glucose, plasma insulin and plasma glucagon levels were monitored. Glucose homeostasis and insulin secretion were assessed by intraperitoneal glucose tolerance test.
Results: Compared to control mice, β-IDE-KO old mice (6 months) showed a significant decrease in insulin secretion after a glucose challenge. These results recapitulate our observations in vitro which showed IDE-knock-down in INS1E cells lead to reduce insulin secretion after glucose overload. Surprisingly, although basal glucose and plasma insulin levels were not altered, β-IDE-KO mice suffer hiperglucagonemia. Furthermore, β-IDE-KO old mice fed a high-fat diet exhibited impaired glucose tolerance compared to control mice.
Conclusion: Whole-body glucose homeostasis is not affected by depletion of IDE in pancreatic β-cells, despite reduced plasma insulin levels. However, the effect of IDE depletion on insulin secretion impairs glucose homeostasis in diet-induced obese mice. Thus, our work has shed light on the physiological role of IDE on the regulation of glucose homeostasis.
Supported by: MINECO SAF2014-58702-C2-1-R
Disclosure: **C. Fernández-Díaz:** None.

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Deletion of beta cell specific K_{ATP} channels causes severe glucose intolerance with impaired insulin secretion and enhanced incretin secretion
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Background and aims: Pancreatic β-cell ATP-sensitive K+(KATP) channel plays an important role in insulin secretion. By studying global KATP knockout mice, we previously reported that the mice exhibited a severe defect in glucose-induced insulin secretion (GIIS) but only a slight impairment in glucose tolerance. However, no β-cell specific KATP channel knockout mice have been reported to date. In this study, we generated and investigated the β-cell specific KATP knockout (βKir6.2 KO) mice to clarify the role of the β-cell KATP channel in insulin secretion and glucose homeostasis
Materials and methods: Mice harbouring a floxed Kir6.2 allele (Kir6.2flox/+) were generated by introducing a targeting vector. Multiple crossing with Ins-Cre mice generated βKir6.2 KO mice. Kir6.2 knockout efficiency was determined by mRNA expression and electrophysiological study of isolated β-cells. Oral glucose tolerance test (OGTT), meal tolerance test and insulin tolerance test (ITT) were performed. Plasma insulin and glucose-dependent insulinotropic polypeptide (GIP) levels were measured. The effects of different anti diabetic drugs on blood glucose levels during OGTT were examined. GIIS in isolated islets was also determined
Results: In comparison with wild-type mice islets, βKir6.2 KO islets had Kir6.2 mRNA expression levels of below 10%, suggesting over 90% knockout efficiency. Washout increase of K+ conductance was observed in whole cell recordings in wild-type mice islets and addition of tolbutamide inhibited this conductance. Conversely, no increase in K+ conductance was observed in βKir6.2 KO β-cells, confirming absence of functional KATP channels. Unlike global Kir6.2 KO mice, βKir6.2 KO mice showed significantly elevated fasting blood glucose levels and their tolerance to both oral glucose loading and meal ingestion was severely impaired. In addition, GIIS in isolated βKir6.2 KO islets was absent. Both glucose- and meal-induced insulin secretions were severely impaired in vivo. Our results suggest that βKir6.2 deletion promotes hyperglycaemia by impairing insulin secretion. However, insulin sensitivity was slightly enhanced as assessed by ITT. Basal insulin level in βKir6.2 KO islets was high.The effects of sulfonylureas (glibenclamide and tolbutamide) were completely lost in both βKir6.2 KO mice in vivo and their isolated islets. Interestingly, pre-treatment with liraglutide, a glucagon-like peptide-1 (GLP-1) receptor agonist, completely normalised glucose tolerance in βKir6.2 KO mice, as assessed by OGTT. In addition, fasting GIP level in the KO mice was significantly elevated, and the high level of GIP was maintained throughout OGTT. These findings suggest that incretin signalling is rather enhanced in βKir6.2 KO mice. This is further supported by the observed propensity of the mice to grow obese as they age, possibly attributable to elevated GIP level, as GIP is known to promote fat deposit.
Conclusion: β-cell specific Kir6.2 deletion impairs GIIS, leading to hyperglycaemia and a marked glucose intolerance enhancing incretin secretion which possibly play a more pronounced and compensatory role for the impaired insulin response to glucose due to the β-cell KATP channel deficiency.
Supported by: MEXT
Disclosure: **O.S. Oduori:** None.

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Long-term study of mice with beta cell specific insulin resistance

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Background and aims: Type 2 diabetes is associated with obesity, insulin resistance, and eventually significant beta-cell failure and loss, although the causal molecular mechanisms driving these defects remain to be fully understood. It has been hypothesized that insulin resistance in beta-cells may be an early defect in type 2 diabetes. Earlier beta-cell specific insulin resistance mouse models, including the so-called BIRKO mice, employed Cre recombinase under the control of a short fragment of the rat insulin 2 promoter. Unfortunately, the rat insulin 2 promoter has been shown to delete alleles in both beta-cells and the brain. We therefore generated two new mouse models to re-address the function of insulin receptors specifically in beta-cells. First, we crossed Insrf/f mice with a mouse line expressing Cre within the endogenous Ins1 gene locus (Ins1Cre/wt), as our group and others have shown little or no Ins1 gene expression in the brain. Second, we employed the inducible Ins1-CreERT transgenic mouse model, known as the MIP-Cre, which has also been shown to have virtually no recombination in the brain.
Materials and methods: We assessed the progression of glucose homeostasis, C-peptide and pro-insulin secretion, islet insulin content, and body weight over a 1-year period in several cohorts of both male and female control Insrwt/wt:Ins1Cre/wt, Insrf/wt:Ins1Cre/wt, and Insrf/f:Ins1Cre/wt mice, fed either a 10% or 60% fat diet. Insrf/f:Ins1-CreERT mice, injected or not with tamoxifen, were assessed for differences in glucose homeostasis, insulin secretion and body weight at 4, 8, 12, 16, and 20 weeks after injection.
Results: We confirmed beta-cell specific Insr gene deletion in at least 95% of beta-cells, with no deletion in the brain. Unlike the original BIRKO model, we did not observe glucose intolerance in Insrf/f:Ins1Cre/wt or Insrf/wt:Ins1Cre/wt mice relative to Insrwt/wt:Ins1Cre/wt littermate controls. In female, high fat fed mice with reduced or eliminated Insr, we observed modestly improved glucose tolerance at 21 weeks of age. This was associated with a modest elevation of insulin levels during a glucose challenge at 10 and 22 weeks of age in Insrf/f:Ins1Cre/wt and Insrf/wt:Ins1Cre/wt mice. Pro-insulin and C-peptide levels were elevated in Insrf/wt:Ins1Cre/wt mice at several time points relative to littermate controls. Interestingly, insulin sensitivity was moderately improved at 5 and 9 months of age. Insrf/f:Ins1Cre/wt and Insrf/wt:Ins1Cre/wt mice were also heavier from 7-16 weeks of age, when compared to Insrwt/wt:Ins1Cre/wt littermates. Insrf/f:Ins1-CreERT mice had significantly improved glucose tolerance 4 weeks after tamoxifen injection relative to both Insrwt/wt:Ins1-CreERT and Insrf/f littermate controls (n = 16,17), although this reverted to impaired glucose homeostasis later in life.
Conclusion: Our data are consistent with the concept that insulin inhibits its own secretion, at least initially. Our data suggest that early beta-cell specific modulation of insulin secretion via insulin receptor deletion can have peripheral consequences for body weight regulation and insulin sensitivity. Together, these data provide new information about the function of beta-cell insulin receptors and will provide more insight into the pathogenesis of type 2 diabetes.
Supported by: CIHR
Disclosure: **S. Skovsø:** None.

OP 29 At last! From GWAs to function

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Coding variation and type 2 diabetes risk in 435,386 individuals

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Background and aims: Changes in protein-coding sequence have been hypothesised to be particularly enriched for variants of large-effect on complex human diseases. Such variants are also more likely to have functional impact and support more direct biological interpretation than non-coding alleles. Here, we aim to evaluate the contribution of coding variation to type 2 diabetes (T2D) risk.
Materials and methods: We aggregated 72,927 cases and 366,189 controls of diverse ancestry including (i) exome array data from 51 studies and (ii) genome-wide association (GWA) data from UK BioBank and Genetic Epidemiology Research on Aging (GERA), supplemented with UK10K and/or 1000 Genomes Project imputation. Within each study, we tested single variants for association with T2D, with/without body-mass index (BMI) adjustment. We combined association summary statistics across studies in a fixed-effects Z-score effective sample size weighted meta-analysis. In established GWA regions, we investigated the relationship between coding variants and previously reported lead SNPs through reciprocal conditional analyses.
Results: We identified 15 entirely novel association signals (outside known T2D GWA regions) at exome-wide significance (P<5x10-7) including POC5 (H36R, P=3.1x10-15, minor allele frequency (MAF) = 44%), ZZEF1 (L1972P, P=8x10-12, MAF=24%), PNPLA3 (I148M, P=7x10-9, MAF=42%), and FAM63A (Y285N, P=9x10-8, MAF=1%). A further 38 significant coding associations mapped within 20 established common variant GWA loci. All but four were common (MAF>5% in the ethnic group driving the association), and at 14 of these 20 loci, previous lead SNPs were non-coding. For 10 of these 14, the lead GWA SNP or a close proxy (r2>0.8) was typed on the exome array, enabling reciprocal conditional analyses to assess their relationships to the coding variant signal. At two, including TM6SF2 E167K at the CILP2 locus, coding variant and GWA SNP association signals were indistinguishable. At a further six, association signals at the established GWA SNP and coding variant (including COBLL1 N939D P=2x10-19 and GIPR E354Q, P=3x10-12) were conditionally distinct. We confirmed these relationships using UK Biobank and GERA GWA data with denser variant coverage, demonstrating that for these eight loci, the association evidence was at least consistent with a causal role for the associated coding variants. Overall, our analysis of coding variation points to candidate effector transcripts at ~25% of the >80 T2D GWA loci because: (i) the coding variant drives the GWA signal (e.g. KCNJ11, SLC30A8, GCKR, TM6SF2); (ii) the coding variant association is distinct from the common variant GWA SNP (e.g. COBLL1, RREB1, GIPR, HNF4A); or (iii) the GWA signal overlaps a gene directly implicated in monogenic diabetes (e.g. HNF1B, GCK, PAX4, INS).
Conclusion: Our large-scale association analysis of coding variants has enabled identification of several novel T2D susceptibility loci. These analyses indicated that low-frequency and rare coding variants of large effect do not make a major contribution to T2D risk and highlighted candidate effector transcripts at ~25% of the >80 T2D previously established GWA loci.
Disclosure: **A. Mahajan:** None.

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Prioritising causal genes at type 2 diabetes risk loci through high-throughput screening for human beta cell dysfunction

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Background and aims: Most genetic association signals for type 2 diabetes (T2D) risk are located in non-coding regions of the genome, hindering translation into molecular mechanisms. Physiological studies have shown pancreatic β-cell dysfunction to be a central mechanism through which risk alleles exert their effects, but uncertainty remains over the causal gene in the vast majority of cases. Systematically characterizing the role of regional transcripts in β-cell function could point to the underlying disease-causing genes. However, previous high-throughput screening efforts have been limited by inadequate cellular models and prohibitively high costs of insulin immunoassays. Our aim was to design a scalable assay for β-cell dysfunction in a human model system, and to apply this for accelerated discovery of causal genes for T2D risk loci.
Materials and methods: To overcome previous limitations, we developed a strategy based on arrayed gene silencing in the human β-cell line EndoC-βH1. Insulin immunoassays were miniaturized using acoustic liquid handling to achieve a ten-fold cost reduction while maintaining high sensitivity (coefficient of variation < 3%). We performed a primary loss-of-function screen in triplicate on 300 positional candidates selected from 75 T2D-associated regions. Each gene was assayed for effects on cell count (a function of viability and proliferation) and insulin secretion under four different conditions (basal glucose, high glucose, IBMX, and tolbutamide).
Results: Applying a false discovery rate threshold ($q < 0.05$), we identified a total of 67 significant effects (15 for cell count and 52 for insulin secretion phenotypes) among 45 genes at 37 disease-associated loci. The hits showed a strong enrichment for genes known to be implicated in monogenic diabetes (odds ratio = 14.6; $p = 5.5 \times 10^{-9}$), driven by significantly altered insulin secretion rather than differences in cell counts ($p < 2 \times 10^{-16}$ versus $p = 0.67$). Selected effects were successfully validated in a follow-up study ($\rho = 0.85$, $p = 6.7 \times 10^{-10}$), including several genes (*ARL15*, *ZMIZ1* and *THADA*) with previously unknown or poorly described roles in β-cell biology. Loss-of-function of the known monogenic diabetes gene *HNF4A* was found to cause a paradoxical increase in insulin secretion across conditions (q-values < 0.001). This is consistent with the previously unexplained observation that loss-of-function *HNF4A* mutations are associated with congenital hyperinsulinism in early infancy.
Conclusion: We have identified genes at half of the T2D-associated loci studied (37 of 75) where loss-of-function in EndoC-βH1 resulted in β-cell dysfunction. This demonstrates, for the first time, the feasibility of performing systematic screening for insulin secretion defects in human β cells. Our data confirmed known regulators of β-cell function, and also highlight possible roles for genes with no or limited prior evidence. These results can be integrated with complementary lines of genomic and epigenomic evidence to shed light on molecular mechanisms for insulin insufficiency and type 2 diabetes.
Supported by: Wellcome Trust (095101/Z/10/Z and 098381)
Disclosure: **S.K. Thomsen:** None.

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Deep functional profiling of 40 MTNR1B mutants reveals the involvement of Gαi1 and Gαz proteins in the association between melatonin signalling and type 2 diabetes

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Background and aims: G protein-coupled receptors (GPCRs) activate multiple distinct downstream pathways, but the specific impact of these pathways on disease risk is unknown. Previously, we reported that rare mutations in MTNR1B (encoding the GPCR melatonin receptor 1B) which damage the melatonin binding, as well as the melatonin-induced signalling of ERK and/or cyclic AMP (cAMP), contribute to increased type 2 diabetes (T2D) risk. However, other downstream pathways were not analyzed. Here, we performed a deep functional profiling of 40 MTNR1B mutants by investigating five downstream signalling pathways including basal or melatonin-induced Gαi1 activation, Gαz activation, cAMP inhibition, β-arrestin-2 recruitment and ERK activation.
Materials and methods: The effects of the mutations on these pathways were assessed using bioluminescence resonance energy transfer-based assays, protein-complementation assays and alphascreen technology. The relative melatonin-induced responses were determined using the operational model and were expressed as $\Delta\log(\tau/KA)$. The association analyses were performed on a case-control study including 2,186 patients with T2D and 4,804 controls. Rare variants (with a frequency < 1%) were analyzed by pooling them on the basis of their functional consequences. We assessed the effect of these pooled variants on T2D risk via the KBAC method embedded in a logistic regression model adjusted for age, gender and body mass index.
Results: We found that several MTNR1B variants led to defects in Gαi1 activation, Gαz activation, cAMP inhibition, β-arrestin-2 recruitment and/or ERK activation. Of note, the vast majority of these loss-of-function variants were rare, except for the K243R variant that is frequent and led to defects in β-arrestin-2 recruitment. We found that the variants causing defects in melatonin-induced Gαi1 or Gαz activation were the most strongly and significantly associated with increased T2D risk (OR=3.25 or 2.92, respectively; $p<0.001$; Table 1). The rare variants causing defects in melatonin-induced β-arrestin-2 recruitment only modestly contributed to T2D; this is in line with the fact that K243R is not associated with T2D (either in this study or in the consortia data). We found that the "neutral" variants did not associate with T2D.
Conclusion: Through this strategy of deep functional profiling of MTNR1B mutants, we found that melatonin-induced defects in Gαi1 and Gαz activation strongly contribute to the association between MTNR1B loss of function and increased T2D risk; while defects in β-arrestin-2 recruitment seem less important. The present study highlights the high complexity of GPCR signalling and demonstrates that only some of these pathways are involved in specific disease physiology, opening avenues to smarter drug design targeting GPCRs.

Table 1. Association between rare *MTNR1B* variants and T2D risk on the basis of their functional consequences

Consequences on MTNR1B function (in response to melatonin)	Rare *MTNR1B* variants	Odds Ratio	*P*-value
Defects in $G\alpha_{i1}$ activation	A42P, L60R, A74T, P95L, S123R, V124I, R138C/H/L, Y141F, R154H, T201M, R222H, I223T, F250V, Y308S, R316H, R330W, A342V	3.25	<0.001
Defects in $G\alpha_z$ activation	A42P, L60R, A74T, P95L, G109A, M120I/V, S123R, V124I, R138C/H/L, Y141F, T201M, R222H, I223T, D246N, F250V, Y308S, R316H, R330W, A342V	2.92	<0.001
Defects in cAMP inhibition	A42P, L60R, P95L, G109A, M120V, S123R, R138C/H/L, R222H, I223T, F250V, Y308S, R316H, R330W	2.82	<0.01
Defects in β-arrestin-2 recruitment	A42P, L60R, A74T, P95L, G109A, M120V, S123R, V124I, R138C/H/L, L166I, T201M, R222H, I223T, R231H, E237K, S238G, K243R, D246N, F250V, Y308S, R316H, A342V	2.13	<0.01
Defects in ERK activation	A42P, L60R, A74T, P95L,M120I, S123R, V124I, R138C/H/L, M146V, R154H, T201M, R222H, I223T, F250V, Y308S, R316H	2.48	<0.01
Neutral variants	A8S, A13V, G21S, W22L, G24E, A25T, P36S, A52T, A234T, I353T, A359E	-	0.20

Supported by: French National Research Agency
Disclosure: **A. Bonnefond:** None.

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Coding variants in G6PC and G6PC2 identified from large-scale exome array meta-analysis impact on fasting glucose homeostasis through effects on different tissues

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Background and aims: We previously reported multiple coding variants in the pancreatic islet-specific glucose-6-phosphatase gene (*G6PC2*) which are independently associated with fasting glucose (FG) levels. We showed that these variants result in loss of function (LOF) through reduced protein stability. Given the central role of glucose-6-phosphatase in glucose metabolism, we hypothesised that additional rare coding variants in both islet and liver/kidney forms of the protein (encoded by *G6PC2* and *G6PC* respectively) may influence glycemic traits through altered protein function in their respective tissues. We now extend our studies to investigate the role of coding variation in both genes on glycemic traits using exome array genotyping in up to 130K normoglycemic individuals from multiple international consortia (T2D-GENES and MAGIC), and coupled this with functional evaluation.
Materials and methods: Single-variant results from linear mixed models across studies were combined by inverse-variance weighted fixed-effect meta-analysis. Gene-based analyses were performed using sequence kernel association (SKAT) and burden tests. Nonsynonymous variants in *G6PC* and *G6PC2* were assessed *in vitro* for protein expression (western blot) and enzymatic activity (phosphatase assay).
Results: We identified a gene-based signal with exome-wide significance for FG and fasting insulin (FI) at the *G6PC* locus ($P_{burden}<2.5 \times 10^{-6}$) that was driven by missense variants A204S and R83C, and protein-truncating variant (PTV) Q347X. All 3 variants are rare (minor allele frequency<0.01). A204S-G6PC displayed reduced protein levels (33% of wild type, P<0.05) when expressed in HEK293 cells. p.R83C maps to the enzyme's active site and R83C-G6PC demonstrated both a complete loss of the glycosylated form of the protein in HEK293 and HepG2 cells as well as abolished phosphatase activity. Likewise, Q347X-G6PC lacked any detectable activity. Within the known glycemic trait locus *G6PC2*, 5 coding variants (common variant V219L, rare variants Y207S, H177Y, S324P, R283X) now each achieve single variant exome-wide significance ($P<2.07 \times 10^{-7}$) for FG. All missense variants resulted in both reduced protein expression and glycosylation in INS-1 832/13 cells (P<0.01), whereas R283X displayed null phosphatase activity. In addition, we identified 2 rare variants with nominal significance for FG at single variant level (P<0.05) at the same amino acid position (I171) which resulted in opposite directions of effect on FG (β_{I171T}=-0.179 mmol/l; β_{I171V}=+0.263 mmol/l). *In vitro* analysis supported this observation as the Thr substitution gave rise to decreased activity whereas the Val substitution enhanced phosphatase activity (both P<0.05).
Conclusion: We identified a gene-based association in *G6PC* driven by LOF coding variants influencing FG and FI. We characterised additional functional rare *G6PC2* coding variants and described the first activating variant consistent with elevated FG. Our study highlights the critical role that glucose-6-phosphatase plays in both hepatic glucose metabolism and pancreatic beta cell function.
Supported by: Wellcome Trust
Disclosure: **N.H. Ng:** None.

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Deletion of the type 2 diabetes-associated gene product StarD10 in mice impairs insulin secretion and action

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Background and aims: Genome-wide association studies have identified more than 90 loci associated with type 2 diabetes risk. Common genetic variants in the ARAP1/STARD10 locus on chromosome 11 affect fasting proinsulin levels (rs11603334) and glucose-induced insulin secretion (rs1552224). The product of this gene, StAR-related lipid transfer (START) domain containing 10, is involved in the intracellular transport of phospholipids. Here we compare human islet transcriptomes and use mouse genetics to explore the role of the STARD10 gene in insulin action and secretion.
Materials and methods: Null (tm1a, bearing a LacZ-STOP cassette downstream of exon 2) and conditional (tm1c) alleles of StarD10, were provided through the International Mouse Phenotyping Consortium (IMPC) and maintained on a c57bl/6 background. Loss of StarD10 mRNA from liver and islets was confirmed by qRT-PCR and western blotting. Expression in cadaveric human islets was examined using oligonucleotide arrays.
Results: We studied the transcriptional activity of the three most likely causal variants identified by Metaboship at the ARAP1/STARD10 locus in luciferase constructs. The protective allele in variant rs1401030268 displayed greater enhancer activity than the risk form in INS1 cells (p<0.05). These variants were found able to physically associate with the promoter of STARD10 by chromatin conformation capture analysis in EndoC-βH1 cells (p<0.05). Compared to wild type (WT) littermates, mice deleted globally for StarD10 (KO), and assessed at 14-16 weeks of age, displayed increased fed glycemia (WT, 8.1 ± 0.1 mmol/L vs KO, 9.7 ± 0.3, p<0.001), intraperitoneal glucose (1g/kg; AUC: WT, 1169 ± 34 WT vs KO, 1274 ± 30 mmol/l*min., p=0.034) and insulin (0.75U/kg; AUC: WT, 875 ± 62 vs KO, 670 ± 34 pM*min., p<0.01) tolerance. Glucose- (3g/kg) stimulated insulin secretion was also sharply reduced in vivo in knockout mice (AUC: WT, 50.37 ± 22.53 vs KO, 40.57 ± 6.65 pM*min., p=0.026). Correspondingly, glucose- (17 vs 3 mM) induced insulin secretion (WT, 0.86 ± 0.05%; KO, 0.21 ± 0.03% p<0.001) and cytoplasmic Ca2+ increases, assessed using the fluorescent probe fluo-2, and confocal imaging of whole islets (vs 3G: WT, 23.5% ± 1.7 vs KO, 15.4% ± 1.9, p<0.001) were reduced in islets from KO mice versus those from WT littermates. ß-cell-selective inactivation of StarD10 in mice (βKO) led to increased fed glycemia and impaired glucose-stimulated cytoplasmic Ca2+ increases and insulin secretion in vitro (p<0.05). In addition, both StarD10 KO and ßKO mice presented a reduced [proinsulin]/[insulin] ratio (p<0.05), as observed in human carriers of the risk allele. eQTL analysis revealed a significant association between lowered human islet STARD10 mRNA levels and the possession of risk alleles at rs11603334, p<0.001).
Conclusion: These data indicate that lowered expression of StarD10 gene in risk allele carriers may contribute to exaggerated disease risk, and may act both via changes in insulin secretion and action. Future studies will be required to test this hypothesis.
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Disclosure: **G.R. Carrat:** None.

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A new PPARγ truncated isoform induced by a regulatory feedback: Is alternative splicing crucial in adipocyte differentiation and defective neoadipogenesis?

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Background and aims: The current global epidemic of type 2 diabetes (T2D) is driven by obesity. The impairment of adipogenesis is a hallmark of hypertrophic obesity. The adipogenesis is crucially regulated by the

peroxisome proliferator-activated receptor γ (PPARγ). Loss-of-function (LOF) mutations in PPARG gene are associated with insulin resistance and T2D and dominant negative mutations (i.e. E471A, L466A) in the ligand-binding domain (LBD) block adipocyte differentiation. A systematic analysis of PPARG alternative splicing has not yet been performed. Here we aim to analyze the impact of a newly identified isoform lacking LBD (PPARγΔLBD) on PPARγ activity during adipogenesis and in adipose tissue of obese patients.

Materials and methods: In vitro, ex vivo and in silico analyses have been carried out. HEK293 and hTERT-AdMSCs have been used as in vitro models. Primary AdMSCs - isolated from subcutaneous adipose tissue of class III obese patients (n=6, BMI≥40) - and hTERT-AdMSCs have been differentiated in adipocytes. Glucose tolerance (GT) of obese patients has been determined by OGTT. Drug treatments (rosiglitazone, troglitazone and GW9662) on the above-mentioned cells have been combined to confocal microscopy, luciferase-based transactivation assays, CoIP assays, RNA-Seq, qRT-PCR and western blot.

Results: We demonstrated that ligand-induced activation of PPARγ induces a new event of splicing (exon skipping) of its own pre-mRNA (increase of 80-100%;pval≤0,01). Knockdown of ASF/SF2 significantly prevents it (n=5; pval≤0,01), suggesting that this splicing factor mediates such a process. This splicing event leads to a truncated protein lacking LBD (PPARγΔLBD), similarly to LOF PPARγ mutants. Interestingly, it is not able to transactivate a reporter gene and acts as a dominant negative, reducing of about 60% the activity of the canonical PPARγ (n=3; pval≤0,01). Its ability to bind the partner RXRα suggests a potential competition with PPARγ. RNA-Seq in HEK293 revealed that its overexpression impedes the transcription of key genes, induced by PPARγ in adipocytes (e.g. LIPA, STARD4 and SLC38A9, pval≤0,01). Notably, PPARγΔLBD levels are increased in subcutaneous adipose tissue of obese patients with impaired GT vs obese with normal GT (n=10 and n=12, respectively, FC mean=6,7). Furthermore, preliminary data reveal that hAdMSCs with high PPARγΔLBD levels display a reduced ability to differentiate in adipocytes.

Conclusion: PPARγ activation promotes a new splicing event on its own pre-mRNA, triggering a negative regulatory feedback on its function. This event is particularly relevant during adipogenesis, where we propose it is a physiologic mechanism necessary to modulate PPARγ activity . Preliminary data showed that precursors cells with high levels of this new isoform have a reduced differentiation ability, resembling the effects of PPARG LOF mutations. Then, the alteration of this physiological mechanism may compromise the commitment of adipogenic precursors contributing to the defective neoadipogenesis in hypertrophic obesity and predisposition to T2D onset.

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Disclosure: **M. Aprile:** None.

OP 30 Exercise effects: from molecules to humans

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Multimodal live-imaging analysis of GLUT4 behaviour and exercise effects in mouse skeletal muscle

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Background and aims: Exercise exerts a beneficial effect on insulin potency (exercise effects) via augmented GLUT4 translocation in skeletal muscles, but the molecular details of this regulating effect remain unclear. Employing our GLUT4 nanometry combined with a cell-based reconstitution model, we demonstrated the respective functional roles of AS160 and Tbc1d1, proteins of the TBC1D Rab GTPase-activating (RabGAPs) family, implicated in the key nexus deciphering biochemical phosphorylation signals into physical processes of GLUT4 translocation in response to insulin and exercise. Our results suggest involvement of RabGAPs in exercise effects (MBoC 24: 806, 2013). AS160 mediates insulin-responsive GLUT4 release from its static storage sites, whereas Tbc1d1 temporally acquires insulin-responsiveness which triggers GLUT4 release only after exercise-mimetic stimuli such as AICAR-pretreatment. Unlike adipocytes which express only AS160, skeletal muscle expresses both RabGAPs and their relative abundances vary among muscles. We herein analyzed GLUT4 behavior in mouse skeletal muscle and combinatorial regulatory mechanisms mediated by the two RabGAPs employing several live-imaging techniques.

Materials and methods: Myc-GLUT4-EGFP transgenic mice were generated using the cDNA under control of the human α-actin promotor. Single myofibers were isolated by collagenase digestion. The cell-based reconstitution model (3T3-L1 fibroblasts exogenously expressing myc-GLUT4, HA-sortilin, AS160 and Tbc1d1) was also used. Intracellular movements of individual myc-GLUT4 were tracked by labeling the protein with Quantum dot-conjugated anti-myc antibodies.

Results: In vivo imaging with multiphoton microscopy allowed observation of global GLUT4 trafficking processes such as contraction- and insulin-responsive translocation to both sarcolemma and t-tubules in quadriceps of myc-GLUT4-EGFP transgenic mice. We also visualized, tracked and quantified individual GLUT4 behaviors such as insulin-responsive release of static GLUT4 in single myofibers isolated from these transgenic mice. Detailed analyses with the cell-based reconstitution model revealed coordinated actions of AS160 and Tbc1d1 on GLUT4 behavior in the co-presence of both RabGAPs. Importantly, AS160 modulates sensitivity to external stimuli in Tbc1d1-mediated activation of GLUT4 trafficking. For example, acquired insulin responsiveness after AICAR-pretreatment is more obvious in cells expressing both Tbc1d1 and AS160, even after acute (5 min) stimulation, than in cells expressing only Tbc1d1. This modulatory action of AS160 was completely abolished by Tbc1d1 mutants with phosphotyrosine-binding (PTB) domain impairments, including an obesity-related mutation (R125W).

Conclusion: Multimodal live-imaging techniques, including in vivo imaging and single molecule nanometry, enabled high-accuracy measurement of GLUT4 behavior in mouse skeletal muscle. We also found that

AS160 potentiates insulin sensitivity in Tbc1d1-mediated GLUT4 releasing activity after exercise-mimetic stimuli, apparently mediated through functional interaction with the PTB domain of Tbc1d1. This modulatory action might be involved in the insulin sensitivity differences among skeletal muscles and beneficial effects of exercise on muscle insulin potency. We are now examining GLUT4 behavior in isolated skeletal myofibers to verify further details.
Disclosure: **H. Hatakeyama:** None.

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Impaired exercise performance in Tbc1d1/Tbc1d4-deficient mice is rescued by high-intensity interval training

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Background and aims: The two Rab-GTPase-activating proteins (RabGAPs) TBC1D1 and its close homologue TBC1D4 (AS160) are important regulators of insulin- and contraction-mediated glucose uptake and lipid metabolism in skeletal muscle. Moreover, deletion of TBC1D1 has been shown to result in an impaired exercise performance. Whole-body glycaemia, however, was not changed upon lack of TBC1D1, presumably due to compensatory mechanisms involving TBC1D4. Our aim was to elucidate the role of both RabGAPs in glycaemic control in response to exercise in Tbc1d1/Tbc1d4-deficient mice.
Materials and methods: Wildtype (WT) and Tbc1d1/Tbc1d4-deficient (D1/4) mice were fed a high-fat diet (HFD) with 60% fat from calories after weaning and subjected to forced high-intensity interval training (HIIT) on treadmills for six weeks starting from week 14. Body composition, glucose sensitivity and physical condition were determined and compared with sedentary controls. Statistical analysis was performed using student's t-test and two-way ANOVA.
Results: Sedentary D1/4 mice showed impaired physical activity. Compared to WT controls, knockouts displayed reduced voluntary activity measured in metabolic cages (801 ± 73 vs 533 ± 67 (a.u.), n = 8-14, $p<0.05$), and reduced time to exhaustion during an acute exercise exhaustion test on treadmills (10.5 ± 0.4 min vs 9.2 ± 0.3 min, n = 17-19, $p<0.05$). D1/4 mice also exhibited a markedly impaired glucose tolerance compared to WT controls (AUC 49169 ± 2177 vs 60876 ± 2601, n = 11-14, $p<0.01$) and a trend for increased fasting (6h) plasma insulin concentrations (AUC 31377 ± 2935 vs 43438 ± 5707, n = 10-14, p = 0.08). After six weeks of HIIT, WT and D1/4 mice exhibited reduced body weight compared to sedentary controls (sedentary vs trained; WT: 41.0 ± 1.0 g vs 36.3 ± 1.7 g, n = 10-14, NS; D1/4: 40.3 ± 2.1 g vs 34.6 ± 1.5 g, n = 9-11, $p<0.05$). Both trained WT and D1/4 mice significantly improved their exercise performance after six weeks of HIIT during an acute exercise exhaustion test (pre vs post training; WT: 10.5 ± 0.4 min vs 13.2 ± 0.8 min, n = 9-19, $p<0.001$; D1/4: 9.2 ± 0.3 min vs 11.9 ± 0.4 min, n = 9-17, $p<0.001$). In contrast to WT mice, trained D1/4 mice showed significantly reduced blood glucose concentrations during glucose tolerance tests compared to sedentary controls (AUC sedentary vs trained; WT: 49169 ± 2177 vs 46078 ± 1521, n = 10-14, NS; D1/4: 60876 ± 2601 vs 50469 ± 2986, n = 9-11, $p<0.05$).
Conclusion: In summary, our results demonstrate that deletion of the RabGAPs TBC1D1 and TBC1D4 leads to impaired physical activity, probably due to reduced glucose uptake into skeletal muscle and adipose tissue and redistribution of glycogen and triglycerides between liver and skeletal muscle. After six weeks of HIIT, D1/4 mice were able to normalise both, exercise performance and glucose tolerance, respectively, to a degree comparable to WT controls. This suggests a RabGAP-independent mechanism highly sensitive to exercise intervention. It remains to be investigated which pathways account for the improved exercise performance in trained D1/4 mice.
Disclosure: **C.A. Springer:** None.

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A surge of appetite regulating hormone, ghrelin, and its relevance to motivation for the initiation of voluntary exercise in mice

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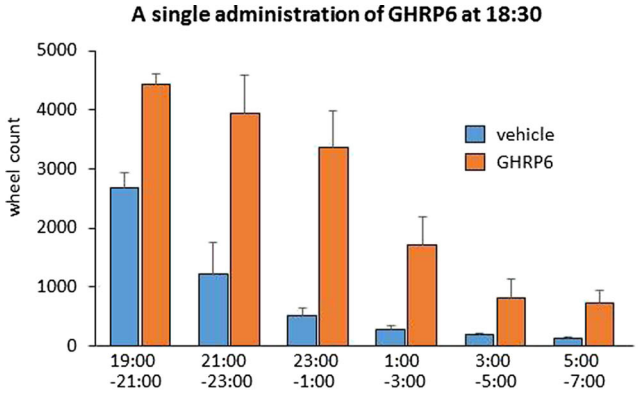
Background and aims: We previously reported that voluntary exercise contributed to an amelioration of abnormal feeding behavior with a concomitant restoration of ghrelin production in high fat diet (HFD)-induced obese rats (Peptides 71: 49, 2015), suggesting a possible relationship between exercise and appetite regulating hormone. Because ghrelin is related to higher motivation and hyperactivity as an exploring behavior for food, we investigated the relevance of ghrelin as an initiator of voluntary exercise as well as feeding behavior.
Materials and methods: The animals were housed under a 12 h light dark cycle (light on 7:00-19:00). Four-week-old male wild type mice were either fed control chow diet (WT-CD) or high fat diet (60kcal% fat: WT-HFD) for 12 weeks. Ghrelin knockout mice at the same age were fed CD for the same period (GKO-CD). At 16weeks old, they were moved individually into acrylic metabolic chambers equipped with running wheel for the measurement of food intake (FI) and wheel running count (COUNT) as voluntary exercise performance on a minute by minute basis. After all measurements, they were sacrificed under isoflurane anesthesia and blood samples were collected at 8 time points (7:00, 9:30, 13:00, 15:30, 19:00, 21:30, 1:00, 3:30) for the measurement of plasma active ghrelin concentrations by RIA.
Results: WT-HFD revealed an obvious weight gain and abnormal feeding behavior as an increase of FI during light phase compared to WT-CD. Plasma ghrelin levels in WT-CD showed a bimodal diurnal rhythm with its peaks at 7:00 and 19:00. In WT-HFD, however, those peaks shifted to 13:00 and 1:00, respectively. In WT-CD, a marked increase of COUNT was observed both at the beginning and at the end of dark phase concomitant with an increase of FI in these periods. These increases were weakened in WT-HFD and markedly reduced in GKO-CD. To verify a role of ghrelin as an initiator of voluntary exercise, we further tested effects of ghrelin agonist (GHRP6) injection (ip) on COUNT in GKO-CD at 16 weeks old. A single injection of GHRP6 (1mg/kg) at 18:30 for 2 weeks brought about a significant enhancement of COUNT during dark phase in spite of no effect of continuous administration of this agent by osmotic pumps at the same dose (Figure).

Conclusion: It was clearly demonstrated that ghrelin surges at 7:00 and 19:00 observed in WT-CD play a crucial role in the initiation and motivation of voluntary exercise in these periods. Because diurnal ghrelin rhythm were disturbed in WT-HFD concomitant with the decrease of COUNT, therapeutic properties of this peptide are to be further elucidated in future investigations.



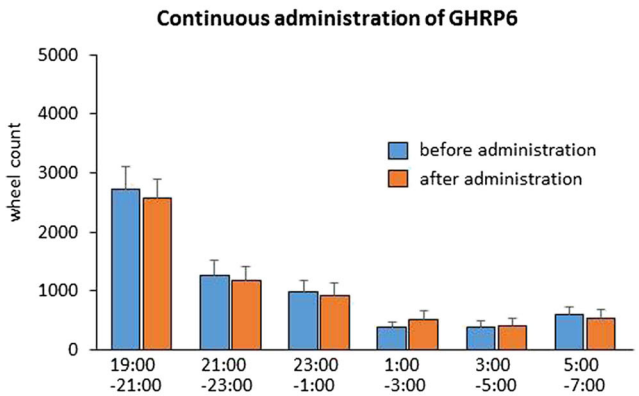


Figure. Effects of ghrelin agonist on voluntary exercise

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Disclosure: **Y. Tajiri:** None.

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Dual specificity phosphatase 5 and 6 are oppositely regulated in human skeletal muscle by acute exercise

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Background and aims: Dual specificity phosphatase (DUSP) 5 and 6 specifically dephosphorylate ERK1/2; thus, they are important regulators of ERK1/2 signaling. DUSP5 is located in the nucleus, whereas DUSP6 is found in the cytoplasm. Both DUSPs bind also unphosphorylated ERK1/2 and sequester it in nucleus and cytoplasm, respectively. Depending on the stimulus, e.g. growth factors, DUSP5 and/or DUSP6 expression is increased or reduced. Thereby, a stimulus-dependent pattern of DUSP5 and DUSP6 determines the activity of ERK1/2 in a compartment-specific manner. This might be an important regulatory mechanism in determining physiological consequences of ERK1/2 signaling. In human skeletal muscle, ERK1/2 activity is increased by acute exercise and rapidly reduced upon cessation. However, the regulation and importance of DUSP5 and DUSP6 by exercise has not been studied so far.
Materials and methods: Healthy sedentary men categorized either as control (BMI = 23.5 ± 2.0 kg/m2; normal fasting and 2 h serum glucose levels; n = 13) or dysglycemic (BMI = 28.9 ± 2.5 kg/m2; fasting glucose ≥ 5.6 mmol/L and/or 2 h serum glucose ≥ 7.8 mmol/L; n = 11) were subjected to acute cycling (45 min, 70% VO2max). Skeletal muscle biopsies were taken before, after, and 2 h after exercise. RNA was isolated and analyzed by high throughput mRNA sequencing followed by differential gene expression analysis. Primary human myotubes were acutely exposed to dexamethasone (dex) mimicking the increase of cortisol during exercise, and ionomycin (iono), which increases intracellular Ca2+ concentrations. ERK1/2 phosphorylation was analyzed by Western blot and expression of DUSP5 and DUSP6 by qRT-PCR.
Results: Acute exercise promoted DUSP5 expression 9.1-fold ($p<0.001$) directly after exercise while DUSP6 was reduced by 43%, with no difference between the groups. After 2 h rest DUSP5 returned to basal values whereas DUSP6 was still 30% lower compared to baseline. Basal DUSP5 and DUSP6 levels were not different between the groups. During in vitro differentiation of human skeletal muscle cells, DUSP5 expression decreased by 50% from day 0 to day 1 and remained stable thereafter. DUSP6 expression increased during differentiation (day 6 vs. day 0: 3.5-fold, $p<0.005$, n=3) in parallel with enhanced expression of myogenin (4-fold) and MHCIIa (10-fold). Incubation with dex increased ERK1/2 phosphorylation after 15 min (1.6-fold, $p<0.001$, n=3), which returned to basal level after 30 min. Moreover, dex stimulation promoted DUSP5 expression 2.6-fold ($p<0.05$, n=4-6) after 1 h, which returned to basal level after 4 h. DUSP6 expression was significantly reduced by 42% after 2 h and 4 h, and returned to basal level after 6 h. A similar pattern but with different kinetics was observed for iono, which increased DUSP5 expression 2-fold after 6 h, whereas DUSP6 was reduced by 40% after 3 h and returned to basal level after 6 h.
Conclusion: The prominent regulation of DUSP5 and DUSP6 expression by acute exercise in human skeletal muscle indicates an important role in generating a specific spatio-temporal pattern of ERK1/2 activity during exercise. Stimulation of human myotubes with dex and iono mimicked this pattern of DUSP5 and DUSP6 in vitro, and enables future studies aiming to decipher the physiological consequences of a proper regulation of DUSP5 and DUSP6.

Clinical Trial Registration NumbeR: NCT01803568
Supported by: DFG, Johan-Throne-Holst Foundation, Helse Sør-Øst, NutriTech

Disclosure: **K. Eckardt:** None.

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High-intensity interval training changes insulin stimulated cerebral glucose uptake of in subjects with impaired glucose tolerance

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Background and aims: Obesity and insulin resistance are associated with increased insulin-stimulated glucose uptake (GU) in the brain, which is reversed by bariatric surgery. Whether exercise training affects brain GU is unclear. The aim of this study was to compare two-weeks training responses of moderate intensity continuous training (MICT) and high intensity interval training (HIIT) on brain GU as measured using PET under insulin stimulation in subjects with impaired glucose tolerance (IGT).
Materials and methods: Sedentary subjects (n= 21, BMI 23.7-34.3 kg/m^2, age 43-55 y) with IGT were randomized into MICT (n=11) and HIIT (n=10) groups. Two-week training intervention included six supervised training sessions. The brain GU was measured using [18F]FDG-PET during hyperinsulinemic euclycemic clamp before and after the training intervention.
Results: Whole-body insulin sensitivity improved with both training modes (from 16.0 [12.2;20.9] to 19.8 [15.0;26.1] μmol/kg/min, time p=0.02) while only HIIT led to improved aerobic capacity (from 26.6 [24.2;29.0] to 27.8 [25.4;30.3] ml/kg/min, time p=0.03). HIIT led to reduced insulin-stimulated brain GU both in global (from 15.9 [13.7;18.2] to 14.0 [11.6;16.4] μmol/100 g/min, time p=0.03) and regional level (time p<0.05, all areas expect occipital cortex), whereas no change was observed with MICT. Both global and regional brain GU was strongly negatively correlated with whole-body insulin sensitivity in both groups (p<0.05, all areas).
Conclusion: In addition to the well-known beneficial effects of exercise on whole body insulin sensitivity training with high intensity intervals changes brain metabolism during insulin stimulation in subjects with impaired glucose tolerance.
Clinical Trial Registration Number: NCT01344928
Supported by: EFSD/NovoNordisk, AF, ERVA, Ministry of educationFinland, EA, Diabetes foundation
Disclosure: **S.M. Honkala:** None.

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High intensity interval training causes sustained improvement of maximal mitochondrial respiration in patients with type 2 diabetes and healthy controls

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Background and aims: High intensity interval training (HIT) represents a time-efficient training concept, shown to stimulate mitochondrial biogenesis and increase mitochondrial respiratory chain enzyme content in healthy populations. We hypothesized that HIT would increase skeletal muscle insulin sensitivity due to improved skeletal muscle mitochondrial function in type 2 diabetes patients and age- and BMI- matched controls and that 4 weeks of detraining would abolish these improvements.
Materials and methods: We examined 11 sedentary male patients with type 2 diabetes and 5 healthy male controls (age: 58±6 vs 55±2 years, p=0.25, BMI: 31.3±3.0 vs 30.5±2.5 kg.m-2, p=0.56 and VO2max: 24±4 vs 27±5 ml.min-1.kg-1, p=0.23) that were enrolled in a 12-week cycling protocol (4 x 4 min intervals at 85% of maximal heart rate, three minutes recovery, three days weekly) at baseline, after one training bout, after 12 weeks of training and following 4 weeks of training pause. Two-step hyperinsulinemic-euglycemic clamps with somatostatin infusion and skeletal muscle biopsies for high resolution respirometry measurements were performed. Amplex Red was used to measure muscle H2O2 emission, reflecting production of reactive oxygen species (ROS) from complexes I+III.
Results: After 12 weeks of HIT insulin sensitivity (in mg.kg-1.min-1) increased (2.7±1.6 vs. 12 weeks: 3.9±1.9, p=0.02) and fasting blood glucose as well as free fatty acids were reduced (156±42 vs.12 weeks: 144±35 mg/dl, p=0.004 and 441±161 vs. 12 weeks: 293±52 μmol/l, p=0.008, respectively) among patients with type 2 diabetes but these improvements were abolished after 4 weeks of training pause. Glycemic control and BMI remained unchanged (HbA1c: 7.2±0.9 vs 12 weeks: 7.1±0.9%, p=0.73, BMI: 31.6±2.5 vs 12 weeks: 30.9 ±2.3 kg.m-2). Maximal uncoupled respiration increased at 12 weeks and remained increased after the detraining phase (baseline: 67±11, 12 weeks: 89 ±20 vs. detraining: 83 ± 21 pmol/mg wet tissue/s, p<0.01). There was no change in leak control ratio (p=0.44), respiratory control ratio (p=0.76) or ROS production (p=0.20). The change of insulin sensitivity did not correlate with changes in maximal uncoupled respiration (r=0.31, p=0.37) or VO2max (r=0.40, p=0.24). Among controls, insulin sensitivity remained unchanged after 12 weeks (6.6±2.6 vs. 12 weeks: 8.0±1.7, p=0.23), but an increase in maximal uncoupled respiration was also observed and present after training abstinence (baseline: 81 ±22, 12 weeks: 106±28 vs. detraining: 95 ±27 pmol/mg wet tissue/s, p<0.01).
Conclusion: 12 weeks of HIT training can improve insulin sensitivity and free fatty acid levels in patients with type 2 diabetes despite unchanged body weight and glycemic control, however 4 weeks of training abstinence are potent to abolish these improvements. Sustained increases in maximal uncoupled respiration after detraining were present among type 2 diabetes patients but also healthy controls.
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Disclosure: **M. Apostolopoulou:** None.

OP 31 SCLT-2 inhibitor trials

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Efficacy and safety of ertugliflozin in subjects with type 2 diabetes mellitus inadequately controlled on the dual combination of metformin and sitagliptin: the VERTIS SITA2 trial

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Background and aims: Ertugliflozin (ERTU) is an oral sodium/glucose cotransporter 2 (SGLT2) inhibitor in development for treatment of patients with T2DM. This study assessed the safety and efficacy of adding ERTU 5 mg or 15 mg compared with placebo (PBO) to the dual combination of metformin (MET) and sitagliptin (SITA), after 26 weeks of treatment.
Materials and methods: In a double-blind, randomised Phase 3 trial, subjects (n=463) with HbA1c 7.0-10.5% on stable MET ≥1500 mg/day and SITA 100 mg/day were randomised to ERTU 5 mg, ERTU 15 mg, or PBO. Data were analysed with a constrained longitudinal model for continuous endpoints and logistic regression for binary endpoints.
Results: Baseline characteristics were comparable between groups (overall mean age 59.1 years, HbA1c 8.0%), except for higher proportion of males in the PBO group (65.4%) compared with the ERTU 5 and 15 mg groups (51.9 and 53.6%, respectively). After 26 weeks, ERTU 5 and 15 mg were significantly more effective than PBO in reducing HbA1c, fasting plasma glucose (FPG), body weight and systolic blood pressure (BP), and a greater proportion of subjects were at the target of HbA1c <7.0% (Table) in the ERTU 5 and 15 mg groups than in the PBO group. Incidence of AEs was generally similar among treatment groups, except for higher rates of genital mycotic infections with ERTU 5 and 15 mg (males: 4.9% and 3.7% vs no events with PBO; females: 8.0% and 12.7% vs 1.9% with PBO). Urinary tract infection rates were similar in PBO and ERTU 5 mg groups (2.0% and 2.6%) and higher in the ERTU 15 mg group (4.6%). Across groups, rates were similar for symptomatic hypoglycaemia (PBO, 2.6%; ERTU 5 mg, 3.2%; ERTU 15 mg, 0.7%) and hypovolaemia AEs (PBO, 0.7%; ERTU 5 mg, 0.6%; ERTU 15 mg, no events).
Conclusion: Addition of ERTU 5 mg or 15 mg to MET and SITA provided more effective glycaemic control compared with addition of PBO after 26 weeks of treatment. ERTU was generally well-tolerated.

Table. Summary of key efficacy endpoints at Week 26

		PBO (n=153)	ERTU 5 mg (n=156)	ERTU 15 mg (n=153)
Change from baseline, LS mean (95% CI)	HbA1c (%)	−0.10 (−0.23, 0.04)	−0.78 (−0.91, −0.65)	−0.86 (−0.99, −0.72)
	Difference vs PBO	----	−0.68 (−0.87, −0.50)*	−0.76 (−0.95, −0.58)*
	FPG (mg/dL)	−1.8(−7.7, 4.1)	−26.9 (−32.5, −21.2)	−33.1 (−38.7, −27.4)
	Difference vs PBO	----	−25.1 (−32.7, −17.5)*	−31.3 (−38.9, −23.6)*
	FPG (mmol/L)	−0.1 (−0.4, 0.2)	−1.5 (−1.8, −1.2)	−1.8 (−2.1, −1.5)
	Difference vs PBO	----	−1.4 (−1.8,−1.0)*	−1.7 (−2.2, −1.3)*
	Body weight (kg)	−1.3 (−1.8, −0.9)	−3.3 (−3.8, −2.9)	−3.0 (−3.5, −2.6)
	Difference vs PBO	----	−2.0 (−2.6, −1.4)*	−1.7 (−2.3, −1.1)*
	Systolic BP (mmHg)	−0.9 (−2.7, 1.0)	−3.8 (−5.5, −2.1)	−4.8 (−6.6, −3.1)
	Difference vs PBO	----	−3.0 (−5.4, −0.5)†	−4.0 (−6.4, −1.5)‡
HbA1c <7.0%, n (%)		26 (17.0)	50 (32.1)	61 (39.9)
Adjusted odds ratio (95% CI) vs PBO§		----	3.1 (1.7, 5.7)*	4.4 (2.4, 8.0)*

*P<0.001 vs PBO; †P=0.018 vs PBO; ‡P=0.002 vs PBO.
§Logistic regression model fitted with terms for treatment, baseline HbA1c, and baseline eGFR (continuous).
FPG values in mmol/L were converted from mg/dL by dividing by 18.

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Empagliflozin (EMPA) as add-on to linagliptin (LINA) and metformin in patients with type 2 diabetes (T2DM): a 24-week randomised, double-blind, parallel-group trial

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Background and aims: This Phase III study investigated the efficacy and safety of EMPA 10 mg and 25 mg vs placebo (PBO) as add-on to LINA 5 mg and metformin in patients with T2DM.
Materials and methods: Patients with HbA_{1c} ≥8.0 and ≤10.5% while receiving stable-dose metformin were treated with open-label LINA 5 mg (n=606) for 16 weeks. Subsequently, those with HbA_{1c} ≥7.0 and ≤10.5% were randomised to double-blind, double-dummy treatment with a single-pill combination of EMPA 10 mg/LINA 5 mg (n=112) or EMPA 25 mg/LINA 5 mg (n=111), or PBO plus LINA 5 mg (n=110) for 24 weeks. Endpoints included changes from baseline (randomisation) in HbA_{1c} (primary endpoint), fasting plasma glucose (FPG), and weight (key secondary endpoints) after 24 weeks of double-blind treatment.
Results: At week 24, EMPA 10 mg and 25 mg significantly reduced HbA_{1c}, FPG, and weight from baseline compared with PBO as add-on to LINA 5 mg and metformin (Table). Greater proportions of patients reached HbA_{1c} <7% at week 24 with EMPA 10 mg (37.0%) and 25 mg (32.7%) than PBO (17.0%; odds ratio [95% CI] vs placebo: 4.0 [1.9, 8.7] and 2.9 [1.3, 6.1], respectively; both p<0.01). Open-label LINA 5 mg reduced HbA_{1c} by -1.16 (SD 1.10)%. Adverse events are presented in the Table.
Conclusion: EMPA 10 mg and 25 mg improved glycaemic control and weight vs PBO as add-on to LINA 5 mg and metformin for 24 weeks and were well tolerated in patients with T2DM.

	Linagliptin 5 mg and metformin		
	Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
HbA_{1c} (%)			
Baseline	7.96 (0.08)	7.97 (0.08)	7.97 (0.08)
Change from baseline at week 24	0.14 (0.09)	−0.65 (0.08)	−0.56 (0.08)
Difference vs PBO at week 24		−0.79	−0.70
(95% CI)	–	(−1.02, −0.55)	(−0.93, −0.46)
p-value		<0.0001	<0.0001
FPG (mmol/L)			
Baseline	9.0 (0.2)	9.3 (0.2)	9.4 (0.2)
Change from baseline at week 24	0.3 (0.2)	−1.5 (0.2)	-1.8 (0.2)
Difference vs PBO at week 24		−1.8	−2.1
(95% CI)	–	(−2.3, −1.3)	(−2.6, −1.6)
p-value		<0.0001	<0.0001
Weight (kg)			
Baseline	82.3 (1.9)	88.4 (2.0)	84.4 (1.8)
Change from baseline at week 24	−0.3 (0.3)	−3.1 (0.2)	−2.5 (0.2)
Difference vs PBO at week 24		−2.8	−2.2
(95% CI)	–	(−3.5, −2.1)	(−2.9, −1.5)
p-value		<0.0001	<0.0001
Adverse events, n (%)*			
Any adverse event	75 (68.2)	62 (55.4)	57 (51.8)
Confirmed hypoglycemia†	1 (0.9)	0	3 (2.7)
Events requiring assistance	0	0	1 (0.9)
Events consistent with urinary tract infection‡	8 (7.3)	8 (7.1)	4 (3.6)
Events consistent with genital infection§	2 (1.8)	2 (1.8)	5 (4.5)

Baseline values are mean (SE). Changes are adjusted mean (SE) based on MMRM (including treatment, baseline estimated glomerular filtration rate, region, visit, and visit by treatment as fixed effects and baseline values for HbA_{1c} and the endpoint in question as linear covariates) in patients who received ≥1 dose of study drug during the double-blind period and had a baseline HbA_{1c} and ≥1 on-treatment value (observed cases, excluding values after initiation of rescue therapy). *Patients who received ≥1 dose of study drug during the double-blind period (n=110 for placebo, n=112 for empagliflozin 10 mg, n=110 for empagliflozin 25 mg). †Plasma glucose ≤3.9 mmol/L and/or requiring assistance. ‡Based on 79 MedDRA preferred terms. §Based on 138 MedDRA preferred terms. MedDRA v. 17.1.

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DPP4-i/SGLT-2i combination as add-on to metformin for type 2 diabetes mellitus: comparison to OADs, GLP-1RAs or basal insulin

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Background and aims: To compare the combination dipeptidyl peptidase-4 inhibitors/sodium glucose co-transporter-2 inhibitors (DPP-4i/SGLT-2i) against DPP-4i or SGLT-2i alone, or glucagon-like peptide-1 receptor agonists (GLP-1RA), thiazolidinediones (TZD), or sulfonylureas (SU) (Network 1) and as an alternative to initiating basal insulin (Network 2) in type 2 diabetes mellitus (T2DM) patients inadequately controlled on metformin (HbA1c% >6.5).

Materials and methods: We conducted a literature search and systematic review to identify suitable randomised-controlled studies published up to October 2015 including one or more treatments licensed (or pending) in the US or EU. We conducted a network meta-analysis (NMA) in WinBUGS using the methodology recommended by the NICE Decision Support Unit and pairwise direct meta-analyses with indirect treatment comparisons using the Bucher method. Network 1 included all relevant studies with one or more treatments of interest including both placebo and active controlled (head-to-head) studies. Network 2 included basal insulin studies and SU and DPP-4i as additional comparators, with DPP-4i acting as a common control treatment to enable comparisons to be made with DPP-4i/SGLT-2i. Results presented are the basecase analysis after 24(+/-6) weeks of follow-up.

Results: Network 1 included 44 studies: the average patient age was 56 years, duration of diabetes 6.4 years, baseline HbA1c 8.1%, weight 86 kg and 48% were female. Based on the random-effects model adjusted for baseline HbA1c, DPP-4i/SGLT-2i, when added to metformin, results in significantly lower HbA1c than DPP-4i, SGLT-2i, TZD, SU and placebo. The DPP-4i/SGLT-2i combination provides statistically significant benefits in terms of weight loss when compared with DPP-4i, TZD, SU or placebo. DPP-4i/SGLT-2i results in a significant reduction in systolic blood pressure compared with placebo and SU. The odds of hypoglycemia for the DPP-4i/SGLT-2i combination are not significantly different from placebo, SGLT-2i or DPP-4i alone, GLP-1RA or TZD but are significantly lower than SU. Network 2 included 7 studies with similar study population characteristics as Network 1. Based on the fixed effect NMA results, DPP-4i/SGLT-2i is non-inferior to basal insulin in reducing HbA1c but DPP-4i/SGLT-2i results in a significant reduction in weight. There were insufficient data to conduct an NMA for the systolic blood pressure or hypoglycemia outcomes. Based on direct estimates, the odds of hypoglycemia with DPP-4i/SGLT-2i is significantly lower than SU or insulin.

Conclusion: The results indicate that the addition of DPP-4i/SGLT-2i to metformin will provide improved glycemic control and beneficial blood pressure and weight effects, without increasing hypoglycemia risk.

Network 1: DPP-4i/ SGLT-2i/MET versus	Difference in HbA1c %‡	Difference in weight kg	Difference in SBP mmHg*	Odds ratio, hypo†
SGLT-2i/MET	-0.44 (-0.66, -0.22)†	0.10 (-0.50, 0.71)	1.10 (-1.47, 3.72)	0.78 (0.22, 2.51)
GLP-1RA/ MET	-0.26 (-0.52, 0.01)	-0.42 (-1.12, 0.30)	-2.02 (-4.67, 0.76)	0.58 (0.13, 2.32)
DPP-4i/MET	-0.49 (-0.72, -0.26)†	-2.04 (-2.68, -1.41)†	-1.92 (-4.51, 0.69)	0.91 (0.25, 3.21)
TZD/ MET	-0.33 (-0.60, -0.04)†	-4.27 (-5.00, -3.55)†	-2.13 (-5.55, 1.37)	1.86 (0.37, 8.79)
SU/ MET	-0.32 (-0.59, -0.05)†	-4.09 (-4.82, -3.35)†	-5.17 (-8.05, -2.18)†	0.15 (0.03, 0.70)†
placebo/MET	-1.13 (-1.37, -0.89)†	-1.81 (-2.45, -1.17)†	-3.55 (-6.18, -0.89)†	0.88 (0.24, 3.15)
baseline	-1.21 (-1.46, -0.96)†	-2.70 (-3.36, -2.03)†	-3.44 (-6.35, -0.57)†	1.9% (0.5%, 6.8%)*
Network 2: DPP-4i/ SGLT-2i/MET versus	Difference in HbA1c %	Difference in weight kg	Difference in SBP mmHg	OR of hypo‡ (Direct analysis)
Insulin/MET	0.11 (-0.10, 0.31)	-3.63 (-4.40, -2.86)†	-	0.18 (0.05, 0.68)†
DPP-4i/MET	-0.48 (-0.60, -0.36)†	-2.10 (-2.60, -1.60)†	-	0.97 (0.27, 3.54)
SU/ MET	-0.35 (-0.48, -0.22)†	-3.82 (-4.37, -3.27)†	-	0.26 (0.07, 0.99)†

Median (95% credible interval) or summary weighted mean (95% confidence interval) for direct analysis; † statistically significant result based on 95% credible interval/confidence interval; ‡ adjusted for baseline HbA1c%; * DPP-4i/SGLT-2i SBP data reported in one trial (saxagliptin/dapagliflozin data); ‡ symptomatic or reported hypoglycemia; * Absolute risk of hypoglycemia during follow-up; DPP-4i, dipeptidyl peptidase-4 inhibitors; GLP-1RA, glucagon-like peptide-1 receptor agonist; HbA1c, glycated hemoglobin; MET, metformin; SBP, systolic blood pressure; SGLT-2i, sodium glucose transporter-2 inhibitor; SU, sulfonylurea; TZD, thiazolidinedione

Basecase meta-analysis at 24 +/- 6 weeks: DPP-4i/SGLT-2i/MET compared with other drug classes + MET for T2DM patients inadequately controlled on MET

Disclosure: **L. Qin:** Employment/Consultancy; AstraZeneca.

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Canagliflozin improves risk factors of metabolic syndrome versus sitagliptin in patients with type 2 diabetes and metabolic syndrome on background metformin + sulphonylurea

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Background and aims: Metabolic syndrome (MetS) is defined as a combination of factors associated with increased risk of cardiovascular disease and type 2 diabetes mellitus (T2DM). Canagliflozin (CANA), an SGLT2 inhibitor, lowers plasma glucose by decreasing renal glucose reabsorption, thereby increasing urinary glucose excretion and leading to a net caloric loss. In patients with T2DM on background metformin (MET) and sulphonylurea (SU), CANA provided reductions in HbA1c, body weight, and blood pressure (BP) compared with sitagliptin (SITA) over 52 weeks. This post hoc analysis assessed the effects of CANA versus SITA on the components of MetS in patients with T2DM and MetS.

Materials and methods: In this randomised, double-blind study, patients (N = 755; mean age, 57 y; HbA1c, 8.1%; body mass index [BMI], 32 kg/m^2) received CANA 300 mg or SITA 100 mg as add-on to MET + SU for 52 weeks. MetS was diagnosed if patients met ≥2 of the following criteria: triglycerides ≥1.7 mmol/L; high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/L (men), <1.3 mmol/L (women); waist circumference ≥102 cm (non-Asian men), ≥88 cm (non-Asian women), >90 cm (Asian men), >80 cm (Asian women); diagnosis of hypertension or BP-related criteria (systolic BP [SBP] ≥130 mmHg or diastolic BP [DBP] ≥85 mmHg). Changes from baseline in HbA1c, fasting plasma glucose (FPG), BP, waist circumference, body weight, BMI, and lipids were assessed at Week 52.

Results: At baseline, 78% (n = 586) of patients with T2DM met the criteria for MetS; proportions were similar across treatment groups. Among patients with data available for all MetS criteria at baseline (n = 584), 37%, 37%, and 18% met 3, 4 or 5 criteria, respectively. CANA 300 mg provided greater reductions in HbA1c, fasting plasma glucose (FPG), SBP, DBP, body weight, waist circumference, and BMI versus

SITA 100 mg over 52 weeks (Table). Increases in low-density lipoprotein cholesterol (LDL-C) and HDL-C, and reductions in triglycerides were seen with CANA versus SITA. CANA was generally well tolerated.

Conclusion: CANA improved all components of MetS versus SITA over 52 weeks in patients with T2DM and MetS on background MET + SU.

Table. Changes From Baseline in MetS Risk Factors at Week 52 (mITT, LOCF)*

Parameter	CANA 300 mg (n = 289)	SITA 100 mg (n = 297)	Parameter	CANA 300 mg (n = 289)	SITA 100 mg (n = 297)
HbA1c change, %	−1.07 (0.05)	−0.59 (0.06)	Waist circumference change, cm	−2.1 (0.3)	−0.02 (0.3)
Difference vs SITA	−0.48 (−0.62, −0.34)		*Difference vs SITA*	−2.1 (−2.9, −1.3)	
FPG change, mmol/L	−1.8 (0.1)	−0.3 (0.1)	BMI change, kg/m^2	−0.8 (0.1)	0.1 (0.1)
Difference vs SITA	−1.5 (−1.9, −1.2)		*Difference vs SITA*	−0.9 (−1.1, −0.7)	
SBP change, mmHg	−5.5 (0.8)	1.1 (0.8)	LDL-C change, mmol/L	0.19 (0.05)	0.00 (0.05)
Difference vs SITA	−6.6 (−8.6, −4.6)		*Difference vs SITA*	0.18 (0.06, 0.30)	
DBP change, mmHg	−3.2 (0.5)	−0.1 (0.5)	HDL-C change, mmol/L	0.07 (0.01)	−0.00 (0.01)
Difference vs SITA	−3.1 (−4.3, −1.9)		*Difference vs SITA*	0.07 (0.04, 0.10)	
Body weight change, kg	−2.4 (0.2)	0.3 (0.2)	Triglycerides change, mmol/L	−0.04 (0.06)	0.02 (0.07)
Difference vs SITA	−2.6 (−3.2, −2.0)		*Difference vs SITA*	−0.07 (−0.24, 0.10)	

mITT, modified intent-to-treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; CI, confidence interval.
*Data are LS mean (SE) change from baseline and SITA-subtracted LS mean difference (95% CI).

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Canagliflozin and dapagliflozin, but not liraglutide, reduce Alanine Aminotransferase (ALT) levels in type 2 diabetes: a difference not explained by HbA_{1c} or weight change

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Background and aims: Sodium Glucose Transporter-2 (SGLT2) inhibitors and Glucagon Like Peptide-1 receptor agonists (GLP-1 RA) are both contemporary antidiabetic agents (ADA), with added benefits of weight loss. The effects of these ADAs on markers of fatty liver disease are uncertain and may be influenced by their opposing effects on glucagon. The present retrospective study investigates the effects of canagliflozin (Cana), dapagliflozin (Dapa) and liraglutide (Lira) on Alanine Aminotransferase (ALT) levels.

Materials and methods: Electronic medical records of patients followed in seven diabetes and endocrinology clinics in Ontario, Canada were queried. Study criteria were type 2 diabetes; initiation of Lira, Cana or Dapa between 2011 and 2015; drug persistence > 3 months; non-pregnant; no history of gastric bypass surgery, liver transplant, or hepatitis B or C. Subjects without a baseline and follow-up ALT were excluded. Baseline ALT was defined as the closest measurement prior to ADA initiation. Follow-up ALT was the last measurement, while the patient was still on the ADA, within 1 year after drug initiation. Within group comparisons were performed using paired t-tests. Differences in ALT change between the 3 drugs were assessed with analysis of variance (ANOVA), with a Bonferroni adjustment for multiple group comparisons. Analysis of covariance (ANCOVA) was used to investigate the association between ALT change between the 3 drugs, adjusting for A1C change and weight change.

Results: We identified a total of 2,820 subjects who met study inclusion and exclusion criteria. 56% of the subjects were male. Baseline characteristics of the cohort are outlined in Table 1. Compared to baseline, follow-up ALT levels were lower in Cana (-4.3 U/L, p <0.01) and Dapa (-4.1 U/L, p <0.01), with no significant differences in Lira (-0.06 U/L, p=0.22). Between group differences were significant for Cana vs. Lira (mean = -3.7 U/L, Confidence Interval (CI) = -5.4 to -2, p<0.05) and Dapa vs. Lira (-mean = 3.5 U/L, CI = -5.5 to -2, p<0.05), but not for Cana vs. Dapa comparison. After adjustment for A1C and weight change, both the Cana (-2.7, p<0.001) and Dapa (-2.5, p<0.001) cohorts maintained significantly lower ALT levels compared to the reference group of Lira.

Conclusion: Subjects with type 2 diabetes initiated on Canagliflozin and Dapagliflozin were observed to have significantly lower levels of ALT in this study, compared to no significant change seen among those initiating Liraglutide. Additionally, this differential effect on ALT for SGLT2 inhibitors and GLP-1 RA in our study does not appear to be explained by A1C or weight change. Effects of these two drug classes on markers of fatty liver disease should be investigated further in future studies in addition to glucagon stimulation/suppression as a potential mechanism of action to explain these differential liver effects.

Table 1. Baseline characteristics and comparison of Alanine Aminotransferase (ALT), Glycated hemoglobin (A1C) and body weight changes within the three study cohorts

N=Subjects meeting study inclusion and exclusion criteria	Canagliflozin (N=1388)	Dapagliflozin (N=692)	Liraglutide (N=740)
Age (years)	58.2 ± 10	57.4 ± 10	57.0 ± 9
Duration of type 2 diabetes (years)	13 ± 7	12.7 ± 8	13.4 ± 7
Baseline ALT (U/L)	31.2 ± 21	31 ± 23	31.4 ± 19
Follow-up ALT (U/L)	26.9 ± 17	26.9 ± 15	30.8 ± 19
p-value for change in ALT	<0.01	<0.01	0.22
Baseline A1C (%)	8.4 ± 1.3	8.5 ± 1.3	8.3 ± 1.5
Follow-up A1C (%)	7.6 ± 1.1	7.7 ± 1.2	7.9 ± 1.5
p-value for change in A1C	<0.01	<0.01	<0.01
Baseline body weight (kg)	92.8 ± 22	89.1 ± 21	101.8 ± 23
Follow-up body weight (kg)	90 ± 22	88.3 ± 21	99.4 ± 23
p-value for change in body weight	<0.01	<0.01	<0.01

Disclosure: **H.S. Bajaj:** None.

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Effects of dapagliflozin on insulin-requirement, glucose excretion and beta-hydroxybutyrate levels is not related to baseline A_{1c} in youth with type 1 diabetes

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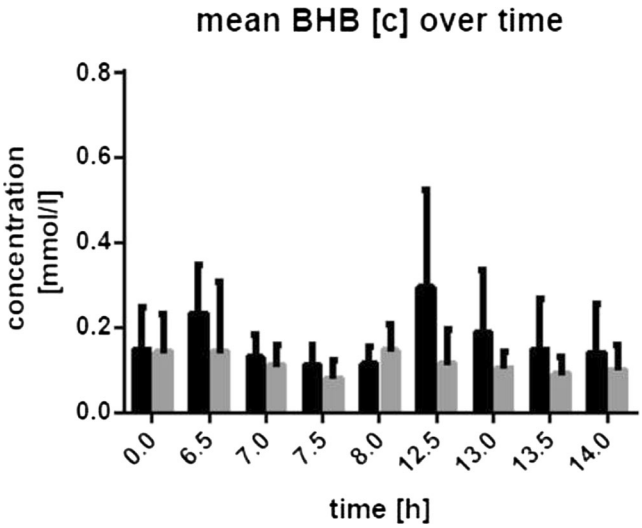
Background and aims: Youth with type 1 diabetes (T1D) infrequently achieve A1c targets. This is the first study to assess the safety, tolerability, and pharmacokinetics of a SGLT-2 inhibitor as add on to insulin in relationship to A1c in youth.

Materials and methods: In a placebo-controlled, randomized, crossover study, the effect of a single dose of 10mg dapagliflozin (DAPA) on the insulin dose administered i.v. during a glucose-infusion for the ensuing 24 hours with blood glucose kept between 160 - 220 mg/dl was studied in 33 youth (14 males, age: 16 (12-21) [median(range) years], diabetes duration 8 years (2-16)), stratified according to glycemic control being within target (n=11, A1c 5.5 to 7.4%), moderately elevated (n=11, 7.5 - 9.0%) or clearly elevated (n=11, 9.1 - 12.5%)(DCA, Siemens).

Results: DAPA reduced mean i.v. insulin dose by 13.6% (P<0.0001 by ANOVA). This was irrespective of baseline A1c (mean [CI 95%] DAPA vs.Placebo: in target: 0.87 [0.81-0.92] vs. 0.99 [0.93-1.05] U/kg/24h; moderately elevated: 0.90 [0.81-0.92] vs. 1.02 [0.95-1.09] clearly elevated: 0.99 [0.91-1.06] vs. 1.17 [1.09-1.25]). Urinary glucose excretion was A1c-independently increased by 610% (143.12 [128.39-157.84] vs. 22.40 [7.68-37.13]; P<0.0001). 6 independent episodes in 6 patients with plasma ß-hydroxybutyrate levels between ≥0.6 and <1.0 mmol/l have been observed after a standardized liquid meal challenge, 5 episodes in the DAPA and 1 in the placebo group (Figure). Although the amount of liquid

meal (6 ml/kg BW) was set to a maximum of 360 ml per dose and only 4 patients did not receive the maximum, there was no correlation between the amounts of meal intake per weight compared to excess of ß-hydroxybutyrate: correlation coefficient (with 95% CI) for placebo was -0.01 [-0.13 - 0.11], p=0.25), for DAPA: -0.07 [-0.19 - 0.05], p=0.88).
Conclusion: In youth with T1D, DAPA led to a significant reduction of insulin needed to achieve target glucose by increasing glucose excretion irrespective of preexisting A1c levels. In previous studies positive urine ketones were observed more frequently with DAPA 5 mg and DAPA 10 mg during study drug treatment, yet were similar to baseline. In the present study, slightly elevated ß-hydroxybutyrate levels were seen with DAPA. The amount of standardized meal intake or baseline A1c had no influence on ß-hydroxybutyrate levels. Although these mildly elevated levels were far below those associated with clinical diabetic ketoacidosis further mechanistic studies are necessary to elucidate the underlying pathophysiology. Nevertheless, this study provides a proof of concept for adjunct SGLT-2 inhibitor therapy in the pediatric age group.



Clinical Trial Registration Number: NCT02325206
Disclosure: **T. Biester:** Honorarium; Astra Zeneca.

OP 32 "Devices and desires" (Thomas Cranmer, 1548)

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Accuracy and longevity of an implantable continuous glucose sensor in the precise study: a 180 day, prospective multi-centre pivotal trial
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Background and aims: The accuracy, longevity and glycaemic effects of a new long-term implantable continuous glucose monitoring system (CGM) was investigated over a 6 months period.
Materials and methods: The Eversense® CGM System (Senseonics Inc. MD, USA) is composed of an implantable, fluorescence-based glucose sensor and a wearable transmitter that enables on-body vibration alerts and wirelessly communicates with a smartphone-based medical app to display glucose results. In this prospective, single-arm investigation 71 adult subjects with T1DM and T2DM were enrolled at 7 clinical sites. Patients used the CGM system at home and in-clinic. During 8 in-clinic visits (8h-24h) venous reference glucose measurements were taken (YSI2300 Stat plus). Mean Absolute Relative Difference (MARD) and Mean Absolute Difference (MAD) between YSI and CGM measured glucose was calculated. Change in HbA1c from baseline to last visit was assessed for those continuing beyond 90 days in the study. After the study, improvements were made to the glucose calculation algorithm.
Results: MARD over the full glycaemic range (2.2 − 22.0 mmol/L; 40 − 400 mg/dL) was 11.6% (n=21527, SD 11.2%, 95%CI 10.9% − 12.2%). MARD for reference glucose >4.2 mmol/L (75 mg/dL) was 11.1% (n=20470, SD 10.1%, 95%CI 10.5% − 11.7%). Mean Absolute Difference (MAD) for reference glucose ≤4.2 mmol/L (75 mg/dL) was 0.8 mmol/L (14.2 mg/dL) (n=1057, SD 0.75 mmol/L, 95%CI 0.7 − 0.9 mmol/L; SD 13.5 mg/dL, 95%CI 12.1 − 15.4 mg/dL). A Kaplan-Meier analysis for survivability of the sensors found 82% and 40% functioning through the 90 day and 180 day in-clinic evaluation sessions, respectively (median survival: 149 days, IQR 97 - 180). The participant median CGM wear duration was found to be 23.5 hours per day. HbA1c decreased from baseline to last visit from 7.5% to 7.2% (n=55, paired Student's t-test, p<0.001). Upon applying the algorithm improvements to the study raw measurement data set, the full glycaemic range MARD decreased from 11.6% to 10.5% (95%CI 9.9% − 11.1%, p<0.001).
Conclusion: The Eversense® implantable CGM system was accurate with a MARD of 11.1% for reference glucose >4.2 mmol/L (75 mg/dL) and had a median sensor life of 149 days. Wear time was over 23 hours per day. Use of the implantable glucose sensor was found to be associated with decreased HbA1c.
Clinical Trial Registration Number: NCT02154126
Supported by: Senseonics Incorporated
Disclosure: **J. Kropff:** Employment/Consultancy; J.K. is a consultant/advisor on the speaker bureaus for Senseonics and Dexcom.

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Hybrid closed-loop (HCL) pivotal trial in type 1 diabetes

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Background and aims: A hybrid closed-loop (HCL) system was examined in a pivotal trial with the aim to establish the system's safety for use in adolescents and adults (age 14-75 years) with type 1 diabetes.
Materials and methods: The system included the Medtronic MiniMed 670G pump, a fourth-generation glucose sensor, and a proportional-integral-differential control algorithm. Following a 2-week run-in phase to establish baseline parameters, 124 subjects (55 male), with age 37.8 ±16.5 (range, 14-75) years, diabetes duration 21.7±13.7 years, and HbA1c 7.4±0.9% entered a 3-month study phase. The study phase was followed by an optional continued-access program. Subjects calibrated the sensors periodically and administered mealtime and correction boluses as needed. Sensor glucose (SG) and HbA1c values from baseline and study phases were compared. Glycemic variability was measured by the standard deviation (SD) and coefficient of variation (CV) of SG values. Subjects were categorized according to baseline HbA1c values and within each category, the change in HbA1c was calculated. The change in GV from baseline to the final 2 weeks of the study was also calculated for subjects in each starting HbA1c category.
Results: The HCL auto-mode was used for a median of 87.2% (IQR, 75.0% to 91.7%) of the time after first start. There were higher percentages of SG 71-180 mg/dL, lower percentages of SG ≤70 mg/dL, and lower percentages of SG ≤50 mg/dL during 24 hours and at night ($p<0.001$ for each) in the study phase compared to baseline (Table). Mean HbA1c decreased from 7.4±0.9% to 6.9±0.6% (overall ΔHbA1c = -0.5 percentage points) and the proportion of subjects with HbA1c <7% increased from 33.1% to 55.3%. Overall SD decreased from 56.5 mg/dL to 52.5 mg/dL, and CV decreased from 37.6% to 34.7% ($p<0.001$ for each). For subjects with starting HbA1c <7.0%, ΔHbA1c and ΔSD were -0.1 percentage points and -4.5 mg/dL. For subjects with starting HbA1c from 7.0% to 7.5%, ΔHbA1c and ΔSD were -0.3 percentage points and -2.1 mg/dL. For subjects with starting HbA1c >7.5%, ΔHbA1c and ΔSD were -1.0 percentage points and -4.7 mg/dL. There was no DKA, severe hypoglycemia, or serious device-related adverse event during 12,389 patient-days of use. At study's end, 99 patients entered the continued-access program.
Conclusion: Subjects with the highest baseline HbA1c values experienced the largest reductions in HbA1c and glycemic variability. The HCL system was safe, acceptable, and associated with improved glucose control during day and nighttime periods during extended at-home use.

Table. Sensor glucose (SG) values in the hybrid closed-loop (HCL) study

Parameter	All 24 Hours (mean±SD, (median))			10:00PM to 7:00AM (mean±SD, (median))		
	Baseline	HCL Period	p	Baseline	HCL Period	p
SG, mg/dL	150.2±22.7 (150.1)	150.8±13.7 (149.9)	0.644	148.8±25.1 (146.9)	147.5±14.1 (146.3)	0.380
SG>180 mg/dL (%)	27.4±13.7% (26.7%)	24.5±9.2% (24.1%)	< 001	26.9±15.2% (26.4%)	21.6±9.9% (20.6%)	< 001
70 < SG ≤ 180 mg/dL (%)	66.7±12.2% (67.8%)	72.2±8.8% (73.4%)	< 001	66.8±14.0% (67.0%)	75.3±9.8% (76.4%)	< 001
SG ≤70 mg/dL (%)	5.9±4.1% (5.25%)	3.3± 2.0% (2.9%)	< 001	6.4±5.3% (5.3%)	3.1±2.2% (2.6%)	< 001
SG ≤50 mg/dL (%)	1.0±1.1% (0.6%)	0.6±0.6% (0.4%)	< 001	1.1±1.5% (0.6%)	0.6±0.7% (0.5%)	< 001

Clinical Trial Registration Number: NCT02463097
Disclosure: **R.M. Bergenstal:** Grants; Medtronic, Inc.

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Home use of a bihormonal bionic pancreas vs conventional insulin pump therapy in adults with type 1 diabetes: a multicenter randomised clinical trial

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Background and aims: The safety and effectiveness of continuous, multi-day, automated glycemic control using insulin and glucagon have not been tested in a home-use setting. We evaluated the efficacy of a bihormonal bionic pancreas in adults with type 1 diabetes living at home and performing their normal activities without restrictions on diet or exercise.
Materials and methods: We performed a random-order cross-over study comparing glycemic regulation with a bihormonal bionic pancreas versus conventional insulin pump therapy over 11 days each. During the bionic pancreas arm, data from a continuous glucose monitor was used by an autonomously adaptive algorithm to control subcutaneous delivery of insulin and glucagon. During the comparator arm, participants managed their own conventional insulin pump therapy. The co-primary outcomes were the mean glucose level and time <60 mg/dl by continuous glucose monitoring analyzed over days 2-11 in participants completing both arms of the study according to a modified intention-to-treat principle.
Results: The bionic pancreas was associated with a reduction in the mean glucose level and a reduction in hypoglycemia when compared to conventional insulin pump therapy in adults with type 1 diabetes living at home and participating in their normal daily activities. The bionic pancreas was associated with a reduction in the mean glucose level (162±29 versus 141±10 mg/dl, $p<0.0001$) and reduced time <60 mg/dl (1.9±1.7 versus 0.6±0.6%, $p<0.0001$) relative to the comparator.
Conclusion: The bionic pancreas was associated with a reduction in the mean glucose level and a reduction in hypoglycemia when compared to conventional insulin pump therapy in adults with type 1 diabetes living at home and participating in their normal daily activities.
Clinical Trial Registration Number: NCT02092220
Supported by: NIDDK 1R01DK097657-01 and 1DP3DK101084-01, NIH-NCATS-CTSA grants UL1 TR001
Disclosure: **S.J. Russell:** Honorarium; Tandem, Novo Nordisk. Lecture/other fees; Tandem, Sanofi, Dexcom, Eli Lilly. Non-financial support; Dexcom, Tandem Diabetes, SweetSpot Diabetes, International Biomedical, Abbott Diabetes Care, Insulet Corporation, Medtronic. Stock/Shareholding; Companion Medical. Other; patents pending on bionic pancreas.

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Telemedicine and continuous glucose monitoring in paediatric type 1 diabetes care

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Background and aims: Attaining and maintaining glycemic control remains burdensome and elusive in insulindependent children, even with

the use of continuous glucose monitoring (CGM) systems. Retrospective analysis of glucose monitoring data by a healthcare professional during regular check-ups are valuable but lack the element of opportunity, decreasing the value of medical intervention. We aimed to evaluate if the use of telemedicine with continuous glucose monitoring for real-time visualisation of glucose values by a medical professional and consequently real time adjustments of diabetes treatment under medical supervision is feasible and capable of adding benefit to paediatric diabetes care in an outpatient setting.

Materials and methods: In this six-month, prospective, open-label study we enrolled 30 children with type 1 diabetes (T1D) using a combined telemedicine-CGM system continuously supervised by a medical professional, and 30 children using just CGM (the control group), with historical data analysis during regular check-ups. Children had diabetes for more than 1 year and were treated with a basal-bolus insulin regimen (>6 months). The CGM system used was Dexcom G4® PLATINUM (Dexcom, San Diego, CA). All children were adherent to ongoing use of the devices. We developed a centralised telemedicine system integrated with the CGM devices, in order to visualise real-time CGM data simultaneously by family, caregivers and medical staff. A healthcare professional was in charge 24/24 hours to supervise children' glycemic excursions and to intervene in case of dangerous glycemic values and detected patterns of hypo/hyperglycemia, in the telemedicine group. The variables studied were the difference in time spent in and outside a glucose target of 90-160 mg/dL (5.0- 8.8 mmol/L), mean glycemic value, HbA1c and average daily insulin dose in the telemedicine group as compared to CGM control group. Data were analysed at 3 and 6 months of intervention.

Results: Study groups characteristics were: telemedicine group (n=30), 53% boys, mean age 7.5 ± 2.7 years, T1D of 2.4 ± 0.9 years; control group (n=30), 50% boys, mean age 7.6±2.5, T1D of 2.1±0.7 years. There were no significant differences between groups regarding BMI, daily insulin dose and HbA1c at inclusion (7.8±0.4% telemedicine vs 7.7±0.3% control group, p=0.32). Use of telemedicine was associated with a significant increase in time spent in normal glycemic range (78% versus 70%, p<0.001), less time spent in hypoglycaemic range (5% vs 9%, p< 0.001),improved mean glycemia (138±16 mg/dl vs 149±14 mg/dl, p<0.001) and HbA1c (6.93%±0.4% vs 7.2±0.5%, p<0.001) (all data showed at 6 months).We noted a daily insulin dose increase in telemedicine group (17±5,4units at start vs 19.4±6.2units at 6 months, p<0.01), maybe due to a more "ambitious" approach of adjusting insulin dose under the surveillance of medical staff. There were no severe hypoglycaemias in either groups.

Conclusion: Our study showed that just viewing and collecting data is not enough. Telemedicine and medical supervision provide improvement in glycemic control and decrease hypoglycaemic burden but, most important, enable the process of active learning, real life adaptation and self-care skills acquisition. Using telemedicine with CGM systems not only allowed to reach more patients, from remote areas, but mainly enabled continuous care and enhanced the clinical utility of these monitoring systems, providing progressive refinement and fine tuning in paediatric diabetes treatment

Disclosure: **M.V. Vlaiculescu:** None.

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Real-time continuous glucose monitoring improves time spent in euglycaemia and prevents severe hypoglycaemia in type 1 diabetes mellitus patients with impaired awareness of hypoglycaemia

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Background and aims: Real-time continuous glucose monitoring (RT-CGM) reduces HbA1c without increasing hypoglycaemia. Type 1 diabetes mellitus (T1DM) patients with impaired awareness of hypoglycaemia (IAH) have a threefold to sixfold increased risk of severe hypoglycaemia. Whether RT-CGM prevents hypoglycaemia in this high risk (IAH) population has yet to be established.

Materials and methods: Fifty-two adult T1DM patients with IAH as confirmed by a Gold score ≥4 were enrolled in a two-centre, randomised, cross-over trial with a 12-week wash-out period in between 16-week intervention periods comparing RT-CGM (without low-glucose suspend) with self-monitoring of blood glucose (SMBG). During SMBG, participants wore a blinded CGM continuously. CGM data were analysed on an intention-to-treat basis with time spent in euglycaemia [4-10 mmol/L] as primary endpoint. Severe hypoglycaemia (requiring third party assistance) was a secondary endpoint. Mixed model analysis was used to analyse time spent in euglycaemia. The Wilcoxon matched-pair signed-rank test was used to analyse the incidence rates of severe hypoglycaemic events. The proportion of patients experiencing at least one severe hypoglycaemic event was analysed with a generalized estimating equation model.

Results: Twenty-eight (53.8%) of enrolled patients were male, HbA1c was 7.5% ±0.8%, age 48.6 ±11.6 years, Gold score 5.4 ±0.7 and 23 (44.2%) patients were treated with continuous subcutaneous insulin infusion (CSII). Time spent in euglycaemia was significantly higher during RT-CGM: 65.0% vs. 55.4% (difference 9.6%, 95%CI 8.0-11.2, p<0.0001). In addition, both time spent ≤3.9 mmol/L (6.8% vs. 11.4%, difference 4.7%, 95%CI 3.4-5.9, p<0.0001) and time spent >10 mmol/L (28.2% vs. 33.2%, difference 5.0%, 95%CI 3.1-6.9, p<0.0001) were lower during RT-CGM. Also, less time was spent ≤3.9 mmol/L at night-time (0000-0600h) during RT-CGM (7.6% vs. 13.3%, difference 5.7%, 95%CI 3.2-8.2, p<0.0001). During RT-CGM, the number of severe hypoglycaemic events was lower (14 events vs. 34 events, p=0.03) and also the proportion of patients experiencing at least one severe hypoglycaemic event was lower (21% vs 38%, OR 0.48, 95%CI 0.22-1.04, p=0.06), with no interaction for treatment modality (CSII vs. multiple daily injection), p=0.4. HbA1c at the end of each period was similar (7.3% and 7.3%, p=0.8).

Conclusion: Real-time continuous glucose monitoring diminished severe hypoglycaemia in T1DM patients with IAH. Moreover, RT-CGM improved glycaemic control by decreasing both time spent in hypoglycaemia and time spent in hyperglycaemia. These data support the use of RT-CGM in this high risk population.

Clinical Trial Registration Number: NCT01787903

Supported by: Unrestricted grant from Eli Lilly and Sanofi

Disclosure: **C.A.J. van Beers:** Grants; The research leading to these results has received unrestricted grants from Ely Lilly and Sanofi.

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Faster-acting insulin aspart: faster offset of exposure and action in addition to faster onset in subjects with type 1 diabetes using continuous subcutaneous insulin infusion

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Background and aims: In an insulin pump setting, the ideal insulin would have a fast onset as well as a fast offset of glucose-lowering effect in order to provide effective post-prandial glucose control

without the risk of late post-meal hypoglycaemia. Faster-acting insulin aspart (faster aspart) is insulin aspart (IAsp) in a new formulation containing two well-known excipients, L-arginine and niacinamide, which result in a stable formulation with faster initial absorption. Faster aspart has demonstrated onset twice as rapid producing greater early exposure and glucose-lowering effect compared with IAsp in subjects with type 1 diabetes (T1D) using continuous subcutaneous insulin infusion (CSII) or subcutaneous injection. Here, we present the results of a *post-hoc* analysis evaluating the offset of exposure and glucose-lowering effect with faster aspart in an insulin pump setting.

Materials and methods: In a randomised, double-blind, crossover trial, 48 subjects with T1D (mean±SD age 46±9 years, HbA_{1c} 7.4±0.6%) received faster aspart or IAsp as a 0.15 U/kg bolus dose using CSII on top of a basal rate (0.02 U/kg/h). An automated glucose clamp (target 5.5 mmol/L) was conducted from 13 hours pre-bolus dose to 14 hours post-bolus dose.

Results: The mean serum insulin aspart concentration profile following a bolus dose administered by CSII was shifted to the left for faster aspart compared with IAsp. The left-shift for faster aspart vs. IAsp was seen in the late part as well as in the early part of the profile (Figure). The offset of insulin exposure, as measured by time to 50% of maximum insulin concentration in the late part of the pharmacokinetic profile ($t_{Late\ 50\%\ Cmax}$), occurred 35.4 min earlier for faster aspart than for IAsp (estimated treatment difference faster aspart - IAsp [95% CI] -35.4 min [-47.0;-23.8]), and the time to 50% of maximum insulin concentration in the early part of the pharmacokinetic profile ($t_{Early\ 50\%\ Cmax}$) was 11.8 min shorter for faster aspart than for IAsp (estimated treatment difference -11.8 min [-14.4;-9.2]). The findings for onset and offset of insulin exposure also translated to similar findings for glucose-lowering effect. There was a faster offset of glucose-lowering effect ($t_{Late\ 50\%\ GIRmax}$; estimated treatment difference -24.0 min [-38.9;-9.1]) as well as a faster onset of glucose-lowering effect ($t_{Early\ 50\%\ GIRmax}$; estimated treatment difference -11.1 min [-15.4;-6.9]) with faster aspart vs. IAsp.

Conclusion: In addition to a faster onset and greater early exposure and glucose-lowering effect with faster aspart vs. IAsp, faster aspart also provides a faster offset of exposure and glucose-lowering effect in subjects with T1D using CSII, thereby better mimicking the physiologic insulin action profile. Thus, using CSII faster aspart has the potential to improve post-prandial glucose control with lower risk of late post-meal hypoglycaemia.

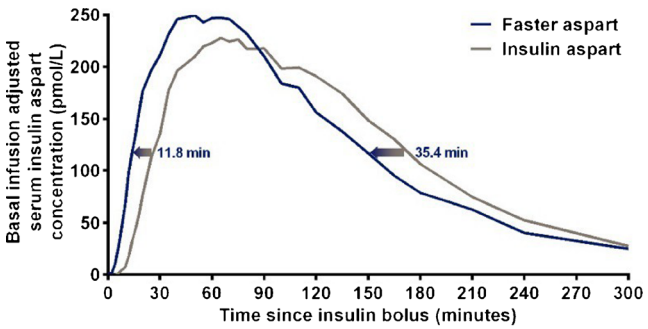

Figure: Mean serum insulin aspart concentration following a bolus dose of 0.15 U/kg faster aspart or insulin aspart administered by continuous subcutaneous insulin infusion.

Clinical Trial Registration Number: NCT01992588
Supported by: Novo Nordisk
Disclosure: **E. Zijlstra:** Grants; Adocia, AstraZeneca, Becton Dickinson, Biocon, Boehringer Ingelheim, Dance Biopharm, Eli Lilly, Grünenthal, Gulf Pharmaceutical Industries, Johnson & Johnson, Marvel, MedImmune, Medtronic, Novartis, Novo Nordisk, Roche Diagnostics, Sanofi, Senseonics, Zealand Pharma. Other; Novo Nordisk.

OP 33 Vascular disease: mechanisms and management

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The reduction in small dense low-density lipoproteins by liraglutide is independently associated with reduced carotid atherosclerosis in patients with type 2 diabetes

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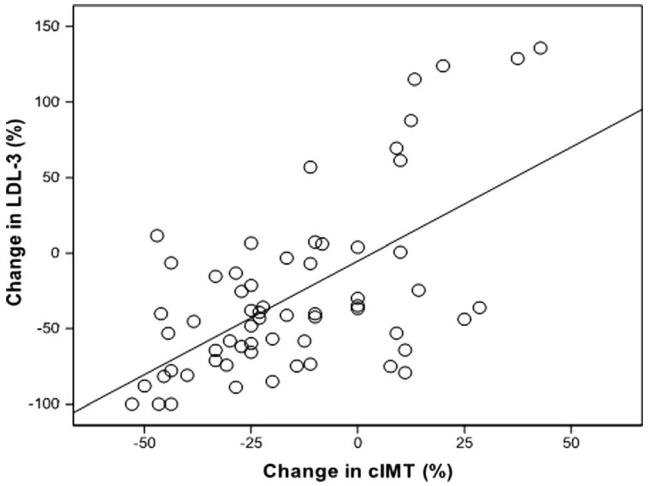
Background and aims: Liraglutide has a number of significant non-glycemic effects, including those on plasma lipids; however, its effect on distinct lipoprotein subclasses is still unknown. In recent years it became evident that the "quality" and not only the "quantity" of cholesterol is strongly associated with cardiovascular (CV) risk, and the presence of small dense (sd) low-density lipoproteins (LDL) is a novel CV risk marker.

Materials and methods: 62 patients with type-2 diabetes (T2DM) (31 men, 31 women; age: 61±9 years) naïve to incretin-based therapies, were treated with liraglutide (1.2 mg/day) as add on therapy to metformin (1500-3000 mg/day) for 4 months. Laboratory analyses included the assessment by gel electrophoresis (Lipoprint, Quantimetrix, USA) of the full LDL subclass profile: from larger and less atherogenic LDL-1 and LDL-2, to smaller denser LDL-3 to LDL-7. Carotid-intima media thickness (cIMT), as a marker of subclinical atherosclerosis was assessed by color doppler ultrasound.

Results: Statistical analysis was performed using paired t-test, Spearman correlation and regression analysis. After 4 months of liraglutide therapy we found significant reductions in fasting glycemia (from 9.1±4.0 to 7.3±2.0 mmol/L, p<0.0010), A1c (from 8.4±1.5 to 6.9±1.1%, p<0.0001), body mass index (from 30±5 to 29±5 kg/m2, p<0.0001), waist circumference (from 106±13 to 103±12 cm, p<0.0001), total-cholesterol (4.7±1.1 to 4.2 ±0.9 mmol/L, p=0.0020), triglycerides (from 1.9±1.1 to 1.6±0.7 mmol/L, p=0.0061) and LDL-cholesterol (from 2.7±1.1 to 2.3±0.8 mmol/L, p=0.0089), while no significant changes were found in high density lipoprotein-cholesterol. We calculated the cholesterol content (in mmol/L) in LDL subclasses and we found an increase in LDL1 (from 0.98±0.45 to 1.12 ±0.46, p=0.0401), with a concomitant reduction in LDL3-C and LDL4-C (from 0.55±0.34 to 0.25±0.14, p<0.0001 and from 0.15±0.20 to 0.07±0.10, p=0.0013, respectively). Liraglutide also reduced cIMT (from 1.13±0.29 to 0.92±0.24 mm, p<0.0001), and a significant correlation was observed between changes in cIMT and those in LDL-3 (r=0.501; p<0.0001, Figure). At multivariate analysis, including anthropometric parameters, glycemia, A1c, plasma lipids and LDL subclasses, only sdLDL-3 were found independent predictors of changes in cIMT (p<0.0001).

Conclusion: We provide the first evidence showing that treatment with liraglutide significantly reduces sdLDL. Importantly, this reduction was a major contributor to reduction of carotid atherosclerosis. This favorable modulation of atherogenic lipoproteins, independently of several confounders including glycemic control, may contribute to explain the potential positive effect of liraglutide on major CV outcome.



Clinical Trial Registration Number: NCT01715428
Disclosure: **D. Nikolic:** Other; DN has participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

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The dynamics of endothelial progenitor cells and vascular endothelial growth factor in patients with type 2 diabetes after endovascular interventions

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Background and aims: To study dynamics of endothelial progenitor cells (EPCs) and vascular endothelial growth factor A (VEGF-A) in patients with type 2 diabetes mellitus (T2DM) after endovascular interventions on coronary and peripheral arteries.
Materials and methods: We observed 68 patients (40 men, mean age 66 ± 8.4 years), admitted for elective percutaneous coronary intervention (PCI) or endovascular revascularization of lower extremity. Number of CD34 + VEGFR2 + CD45- cells and CD34 + CD133 + CD45- cells, and level of VEGF-A were determined 1-2 days before endovascular intervention and in 2-4 days after the surgery. Thirty nine patients with T2DM were included in group 1 (22 women, mean age 66.5 ± 7.9 years), 29 patients without T2DM were included in group 2 (21 men, mean age 65.5 ± 9.1 years). PCI was done for stable angina. Endovascular revascularization of lower extremity was done for critical limb ischemia (CLI). In group 1 PCI was performed in 18 patients, endovascular limb revascularization was performed in 21 patients. In group 2 PCI was performed in 18 patients, endovascular limb revascularization was performed in 11 patients.
Results: We found that in non-diabetic patients levels of EPCs have increased significantly after endovascular interventions (CD34 + VEGFR2 + CD45- cells: 0.011 ± 0.005% and 0.015 ± 0.006% before and after procedure, respectively; $p < 0.001$ and CD34 + CD133 + CD45- cells: 0.018 ± 0.010% and 0.022 ± 0.007% before and after procedure, respectively; $p = 0.041$). Levels of EPCs in the peripheral blood of patients with T2DM before and after endovascular interventions did not significantly differ. Analysis of VEGF-A showed the statistically significant increase after intervention in patients with T2DM (461.3 ± 262.4 pg/ml and 569.7 ± 451.2 pg/ml before and after procedure, respectively, $p < 0.05$). We divided patients with T2DM into 2 subgroups: the 1st subgroup included 17 patients with HbA1c≤7.5%, the 2nd subgroup included 22 patients with HbA1c≥7.5%. In the 1st subgroup the increase of CD34 + VEGFR2 + CD45- cells ($p = 0.001$) and CD34 + CD133 + CD45- cells ($p = 0.003$) were observed after endovascular intervention.While EPCs levels in the peripheral blood of patients in the 2nd subgroup before and after endovascular interventions did not significantly differ.
Conclusion: The study has shown that diabetic patients have impaired of EPCs mobilization after endovascular intervention, despite the significant increase of the factor, stimulating their mobilization (VEGF-A). In addition, the dynamics of EPC levels depended on the glycaemic control. Thus, levels of EPCs were significantly higher after endovascular interventions in diabetic patients with good glycaemic control (HbA1c≤7.5%).
Disclosure: **M.S. Michurova:** None.

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Levels of Anti-TPO correlate significantly with carotid Intima-Media Thickness (IMT) in type 2 diabetic patients with subclinical hypothyroidism

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Background and aims: Recent studies have shown that immune processes contribute to atherosclerosis. The aim of this study was to investigate the relationship between carotid IMT and the levels of anti-peroxidase autoantibodies (Anti-TPO) in type 2 diabetic subjects with subclinical hypothyroidism (SCH).
Materials and methods: A total of 109 non-smoking diabetic subjects with SCH were recruited for this study from June 2013 to May 2015. We used the analysis of variance to compare the differences of IMT among the groups with different Anti-TPO levels (from 64-1900 IU/ml). Carotid IMT was evaluated using high-resolution B-mode ultrasonography. Measurements of mean IMT were taken from distal common carotid artery, carotid bifurcation and proximal internal carotid artery (right and left). Statistical analysis was performed by using SPSS Inc., Chicago, IL, USA version 18 (P value <0.05 was considered significant).
Results: The mean age of the participants was 54.3 ± 5.06 years with diabetes duration 4.6 ± 3.21years. A positive correlation was found between the mean IMT of 6 segments of carotid artery and the levels of Anti-TPO (Anti-TPO levels more than 350 IU/ml; r=0.58). Carotid IMT levels were also significantly and positively associated with TSH (Thyroid-stimulating hormone), FPG (Fasting plasma glucose) and HbA1c levels, whereas negatively with HDL-C. There were no statistically significant differences in the lipid profile and blood pressure levels between the groups with different Anti-TPO levels.
Conclusion: In type 2 diabetic subjects with SCH we found significant association between levels of Anti-TPO and carotid IMT. Measurement of Anti-TPO levels can be useful risk factor for progression of carotid IMT in type 2 diabetic subjects.
Disclosure: **L. Petrosyan:** None.

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Impaired autophagic flux involves in advanced glycation end products-induced apoptosis of vascular endothelial cells

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Background and aims: Dysfunction of vascular endothelial cells (ECs) elicited by advanced glycation end products (AGEs) is the main cause of diabetic vascular complications. Recent data found that inadequate autophagy contributed to endothelial dysfunction in patients with diabetes. However, the underlying mechanisms remain unknown. The aim of this study is to investigate the effect of AGEs on ECs autophagy and its impact on AGEs-induced ECs apoptosis.
Materials and methods: Cultured human aortic vascular ECs (HAECs) were stimulated with AGEs-bovine serum albumin (AGEs-BSA) or BSA. Autophagosomes were observed by electron microscopy. The apoptosis rate was evaluated by flow cytometry. The expression levels of LC3-II, P-62, Rab7, cleaved-caspase-3, Bcl-2, SIRT1, p-SIRT, FoxO1, Ac-FoxO1, p-FoxO1, AKT and p-AKT were determined by western blotting.
Results: AGEs induced HAECs autophagy in a time-dependent manner. For 24h, LC3-II expression and the number of autophagosomes were gradually increased with no change of P-62, Rab7 expression and apoptosis rates. For 48h, AGEs markedly upregulated LC3-II expression and the number of autophagosomes with increased level of P-62 which indicated the reduced autophagic flux. The apoptosis rates were significantly increased with elevated cleaved-caspase-3 level and declined Bcl-2 expression. Western blotting showed that expression of p-SIRT, Ac-FoxO1, Ac-FoxO1/Atg7 and p-AKT was strikingly increased while expression of p-FoxO1 was obviously decreased. Inhibition of autophagy with 3-MA could reduce AGEs-induced HAECs apoptosis.
Conclusion: Impaired autophagic flux involves in AGEs-induced ECs apoptosis via SIRT1/Foxo1 pathway and may be a target for therapy of diabetic vascular disease.
Supported by: NSFC(81261120566)
Disclosure: **H. Zhang:** None.

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Preclinical carotid atherosclerosis in asymptomatic individuals with latent autoimmune diabetes in adults (LADA)

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Background and aims: The aim of this pilot study was to investigate the prevalence of carotid atherosclerosis in patients with LADA and to compare it with the prevalence in patients with type 1 diabetes (T1DM) and type 2 diabetes (T2DM).
Materials and methods: In this cross-sectional study, 71 patients with LADA, 191 patients with type 2 diabetes and 116 patients with type 1 diabetes without clinical macrovascular disease underwent B-mode carotid ultrasound to detect the presence of atheroma plaques. Mean ± standard deviation and the Mann-Whitney test as well as percentages and the Chi-squared test were used to assess differences between groups. Univariate and multivariate logistic regression models were computed to evaluate the presence of atheromatosis All analyses were obtained using R, setting a threshold for significance at 5% ($\alpha = 0.05$).
Results: There were no differences in age and sex distribution between T1DM, LADA and T2DM patients: 55.2%, 47.9% and 45% females respectively ($p = 0.224$), mean age 56.5, 58.2 and 58.3 years respectively ($p = 0.321$). Diabetes duration was different between groups: T1DM 23.7 ± 12.4, LADA 13.1 ± 9.7 and T2DM 8.7 ± 7.8 years ($p < 0.001$). Patients with LADA showed an intermediate clinical and biochemical phenotype between T1DM and T2DM (Table 1). Patients with T1DM and LADA were more frequently treated with aspirin and statins than T2DM. Diabetic retinopathy was more prevalent in T1DM than in LADA and T2DM (44.8, 18.3 and 18.8% respectively, $p < 0.001$). Carotid atherosclerosis was more prevalent in patients with LADA compared with patients with type 2 and type 1 diabetes (T1DM 56.9%, LADA 73.2%, T2DM 57.1%; $p = 0.042$). In addition, LADA patients had a more diffuse carotid atherosclerosis (multiple plaques): T1DM 33.6%, LADA 45.1%, T2DM 27.2%; $p = 0.022$. Age ($p < 0.05$) and tobacco ($p < 0.05$) were factors independently associated with carotid plaque. In the analysis between LADA and T2DM, we found an age-adjusted significant interaction between the disease duration and type of diabetes ($p = 0.02$), indicating that the prevalence of carotid atherosclerosis increased with diabetes duration in patients with LADA but not in patients with type 2 diabetes.
Conclusion: Patients with LADA, especially those with a longer duration of diabetes, had an unexpectedly high prevalence of preclinical atherosclerosis. These differences in carotid plaque prevalence occur even though patients with LADA exhibit an intermediate cardiovascular risk profile.

Variables	T1DM (n = 116)	LADA(n = 71)	T2DM(n = 191)	p
Body mass index (kg/m2)	26.5 ± 10.8	27.4 ± 4.9	31.3 ± 5.2	<0.001
Waist circunference (cm)	91.5 ± 13.3	96.2 ± 14	104.8 ± 11.7	<0.001
HbA1c (%)	7.7 ± 1	7.7 ± 1.1	7.4 ± 1.2	0.07
Cholesterol (mg/dl)	184.9 ± 29.8	178.9 ± 37.8	185.6 ± 35.2	0.357
HDL cholesterol (mg/dl)	65.8 ± 14.5	61.4 ± 18.1	48.3 ± 12	<0.001
LDL cholesterol (mg/dl)	102.5 ± 25.4	101.9 ± 24.8	110.5 ± 29.3	0.015
Triglycerides (mg/dl)	79.8 ± 36.4	100.5 ± 72.9	141.2 ± 78.7	<0.001
Albumin to creatinine ratio (mg/g)	12.1 ± 29.7	20.1 ± 69.6	14.5 ± 32.8	0.451
Current smokers (%)	18.1	23.9	21.5	0.647
Antiplatelet treatment (%)	43.1	56.3	30.9	0.002
Statin treatment (%)	62.9	59.2	37.2	<0.001

Disclosure: **C. López Cano:** None.

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Glucose metabolism status is associated with impaired microvascular function: the Maastricht study

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Background and aims: Type 2 diabetes (T2DM) is associated with a nearly 2-fold increased risk of cardiovascular disease (CVD). This can be partly explained by large artery dysfunction, which already occurs in prediabetes ('ticking clock hypothesis'). Whether a similar phenomenon also applies to microvascular dysfunction (MVD) is not known. We therefore tested the hypothesis that MVD is already present in prediabetes and deteriorates in T2DM. To do so, we investigated the association between glucose metabolism and MVD using the retinal arteriolar dilator response to flicker light and skin heat-induced hyperemic response.
Materials and methods: In a population-based cohort study (n=2213, 51% men, aged 60±8 years with oversampling of T2DM), we determined flicker-light-induced retinal arteriolar %-dilation (Dynamic Vessel Analyzer), skin heat-induced hyperemia (Laser-Doppler Flowmetry) and glucose metabolism status (OGTT; NGM, (n=1269), prediabetes (n=335) or T2DM (n=609)). Differences were assessed with multivariable regression analyses adjusted for age, sex, BMI, smoking, systolic BP, lipid profile, retinopathy, eGFR, (micro)albuminuria, the use of lipid-modifying and/or blood-pressure-lowering medication, and prior cardiovascular disease (CVD).
Results: Retinal arteriolar %-dilation was 3.4±2.8 in NGM, 3.0±2.7 in prediabetes, and 2.3±2.6 in T2DM. Adjusted analyses showed a lower %-dilation in prediabetes (β=-0.20,[CI95% -0.56;0.15]), with further deterioration in T2DM (β=-0.61,[-0.97;-0.25]) vs NGM, p for trend=0.001. Skin %-hyperemia was 1234±810 in NGM, 1108±748 in prediabetes, and 937±683 in T2DM. Adjusted analyses showed a lower %-hyperemia in prediabetes (β=-46,[CI95% -163;72]), with further deterioration in T2DM (β=-184,[-297;-71]) vs NGM, p for trend=0.001. Higher HbA1c was associated with lower arteriolar %-dilation and skin %-hyperemia in fully adjusted models (β=-0.32,[-0.48;-0.16], p<0.001 and β=-108, [-158;-57], p<0.001, respectively).
Conclusion: Glucose metabolism status and HbA1c are independently associated with impaired microvascular function in the retina and skin. These findings support the concept that MVD precedes and thus may contribute to T2DM-associated CVD and other complications such as impaired cognition and depression.
Supported by: EFRO, MUMC+
Disclosure: **B.M. Sörensen:** None.

OP 34 On the fate of the beta cell: from beginning to end

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Increase in insulin and proinsulin positive area and preservation of beta cell mass during the pre-diabetic phase in type 1 diabetes

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Background and aims: Type 1 diabetes (T1D) is characterized by the loss of insulin production due to beta-cell destruction. Recent studies have shown that pancreatic beta cell loss is heterogeneous and is influenced by multiple parameters like metabolic components and the age at onset. Furthermore, it has been assumed in the past that in most patients all beta cells are lost at diagnosis or shortly thereafter. Recently, this paradigm has been overturned since now we know that some patients retain beta cell function over many years post-onset. Conversely, little is known about beta cell loss during the pre-diabetic period and if seroconversion is in any way linked to beta cell destruction in the pancreas or not.
Materials and methods: Formalin-fixed paraffin-embedded (FFPE) tissue sections were provided by the Network for Pancreatic Organ Donors with Diabetes (nPOD). Sections from the head, body and tail regions of the pancreas were obtained from 10 controls and 10 autoantibody positive donors (5 single and 5 double autoantibody positive). Sections were stained for insulin, glucagon and proinsulin following a standard immunofluorescence protocol. Beta and alpha cell numbers, islet size and distribution, as well as insulin, proinsulin and glucagon areas were determined.
Results: We provide data to challenge the assumption that beta cell loss occurs in a linear fashion over time beginning early during the pre-diabetic phase. Our findings on pancreas sections from autoantibody positive donors show instead that insulin and specially proinsulin areas increase before diagnosis, and that there is a significant inversion of the insulin to proinsulin ratio in these individuals compared to non-diabetic donors. This could indicate that there is a high accumulation of immature vesicles in beta cells containing both insulin and proinsulin or it could be due to an increase in the metabolic state of the islet. In addition, beta cell mass was not reduced in autoantibody positive donors. Interestingly, we observed a very good correlation between the presence of alpha and beta cells in the islets of non-diabetic donors while this correlation was weaker in autoantibody positive individuals, suggesting a possible disruption of the homeostatic balance of these 2 cell populations in the islets, long before the onset of disease. Lastly, important regional differences in islet size and distribution were noticed between head, body and tail sections of the pancreas. Both non-diabetic and autoantibody positive donors presented a different islet size distribution in the tail region compared to head and body. Moreover, double autoantibody positive donors showed a differential distribution in head, body and tail compared to single and non-diabetic donors.
Conclusion: Taken together, our data suggest that the normal metabolism of beta cells might be altered many years before the onset of type 1 diabetes but that beta cell mass and insulin secretion seem to be maintained until shortly before diagnosis, declining more precipitously and rapidly than previously assumed just around the time of diabetes onset. This suggests that secondary prevention before onset, when beta cell mass remains intact, could yield strong benefits; and therefore, means to universally detect type 1 diabetes at this stage should be generally put in place.
Disclosure: **T. Rodriguez-Calvo:** None.

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Expression of the transcriptional activator, CIITA, correlates with aberrant expression of MHC class II in in the beta cells of individuals with type 1 diabetes

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Background and aims: A hallmark feature of type 1 diabetes is hyperexpression of the major histocompatibility complex (MHC) Class I within insulin-containing islets. This phenomenon probably contributes to the increased visibility of pancreatic islet cells to the immune system in type 1 diabetes thereby promoting β-cell loss. Interestingly, however, a number of studies have suggested that MHC class II (MHCII) molecules are also expressed aberrantly within a subset of islet cells in type 1 diabetes but this notion remains controversial. In the current study, we have examined the expression of the principal transcriptional regulator of MHCII, CIITA, in the islets of patients with recent-onset type 1 diabetes and have correlated this with the presence of MHCII.
Materials and methods: Formalin-fixed, paraffin-embedded pancreas sections from 6 healthy control individuals and 10 age-matched patients with type 1 diabetes (8/10 with disease duration <10w) were investigated from within the Exeter Archival Diabetes biobank, nPOD and DiViD cohorts. The expression of CIITA and other antigens was examined using either immunoperoxidase or immunofluorescence (IF) methods.
Results: CIITA was either absent or, on occasion, detected at very low levels in the islets of control individuals. However, the pattern of expression was clearly different in islets from type 1 diabetes patients. 6/10 of the patient samples studied contained islets (>3 per section) in which a subset of discrete endocrine cells stained strongly for CIITA. Despite this, it was clear that the majority of islet cells did not express CIITA. IF staining confirmed that the majority of cells expressing CIITA also contained insulin, suggesting that this transcription factor can be expressed in β-cells. We then assessed whether CIITA was associated with aberrant expression of MHCII in islet cells. Using 4 T1D cases in which CIITA expression had been detected, it was shown that MHCII and CIITA co-localised within the same islet cells. These were confirmed as insulin positive cells by dual IF analysis. There was no evidence of MHCII expression in islet cells which did not stain positively for CIITA. The 4 type 1 diabetes cases in which CIITA was not detected in islets failed to express MHCII. As expected, we found no correlation between the presence of CIITA and the hyperexpression of MHCI in the insulin-containing islets of patients with type 1 diabetes. Finally, we demonstrated that CIITA positive β-cells did not express CD68, confirming that these are unlikely to represent macrophages which were mistakenly identified as β-cells by virtue of having phagocytosed insulin during β-cell clearance.
Conclusion: Our data provide strong evidence supporting the proposal that aberrant expression of MHCII occurs in a subset of β-cells in patients with type 1 diabetes. They also reveal that this is likely to be driven by induction of expression of the transcription factor CIITA in these same cells. This upregulation of CIITA and MHCII may then contribute to altered antigen presentation during the development of autoimmunity in type 1 diabetes. Indeed, these data imply that certain β-cells may play a direct role in antigen presentation to immune cells by virtue of their aberrant MHCII expression.
Disclosure: **M.A. Russell:** None.

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Significance of microRNAs for the differentiation of human embryonic stem cells into definitive endoderm and mesoderm

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Background and aims: Pluripotent stem cells are able to generate insulin-producing surrogate cells and thus hold great promises for a potential cell replacement therapy of type 1 diabetes. One important class of post-transcriptional regulators that influence the gene expression are microRNAs (miRNAs). This study analyzed the miRNA expression profiles of purified definitive endoderm (DE), mesoderm and undifferentiated embryonic stem cells (ESCs) to identify important early cell fate regulators. In addition, the function of miRNA candidates was studied during differentiation.
Materials and methods: The Human ESC lines (HUES8, HES3) were differentiated towards DE (CHIR-99021/ActivinA) and mesoderm (CHIR-99021/BMP4). For cell sorting of differentiated populations via FACS the surface proteins CXCR4, EpCAM and NCAM were used. Expression of specific marker genes was analyzed by RT-qPCR and miRNA expression profiles of 754 miRNAs were measured by qPCR-based miRNA-Arrays. The function of identified miRNAs was analyzed upon transient transfection with mimics or inhibitors during differentiation.
Results: Expression of FOXA2 and SOX17 (DE-marker genes) was significantly higher in FACS-sorted CXCR4-positive cells, while VEGFR2, PDGFRa and CD34 (mesoderm markers) were highly expressed in sorted EpCAM-negative/NCAM-positive mesodermal cells. Data from miRNA-arrays revealed 19 DE-specific (e.g. miR-371, miR-489 or miR-1263) and 28 mesoderm-specific miRNAs (e.g. miR-10a, miR-196b, or miR-483) differentially expressed in mesoderm and endoderm, respectively. Validation of selected candidates showed that the miR-371-373 cluster, miR-1263 and miR-489 were highly enriched specifically in DE cells. Functional analysis of endodermal miRNAs revealed that upon transfection with miR-1263 mimic CXCR4-positive DE-cells arise earlier in a significantly increased quantity. In silico target prediction revealed KLF4 as potential target of miR-1263 indicating that this effect is potentially mediated by regulation of the pluripotent transcriptional network. Out of 7 mesodermal enriched miRNAs the candidate's miR-199a, miR-214 and miR-483 were used for functional tests. The analysis of these miRNAs showed that transfection of miR-483 mimic during mesoderm differentiation yielded in higher amounts of mesodermal PDGFRa-positive cells, whereas inhibition of miR-483 reduced their quantity. PDGFRa-positive cells are considered as paraxial mesoderm, the progenitor population for cardiac, smooth muscle and mesenchymal lineages.
Conclusion: This study identified novel miRNAs exhibiting particular functions for the early lineage formation of DE and mesoderm. The miR-1263 is facilitating the endodermal differentiation, whereas miR-483 was identified as important regulator of the paraxial mesodermal PDGFRa-positive subpopulation. Thus, miR-1263 is a potential tool that can be used to further increase the efficiency of beta cell differentiation protocols for human embryonic stem cells.
Disclosure: **U. Diekmann:** None.

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A microRNA atlas of human insulin-producing cells

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Background and aims: MicroRNAs are ~20-nucleotide long non-coding RNAs that are emerging as a promising biomarker for multiple diseases. Since a single microRNA can target multiple messenger (m)RNAs and regulate gene expression post-transcriptionally, they are becoming increasingly important in understanding the regulation of gene expression. Through our

preliminary analysis of developing and adult rodent and human tissues, we identified that although the mammalian pancreatic beta cells are factories of insulin production, the human gallbladder as well as a very limited number of cells in the brain also produce insulin. With our understanding of the role of microRNAs in mammalian pancreas development and function, we find it increasingly important to generate database / resource of microRNA expression profiles in human insulin-producing tissues. The aim of this research is to generate a miRNome of the human brain, gallbladder and pancreatic islets; tissues that naturally produce insulin, as well as other tissues that do not produce insulin (muscle, endothelium, skin, liver).

Materials and methods: We investigated a biobank of 284 different human tissues collected by the team over the past 15 years. RNA isolation was carried out using either a Trizol®-based protocol that we optimized in our laboratory or following manufacturer's protocol using a mirVana microRNA isolation kit. Quality of the isolated RNA was confirmed using a Bioanalyzer RNA nano or smallRNA chip (Agilent Technologies CA) and a set of eight mRNA transcripts (ins, gcg, sst, pdx1, mafA, ngn3, 18s and gapdh) as well as a set of 754 known and validated mature microRNAs (Thermo Fisher Sci., CA) were profiled using TaqMan™ real-time quantitative PCR (qPCR) technology on a ViiA7 or QuantStudio12K platform. Data were normalized to housekeeping genes (for mRNA) or using a global normalization protocol (for microRNAs) and analysed using a penalized logistic regression. Islet hormones were also confirmed by immunostaining followed by confocal microscopy.

Results: Confocal microscopy and qPCR analysis for gene transcripts confirmed that human endothelium, muscle, liver and skin do not contain any insulin transcripts, whilst the brain, gallbladder and islets transcribed insulin gene with increasing efficiency. Bidirectional hierarchical clustering was able to identify microRNAs that were enriched in each of the tissues as well as those that are associated with high level of insulin transcript. Logistic regression analysis of 150 human islet samples (compared to insulin negative tissues) led to selection of a set of 12 microRNAs that are associated with high levels of insulin transcript. Furthermore this signature of microRNAs could predict insulin expression in a validation set of 88 tissue samples with 97% efficiency.

Conclusion: We present the first largest of resource of microRNAs from human insulin-producing tissues. Our analyses of microRNA signatures when tested in wet lab setting provide key evidence to understand the role of microRNAs in influencing insulin gene expression. Generation of such expressome maps will help further research in the field and may also guide the differentiation of human progenitors to insulin-producing cells for potential use in replacement therapy for diabetes.

Supported by: AAH-Australian Research Council (ARC), JDRF Australia & Rebecca Cooper Fndn

Disclosure: **W. Wong:** None.

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Betatrophin: A new potential biomarker of beta cell function and autoimmunity in diabetes?

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Background and aims: The potential effect of betatrophin as a stimulator of beta cell proliferation is a matter of debate and information on the protein level and meaning in human metabolic disease is still scanty. In the attempt to explore the potential meaning of BT in diabetes we have recruited a mixed population to encompass diabetes variety.

Materials and methods: We recruited 24 non-diabetic (ND; 46±12 yrs; 9M/15F; BMI 25.1±1.9 Kg/m2, Hb1Ac 5.1±0.5%), 22 Type 1 diabetic (T1DM; 43±7 yrs; 10M/12F; BMI 24.5±1.4 Kg/m2, Hb1Ac 7.9±1.1%, diabetes duration 10.3±10.1 yrs) and 15 pancreas-transplanted T1DM (TX; 42±4 yrs; 6M/9F; BMI 21.3±2.1 Kg/m2, Hb1Ac 5.9±0.7%, diabetes duration 28.9±2.1 yrs, time since transplantation 5.5±4.2 yrs) subjects. Blood samples for BT determination were collected in the fasting state in tubes containing Aprotinin and BT and C-peptide levels were determined by ELISA technique.

Results: In the population as a whole, BT levels were directly associated with plasma glucose levels ($r=0.585$, $p<0.001$) and HBA1c ($r=0.524$, $p=0.0004$) and inversely correlated with C-peptide levels ($r=0.471$, $p=0.0004$). BT was higher in T1DM (873±42 pg/ml; $p<0.05$, Bonferroni test) than in ND (386±24 pg/ml). In TX, BT concentration was similar to that of ND (474±20 pg/ml). In T1DM BT was also associated with anti-GAD ($r=0.455$, $p=0.006$) and anti-IA2 ($r=0.551$, $p=0.006$) antibody titer.

Conclusion: The correlation between BT and C-peptide concentrations, together with normalization of BT in TX patients, suggests a potential role of the hormone as a biomarker of beta cell function (or mass). Similarly, the positive correlation with autoantibody titer may reflect an activation of beta-cell regeneration processes.

Disclosure: **R. Lupi:** None.

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Molecular markers of beta cell mortality

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Background and aims: The loss of insulin-producing (beta) cells is central to the development of Type 1 diabetes (T1D), and a common feature in islet transplantation, Latent Autoimmune Diabetes of Adults (LADA) and Type 2 diabetes. Beta cell death occurs months to years prior to clinical T1D. Currently, we lack diagnostic tools to quantitate beta cell loss prior to clinical onset of T1D. Circulating microRNAs (miRs) are highly stable, non-coding RNAs that are increasingly recognised as biomarkers of disease progression. Similarly, insulin cell-free (cf)DNA is a potential biomarker of beta cell death. Insulin DNA in beta cells is unmethylated at cytosine islands at 8 different positions downstream of the transcriptional start site. These are methylated in insulin DNA from non-beta cells. We have optimised a digital droplet (dd)PCR method to reliably identify unmethylated or methylated insulin cfDNA in plasma. In addition, our islet miR-profiling studies using next generation discovery analyses followed by qPCR validation identified a set of 50 miRs with potential to predict T1D progression. We hypothesise that circulating concentrations of islet-enriched miRs and insulin cfDNA correlate with islet beta-cell death and diabetes progression, and with other markers such as residual C-peptide.

Materials and methods: This cross-sectional study used plasma from a cohort of 180 T1D individuals (average T1D duration of 20±13yrs), including 58 with microvascular diabetes complications (T1DCx+), and 138 age and gender matched controls. HbA1c levels were 8.0±1.3% in T1D vs. 5.1±0.4% in controls ($P<0.0001$). Circulating miR profiling was undertaken via high-throughput qPCR, whilst cfDNA quantitated using ddPCR. C-peptide levels were measured using ultra-sensitive ELISA (detection limit 1.25 pmol/L). Data were analysed using Statistica software and results compared using t-test and ANOVA as appropriate. Correlations were assessed using non-parametric Spearman method. Significance $P<0.05$.

Results: Five (10%) miRs were significantly increased in the circulation of T1D individuals (vs. controls) whilst seven miRs (14%) were significantly decreased (>2 Fold Change / FC, $P<0.05$) in T1DCx+ vs. T1DCx-. Five (10%) miRs were significantly increased (>2 FC, $P<0.05$) in T1D individuals with detectable C-peptide vs. non-detectable. There was no correlation between miR abundance and C-peptide level in control subjects. Eight (16%) miRs correlated significantly ($P<0.05$) with C-peptide levels in T1D. Increased C-peptide level was associated with greater abundance for all of these miRs. Two (4%) miRs correlated significantly

(P<0.05) with the age of T1D diagnosis, with increased age correlating with lower miR abundance. Ten (20%) miRs correlated significantly (P<0.05) with T1D duration, with increased duration associated with reduced abundance in all but one miR. T1D subjects were divided into two groups based on T1D duration (≤20yrs and >20yrs). Nine (18%) miRs had significantly lower abundance (>2 FC, P<0.05) in the circulation of subjects with duration >20yrs.

Conclusion: Our studies highlight the potential of circulating miRs and cfDNA in quantifying beta cell mortality. We have shown that as beta cells are lost in established diabetes (reduction in C-peptide) there is a reduction in the abundance of released miRs. These miRNAs as well as insulin cfDNA are robust molecular markers of beta cell death in diabetes.

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Disclosure: **R.J. Farr:** None.

OP 35 Novel genetics discoveries and phenotypic characterisation

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Loss-of-function mutations in DNAJC3 cause young-onset diabetes due to oxidative stress and mitochondrial beta cell apoptosis

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Background and aims: Pancreatic β-cells synthesize and secrete large amounts of insulin. Translation of insulin and other secretory proteins takes place in the endoplasmic reticulum (ER). The canonical ER stress transducer PERK senses unfolded proteins in the ER and phosphorylates eukaryotic translation initiation factor 2α (eIF2α) to attenuate protein translation. A narrow regulation of eIF2α phosphorylation is required to preserve β-cell function and survival. DNAJC3 acts as a co-chaperone in the ER and inhibits PERK. DNAJC3 loss-of-function has been shown to cause diabetes in mouse and man. Here we describe two patients with novel loss-of-function mutations in DNAJC3, and we investigate the pathogenic mechanisms involved.

Materials and methods: Mutations were identified through exome sequencing and confirmed by Sanger sequencing. DNAJC3 was silenced by RNA interference in INS-1E cells. Antioxidant response element (ARE) promoter activity was measured with a luciferase reporter. PERK and eIF2α phosphorylation and DNAJC3 expression were examined by Western blot and mRNA by qPCR. Apoptosis was detected by nuclear dyes and Western blot for cytochrome c.

Results: We performed exome sequencing of selected patients with likely monogenic diabetes and identified homozygous or compound heterozygous mutations of DNAJC3 in two unrelated patients with diabetes, short stature, microcephaly and variable neurodegenerative features. Diabetes was diagnosed at ages 12 and 16 years; autoantibodies were negative and C-peptide was detectable. Patient 1 was compound heterozygous for p.R393X and p.M1? mutations and patient 2 was homozygous for a p.R346X mutation. These mutations are predicted to result in complete loss of protein function. In lymphoblasts from patient 1, DNAJC3 mRNA expression was detectable but protein was not. DNAJC3 silencing in clonal β-cells increased PERK phosphorylation, but eIF2α phosphorylation was unchanged. ARE promoter activity was increased in DNAJC3-deficient cells after exposure to the chemical ER stressor CPA (8±1-fold vs 4±1-fold in DNAJC3-competent cells, p<0.05, n=4), which is suggestive of an antioxidant response due to PERK-mediated NRF2 activation. DNAJC3 deficiency did not affect insulin content or secretion but it induced β-cell apoptosis (13±1% with siDNAJC3 vs 6±1% with control siRNA, p<0.01, n=4). Apoptosis was paralleled by mitochondrial cytochrome c release, indicating activation of the intrinsic pathway of apoptosis.

Conclusion: Loss-of-function mutations in DNAJC3 cause young-onset diabetes with short stature and various neurological features. DNAJC3 deficiency does not affect β-cell function but it induces β-cell death through oxidative stress and activation of the mitochondrial apoptosis pathway.

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Disclosure: **B. Abdulkarim:** None.

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Activating STAT3 mutation leads to pancreatic hypoplasia through premature differentiation

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Background and aims: Permanent neonatal diabetes (PNDM) is caused by mutations impairing the development or function of the pancreatic beta cells. Activating de novo germline mutations in STAT3 were recently identified as a cause of PNDM. The patient with the most activating mutation K392R presented with hypoplastic pancreas and high beta-cell autoantibody levels already at birth. We used patient-derived iPS cells to test the hypothesis that the mutation may cause pancreatic developmental failure.
Materials and methods: Patient's skin fibroblasts were reprogrammed to iPSC and differentiated into pancreatic progenitors through a four-stage, 17 days protocol.
Results: Expression levels of pancreatic progenitor markers, such as PDX1 and NKX6.1, did not differ between STAT3K392R cells and healthy controls. Instead, NEUROG3 expression was upregulated prematurely together with significantly higher NKX2.2 (4-5 fold,) INS (10-fold) and GCG (5-fold) levels in STAT3K392R cells. Thus, overactive STAT3 did not cause a developmental block or inhibit endocrine differentiation. RT-qPCR results were confirmed by immunocytochemistry, with more NEUROG3-positive nuclei after 13 days and markedly more INSULIN-positive area after 17 days (p=0,005), and by RNA-seq, showing robust upregulation of all NEUROG3 downstream targets. The STAT3 mutation was then corrected using CRISPR/Cas9 with guide RNAs targeting next to the mutation site and a double stranded DNA repair template. Corrected isogenic cells differentiated similarly to control cells, showing a complete reversal of the disease phenotype. STAT3K392R activating properties are not explained by an increase in DNA-binding affinity or its phosphorylation status. Instead, protein proximity assay and quantitative immunocytochemistry revealed increased nuclear translocation of STAT3 and increased transcriptional activity in STAT3K392R overexpressing cells
Conclusion: Our data suggest that increased STAT3 localization in the nucleus of STAT3K392R cells leads to early upregulation of NEUROG3 and premature endocrine differentiation, resulting in reduction of the pancreatic progenitor pool, consistent with the pancreatic hypoplasia. Patient-specific iPSC in combination with CRISPR-based genome editing are valuable tools for recapitulating pancreatic developmental defects, enabling the study of pathogenic mechanisms leading to monogenic diabetes.
Supported by: Novo Nordisk Foundation
Disclosure: **J. Saarimäki-Vire:** None.

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Hepatic and biliary findings using MR and MRCP imaging in patients with HNF1B mutations

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Background and aims: Heterozygous mutations in the gene encoding for hepatocyte nuclear factor 1-β (HNF1B) lead to a spectrum of renal and extra-renal manifestations, including cystic renal disease, hypoplastic pancreatic body and tail, urogenital malformations, early-onset diabetes (MODY5) and gout and hypomagnesaemia. HNF1B plays a crucial role during bile system morphogenesis in mice, and conditional knockout mice exhibit abnormalities of intrahepatic bile ducts and the gallbladder epithelium. Data on bile duct defects in humans are scarce and restricted to few case reports in patients with mainly neonatal cholestasis, in whom HNF1B mutations have been associated with a paucity of intrahepatic bile ducts. Our aim was to systematically evaluate bile duct anomalies and malformations using MRI.
Materials and methods: To date, 12 subjects with mutations in HNF1B have participated in the Botnia and FinnMODY Studies (www.botnia-study.org/finnmody). They undergo gadolinium-enhanced MRI of the liver and MRCP (magnetic resonance cholangiopancretography). Blood samples are drawn for creatinine, ALT, AST, ALP, GGT, uric acid, Mg, and triglycerides.
Results: The median age of the patients is 27 years (range 14-57 years; 10 females, 2 males) and the median BMI is 21.7 (kg/m^2, range 16.3 - 24.3). All but one patient have diabetes treated with diet or insulin. Estimated GFR using CKD-EPI spans from 7 to 138 (ml/min/1.73 m^2); four patients have renal insufficiency (GFR < 60) and one has end-stage renal disease. To date, three of the patients have undergone MR imaging and biliary tract abnormalities have been observed in two of them. A fusiformly dilatated common bile duct (type 1C choledochal cyst according to the Todani classification) was observed in a 14-year-old patient who had been diagnosed with cystic kidney disease prenatally; her intrahepatic bile ducts appeared normal. A 22-year-old patient presented with MODY-type diabetes; MRCP imaging revealed multiple dilatations of the branch ducts of the pancreas, caliber variation in the intrahepatic bile ducts reminiscent of sclerosing cholangitis, and focal narrowing of the extrahepatic bile ducts. Imaging of the rest of the patients is scheduled for April-August 2016.
Conclusion: Heterozygous mutations in the HNF1B gene can be associated with developmental and structural abnormalities in the biliary tract. The clinical significance of these changes is unclear. However, considering the wide use of MR imaging, awareness of these changes can aid in differential diagnostics.
Supported by: Helsinki University Hospital research fund (EVO)
Disclosure: **J.L.T. Kettunen:** None.

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Strategies for improving statistical power for detailed physiological characterisation of individuals with type 2 diabetes-associated variants

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Background and aims: More than 70 common genetic variants are associated with type 2 diabetes (T2D). Some variants have been classified into broad categories such as those acting through insulin secretion or insulin resistance, but the physiological mechanisms for most are poorly understood. Detailed clinical studies could provide important mechanistic insights but these studies are expensive and hard to scale-up to the necessary numbers required for the modest effect sizes observed. Recruiting individuals to clinical studies based on their genotype (RBG) is a potentially efficient strategy to increase statistical power without needing the large sample sizes normally used in genetic studies. The aim of this study was to test strategies for improving statistical power for RBG studies. We used the T2D-associated variant at the *ARAP1/STARD10* locus as a proof of principle example. The type 2 diabetes risk allele is paradoxically associated with lower proinsulin to insulin ratios and so may have a specific role in insulin processing.
Materials and methods: We used two cohorts, each of >5000 individuals, consented for RBG studies (the Oxford Biobank and the Exeter EXTEND study), to identify 131 homozygous carriers (2.2% of individuals) of the less frequent *ARAP1* T2D protective allele. We matched these individuals to 131 homozygous carriers of the T2D risk allele, for sex and to within 1.6 kgm^2 BMI (95%<1kgm^2) and 2 years of age (96%<1 year difference). Fasting proinsulin and insulin concentrations were measured using an ELISA based method and log transformed results were compared between groups.
Results: Our RBG approach using 131 matched pairs provided strong evidence that the *ARAP1* T2D risk allele is associated with lower proinsulin levels. Homozygous carriers of the T2D protective allele had higher fasting proinsulin concentrations (0.29, 95%CI[0.44, 0.14], p=0.0002) and proinsulin to insulin ratios (0.07, 95%CI[0.10, 0.04], $p=1.34x10^{-5}$) compared with homozygous carriers of the risk allele.
Conclusion: By reproducing the association between alleles at the *ARAP1* locus and reduced proinsulin concentration, this study demonstrates the statistical power of bespoke recruit-by-genotype studies. The use of RBG studies may enable in depth physiological characterisation of novel T2D variants of moderate effect.
Supported by: MRC
Disclosure: **M.M. Umapathysivam:** None.

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Physical activity energy expenditure attenuates the effect of the TBC1D4 p.Arg684Ter loss-of-function variant on 2-hour plasma glucose among Greenland Inuit

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Background and aims: To investigate whether physical activity energy expenditure (PAEE) attenuates the effect of a type 2 diabetes predisposing common high penetrant Greenlandic TBC1D4 p.Arg684Ter loss-of-function variant on plasma glucose levels obtained after 2 hours during an oral glucose tolerance test (OGTT).
Materials and methods: We performed TBC1D4 x PAEE interaction analysis in the population based Inuit Health in Transition Study, where information on PAEE was estimated subjectively by a modified and to arctic living conditions adapted version of the International Physical Activity Questionnaire (IPAQ, n= 2655) as well as objectively by combined heart rate and accelerometry (ActiHeart, n= 1403). We tested for an interaction effect using a linear mixed model, accounting for admixture and relatedness among individuals. Based on previous results we tested for the interaction effect of being a homozygous TBC1D4 carrier versus being a non-carrier. All quantitative traits were quantile-transformed to a sex-specific standard normal distribution, therefore effect sizes are reported in standard deviations (SD). All analyses are adjusted for age and gender.
Results: Subjectively assessed PAEE significantly (beta-interaction=−0.0054 SD, p-interaction=0.0054) attenuated the association between the TBC1D4 variant and 2 hour plasma glucose (PGLU120) levels after an OGTT. An increase of PAEE by 10 kJ/kg/day (corresponding to 57 min of activity compared to spending those 57 min at rest) decreased PGLU120 by 0.16 mmol/l more in TBC1D4 carriers compared to non-carriers. In fact it appears that highly active TBC1D4 carriers have similar levels of glucose to that of non-carriers. Objectively assessed PAEE was borderline significant and with similar magnitude of effect (beta-interaction=− 0.0051 SD, p-interaction=0.36).
Conclusion: We found that PAEE attenuates the effect of a common Greenlandic TBC1D4 variant on plasma glucose levels 2 hours after an oral glucose load under the assumption of a fullHO inheritance model. This study indicates that Inuit in Greenland who are homozygous carriers of the TBC1D4 risk variant can benefit from physical activity.
Disclosure: **M.E. Jørgensen:** None.

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Clinical utility of a 1-hour oral glucose tolerance test for prediction of type 2 diabetes

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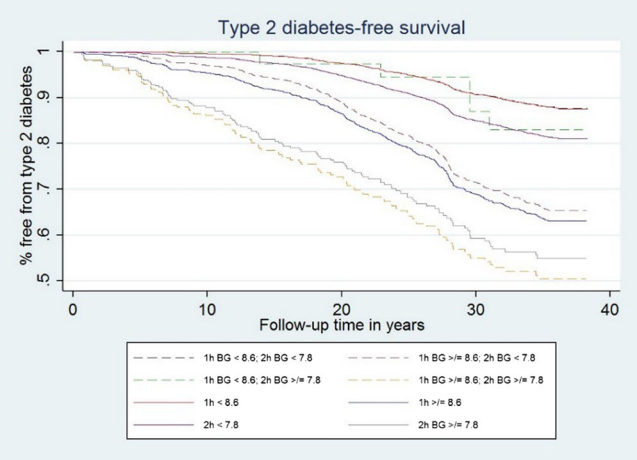
Background and aims: Subjects with prediabetes, i.e. impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) are at increased risk for type 2 diabetes (T2D). However, not all subjects with prediabetes develop T2D, and a significant number without prediabetes proceed to T2D. Blood glucose (BG) at 1h during OGTT with a cut-off ≥ 8.6 mmol/L may be an early marker of IGT and future T2D. The aim of this study was to examine whether 1h BG would be a more suitable screening tool for risk assessment than 2h BG.
Materials and methods: 5,256 men without diabetes from the Malmö Preventive Project, who had BG measured at 0, 1, and 2h during OGTT, were followed for up to 40 years, with registry-based recording of T2D. Discrimination ability and/or improvement in prediction of T2D was evaluated using Cox regression, C-index, and net reclassification improvement (NRI), with BG measurements assessed as binary variables (IFG: fasting BG (FBG) ≥ 5.6 mmol/L; elevated 1h BG: ≥ 8.6 mmol/L; IGT: 2h BG ≥ 7.8mmol/L). All analyses were repeated at 10 and 40 years.
Results: The Kaplan-Meier plot shows the unadjusted T2D free survival. At 10 and 40 years, 99 (2%) and 776 (15%) had developed T2D. The combination of IFG and/or elevated 1h BG was associated with a greater risk of T2D (10 years: hazard ratio (HR) 13.76, p < 0.0001, C-index 0.761; 40 years: HR 3.75, p < 0.0001, C-index 0.670) than IFG and/or IGT (10 years: HR 9.57, p < 0.0001, C-index 0.715; 40 years: HR 3.18, p < 0.0001, C-index 0.587); differences being significant only at 40 years (p = 0.055 at 10 years, p < 0.0001 at 40 years). Addition of IFG and/or elevated 1h BG to IFG and/or IGT, with classification of subjects into two risk categories, was associated with positive NRI at both 10 years (0.093, p = 0.12) and 40 years (0.157, p < 0.0001), significant only at 40 years. There was a greater number of subjects with elevated 1h BG than with IGT: 1) NGT and normal 1h BG: n = 3320 (63%); 2) elevated 1h BG only: n = 1589 (30%); 3) IGT only: n = 43 (1%); 4) both elevated 1h BG and IGT: n = 304 (6%). Incidence rates (10 years and 40 years) per 1000 person-years were greater in subjects with elevated 1h BG only than in those with IGT only: 1) 0.5 and 3.0; 2) 2.9 and 9.5; 3) 0 and 4.2; 4) 13.3 and 16.1.

Conclusion: 1h BG is a powerful predictor of future T2D, with positive NRI and detection rates greater than 2h BG, especially with longer follow-up. The low prevalence of IGT compared to elevated 1h BG as well as the progressive increase in T2D incidence rate over time in the latter group supports the concept that an abnormal 1h BG represents an early manifestation of the metabolic dysfunction eventually leading to IGT and T2D. Therefore, 1h BG may replace 2h BG as the preferred marker of IGT.



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Disclosure: **M. Pareek:** None.

OP 36 Biomarkers and predictors of diabetic nephropathy

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High levels of plasma T cell immunoglobulin and mucin-containing molecule 1 (TIM-1) predict deterioration of kidney function and incidence of chronic kidney disease

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Background and aims: The T-cell immunoglobulin and mucin-containing molecule (TIM-1), has been suggested as a urinary biomarker for kidney injury. It has been shown to be expressed in tubular cells of patients with renal diseases and to be associated with renal fibrosis and inflammation. However, little is known if TIM-1 predicts incidence of chronic kidney disease (iCKD) or type 2 diabetes (T2D). Therefore, we investigated whether fasting plasma levels of TIM-1 associate with kidney function, predict iCKD or hospitalization due to impairment of renal function (hiRF) in the population-based, prospective Malmö Diet and Cancer Study-Cardiovascular Cohort (MDCS-CC). Further, we investigated the association between TIM-1 and incidence of T2D (iT2D), and longitudinal change in eGFR in participants with diabetes at baseline.
Materials and methods: For the analyses of longitudinal change in eGFR and iCKD, our study included 2,799 individuals, aged 46-68 years, with measured TIM-1 concentration at baseline (1991-1994) who attended the follow-up re-examination (2007-2012). TIM-1 was analyzed using Proseek Multiplex CVD I, Olink Bioscience, Uppsala, Sweden. The iCDK was defined as eGFR ≤ 60 ml/min/m2. The odds ratios for iCKD according to sex-specific quartiles (Q1-Q4) of fasting plasma TIM-1 were analyzed by logistic regression adjusting for age, sex, eGFR, fasting glucose, systolic blood pressure, anti-hypertensive medication and BMI at baseline, and for the follow-up time. Among 4,406 MDCS-CC participants, hiRF was recorded using ICD9 585&586 and ICD10 N18&N19 by linkage to national registers for in- and out-patient hospital diagnoses (until end of 2013) and association with TIM-1 was analyzed by multivariate adjusted Cox-regression. Finally, association between TIM-1 and iT2D (until end of 2014) was analyzed among 4,231 participants without diabetes at baseline, and association between TIM-1 and change in eGFR between baseline and follow-up re-examination was analyzed in 76 participants with diabetes at baseline.
Results: During a mean follow-up time of 16.6 years, higher TIM-1 levels associated with higher yearly mean decline of eGFR (β per 1SD TIM-1: -0.081, $P < 0.001$), and higher increase of cystatin C ($P < 0.001$) and creatinine ($P < 0.001$). Of the 2,765 participants free of CKD at baseline, iCKD was present in 32% at follow-up and the odds ratio for iCKD was 1.45 (95%CI 1.10-1.92) comparing TIM-1 in the highest (Q4) compared to lowest (Q1) TIM-1 quartile. The Hazard Ratio (HR) for hiRF was significantly higher for individuals in Q4 compared to Q1 of TIM-1 (HR:2.15; 95%CI 1.09-4.23). During a mean follow-up time of 18.6 years the rate of iT2D was 16.8%. The age and sex adjusted HR for iT2D was 2.20 (95%CI 1.78-2.77) comparing Q4 to Q1 of TIM-1, and attenuated after further adjustment for baseline BMI and fasting glucose (HR=1.59; 95%CI 1.27-1.99). Among patients with diabetes at baseline, the mean decline of eGFR was > 4-times higher as compared to the whole cohort (β per 1SD TIM-1: -0.34, P=0.0040) and patients in Q4 of TIM-1 had a greater decline in eGFR compared to patients in Q1 (-2.34 vs -1.45 ml/min/1.73m2, P=0.0068), adjusted for age, sex and baseline eGFR.
Conclusion: Our prospective study suggests higher circulating TIM-1 levels as a potential biomarker to predict iCKD and hiRF, as well as iT2D and a markedly greater longitudinal decline of eGFR in patients with diabetes.
Supported by: Swedish Research Council, Swedish HLF, ERC, Novo Nordic Foundation
Disclosure: **C. Schulz:** None.

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MicroRNA-145 and micro/macrovascular complications of type 1 diabetes in the EURODIAB Prospective Complications study

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Background and aims: MicroRNAs (miRNAs) are small non-protein-encoding RNAs that post-transcriptionally regulate gene expression via suppression of target mRNAs. Specifically, miRNAs bind through canonical base pairing to a complementary site in the 3' untranslated region of their target mRNAs and can direct the degradation or translational repression of these transcripts. MiRNAs are critically involved in many biological processes in health and diseases and there is preliminary evidence of a role of miRNAs in cardiovascular diseases and diabetic complications. MiRNAs are also present in the circulation in a remarkably stable form and a recent study has shown that miRNA-145 levels were significantly reduced in patients with stable of coronary artery diseases (CAD) compared to healthy controls; however, a systematic analysis of circulating miRNA-145 in DM1 patients with vascular complications has not yet been performed.
Materials and methods: We performed a cross-sectional nested case-control study from the EURODIAB Prospective Complications Study. A total of 450 DM1 patients, diagnosed at <36 years of age, were studied. Cases (n=308) were defined as those with one or more complications of diabetes and control subjects (n=142) were those with no evidence of any complication. Total RNA was extracted from individual serum samples using the Triazol reagent. Synthetic C. elegans miR-39 was added to all samples prior to RNA extraction. Total RNA was reverse transcribed and pre-amplified. Mir-145 levels were quantified by qPCR and association with diabetic complication investigated. Values were normalised using the external control c.ele-miR-39 and the endogenous controls U6 snRNA.
Results: miR-145 was measurable in all 450 samples with a right-skewed distribution of values. qRT-PCR analysis showed that miR-145 levels were similar between cases and control subjects [controls: 0.056 (0.068-0.44); cases: 0.042 (0.035-0.68), p=0.11; geometric mean (95% CI)]. Subgroup analysis revealed that miR-145 levels were significantly lower in cases with retinopathy (p=0.038) or in macroalbuminuric subjects (p=0.002). In logistic regression analysis, miR-145 levels were associated to a 52% risk reduction of macroalbuminuria independent of age, sex, diabetes duration, TGF-β levels, and AGEs.
Conclusion: In this large cohort of type 1 diabetic subjects, we found that miR-145 levels are associated with macroalbuminuria in type 1 diabetic patients.
Supported by: EFSD/Sanofi
Disclosure: **F. Barutta:** None.

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Urinary tubular biomarkers and the slope of renal function decline in macroalbuminuric patients with type 1 diabetes mellitus

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Background and aims: Tubular dysfunction increases along with diabetic nephropathy severity, but it is not clear if it predicts the loss of renal function. Our aim was to investigate three common tubular markers as potential predictors of the loss of renal function based on eGFR slopes in patients with type 1 diabetes and macroalbuminuria.
Materials and methods: This study is part of the Finnish Diabetic Nephropathy Study a nationwide, multicenter prospective study aiming to identify all potential risk factors for diabetic complications. We used 315 patients with type 1 diabetes and macroalbuminuria (AER >300mg/24h) from this cohort with a baseline eGFR ≥ 30 ml/min. Patients were required to have at least two eGFR measurements after the baseline visit to calculate the slope. A follow-up time of at least 3 years was also required. The median follow-up in this study was 9.1 years (IQR 6.2 - 12.4). Urinary L-FABP, KIM-1 and adiponectin concentrations were measured from baseline 24 hour urine samples using ELISA and then normalized with urinary creatinine. All other blood and urinary tests were performed by standard methods. Because the slope of eGFR decline was a negative number we used for the analysis ln(1-slope). All the non-normaly distributed variables used in the analysis were also ln transformed. First, we built basic linear regression models with forward selection of covariates to select the best predictors of these slopes from all available clinical and biochemical variables except the tubular markers. Then simple and adjusted linear regressions were used to assess the independent association of markers with the eGFR slopes.
Results: The best predictors of the slopes of renal function decline formed the basic model and were the baseline eGFR ($\beta = -0.26$, $p < 0.05$), AER ($\beta = 0.24$, $p < 0.0001$) and glycated haemoglobin A1c ($\beta = 0.12$, $p = 0.0001$). In simple linear regression analysis all tubular markers were associated with the slope of renal function decline (βAdiponectin = -1.05, $p < 0.0001$; βKIM-1 = -1.13, $p < 0.0001$; βL-FABP = -1.07, $p < 0.0001$). However, when adjusted for the basic model only urinary adiponectin was independently associated with the slopes of eGFR decline (βAdiponectin = 0.08, $p = 0.038$; βKIM-1 = 0.03, $p = 0.54$; βL-FABP = 0.06, $p = 0.14$).
Conclusion: Urinary adiponectin predicts the slope of renal function decline independent of the baseline eGFR, AER and glycated haemoglobin A1c, in patients with type 1 diabetes and macroalbuminuria.
Disclosure: **N.M. Panduru:** None.

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Implication of low muscle mass on albuminuria and chronic kidney disease in patients with type 2 diabetes: the Korean Sarcopenic Obesity Study (KSOS)

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Background and aims: Previous studies have shown that chronic kidney disease (CKD) in patients on dialysis is associated with accelerated loss of skeletal muscle. However, the relationships of sarcopenia with albuminuria and early stage CKD in patients with type 2 diabetes have not been examined.
Materials and methods: We analyzed diabetic subgroup data from 409 patients with type 2 diabetes from the Korean Sarcopenic Obesity Study (KSOS). Sarcopenia was defined as a skeletal muscle mass index (SMI) [SMI (%) = total skeletal muscle mass (kg) / weight (kg) x 100] less than 2 standard deviations (SDs) below the sex-specific mean for a younger reference group. The estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR) were used to assess renal function and albuminuria, respectively.
Results: The prevalence of sarcopenia was significantly increased in the albuminuria group compared with the normo-albuminuria group (26.7% vs. 12.6%, P = 0.001), as well as in the CKD 3 group compared with the CKD 1-2 group (46.7% vs. 15.1%, P = 0.005). After adjusting for age,

SMI was negatively correlated with urinary ACR and positively correlated with AST, ALT, total cholesterol, high-density lipoprotein (HDL)-cholesterol, and low-density lipoprotein (LDL)- cholesterol levels. Multiple logistic regression analysis revealed that the odds ratio for albuminuria risk was 3.03 (95% confidence interval [CI] 1.38-6.65) in the lowest tertile of SMI compared with the highest tertile after adjusting for various confounding factors.
Conclusion: This is the first study to demonstrate that an increased risk of albuminuria is independently associated with low muscle mass in patients with type 2 diabetes.
Supported by: NRF
Disclosure: **H. Chung:** None.

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Performance of different estimated glomerular filtration rate equations in predicting all-cause mortality in Chinese subjects with type 2 diabetes

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Background and aims: Evaluation of kidney function, in terms of estimated glomerular filtration rate (eGFR), is important in the management of type 2 diabetes mellitus (T2DM). Although current KDIGO guidelines recommend using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation over the Modification of Diet in Renal Disease (MDRD) equation in general, their differential performance in predicting all-cause mortality, in particular among subjects with T2DM remains undefined. Recently, a modified CKD-EPI equation for Chinese subjects with T2DM (CKD-EPI Chinese T2DM; Liu et al, 2014) has been developed. This study aims to compare the performance of MDRD, CKD-EPI, the Chinese specific equations of MDRD and CKD-EPI, and the CKD-EPI Chinese T2DM in predicting all-cause mortality in Chinese subjects with T2DM.
Materials and methods: This study included all subjects with T2DM from the Hong Kong West Cluster who attended a diabetes complication screening assessment in 2008. A cohort of 5,082 Chinese subjects with T2DM, with available serum creatinine at baseline, was followed up to January 2016. eGFR was calculated using different eGFR equations. Mortality was identified based on all registered deaths. Concordance index (C-index) was applied to assess the discriminatory accuracy in all-cause mortality using each eGFR equation.
Results: Based on different eGFR equations, there were 17.9% (MDRD), 19.5% (CKD-EPI), 10.0% (Chinese MDRD), 14.9% (CKD-EPI Chinese) and 22.9% (CKD-EPI Chinese T2DM) of subjects who had eGFR < 60 mL/min/1.73 m2 at baseline. After a mean follow up of 7 years, there were 747 deaths (All-cause mortality was 14.7%). Cox regression analysis showed significant associations between eGFR, calculated by each equation, and the risk of all-cause mortality in both univariant and multivariant models, after adjustment for gender, age, BMI, HbA1c, diabetes duration, smoking, presence of dyslipidaemia and hypertension (all $p < 0.005$). In discriminating all-cause mortality among Chinese subjects with T2DM, the C-index of the CKD-EPI equation was better than that of MDRD equation (0.734 vs. 0.707; $p < 0.001$). There were no differences in the C-indices of either the Chinese-specific MDRD or CKD-EPI equations from their respective original eGFR equations. However, the C-index of CKD-EPI Chinese T2DM equation was higher than that of the CKD-EPI Chinese equation (0.740 vs. 0.734; p=0.010).
Conclusion: Our results demonstrated that, in keeping with current recommendations, the CKD-EPI equation is superior to the MDRD equation, for the prediction of all-cause mortality. In addition, among the five eGFR equations evaluated, we showed that the CKD-EPI Chinese T2DM equation provided the best performance in predicting all-cause mortality in Chinese subjects with T2DM.
Disclosure: **A.Z.L. Shih:** None.

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Pleiotropic effects of GLP-1 treatment on renal risk factors in type 2 diabetes

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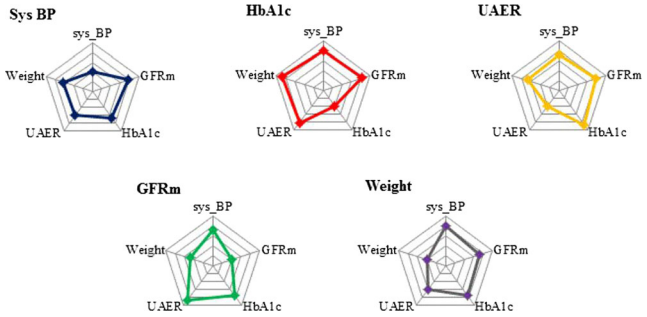
Background and aims: Current management of diabetic nephropathy in type 2 diabetes includes control of blood pressure, lipids and glucose, reduction of albuminuria, and weight loss. Evidence suggests GLP-1 treatment to possess these pleiotropic effects. The objective was to elucidate the individualised GLP-1 treatment response on multiple renal risk factors. The aim was to determine if the "high" responders (highest reduction) on each risk factor also had the highest response on other renal risk factors (cross-dependency). Further, we analysed the effects of treatment/off treatment/re-treatment (the off-on/off-on effect) in the individual patient to account for potential random effects.
Materials and methods: Open-label study including 31 type 2 diabetic patients treated with GLP-1 receptor agonist liraglutide for 7 weeks followed by a 3 week washout period. 23 patients (74%) re-started liraglutide treatment after the primary study and a 1 year follow-up visit was included. Outcome measures were changes in HbA1c, weight, systolic blood pressure, UAER and mGFR (51Cr-EDTA plasma clearance) after 7 weeks of GLP-1 treatment. Changes in high responders (Q4) vs. low responders (Q1-Q3) were compared for each outcome measure. The off-on/off-on effect was evaluated by determination of the changes after 1 year in patients re-starting treatment.
Results: After 7 weeks of treatment, HbA1c was reduced by mean 6 (95% CI: 3 to 9) mmol/mol, weight by 2.5 (1.8 to 3.2) kg, systolic blood pressure by 4 (-1 to 9) mmHg and UAER by 30 (12 to 44) %. mGFR was reduced by 11 (7 to 14) ml/min/1.73m2, previously shown to be reversible and considered a renal haemodynamic effect with this intervention. Individuals with the highest reduction in mGFR had a significant reduction in weight when compared to low responders (4.3 kg vs. 1.9 kg; p=0.002). In individuals with the highest reduction in weight and systolic blood pressure, there was a tendency of a higher reduction in UAER compared to low responders (47% vs. 23% (for both), $p \geq 0.12$). No cross-dependency was observed in any of the other outcome measures ($p \geq 0.16$). The individual treatment response (the off-on/off-on effect) on each outcome measure did not differ after 7 weeks and after 1 year of GLP-1 treatment ($p \geq 0.12$).
Conclusion: GLP-1 treatment possesses pleiotropic effects on renal risk factors. Moreover, the patients experienced same response when re-starting treatment, indicating that our primary findings are not caused by random effects. However, on the patient-level, the effect on the individual risk factor cannot be anticipated based on response in other risk factors.



Clinical Trial Registration Number: NCT01499108
Disclosure: **E.H. Petersen:** None.

OP 37 Novel approaches to study insulin action: omics and beyond

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Multigenerational metabolic reprogramming in the offspring of a non-dietary model of insulin resistance revealed by transcriptomics

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Background and aims: Several studies have focused on investigating the effects of early nutritional insults that increase the likelihood of developing type 2 diabetes (T2D) in the offspring. Virtually none include the use of non-dietary models manifesting hyperglycemia and hyperinsulinemia - two hallmarks of T2D. We aimed to determine the effects of paternal versus maternal genetic insulin resistance on developmental programming in the offspring of the liver-specific insulin receptor knockout (LIRKO) mice.
Materials and methods: Male control F1 offspring from father LIRKO (F1FL), mother LIRKO (F1ML) or control mothers and fathers (C) were weaned on a chow (21%) or high-fat-diet (HFD) (60%) and followed for 3 months. An independent cohort was followed on chow for 12 months. To investigate the presence of maternal multigenerational reprogramming, F2 male offspring from father F1ML (F2ML) and control mothers were weaned on a chow or HFD and followed for 3 months. Phenotypic characterization was accessed in randomly selected mice from at least 3 different offspring per group. Total RNA was isolated from livers of 3 months of age mice on chow and paired-end RNA-sequencing was performed.
Results: At 3 months of age LIRKOs are insulin resistant and glucose intolerant. F1FL, F1ML and F2ML showed impaired growth until 2 months of age on chow exhibiting catch-up growth onwards. After 3 weeks on HFD, F1ML and F2ML but not F1FL exhibit increased body weights compared to C. Total fat mass was 1.4 fold higher in F1FL and F1ML on chow ($p<0.01$; n=5; 12 months of age) and HFD ($p<0.05$; n=4-5, 3 months of age) compared to C. On HFD, F2ML also presented increased fat mass compared to C ($p<0.01$; n=5-8, 3 months of age). Indirect colorimetry revealed metabolic inflexibility in F1FL and F1ML on HFD ($p<0.01$; n=4). F1FL and F1ML exhibited insulin resistance on chow and HFD, while F2ML only developed insulin resistance on HFD. F1FL and F1ML developed fasting hyperglycemia with aging (F1FL: 100.6±10; F1ML: 102.8±8.1; C: 67.6±5.2 mg/dL, F1FL vs C: $p<0.05$; F1ML vs C: $p<0.01$; n=5; 12 months of age) and on HFD (F1FlL: 110.2±6.2; F1ML: 107.2±4.1; C: 78.3±6.9 mg/dL; F1FL vs C and F1ML vs C: $p<0.01$; n=4; 3 months of age). F1FL and F1ML became hyperinsulinemic with aging (F1FL: 5.3±1.54; F1ML: 5.9 ±1.59; C: 1.9±0.52 ng/mL; F1FL vs C: $p=0.07$; F1ML vs C: $p<0.05$; n=5; 12 months of age) and F1ML on HFD (F1FL: 1.62±0.67; F1ML: 2.63±0.66; C: 0.52±0.11 ng/mL; F1ML vs C: $p<0.05$; n=4). At 3 months of age F2ML presented hyperglycemia on chow ($p<0.05$, n=6-7) and hyperinsulinemia on HFD ($p<0.05$; n=5). F1FL, F1ML and F2ML developed prominent hepatic steatosis compared to C on HFD. F1FL and F1ML on HFD presented increased hepatic gene expression of fatty acid transports and lipogenesis-associated genes. Transcriptomic analysis of F1FL and F1ML livers on chow presented down-regulation in pathways associated with insulin and MAPK signaling ($p<0.001$, n=4), while F1ML presented increased regulation in pathways associated with non-alcoholic fatty liver disease ($p<0.001$, n=4).

Protein analysis of insulin signaling in the liver extracts of F1FL and F1ML after in vivo insulin stimulation revealed decreased p-GS3Kβ (F1FL vs C and F1ML vs C: $p<0.05$; n=3; 3 months of age).
Conclusion: These data suggest that prenatal insulin resistance have detrimental effects on the metabolic adaptation and transcriptional regulation of hepatic metabolism in the offspring that contribute to impaired growth and metabolic response to dietary challenges.
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Disclosure: **D.F. De Jesus:** None.

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A proteomic signature that reflects pancreatic beta cell function

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Background and aims: Proteomics in the study of pancreatic beta-cell function and type 2 diabetes mellitus (T2DM) has the potential to be a highly useful technique in both improving the diagnosis of T2DM and monitoring the various stages of disease progression. Gaining knowledge of the proteome may also aid in the development of new targets/ lifestyle interventions in the prevention of T2DM. The objectives of this research are to 1) uncover protein signatures and pathways related to pancreatic beta-cell function 2) validate findings where possible.
Materials and methods: This research focuses on data obtained from the Metabolic Challenge (MECHE) study. Healthy participants aged between 18-60 years were recruited, and both beta-cell function adjusted for homeostatic model assessment of insulin resistance (HOMA-IR) and the disposition index (DI) were calculated for participants who completed an OGTT and had complete proteomic data (n=100). The MECHE proteomic dataset contains information on 1129 proteins which were measured using the SOMAscan assay. Pearson's correlations and linear regression analysis examined the relationship between the proteomic data and beta-cell function measures. Pathway analysis was performed by PathVisio. The BRIN-BD11 rat pancreatic beta-cell line was used to perform in vitro verification. Ethical approval was obtained from the Research Ethics Committees in our University College and the study was performed according to the Declaration of Helsinki.
Results: Beta-cell function/HOMA-IR was significantly correlated with 27 proteins and DI was significantly correlated with 20 proteins ($p< 0.01$). Stepwise linear regression analysis determined the strongest predictors of beta-cell function/HOMA-IR to be tissue factor pathway inhibitor (TFPI) and interleukin-17F (IL-17F) (β= -0.324 and -0.273 respectively) whilst beta-endorphin was positively correlated with beta-cell function/HOMA-IR (β= 0.306) (overall p= 0.04). Calcineurin and cytotoxic and regulatory T-cell molecule (CRTAM) were the strongest predictors of the DI (β= 0.290 and 0.291 respectively) (overall p= 0.036). Pathway analysis identified the biological processes related to beta-cell function. In vitro experiments confirmed that IL-17F modulated insulin secretion in the BRIN-BD11 cell line, with the lower concentration of 10ng/ml significantly increasing glucose stimulated insulin secretion (p= 0.004). Calcineurin was validated in a smaller second human cohort and was significantly related to both beta-cell function measures (beta-cell function/HOMA-IR (β= 0.299, p= 0.046), DI (β= 0.316, p= 0.035).
Conclusion: In conclusion this analysis uncovered a protein signature related to beta-cell function. Early detection of decreased pancreatic beta-cell function would allow for implementation of nutritional and lifestyle interventions before progression into T2DM status. To further understand the biological basis of the altered proteomic signatures pathway was performed. Future work will involve validation of remaining proteins of the proteomic signature and investigation of potential modulation of these proteins to enhance beta-cell function.
Clinical Trial Registration Number: NCT01172951
Supported by: TC20130001, 07FHRIUCD1
Disclosure: **A. Curran:** None.

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Pathway-based approach to reveal the gene-brain interactions for the cognitive impairments of patients with type 2 diabetics

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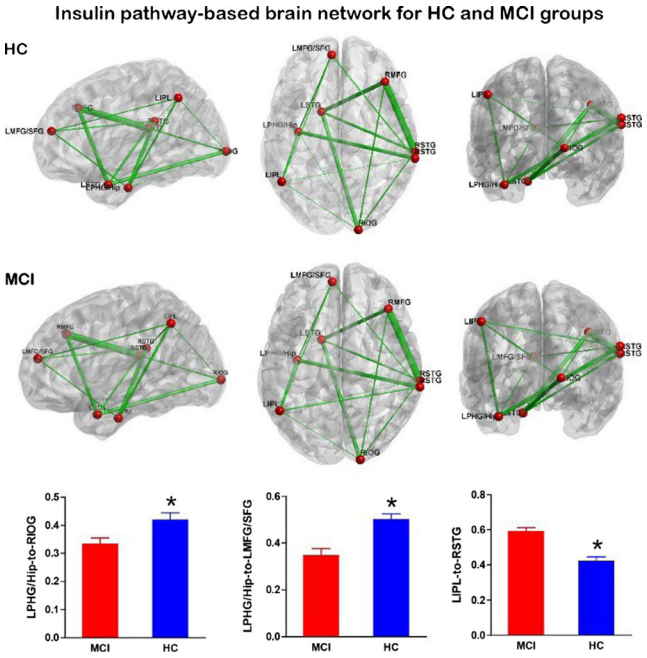
Background and aims: Pathway-based approach has been suggested to comprehensively evaluate to gene-disease interactions by integrating the combined effects of multiple variances within genes known to have biological relationship with one another. Using brain MRI imaging as phenotypes in the gene-association analyses could in vivo reveal the genetic bases for brain dysfunction. Brain insulin dysfunctions represent key mechanisms underlying the cognitive impairments of patients with diabetics, while the brain functional alterations based on the heredity of insulin pathway were not understood
Materials and methods: 168 patients with type 2 diabetic were enrolled, and they went through comprehensive evaluations of multiple cognitive domains. 101 controls were free of cognitive complaint and had normal cognition, and the other 67 participants had cognitive complaint and mild cognitive impairment (MCI). Resting-state functional magnetic resonance imaging was used to assess the neural activity. Genetic variances located in gene involved in brain insulin dysfunction were sequenced via high-throughput technology, and 278 single nucleotide polymorphisms within 17 genes were included in analyses after quality control. The cognitive significances of each gene were explored by set-based analyses, and the SNPs showed interactions with cognitive performances were selected in further MRI analyses. The mass univariate modeling was established and a pathway-based imaging genetics approach was utilized to investigate the disease-related distinctions of genotype-by-MCI interactions between controls and MCIs.
Results: Widespread changes of neural activities were related to the insulin dysfunction pathway genes in MCI subjects, mainly within inferior parietal lobule (IPL), middle frontal gyrus (MFG), superior frontal gyrus (SFG) hippocampus/parahippocampal (PHG/Hip), temporal cortex and inferior occipital gyrus (IOG). With respect to the disease-related differences in the networks, MCI subjects showed reduced connectivity of two edges, including LPHG/Hip-to-RIOG ($T = -4.29$, $P = 0.023$) and LPHG/Hip-to-LMFG/SFG ($T = -3.91$, $P = 0.035$). Meanwhile, increased connectivity was also detected for LIPL-to-RSTG in MCI ($T = 4.02$, $P = 0.029$). Furthermore, for MCI subjects, the more the connectivity increased between LIPL and RSTG, the more the general cognition injured ($r = 0.51$, $P = 0.002$).
Conclusion: The insulin pathway based imaging genetics approach expand our understanding for the mechanisms underlying the diabetics-related injury of cognition, and alleviating the brain insulin resistance may be promising treatment targets to prevent the cognitive decline.



Disclosure: **F. Su:** None.

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Life long moderate insulin reduction extends mammalian lifespan, and demonstrates a causal role for hyperinsulinaemia in insulin resistance

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Background and aims: Hyperinsulinemia is commonly considered to be a downstream consequence of insulin resistance and obesity, but newer models where insulin production can be directly modulated are challenging the prevailing paradigm. Genetic down-regulation of insulin/insulin-like growth factor (IGF)-1 signaling extends lifespan in invertebrates and mammals, but the role of the insulin ligand itself in altering mammalian longevity has remained controversial, in part because few models are available that can distinguish the effects of these two related hormones. A prominent role for insulin in longevity has been generally disregarded, as it is essential for glucose homeostasis. In some cases decreased insulin signaling has been associated with greater risk of age-related disease instead of lifespan extension. Because there has been no way to separate the effects of hyperinsulinemia from insulin resistance, the physiological effects of insulin levels on mammalian healthspan and lifespan were unresolved.
Materials and methods: Throughout their natural lifespans, we characterized insulin levels, glucose tolerance, insulin sensitivity, body composition, and strength in large cohorts of male and female Ins1-/-:Ins2+/- experimental mice and their Ins1-/-:Ins2+/+ control littermates that were fed one of two distinct diets.
Results: At 2 years of age, female Ins1-/-:Ins2+/- experimental mice had ~20% reduced circulating insulin regardless of diet when compared with Ins1-/-:Ins2+/+ control littermates. We report that rather than having negative repercussions on glucose homeostasis, decreasing insulin led to fasting glucose that was 1 mM lower, with a significantly reduced HOMA-IR, and ~15% improved insulin sensitivity in insulin tolerance tests in aged, experimental mice across both diets. Body mass and fat mass were reduced by 10% across both diets, while fat-free mass and grip strength were unchanged in mice with modestly reduced insulin. We also find that a modest reduction in circulating insulin was sufficient to impart significant lifespan extension in female mice. The pro-longevity effect of reduced insulin was not associated with altered IGF-1, and was robustly observed across two diverse diets. Autopsy analyses of each mouse suggested a generalized improvement in health span in Ins1-/-:Ins2+/- mice, rather than protection from a specific cause of death. RNA-sequencing of liver samples from 30 week old mice demonstrated both pan-diet and diet-specific changes in several key growth, metabolism, and longevity related pathways. In male littermates, the reduction of insulin gene dosage was not sufficient to consistently reduce circulating insulin, so we were unable to test our primary hypotheses.
Conclusion: Our results demonstrate that a component of the insulin resistance that is observed with aging is caused by excessive insulin secretion. This is incompatible with the current dogma. Our study shows that moderately lowering circulating insulin, without changing IGF-1, can promote healthier aging and extend lifespan in mammals. These results have implications for the choice of individualized diets that can maintain insulin levels within a healthy range.
Supported by: CIHR Operating Grant, Novo-Nordisk Innovation Grant
Disclosure: **J.D. Johnson:** None.

OP 38 Novel mechanisms of complications

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Slow breathing-induced improvement in subclinical hypoxia, autonomic and vascular function is associated with acute reduction in reactive oxygen species in type 2 diabetes

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Background and aims: Oxidative stress is an established cause of autonomic and vascular abnormalities in diabetes, but has also a negative impact in cardiovascular diseases. In heart failure, hypertension and stroke oxidative stress could be improved by implanted vagal stimulators. In healthy subjects, if oxidative stress was transiently increased by fat meal, similar effects were obtained with slow-deep breathing, which improves parasympathetic function, blood oxygenation and vascular function. We tested whether the antioxidant defense and reactive oxygen species (ROS) production could be modified by slow breathing in patients with type 2 diabetes, and whether this was associated with an improvement in autonomic (heart rate variability [standard deviation of heart period, SDNN] and baroreflex sensitivity [BRS]) and arterial function (augmentation index [AI75] and pulse wave velocity [PWV]), and in oxygen saturation (SaO2).
Materials and methods: In 25 type 2 diabetic patients and 25 healthy controls (age 57±13, 9 male, vs 60±8 yr, 9 male, mean±SD, diabetes duration 9.3±5.4yr, Hba1c 7.0±1.1 mmol/mol) we measured the biological antioxidant potential (BAP® test, Diacron) and reactive oxygen (hydroperoxide) metabolites (d-ROMs® test, Diacron), on fresh venous plasma samples, together with SaO2, BRS, SDNN, AI75 and the PWV, obtained by continuous non invasive blood pressue, electrocardiogram and pulse oximetry monitoring. Data were obtained at rest (5 min), during slow breathing (6 breath/min, 5 min) and 5 and 10 min thereafter. Differences in response to slow breathing were tested by analysis of variance.
Results: Diabetic patients had lower resting SaO2 (96.0±1.4 vs 97.4 ±1.6%, p=0.004) SDNN (15.7±9.1vs 24.8±16.7) and BRS (4.1±2.6 vs 6.8±3.5 ms/mmHg, p=0.0017) and similar AI75 and PWV. Slow breathing improved oxygen saturation (p<0.0001), SDNN (p<0.0001), AI75 (diabetic group from 24.6±10.2 to 22.1±9.8%, p=0.002 , control group from 20.7±7.7 to 19.4±6.9%, p=0.03) in both groups but more (p<0.05 or better) in the diabetic group, and BRS only in the diabetic group (to 5.1±3.4, p=0.046). No changes occurred in PWV. Five and ten minutes after slow breathing, all these data returned to baseline. In the Diabetic group the d-ROMs test was not higher than normal at rest but started to decrease after slow breathing, reached significance after 5 min (from 553±163 to 529±154 UCARR, p=0.00018) then returned to baseline after 10 min. No changes were observed in the control group. The BAP test remained higher (p=0.044) in the diabetic group at all measures without modification by slow breathing.
Conclusion: Slow breathing acutely reduced ROS without affecting the antioxidant reserve, in conjunction with improvement in oxygen saturation, autonomic and vascular function, with a time delay possibly due to the time needed to build up the hydroperoxide products in the blood. The positive antioxidant effects of slow breathing seems dependent on a preexisting condition of oxidative stress, as it occurred only in the diabetic group. Subclinical hypoxia appears as a possible cause of oxidative stress in diabetes.
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Disclosure: **L. Bianchi:** None.

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Microglial activation prevents vasoregression in degenerative retinopathy

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Background and aims: Inflammation and glial activation are key contributors in the progression of diabetic retinopathy (DR). As such their eligibility as therapeutic targets has to be determined carefully. The non-obese and non-hyperglycemic transgenic polycystic-kidney-disease (PKD) rat shows a retinal phenotype similar to DR. In order to describe the role of the retinal innate immune system in the development of a DR-like retinopathy, CD74+ microglia were identified as possible effector system for vasoregression as well as neurodegeneration. Our aim was to analyze the effects of a microglia modulating intervention.
Materials and methods: BV2 cells were incubated with clodronate-coated liposomes for 24 hours, uncoated liposomes and phosphate buffered saline (PBS) served as controls. Viability of the cells was analyzed using a MTT-assay. 4-weeks old PKD rats were intravitreally injected with 3μL clodronate-coated liposomes (5mg/mL) using untreated PKD rats as control. Animals were sacrificed 7 days after injection. To analyze any additive effects in-vivo, PKD rats were injected twice with 4 and 8 weeks of age. Clodronate-treated Sprague Dawley (SD) rats served as control. These groups were sacrificed 4 weeks after the second injection. Glial activation was assessed using immunofluorescence and qPCR of activation markers. Vasoregression was analyzed using quantitative retina morphometrie (QRM).
Results: Incubation with clodronate-coated liposomes in a concentration comparable to the one used in-vivo reduced the number of BV2 cells by 96.4% (p<0.0001), cells incubated with uncoated-liposomes were not significantly reduced. After a single intravitreal injection the total number of microglia was reduced in the superficial capillary layer of PKD rats by 17% compared to controls (p<0.05). The number of CD74+ microglia increased in the superficial layer 8.8-fold (p<0.001) in injected animals. In the deep capillary layer the number of CD74+ microglia was reduced by 50% (p<0.05) but the total number remained unaffected. The amount of acellular capillaries was reduced only in the deep capillary layer by 28% in the treated group (p<0.05). On mRNA level the expression for microglia markers like C3, CD74 and Arginase-1 did not differ in between the groups. Glial fibrillary acidic protein (GFAP) expression as a marker for gliosis was not affected in these animals. Following two injections of clodronate-coated liposomes the number of CD74+ microglia in the deep capillary layer showed a 3.4-fold increase (p<0.01) without a significant difference in the number of total microglia. The superficial layer showed no differences in the numbers of total and CD74+ microglia. In treated SD rats the immunofluorescence for GFAP revealed gliosis but the number of total and CD74+ microglia were not changed significantly.
Conclusion: Clodronate activates microglia following intravitreal injection. Since the in-vitro experiment showed a depletory effect our data on long-term treatment indicates a stimulatory effect in-vivo. Despite an increase in activation, the fact that vasoregression is prevented and pro-inflammatory markers are not affected further studies have to address the polarization of the microglia, the role of müller cells in the inflammatory cascade and the functional outcome of microglia-based interventions.
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Disclosure: **M. Kolibabka:** None.

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Compound 21, an angiotensin II type 2 receptor agonist, is protective in experimental diabetes-associated atherosclerosis

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Background and aims: Angiotensin (Ang) II is well-recognised to be a key mediator in driving the pathological events of diabetes-associated atherosclerosis (DAA) via its AT1 receptor (AT1R) subtype. However, its biological actions through the AT2 receptor (AT2R) subtype still remain unclear. Thus, this study aimed to investigate the role of the AT2R in an experimental model of DAA, using a novel selective AT2R agonist, Compound 21 (C21).

Materials and methods: Apolipoprotein-E knockout (ApoE-/-) mice were rendered diabetic with streptozotocin (55mg/kg/day) and treated in the absence or presence of C21 (1mg/kg/day) over a 20 week period. Non-diabetic ApoE-/- mice subjected to similar treatment served as controls. The specific role of the AT2R in terms of modulating atherosclerotic pathway was further investigated in vitro using human aortic endothelial cells and monocytes cultures in the absence or presence of hyperglycemia.

Results: C21 treatment significantly attenuated aortic plaque area (Diabetic; 12.5±0.8% vs Diabetic+C21; 6.16±0.4%, $p<0.01$ vs diabetic mice) to a level similar to that seen in non-diabetic mice (Table). RT-PCR analyses also demonstrated that C21 treatment significantly ameliorated diabetes-induced up-regulation of oxidative stress (p47phox, Nox1, Nox4), inflammatory (MCP-1) and fibrotic (TGF-β) markers (all $p<0.05$ vs diabetic mice), while having no effect on any of these markers in non-diabetic mice. These beneficial effects of C21 were found to be independent of blood pressure and glycaemic control. In vitro studies in human endothelial cells and monocytes demonstrated that glucose-induced gene up-regulation of MCP-1, various Nox isoforms and TGF-β was abrogated by C21. Furthermore, the benefits seen with C21 were reversed by the AT2R antagonist, PD123319.

Conclusion: Treatment with C21 appears to be protective against diabetes-associated atherosclerosis. These benefits appear to involve the suppression of oxidative stress, inflammation and fibrosis associated with diabetes.

Measured Parameters	Non-diabetic (control)	Control+C21	Diabetic	Diabetic+C21
Plasma glucose (mmol/L)	10.0 ± 0.4	13.4 ± 0.9	23.5 ± 2.1**	20.3 ± 2.0**
Glycated haemoglobin ($HbA1_c$) (%)	3.9 ± 0.1	4.2 ± 0.1	12.4 ± 0.7**	12.7 ± 0.6**
Total cholesterol (mmol/L)	9.0 ± 0.7	10.0 ± 0.6	14.1 ± 1.0**	15.3 ± 1.7**
Triglycerides (mmol/L)	1.5 ± 0.2	1.2 ± 0.2	2.7 ± 0.4**	3.6 ± 0.2**
Systolic BP (mmHg)	95 ± 1	94 ± 2	96 ± 1	95 ± 2
Plaque area (%)	6.1 ± 0.6	7.5 ± 0.5	12.5 ± 0.8**	6.2 ± 0.4 ¶¶
Atheroma Area ($x10^3$ μm²)	136.5 ± 29.4	128.8 ± 14.3	240.4 ± 6.6**	183.5 ± 24.3¶
VCAM-1 staining ($x10^3$ μm²)	95.0 ± 10.4	101.9 ± 12.4	338.0 ± 95.6**	98.8 ± 21.7¶

**p<0.01 vs vehicle-treated control (non-diabetic) group; ¶p<0.05, ¶¶p<0.01 vs diabetic mice. Data are shown as mean ± SEM. N=14-21 animals per group.

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Disclosure: **T. Allen:** None.

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Intestinal inflammation is evident already before diabetic nephropathy in type 1 diabetes

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Background and aims: Poor glycemic control, dyslipidemia, and low-grade chronic inflammation increase the risk of diabetic nephropathy in patients with type 1 diabetes (T1D). Intestinal alkaline phosphatase (IAP) regulates lipid transport and inflammatory responses in the gastrointestinal tract. Whether IAP is altered in individuals with T1D, thereby affecting gut homeostasis, is not known.

Materials and methods: Fecal samples of 41 non-diabetic controls (NDC), 36 T1D patients with normal albumin excretion rate (T1D_N; AER <20μg/min or <30 mg/24 h), and 10 T1D patients with macroalbuminuria (T1D_M; AER ≥200μg/min or ≥300 mg/24 h) were studied for levels of intestinal inflammatory markers: intestinal alkaline phosphatase (IAP), immunoglobulins, short-chain fatty acids (SCFAs) and calprotectin.

Results: Patients with T1D presented significant changes in fecal inflammatory biomarkers compared to non-diabetic controls. T1D_N subjects, compared to NDC, had lower fecal IAP activity (61 [26-221] vs. 131 [73-837] U/l, p=0.010), butyrate (39 [23-81] vs. 71 [40-101] μmol/g dry weight, p=0.020), and secretory IgA concentrations (1.9 [0.7-3.6] vs. 3.4 [1.5-6.9] μg/g wet weight, p=0.015). Moreover, concentrations of fecal IgA antibodies to oxidized-LDL epitopes (MAA-LDL, malondialdehyde-acetaldehyde modified LDL; CuOX, copper-oxidized LDL) were decreased in T1D_N subjects compared to NCD. Similar trends were observed in patients with macroalbuminuria. Strikingly, individuals with T1D presented higher levels of fecal calprotectin compared to NDC. Based on the cutoff value of 50 μg/g, a mild pro-inflammatory state of the gastrointestinal tract was more frequently seen in T1D_N subjects compared to NDC (50% vs. 25%; p=0.024). Fecal calprotectin concentrations above 200μg/g (clinical inflammatory bowel disease cut-off) was more common among T1D_M subjects compared to those with normal AER (40% vs. 6%; p=0.017).

Conclusion: Deprivation of immunoprotective intestinal factors may increase the risk of inflammation in the gut - a phenomenon which seems to be present already in patients with uncomplicated type 1 diabetes. Low levels of intestinal IgA and antibodies to oxidized lipid epitopes may render such patients susceptible to inflammation-driven complications such as nephropathy and cardiovascular disease.

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Disclosure: **M.I. Lassenius:** None.

OP 39 Sweet and low

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Depressive symptoms in people with diabetes: results from the International Diabetes Management Practices Survey

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Background and aims: Depression is more common in diabetes than in general population and may negatively influence metabolic control. The aim was to examine the frequency and risk factors for depressive symptoms in type-1 (T1DM) and type-2 diabetes (T2DM).

Materials and methods: The International Diabetes Management Practices Survey (IDMPS) is a multinational observational cross-sectional study conducted yearly since 2007 to examine the quality of care in T1DM and T2DM in real-world settings. Demographic, clinical and therapeutic data from the 5th wave performed in 2011 in 21 countries (Africa, Middle East, South Asia, Eurasia, Latin America and Turkey) were analyzed. Depressive symptoms were evaluated using the Patient Health Questionnaire (PHQ)-9. Univariate analyses and multivariate logistic regressions adjusted on countries were performed to identify risk factors of depression.

Results: Of the 9865 people eligible for analysis, 2280 had T1DM and 7585 had T2DM (4729 treated with oral glucose lowering drugs only (OGLD), 1892 with OGLD + insulin and 964 with insulin only). In the T1DM group, mean age was 33.6 years and 52.5% were women. Among the T2DM subgroups, mean age ranged from 56.8 to 60.1 years and female proportion ranged from 48.0% to 54.9%. Mean disease duration was 11.8 years in the T1DM group and 48.1% had diabetes-related complications. In the T2DM groups, mean disease duration ranged from 6.8 to 12.6 years and respectively 43.1% of people with OGLD-only, 70.3% of people with OGLD + insulin and 81.8% of people with insulin-only had complications. In the T1DM group, 22.6% were at treatment goal (HbA1c<7%). In the T2DM group, 41.1% were at goal in the OGLD-only subgroup and <20% in the insulin-only or OGLD + insulin subgroups. In the T1DM group, 30.7% of people reported mild to severe depressive symptoms (PHQ-9 score from 7 to 27). In the T2DM group, the respective proportions were 29.0% in OGLD-only, 36.6% in OGLD + insulin and 46.7% in insulin-only subgroup. On multivariate analysis, depression was associated with women and diabetes complications in both T1DM and T2DM. In T1DM and T2DM OGLD-only subgroup, depression was also associated with poor glycemic control.

Conclusion: Depression was common in both T1DM and T2DM. While the causal nature of the association between glycemic control and depression requires further elucidation, our results support the call for routine screening for depressive symptoms in people with diabetes for early intervention.

Results of multivariate analyses

Risk Factors (OR [95% CI])	T1DM	T2DM with OGLD only	T2DM with OGLD + Insulin	T2DM with Insulin only
	N=2017*	N=3993*	N=1804*	N=929*
Macrovascular complication(s): Yes *vs.* No	2.1 [1.4; 3.2]	1.7 [1.4; 2.2]	2.0 [1.5; 2.6]	2.4 [1.7; 3.4]
Microvascular complication(s): Yes *vs.* No	2.5 [1.9; 3.3]	1.5 [1.2; 1.8]	1.8 [1.3; 2.4]	*NS*
HbA1c: ≥7% *vs.* <7%	1.6 [1.1; 2.1]	1.3 [1.0; 1.5]	*NS*	*NS*
Gender: Women *vs.* Men	2.0 [1.6; 2.6]	2.1 [1.8; 2.5]	1.7 [1.3; 2.2]	2.1 [1.5; 2.9]
Living area: Rural *vs.* Urban/Sub-urban	2.2 [1.5; 3.4]	*NS*	*NS*	*NS*
Age: >65 *vs.* ≤65 years old	*NS*	1.3 [1.0; 1.6]	*NS*	*NS*
Education level:	*NS*	*NS*		*NS*
Illiterate/Primary *vs.* University/Higher			1.4 [0.9; 2.0]	
Secondary *vs.* University/Higher			1.4 [1.0; 2.0]	

OR: Odd Ratio; CI: Confidence Interval; NS: non significant
* **N: Number of subjects included in the model**

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Validation of a psychometric tool to assess diabetes acceptance: the Diabetes Acceptance Scale (DAS)

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Background and aims: Suboptimal diabetes acceptance is associated with reduced self-management and hyperglycaemia. To assess diabetes acceptance, however, only one rather limited tool (AADQ) was available so far. To enable more sophisticated assessments, the Diabetes Acceptance Scale (DAS) was recently developed. This study evaluates its psychometric properties.

Materials and methods: The DAS is a 28-item self-report scale enabling estimation of a total score of diabetes acceptance and four subscales reflecting specific aspects: integration, motivation, avoidance/defence and distress; development described in EASD 2015 abstract #946. In this study we assessed 508 diabetes patients (53% type 1, 46% type 2, 1% other; 52% females; age 51±15 y.; BMI 30±7 kg/m2; diabetes duration 15±11 y.; HbA1c 7.9±1.6%) with the DAS and self-report scales for diabetes non-acceptance (AADQ), diabetes distress (PAID-5), depressive symptoms (PHQ-9) and diabetes self-management (DSMQ); HbA1c was estimated using HPLC; complications were self-reported. We analysed reliability, factorial validity and criterion validity.

Results: All DAS scales showed high reliability (Cronbach's α: total scale 0.96; subscales: integration 0.94, motivation 0.92, avoidance/defence 0.89, distress 0.89). A confirmatory factor analysis supported the four-parted scale structure (SRMR=0.049, CFI=0.936, RMSEA=0.066). The DAS showed good convergence with the parallel non-acceptance measure AADQ (r=-0.65, P<0.001). Higher DAS acceptance scores were associated with lower diabetes distress (r=-0.69), fewer depressive symptoms (r=-0.58), better self-management (dietary control: r=0.53; medication adherence: r=0.53; glucose monitoring: r=0.42; physician contact: r=0.47) and better glycaemic control (HbA1c: r=-0.44); all P<0.001. Patients with reduced acceptance (T scores<40) compared to normal acceptance (T≥40) reported lower engagement in self-management activities and showed poorer glycaemic control (see Fig. 1); they were more likely to have had events of diabetic ketoacidosis in the preceding year (11% vs. 4%) and to be diagnosed with neuropathy (28% vs. 16%); P≤0.01. Subscale results were generally similar.

Conclusion: The present findings support the DAS as reliable and valid measure of diabetes acceptance. The scale may facilitate research in this important field and help identify patients with problems of accepting diabetes, a high-risk group in need of tailored care and support.

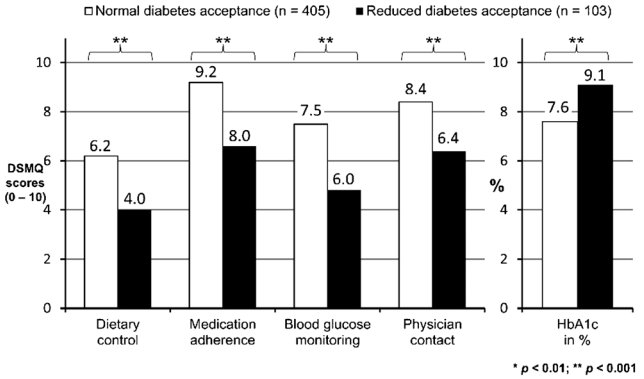

Figure 1. Diabetes self-management activities and glycaemic control of patients with markedly reduced diabetes acceptance (T score < 40) compared to those with normal acceptance (T score ≥ 40) according to the Diabetes Acceptance Scale (DAS)

Supported by: German Center for Diabetes Research (DZD); German Diabetes Foundation (DDS)
Disclosure: **A. Schmitt:** None.

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Generic and disease-specific quality of life in adolescents with type 1 diabetes: comparison to age-matched healthy peers

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Background and aims: There are a few studies examining the health-related quality of life (HRQoL) of adolescents with type 1 diabetes (T1DM), however, different results were demonstrated in surveys in relation to the healthy population. This study aimed to evaluate the HRQoL of adolescents with T1DM on the basis of the Pediatric Quality of Life Inventory™ (PedsQL™) generic and diabetes-specific modules, and compare it to that of healthy peers.
Materials and methods: This study involved 650 participants between ages of 13 -19 including 296 adolescents with T1DM (44.9% females) from 4 diabetes centers and 354 healthy peers (52.3% females) matched for age and gender from three different cities of the country. Participants completed the validated PedsQL™ for assessing the HRQoL. Demographic and clinical data were also recorded. The analysis included independent t-test to compare the means of the total and subscales of the PedsQL™ between boys and girls as well as between groups. Gender differences in exercise (doing regular exercise vs. not regular exercise), intensive insulin therapy modalities (continuous subcutaneous insulin infusion vs. multiple daily injections) were evaluated with the Pearson Chi-square test.
Results: Adolescents with T1DM have similar HRQoL in all domains when compared to their healthy counterparts. Females report impaired HRQoL than males regardless of the presence of the disease (Adolescents with T1DM: boys: 79.74 ±11.16 vs. girls: 75.71 ±12.96; p<.05; Healthy adolescents: boys: 79.52 ±10.41 vs. girls: 74.08 ±10.37; p<.001). Insulin pump therapy (without gender difference) facilitates better glycemic control (F=9,019, p<.01) and HRQoL (F=12,873, p<.001). Regular exercise positively correlates with the HRQoL in both groups (Adolescents with T1DM: F=5,065, p<.05; Healthy control: F=11,978, p=.001); however, it has no relationship with glycemic control. A significant difference was found in regular exercise between the healthy boys and boys with T1DM ($\chi 2(1)=7,756$, p=.006), but it was not observed in the case of girls. Boys with T1DM were less engaged in regular exercise than their healthy peers.
Conclusion: Optimal metabolic control and improved HRQoL are the eventual goals of diabetes management. Despite the difficulties, adolescents with diabetes can manage their disease well and live normal lives, similar to their healthy peers. Although diabetes-related problems exist, it can be eased by encouraging patients to exercise regularly, as well as to promote the insulin pump therapy where it is applicable.
Disclosure: **A. Lukács:** None.

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Increased depression symptom score in newly diagnosed type 2 diabetes patients

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Background and aims: Registries represent a valuable tool to examine the health care and outcomes of type 2 diabetes patients. DIAREG is a novel German diabetes registry with a focus on patient reported outcomes (PRO) and allows consequently to analyze both quality of life (QoL) and the subjective patient well-being of type 2 diabetes patients under real world conditions. The aim of this analysis was to investigate the association between diabetes duration and depressive symptoms. Furthermore, the association of depression with QoL and treatment satisfaction was evaluated.
Materials and methods: DIAREG was initiated as a non-interventional registry study for type 2 diabetes, using a mixed methods approach. Backbone of the registry are retrospective data of an existing nationwide general and internal medicine practice database (IMS Disease Analyzer, Germany) which was augmented by prospective data from physician questionnaires as well as PRO questionnaires. Different patient questionnaires were chosen to measure patients' quality of life multidimensionally. We used the Center of Epidemiological Studies Depression Scale (CES-D) and the Short-Form 36 Questionnaire (SF-36). Furthermore, the Audit of Diabetes-Dependent Quality of Life (ADDQoL) and the Diabetes Treatment Satisfaction Questionnaire (DTSQ) were used to analyze the relationship between depression, QoL and treatment satisfaction. Type 2 diabetes patients were enrolled by general practitioners as well as by diabetologists (ratio 85% to 15%). From August 2013 until March 2016, 2,101 patients were registered in 108 practices and from 386 (18%) patients PRO could be collected, which were similar with respect to age, sex, and diabetes duration to the whole sample.
Results: In a cross-sectional analysis, 270 patients with completed PRO questionnaires could be evaluated. The analysis of the CES-D showed that patients with a shorter diabetes duration less than two years display a trend to an increased depression score (mean CES-D score: 20 (SD=9.3) vs. 13 (SD=9.1) in patients with diabetes duration 2-4 years; p<0.001). Patients with a shorter disease duration also showed a decreased physical sum score of the SF-36 in comparison to patients with a longer diabetes duration. These results persisted after adjustment for age and sex (linear regression). Independent from diabetes duration, depressive patients (based on CES-D score: 0-15 not depressed, ≥16 depressed) showed a significantly decreased QoL (mean ADDQoL score: -1.8 (SD=1.9) vs. -0.9 (SD=1.4); p<0.0001), but no difference in treatment satisfaction (mean DTSQ score: 29 (SD=5.9) vs. 30 (SD=6.6); p=0.5379).
Conclusion: These results show for the first time an elevated depression score in patients with newly diagnosed type 2 diabetes under real world

conditions. Most likely the increased CES-D score is related to an emotional distress due to the diagnosis, therapy and possible complications of the newly diagnosed chronic diabetes disease. Since emotional distress might impair the motivation for self-care and successful self-management it would be advisable to screen newly diagnosed diabetes patients for emotional distress and symptoms of depression in order to support them coping with the emotional burden of diabetes and prevent lasting depression.

Clinical Trial Registration Number: NCT01906294

Disclosure: **M. Hahn:** None.

OP 40 Picturing the brain

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Discordance between central (brain) and pancreatic action of exenatide in lean and obese subjects

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Background and aims: The brain plays a central role in the regulation of appetite and food intake. GLP-1 receptor agonists, in addition to lowering the plasma glucose concentration, suppress appetite and promote weight loss. The aim is to compare the relationship between the central (brain) and pancreatic actions of GLP-1 receptor activation in lean and obese individuals with normal glucose tolerance

Materials and methods: Brain fMRI signal in response to high-calorie-content food pictures was measured with and without intravenous exenatide infusion in 10 lean and 10 obese healthy volunteers. Insulin secretion was measured with two-step (+100 and +200 mg/dl) hyperglycemic clamp with exenatide and with saline infusion

Results: Obese individuals had significantly greater first phase insulin secretion as measured by the incremental area under the plasma insulin concentration during the first 10 minutes of the hyperglycemic clamp (ΔI0-10) (8.0±2.2 and 3.1±0.4 μU/ml, $p<0.05$) performed with saline infusion. Similarly, ΔI(10-60) and ΔI(60-120) were significantly greater in obese compared to lean individuals. Exenatide infusion caused a marked increase in insulin secretion in both obese and lean individuals. The incremental area under the plasma insulin concentration during the hyperglycemic clamp increased from 48 to 801 uU/ml.hour following exenatide infusion in lean individuals and from 179 to 1116 uU/ml.hour in obese subjects. Thus, exenatide caused an 18.5-fold increase in ΔI0-120 during the hyperglycemic clamp in lean individuals compared to an 8.8-fold increase in obese individuals ($p<0.05$). The brain fMRI signal in response to food pictures was significantly higher in obese vs. lean groups in the left insula, right amygdala, right hippocampus and frontal cortex. Interestingly, the fMRI signal in the hypothalamus, which plays a central role in the regulation of food intake, was significantly greater in lean compared to obese individuals ($p<0.05$). Exenatide infusion caused a significant decrease in fMRI signal in response to food pictures in the left insula, right amygdala, right hippocampus and frontal cortex in obese subjects. However, exenatide infusion did not affect the fMRI signal in any brain area in lean individuals. In obese subjects exenatide infusion caused an increase in hypothalamic fMRI signal while it had no effect in lean individuals. During exenatide infusion, the brain fMRI signal in response to food pictures in the hypothalamus and other brain areas associated with the reward system was comparable in lean and obese individuals. There was no correlation between the increase in plasma insulin signal or decrease in the plasma glucagon signal versus the fMRI signal in any of the brain regions. There was no significant correlation between the magnitude of increment in insulin secretion caused by exenatide infusion during the hyperglycemic clamp and the effect of exenatide on fMRI signal in any brain area.

Conclusion: Exenatide causes greater augmentation in insulin secretion in lean compared to obese individuals, while it inhibits the brain response to food pictures only in obese individuals

Disclosure: **G. Daniele:** None.

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Hypothalamic glucose transport kinetics in experimentally induced Hypoglycaemia-Associated Autonomic Failure (HAAF) in humans

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Background and aims: The hypothalamus is thought to play a critical role in glucose sensing and regulating counterregulatory hormone response to hypoglycemia (HG). The mechanisms responsible for the development of HAAF remain uncertain, but some suggest that increased glucose transport may contribute. Hypothalamic glucose transport kinetics have not been studied before in humans. Here we tested the hypothesis that hypothalamic glucose transport will be upregulated in healthy volunteers preconditioned with recurrent HG to induce HAAF compared to preconditioning with euglycemia (EU).
Materials and methods: In these experiments we employed a standard experimental model of HAAF in humans. For preconditioning, subjects underwent 2 hyperinsulinemic EU or HG clamp studies on day 1 and a 3rd on day 2. Hypothalamic glucose transport kinetics were assessed on day 2 after the 3rd preconditioning clamp by measuring hypothalamic glucose concentrations using ^{1}H MRS at 3 tesla over a ~1 hour period during which blood glucose was experimentally increased from 95 mg/dl to one of 3 target hyperglycemic levels (200, 300 or 400 mg/dl). ^{1}H MR spectra were collected from a 10 x 12 x 13 mm^3 hypothalamic voxel. Metabolites were quantified using LCModel. Mathematical modeling was used to calculate maximum transport rate/cerebral metabolic rate of glucose (Tmax/CMRglc).
Results: 11 subjects (9M/2F, age 30±8 yrs) completed the EU preconditioning studies, of whom 7 subjects (6M/1F, age 27±3 yrs) also completed the HG pre-conditioning studies and had successful induction of HAAF. There was no significant difference in Tmax/CMRglc in subjects exposed to recurrent HG with HAAF (1.749 ± 0.143) compared to control subjects exposed to EU(1.619 ± 0.098; p=0.45).
Conclusion: In healthy subjects exposure to recurrent HG with induction of HAAF did not result in upregulation of hypothalamic glucose transport.
Clinical Trial Registration Number: NCT00786825
Supported by: NIH2RO1NS35192, NIH ULTR000114
Disclosure: **E.R. Seaquist:** Grants; NIH 2 RO1NS35192, NIH UL1TR000114.

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Glycogen metabolism measured in the brain of insulin-resistant Goto-Kakizaki rats by ^{13}C magnetic resonance spectroscopy in vivo

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Background and aims: Insulin signalling is involved in metabolic regulation and synaptic plasticity in the central nervous system. In diabetes, reduced insulin signalling may impair brain structure and function leading to behavioural and cognitive alterations. The role of glycogen in the diabetic brain remains to be elucidated. In the present study we investigated insulin resistance-induced alterations of brain glycogen metabolism in the living brain by means of magnetic resonance spectroscopy (MRS).
Materials and methods: MRS experiments in vivo were performed on a 14.1 T spectrometer using a home-built surface coil. [1-^{13}C]glucose was infused into adult Wistar and insulin-resistant Goto-Kakizaki (GK) rats under isoflurane anaesthesia. Localised ^{13}C MRS was performed in a volume of 600 μL within the brain with a modified SIRENE pulse sequence. The ^{13}C MRS experiment measured brain glucose and glycogen signals over at least 8 hours. Then, rats were sacrificed with a focused microwave fixation device, and the brain was stored for extraction of glycogen and water-soluble metabolites. Fractional enrichment (FE) and content of glucose and glycogen were determined by MRS *in vitro*. Time courses of glycogen ^{13}C labelling measured *in vivo* were modelled together with FE and concentration determined in brain extracts to estimate glycogen turnover.
Results: The glucose infusion rate was adjusted to reach similar glucose levels and FE in the plasma of both GK and Wistar rats (~17 mM). Under such conditions, brain glycogen concentrations were similar across both experimental groups, and in accordance to previous reports. Namely. Brain glycogen was 6.7±0.9 μmol/g in controls and 6.7±0.6 μmol/g in diabetic rats. However, FE of brain glycogen was lower in GK rats than in Wistar rats, suggesting that insulin resistance reduces ^{13}C incorporation. Indeed, with a mathematical model of glycogen labelling from [1-^{13}C]glucose, we estimated a glycogen turnover of 0.72±0.33 μmol/g/h in controls and 0.21±0.08 μmol/g/h in GK rats.
Conclusion: These data demonstrate that insulin resistance slows down brain glycogen metabolism despite normal brain glycogen content. This suggests that mobilisation of available glycogen stores in astrocytes is impaired in diabetes, which may have implications for the adequate support of neuronal function, especially during increased brain activity or during reduced energy availability such as hypoglycaemia.
Supported by: CIBM and FNS
Disclosure: **J.M.N. Duarte:** None.

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The ameliorating effect of suvorexant, a dual orexin receptor antagonist, on glucose metabolism in type 2 diabetic db/db mice

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Background and aims: Sleep disturbances and type 2 diabetes are considered to form a vicious cycle. Therefore, it is possible that pharmacological intervention to improve sleep disturbances could ameliorate impaired glucose metabolism in diabetic state. To explore this possibility, we examined the effect of suvorexant, a dual orexin receptor antagonist (an anti-insomnia drug), on the sleep/wake state and glucose metabolism in type 2 diabetic db/db mice.
Materials and methods: Diabetic db/db and non-diabetic db/m+ mice were perorally treated with suvorexant at the beginning of rest phase (light period). Electroencephalogram and electromyogram were recorded to identify the sleep/wake states. To analyze the changes in glucose metabolism, glucose tolerance test, the ELISA analyses of hormonal levels, and biochemical measurements of the expression of key factors related to hepatic gluconeogenesis and chronic inflammation were conducted.
Results: Diabetic db/db mice exhibited sleep disturbances. In particular, the time spent in the awake state during the rest phase was longer than that in db/m+ mice ($P<0.05$). Single or 1-week treatment with suvorexant reduced the wakefulness time and promoted rapid eye movement (REM) and non-REM sleep ($P<0.05$), resulting in the improvement of sleep in db/db mice. Moreover, 3-week treatment with suvorexant ameliorated the impaired glucose tolerance in db/db mice ($P<0.05$), without affecting the body weight, the amount of food intake, serum insulin levels, and systemic insulin sensitivity. Chronic suvorexant treatment reduced the levels of hepatic gluconeogenic factors, including PEPCK, peroxisome proliferators-activated receptor γ coactivator-1α, and phosphorylated cAMP response element binding protein (CREB) in the liver at the rest phase ($P<0.05$). Under such condition, the triglyceride levels in the liver and the levels of proinflammatory cytokines in white adipose tissues, such as TNF-α and interleukin 6, were not altered.
Conclusion: We found that suvorexant improved both sleep disturbances and glucose metabolism in db/db mice. These results suggest that pharmacological intervention for improving sleep/wake rhythm has great potential to treat type 2 diabetes.
Supported by: JSPS KAKENHI Grant Number 15K09380, 15K15599
Disclosure: **H. Tsuneki:** None.

OP 41 Improving clinical beta cell transplantation

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Long-term (10 yr) results of pancreas transplant alone (PTA) in 60 consecutive type 1 diabetic subjects

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Background and aims: The long-term safety and efficacy of PTA in T1D subjects is still debated. Aim of the present study was to evaluate the outcome at 10 yr follow-up of 60 consecutive PTA performed in T1D patients in our center.
Materials and methods: From December 2000 to December 2005, 60 T1D individuals (age: 38±8 yrs; 29 males/31 females; BMI: 23.2±2.8 kg/m2, duration of diabetes: 24±8 yrs) received a PTA. The surgical technique consisted of transplantation of the whole pancreas with the duodenum, with portal or systemic drainage of endocrine secretion and enteric drainage of the exocrine pancreas. Immunosuppression was based on basiliximab or ATG and high dose steroid as induction, whereas maintainance treatment mostly consisted of the calcineurin inhibitor tacrolimus, the anti-proliferative agent mycophenolate (mofetil or sodium) and low dose steroid. Data were analyzed after 10 yrs from transplant.
Results: At the end of follow-up, survival of PTA recipients was 91.7% (55 out of 60), with, therefore, a mortality rate of 0.83% per year. Causes of death (all occurring in patients with functioning graft) were cardiovascular disease (3 cases), viral infection (1 case) and lung cancer (1 case). Graft function at 10 yrs in living recipients was 63.6%, with full insulin independence in 55% of cases, which was accompanied by robust endogenous insulin secretion (C-peptide: 3.1±1.5 ng/ml) and sustained normoglycemia (fasting plasma glucose: 94.6±15.6 mg/dl; HbA1c: 5.7 ±0.5%). Total cholesterol (159±33 vs 204±48 mg/dl) and LDL cholesterol (93±23 vs 129±43 mg) improved significantly (both p<0.01) after transplantation. Two patients developed end-stage renal disease (both at 4 yrs post-transplant). In the remaining recipients with functional graft, the MDRD calculated glomerular filtration rate showed a yearly slope of -2.1±0.4 ml/min/yr.
Conclusion: PTA can be considered a safe and effective procedure in selected T1D patients.
Disclosure: **M. Occhipinti:** None.

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Mathematic analysis of meal tests to evaluate beta cell mass in islet-transplanted patients

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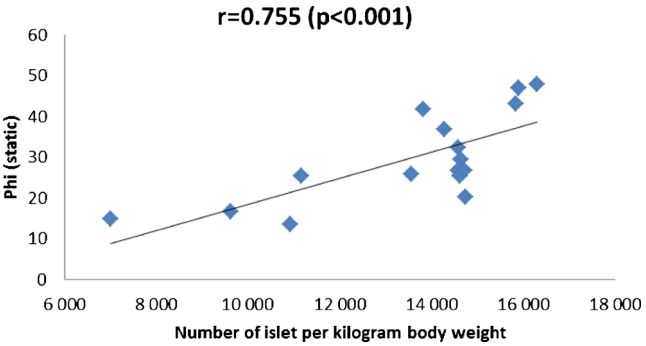
Background and aims: Current model-derived calculations of insulin secretion based on C-peptide kinetics during a meal test allow to measure the two phases of insulin secretion. The second phase has been shown to be a strong predictor of diabetes progression, and recent studies also suggest that it is closely related to the size of pancreatic beta-cell mass. Since β-cell mass is an critical factor for the outcome of pancreatic islet transplantation, we investigated whether these measurements are related to the number of transplanted islets.
Materials and methods: Among a series of subjects treated with islet transplantation we excluded 2 subjects with overt diabetes and included all nondiabetic subjects transplanted less than 2 years ago, and analyzed 16 tests performed in 7 patients (6 F 1 M age 32-60 yr; weight: 51.3-77 kg). All underwent a standardized breakfast test (76 g of carbohydrates) with calculation of insulin sensitivity (Caumo's oral minimal model) and insulin secretion rate (ISR) (Van Cauter's model, with calculation of beta-cell sensitivity to glucose (BCS) and insulin secretion parameters given by the classical models of Breda and of Mari). A simplified model calculating the same parameters from insulin alone without C peptide was also tested.
Results: The best predictors of the number of islets expressed per kg body weight were: Phi(S) (Breda'model index of second phase) r=0.755, p<0.001; Phi(oral) (Breda'model overall index of insulin secretion) r=0.752 p<0.001; Ismax (peak value of prehepatic insulin release) r=0,650; p<0.01; IS total (AUC of prehepatic insulin secretion)) r=0.645; p<0.01; BCS (Mari's model) r=0.611 p<0.01). The prediction of Phi(oral) with the simplified model using insulin rather than C-peptide is also well correlated (r=0.740 p<0,001).
Conclusion: These results show that parameters of insulin secretion calculated from C-peptide kinetics with Breda or Mari's model during a breakfast test are well correlated with the number of islets in successfully islet-transplanted patients. This finding is consistent with reports indicating that these parameters provide a quantitative evaluation of β-cell mass and suggest they may be useful for the follow-up of transplanted patients with a simple and minimally invasive glucose tolerance test. Moreover, an even simpler approach without C peptide seems to give also the same information.



Disclosure: **J.R. Brun:** None.

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Donor-specific alloimmune humoral response after islet grafting: description and impact on graft function

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Background and aims: Pancreatic islets transplantation has been proposed in selected individuals with type 1 diabetes mellitus. However at 3 years after transplantation only 44% are still insulin independent. Graft dysfunction occurs sometimes simultaneously with the appearance of donor specific alloantibodies (DSA). The aim of this study was to evaluate (I) the evolution of the humoral response after islet transplantation and (II) its relationship with graft dysfunction.
Materials and methods: Among 122 patients transplanted between 2000 and 2013 (GRAGIL network), 45 patients which had pre- and post-transplantation sera available for analysis were retrospectively included. DSA were detected by single antigen method (Immucor©) and detection of antibodies able to fix complement was performed by a C3d-binding test. We studied the graft survival time (time until the loss of 50% of the post-transplantation beta-score gain) depending on the appearance or absence of DSA.

Results: Patients baseline characteristics were comparable in the two groups (presence or absence of DSA): IEQ=11991±582IEQ/kg (meanSD), HbA1c =8,1%±0,3% pre-transplantation. 16 patients had a previous transplantation (kidney, pancreas). 13/45 patients had de novo DSA and 2/13 had primary graft dysfunction. There was no survival difference between the two groups. Among 11 patients with functional graft, 5 lost their graft after DSA appearance; 3 produced DSA tardily after the loss of graft function; 3 did not loss their graft at the end of follow-up. The humoral response was heterogeneous (MFI 400-9600) and no antibody was fixing the complement. At 3 years 22.8% of the patients developed de novo DSA and this proportion increases to 80% after immunosuppressive drugs withdrawal.
Conclusion: De novo DSA after islet transplantation appear in one third of our cohort. The presence of de novo DSA has no significant impact on graft survival in our study but could complicate the access to a new graft procedure.
Disclosure: **L. Kessler:** None.

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Is IVGTT a useful predictor of pancreas graft dysfunction?

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Background and aims: In pancreas transplantation the early indicator of graft dysfunction is still missing. The aim of our study was to determine whether a standard IVGTT can be useful in graft biopsy indication.
Materials and methods: We performed 30 examinations in 26 insulin-independent Type 1 diabetic pancreas recipients with some laboratory signs of potential graft dysfunction (increase of serum amylase/ lipase levels): 22 patients with portal venous drainage of the pancreatic graft and 4 subjects with systemic drainage. Fasting glycemia, insulin and C-peptide levels, HbA1 and IVGTT with coefficient of glucose assimilation (KG) calculation were assessed just before ultrasound-guided percutaneous pancreas graft biopsy. Banff classification was used for histological rejection grading.
Results: Despite different grades of acute rejection (AR; moderate in 7 patients, mild in 8, "indeterminate" in 10 and normal histology finding in 5 subjects) parameters of actual glucose metabolism: fasting glycemia, insulin and C-peptide levels did not differ between the groups. In moderate AR group 28% patients had an abnormal response to the glucose stimulus (DM), 42% patients had an impaired glucose tolerance (PGT). In mild AR group both DM and PGT were present in 37% subjects. 40% recipients of "indeterminate" AR group had DM and 50% had PGT. In AR-free group 40% recipients had DM and 50% PGT. We did not find any significant difference in results of IVGTT ($p = 0.68$). Mean KG was 1.16 ± 0.5 [SD] %/min. in moderate AR group, 1.01 ± 0.4%/min. in mild AR group, 0.83 ± 0.3%/min. in "indeterminate" AR group and 0.95 ± 0.3%/min. in AR-free recipients ($p = 0.256$). Serum amylase and lipase levels were significantly increasing with AR grade ($p = 0.0001$ and $p < 0.0001$, respectively).
Conclusion: Though this was a small group study, we conclude that parameters of glucose metabolism measured by IVGTT in time of graft threat probably do not relate directly to the seriousness of acute rejection.
Supported by: NV15-26746A (Czech Republic)
Disclosure: **T. Havrdova:** None.

OP 42 From islet omics to beta cell function

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Human genomic and functional studies of common type 2 diabetes genes demonstrate their role in pancreatic beta cell function

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Background and aims: Genome-wide association studies (GWAS) have identified >100 common genetics variants associated with the risk of Type 2 Diabetes (T2D). Since first GWASs, geneticists have suggested that susceptibility genes for T2D are expressed in pancreatic beta cells where they should play a key role in insulin secretion. However, few studies precisely investigated the tissue expression of these genes as well as their biological function. In the present study, we investigated the expression of T2D susceptibility genes in a large panel of human tissues, and we subsequently performed a comprehensive functional study of the beta cell most expressed/specific genes in the human beta cell line EndoC-BH1.
Materials and methods: We investigated the expression of 105 T2D susceptibility genes in human placenta, whole pancreas, pancreatic islets, laser-capture and FACS-sorted pancreatic beta cells, EndoC-βH1, skeletal muscle, heart, lung, liver, kidney, small intestine, colon, adipose tissue, and several regions from the brain including hypothalamus and substantia nigra. We used the Nanostring technology that enables the simultaneous counting of RNA molecules without PCR in different tissues. Thirty genes were selected for further analysis, according to their specificity and their level of expression in beta cells and EndoC-BH1. Selected genes were investigated in the human beta cell line EndoC-BH1, through transient knock down by siRNA, and we subsequently assessed insulin secretion in response to glucose and other secretagogues.
Results: We identified a very significant enrichment of the expression of T2D susceptibility genes in the whole pancreas and pancreatic beta cells. As positive controls, we demonstrated that the knock down of T2D gene GCK (encoding glucokinase) or KCNJ11 (encoding the pore-forming subunit of ATP-dependent potassium channel in beta cells) significantly decreased insulin secretion from EndoC-BH1in response to glucose with/without IBMX. We also found that the knock down of four other T2D genes (with unknown function in pancreatic beta cells, so far) significantly reduced insulin secretion in response to glucose with/without IBMX.
Conclusion: We demonstrated that most GWAS identified T2D susceptibility genes were significantly expressed and enriched in pancreatic beta cells (compared to other human tissues), even if not fully specific. In four T2D genes with unknown function in human beta cells, we observed a significant reduction in insulin secretion after silencing. RNA-seq assessing affected pathways is in progress. Further investigation on the other beta cell expressed T2D genes are in progress.
Disclosure: **F.K. Ndiaye:** None.

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Single-cell transcriptome profiling of human pancreatic islets from normal and type 2 diabetes mellitus donors

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Background and aims: Although pancreatic islets exhibit considerable cellular heterogeneity, transcriptional analyses of islets from both healthy and Type 2 diabetes mellitus (T2DM) donors to date have been carried out either on whole islets or specific populations enriched for unique cell types. Here we have used single-cell transcriptomics, which allows cell-type comparisons of gene expression programs in healthy and T2DM cells to identify specific alterations that would otherwise be masked in whole islet comparisons. We have built a unique database of pure unbiased transcriptomes by sequencing several thousand individual human islet cells from healthy and with T2DM donors to define cell-type specific transcriptional signatures and examine pathological differences.
Materials and methods: Human islet equivalents were purchased from Prodo Laboratories and glucose-stimulated insulin secretion (GSIS) was performed to assess their function. Hormone protein expression was validated using immunohistochemistry and flow cytometry on pancreatic tissues and dissociated islets, respectively. Islets were dissociated into a single-cell suspension and viable individual cells were sorted using flow cytometry into 384-well plates containing lysis buffer and sequenced using a Smart-seq2 protocol yielding single-cell transcriptomes.
Results: The results, when controlled for inter-individual differences arising from different age, sex and body mass index (BMI), revealed cell-type specific gene expression profiles in 2,209 islet cells. Both novel and previously known genes were found. Interestingly, we found cell type specific expression of several important receptors, known to be involved in glucose regulated insulin-secretion. We also identified novel subpopulations in both endocrine and exocrine cell types, identified genes with interesting and significant correlations to BMI in specific cell types and found alterations in gene expression in T2D. Strikingly, our analyses revealed a down-regulation of INS transcripts levels in b-cells in T2D donors, together with down-regulation of genes involved in regulating proliferation. We also detected subpopulations of a, b-cells and acinar cells with additional proliferative, immune or regulatory transcriptional signatures.
Conclusion: The single-cell RNA-seq technique produced high-resolution transcriptome profiles of the individual cell types of human islets and their pathological transcriptional changes. This is an unprecedented resource that validates previously described attributes of islets as well as revealing novel features and providing a qualitative and quantitative novel definition of the phenotypic cells making up human islets.
Supported by: the Swedish Research Council and Foundationf for strategic research
Disclosure: **P. Eliasson:** None.

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Epigenome-wide RNAi screen identifies an alpha to beta cell transdifferentiation factor

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Background and aims: Pancreatic beta cells represent the sole source of insulin in the human body. They closely interact with their neighbouring cells in the islets of Langerhans, namely the glucagon-secreting alpha cells, to maintain normoglycaemia. Hence, loss of these beta cells, as observed in Type I Diabetes (T1D), results in deregulated glucose homeostasis and severe hyperglycaemia. While treatable with regular insulin injections, patients never reach perfect glycaemic control and are left susceptible to various complications. This underscores the need for a novel insulin cell source in T1D patients. The close developmental link between alpha and beta cells, evident via their common $Ngn3^{+}$ progenitor, make alpha cells a promising candidate. What's more, previous studies have revealed the ability of alpha cells to adopt functional characteristics of beta cells upon epigenetic manipulation. Hence, we are attempting to induce beta cell characteristics in alpha cells, in the hopes of replenishing beta cell mass and gaining a broader understanding of chromatin-mediated transdifferentiation.
Materials and methods: We conducted a chromatin-focused short hairpin RNA screen on the murine alpha cell line, aTC1, in search of proteins repressing beta cell markers. The viral library targeted over 300 potentially druggable chromatin factors. The cells were then screened for changes in their transcription profile, with a particular focus on increased insulin (Ins2) expression. Target hits were validated via rescue experiments and immunofluorescence.
Results: The strongest hit from the screen was a protein involved in post-transcriptional modifications. RNAseq results show a general upregulation in beta cell markers, including Iapp, Gck, Pax4 and Ins2, upon knockdown in aTC1 cells. Knockout experiments reveal the essentiality of the gene for alpha cell survival and proliferation. Its function appears to be conserved in human islets, in which its knockdown induces a significant upregulation of *Pax4* transcription. Currently we are attempting to functionally characterize the encoded protein via affinity proteomics, ChIPseq and mutagenesis experiments.
Conclusion: The ultimate goal of T1D research is to replenish the beta cell pool or restore proper insulin production without triggering a destructive immune response. To address this issue, our group focuses on ways to induce mature, terminally differentiated cells (e.g. alpha cells) to adopt beta-cell characteristics. We have identified a promising candidate whose knockdown in alpha cells yields an increase in beta cell specific markers. It appears to function as a potent repressor of the beta cell master regulatory transcription factor, Pax4. Overall, these experiments could yield valuable information regarding transcriptional regulation in the endocrine pancreas, and potentially a new insulin cell source.
Supported by: JDRF
Disclosure: **T. Casteels:** None.

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Perturbed mitochondrial metabolism in islets from type 2 diabetic donors revealed by integrated analysis of the human islet metabolome and transcriptome

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Background and aims: There is a preponderance for genes involved in ß-cell function among gene variants associated with future risk of type 2 diabetes (T2D). ß-cell function is controlled by metabolism of glucose, yielding signals that trigger and amplify secretion of insulin. Perturbed ß-cell metabolism is a likely, albeit not proven, cause of T2D.
Materials and methods: We profiled metabolites in islets from T2D (n=7) and non-diabetic donors (n=30). Gene expression was examined by RNA sequencing in islets from 131 donors.

Results: Our analyses revealed levels of mitochondrial metabolites to be altered in islets from T2D donors. A subsequent analysis of genes encoding mitochondria localized proteins (MitoCarta) by RNA sequencing revealed genes whose expression depended on glycaemia and/or BMI. Expression of two of these genes, alpha-methylacyl-CoA racemase (AMACR) and methylmalonyl-CoA mutase (MUT) were influenced by genetic variation (cis-eQTL). Silencing of AMACR and MUT in a ß-cell line (INS-1 832/13) resulted in a 40-50% ($p<0.01$) reduction in glucose-stimulated insulin secretion.
Conclusion: In conclusion, we showed that perturbed mitochondrial metabolism is a feature of ß-cell dysfunction in T2D. By linking these metabolite changes to gene expression, we identified two genes that alter islet function.
Disclosure: **P. Spégel:** None.

OP 43 Diabetes and pregnancy

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Maternal neck circumference is associated with glucose tolerance before the 20th week of pregnancy

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Background and aims: A well-established relationship exists between adiposity, especially central adiposity, and abnormal glucose tolerance (AGT). Neck circumference (NC) is a good measure of central obesity with limited data in pregnancy. We aimed to evaluate NC as a risk factor for AGT in the first 20 weeks of pregnancy, compared to body mass index (BMI) and waist circumference (WC).
Materials and methods: 970 pregnant women, recruited to participate in the DALI study for prevention of gestational diabetes (GDM)). Inclusion criteria: BMI ≥29 kg/m2, gestational age at entry <20 weeks, no former diagnosis of diabetes. IADPSG/WHO 2013 criteria were used for diagnosis; HOMA-IR as a measure of insulin resistance. Adiposity measurements: NC, BMI and WC. Other potential risk factors studied: maternal age, ethnicity, obstetric history, socioeconomic condition, height, smoking habit, history of impaired glucose tolerance or previous GDM and family history of diabetes. Statistical analysis: Linear regression; multivariate logistic regression (backward method; Nagelkerke R2). Only results for adiposity measurements are presented.
Results: 85.9% of the participants were Caucasian, mean age was 32±5.3 years, 16.2% were smokers, 11.4% had previously experienced GDM, 21.3% macrosomia and 11.4% stillbirth. Prepregnancy BMI was 34.1±4.5 Kg/m2. At baseline assessment NC was 36.5±2.2 cm, WC 108.1±10.5 cm and 27.1% participants had AGT (0.6% overt diabetes mellitus, 26.5% GDM). B coefficients for association with HOMA-IR were 0.144 for BMI (CI95 01.03, 0.186), 0.358 for NC (CI95 0.275-0.441), and 0.069 for WC (CI95 0.050-0.087). NC was the adiposity measurement selected by the multivariate logistic regression as risk factor for abnormal fasting PG, 1h PG and overall AGT (BMI and WC not included in the models). No adiposity measurement was a risk factor of abnormal 2h PG. The OR for 1 cm of NC, adjusted by the other potential predictors were: For abnormal fasting PG: OR 1.16 (CI95% 1.05-1.27; p=0.002; R2 = 0.096). For abnormal 1h PG: OR 1.19 (CI95% 1.03-1.37; p=0.016; R2 = 0.048). For AGT: OR 1.12 (CI95% 1.03-1.23; p=0.008; R2 = 0.084).
Conclusion: In this study population, NC is the adiposity measurement displaying the best association with HOMA-IR and outdoes BMI and WC as a risk factor for AGT.
Clinical Trial Registration Number: ISRCTN70595832
Supported by: HEALTH-F2—2009-242187
Disclosure: **L.C. Mendoza:** None.

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Role of first trimester HbA_{1c} as a predictor of adverse obstetric outcomes in a multi-ethnic cohort

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Background and aims: In pregnancy risk of obstetric complications increases linearly with rising maternal glycaemia. Determining HbA1C has proved to be an effective option to detect hyperglycemia but its association with adverse pregnancy outcomes remains unclear. A recent study endorsed the usefulness of an early pregnancy HbA1C cut-off point of 5.9% as a predictor for adverse obstetric outcomes. Further studies are needed to verify these results, particularly across different ethnic groups, The aim of this study was to determine the usefulness of a 5.9% first-trimester HbA1C threshold to identify women at increased risk of adverse pregnancy outcomes in a multi-ethnic cohort.
Materials and methods: A prospective study was conducted between April 2013 and September 2015. We included women over 18 years with a singleton pregnancy. Exclusion criteria were: preexisting diabetes, overt diabetes, unavailable delivery data, termination of pregnancy or spontaneous abortion. All pregnant women had an HbA1c measurement added to their first antenatal bloods and were screened for gestational diabetes mellitus (GDM) at 24 to 28 weeks' gestation. Demographic, anthropometric, clinical and analytical variables and pregnancy outcome data were collected.
Results: 1316 pregnancies were included for outcome analysis. Maternal and gestational characteristics stratified according to HbA1C measurement at first antenatal visit are shown in table 1. In univariate analysis, women with HbA1C > 5.9 (n=47) compared with those with HbA1C < 5.9 (n=1266) had a higher rate of macrosomia (17.7% vs 5.9%, p=0.006) and a tendency towards higher rate of preeclampsia (10% vs 3.9%, p=0.081). There were no significant differences in preterm birth (11.3% vs. 7.2%, p=0.368), caesarean section (31.1% vs. 27.3%, p=0.400) or perinatal death (0 vs. 0.8%, p=1.00). In multivariate analysis adjusting for potential confounders (GDM diagnosis, pre-pregnancy body mass index, pregnancy weight gain, ethnicity, nulliparity, previous macrosoma and anemia) a cut-off point of HbA1C > 5.9% was independently associated with higher risk of macrosomia (OR 3.446, 95% CI 1.245 - 9.536, p=0.017) and preeclampsia (OR 3.242, 95% CI 1.005 - 10.463, p=0.049).
Conclusion: In a multiethnic population, a cut off point of HbA1c>5.9% in early pregnancy identifies a group of women at high risk for poorer pregnancy outcomes independently of GDM diagnosis. Further studies are required to assess the impact of early detection and treatment on both maternal and fetal outcomes, as well as the cost-effectiveness of this approach.

MATERNAL AND GESTATIONAL CHARACTERISTICS STRATIFIED ACCORDING TO HbA1C MEASUREMENT AT FIRST ANTENATAL VISIT

	A1c<5.9 N=1266	A1c≥5.9 N=47	P value
Age (years ± SD)	32.72 ± 5.76	33.87 ± 5.16	P 0.180
Pre-pregnancy body mass index (Kg/m² ± SD)	25.27 ± 4.98	27.91 ± 5.29	**P 0.001***
Previous GDM n (%)	45/1166 (3.8%)	11/44 (25%)	**P<0.001***
Previous macrosoma n (%)	32/1166 (2.7%)	2/44 (4.5%)	P 0.353
Multiparous n (%)	674/1234 (54.6%)	31/47 (65.9%)	P 0.137
Ethnicity n (%)			
●Caucasian	672/1204 (55.8%)	17/44 (38.6%)	**P 0.007***
●South-central Asian (India, Pakistan, Bangladesh)	206/1204 (17.1%)	17/44 (38.6%)	
●Moroccan	82/1204 (6.8%)	2/44 (4.5%)	
●Latin American	158/1204 (13.1%)	4/44 (9.1%)	
●East Asian (China, Philippines)	65/1204 (5.3%)	4/44 (9.1%)	
●Other	21/1204 (1.7%)	0	
GDM diagnosis n (%)	134/1146 (11.6%)	21/46 (45.6%)	**P<0.001***
Pregnancy weight gain (Kg ± SD)	10.89 ± 4.64	9.50 ± 5.15	P 0.051
First trimester hemoglobin (g/dl ± SD)	12.59 ± 0.99	12.12 ± 1.21	**P 0.012***

Disclosure: **L. Mañé:** None.

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Elevated levels of circulating FABP4 predict preeclampsia in women with type 1 diabetes

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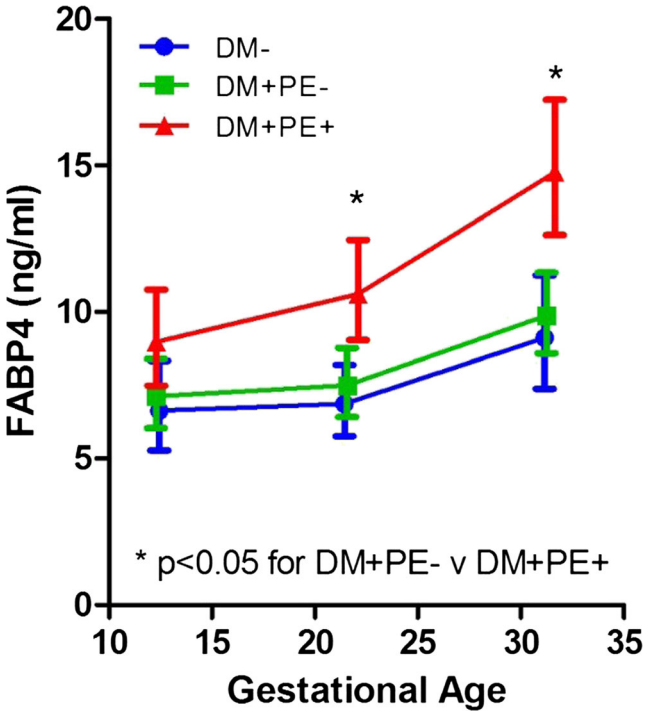
Background and aims: Preeclampsia (PE) is a major cause of morbidity and mortality in pregnant women and infants. It is defined as new onset hypertension occurring after 20 weeks of gestation in a previously normotensive woman, accompanied by either proteinuria or end-organ dysfunction. The incidence of PE in the general population is 4-6%, but in women with pre-gestational type 1 diabetes mellitus (T1DM) it is increased 4-5-fold. Underlying mechanisms for the increased risk in women with diabetes are poorly understood, and predictive measures are inadequate. PE shares many features of the metabolic syndrome. Fatty Acid Binding Protein-4 (FABP4) is a key protein linking metabolic and inflammatory responses. We aimed to determine if plasma FABP4 may serve as a predictive biomarker for PE in women with T1DM. We used samples and clinical data from an established, prospective cohort of pregnant T1DM women.
Materials and methods: The cohort comprised 23 T1DM women who developed PE, 24 T1DM women who remained normotensive, and 19 non-diabetic women. FABP4 was measured by ELISA (R&D Systems) in plasma collected at three study visits (V1 - V3: 12.3 ± 1.8, 21.7 ± 1.4, 31.4 ± 1.5 weeks gestation; mean ± SD). The diabetic groups were matched for age, diabetes duration, HbA1C, and parity. All subjects were free of hypertension and microalbuminuria at V1, and all study visits preceded clinical onset of PE.
Results: Overall, plasma FAPB4 levels (ng/ml) were elevated in T1DM women who later developed PE vs. those who remained normotensive [V1: 9.0 (7.5 - 10.8) vs. 7.1 (6.0 - 8.4), p=0.06; V2: 10.6 (9.0 - 12.5) vs. 7.5 (6.4 - 8.8), p=0.003; V3: 14.8 (12.6 - 17.2) vs. 9.9 (8.6 - 11.3), p<0.001; respectively (geometric mean (95% CI)) [Figure 1: average gestational age vs. FABP4 at three visits]]. FABP4 correlated with maternal age, BMI, and daily total insulin at all visits; with ACR at V2, V3; and with systolic and diastolic BP at V1, V3. Using logistic regression, for every doubling of FABP4, the unadjusted OR for PE was 6.1 (95% CI: 1.7-22.1) at V2, and 9.0 (95% CI: 2.3-35) at V3.

Conclusion: Plasma FABP4 shows promise as an excellent predictor of PE in women with T1DM.



Supported by: JDRF Research Grant
Disclosure: **C.B. Kelly:** None.

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Association of maternal diabetes and diabetes medication treatment during pregnancy with attention-deficit/hyperactivity disorder in offspring

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Background and aims: Pregnancies complicated by maternal pre-existing type 2 diabetes (T2D) or gestational diabetes mellitus (GDM) increase risks of perinatal morbidities and postnatal obesity and metabolic disorders in offspring. Prior studies indicated that exposure to maternal hyperglycemia in utero is associated with an increased risk of autism spectrum disorders (ASD) in offspring. The hypothesis is that abnormal glucose may "disrupt" fetal brain development and maturation and lead to increased risks for offspring to develop neurobehavioral disorders in later life. We assessed whether exposure to maternal T2D or GDM or their medical treatment in utero increased the risk of attention-deficit/hyperactivity disorder (ADHD) in offspring.
Materials and methods: This retrospective longitudinal matched cohort study included 23,888 diabetes-exposed children (n=3,407 for T2D and n=20,481 for GDM) and 110,905 diabetes-unexposed children who were matched with up to 1:5 ratio to the diabetes-exposed children on maternal age at delivery and race/ethnicity. All were singletons born in 1995 to 2009 in hospitals within a single integrated health care system. Children with ASD or born to mothers with type I diabetes were excluded. Children were prospectively followed from age 5 through electronic medical records until the first of the following: date of clinical diagnosis of ADHD; last date of continuous health plan membership; death due to any cause; or December 31, 2014. Relative risks of ADHD were estimated by HR using Cox regression models.
Results: During a median 9.2 (interquartile of 6.7-12.9) years of follow-up after birth, 5,411 children (4501 unexposed, 132 T2D-exposed, and 778 GDM-exposed) were diagnosed with ADHD after age 5. The percentages of children with ADHD were 4.0% overall, 4.1% unexposed, 3.9% T2D-exposed, and 3.8% GDM-exposed. As groups, risk of ADHD was not significantly elevated for T2D-exposed or GDM-exposed compared to the unexposed (p>0.40 before and after adjustment for maternal age, parity, education, household income, race/ethnicity, history of comorbidity, history of maternal ADHD, smoking during pregnancy, and child gender). Gestational age at GDM diagnosis was not associated with risk of ADHD (p=0.47) for the GDM-exposed group. However, we found that diabetes medication dispensed during pregnancy for the T2D or GDM groups (n=7,479, among them, 85% had insulin dispensed), was associated with risk of ADHD in offspring. The HR for ADHD increased in association with increasing duration of medication use (covariate adjusted p=0.03 for trend). The adjusted HR associated with use of anti-diabetes medications for ≥ 60 days during pregnancy was 1.23 (95% 0.99-1.53, p=0.06) compared with no diabetes medication group.
Conclusion: Data from this large, multi-ethnic and population-based clinical care system suggest that risk of ADHD in offspring may be increased for mothers who require diabetes medication for ≥ 2-months to manage hyperglycemia during pregnancy. Studies are needed to understand the causes of the observed elevated risk in the medication group.
Disclosure: **A.H. Xiang:** None.

OP 44 Metformin: old drug, new tricks

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Metformin treatment may impair orthostatic blood pressure recovery

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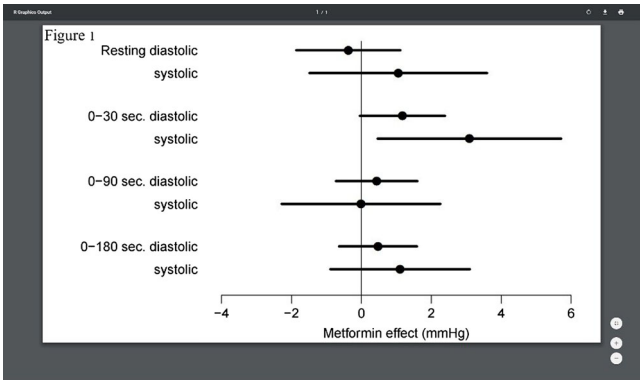
Background and aims: Metformin has been suggested to associate with both neuroprotective and neurodegenerative attributes. The aim of this study is to investigate the effect of metformin in combination with insulin treatment on diabetic peripheral neuropathy (DPN) and cardiovascular autonomic neuropathy (CAN.)
Materials and methods: The study is a substudy of the CIMT trial, a randomized placebo-controlled multicenter study with a 2x3 factorial design where 412 patients with type 2 diabetes (HbA1c > 7.5% (≥58 mmol/mol), Body-Mass Index >25 kg/m2) were randomized to 18 months of blinded one gram twice daily metformin treatment or placebo in addition to an open-labelled insulin analogue regime targeted at HbA1c ≤ 7.0% (≤53 mmol/mol). The outcomes of the present study were measures of autonomic function: changes in heart rate response during deep breathing (beat-to-beat), orthostatic blood pressure (BP) (30, 90 and 180 seconds after standing) the European Society of Cardiology defined orthostatic hypotension diagnosis and vibration detection threshold.as a marker of peripheral nerve function.
Results: 15% of all patients had orthostatic hypotension at baseline. Drop in orthostatic BP increased in the metformin group as compared to placebo for BP measures 30 seconds after rising from lying to standing. Systolic BP drop increased by 3.1 mmHg (95% CI 0.5;5.7, p=0.02) and diastolic BP drop increased borderline significantly by 1.2 mmHg (95% CI -0.1;2.4, p=0.06). Beat-to-beat measures decreased insignificantly in the metformin group by 1.1 beats per minute (CI -2.4;0.1, p=0.07). Resting heart rate did not change in either of the groups. No differences between groups were seen in other orthostatic BP measures nor resting BP as shown in figure 1. There were no change between groups in vibration detection threshold, difference between groups were -0.4 volt (95% CI -1.8;1.1, p=0.61). Metformin did not increase the risk of orthostatic hypotension; odds ratio 1.20 (95%CI 0.72; 1.98, p=0.49).
Conclusion: Metformin in combination with insulin did not improve measures DPN or CAN. Ourresults could indicate that metformin treatment may influence orthostatic blood pressure response as metformin treatment attenuated orthostatic blood pressure recovery at 30 seconds. These adverse changes could be mediated by changes in autonomic function as metformin treatment caused a trend toward a decreased in beat-to-beat measures at follow-up. It is possible that prolonged treatment with metformin could aggravate the pathological changes presented here. Future studies with a primary focus on the effect of metformin on diabetic cardiovascular autonomic neuropathy are needed to confirm our findings before conclusions on adverse effects can be drawn.



Clinical Trial Registration Number: NCT00657943
Supported by: an unrestricted grant from Novo Nordisk A/S
Disclosure: **C.S. Hansen:** Grants; Unrestricted grant from Novo Nordisk.

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No correlation between hepatic volume of distribution for metformin and gene expression of neither organic cation transporter 1 nor multidrug and toxin extrusion 1

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Background and aims: Metformin is the most widely used oral antidiabetic drug worldwide. It has well-documented effects on glycemic control, and reduces diabetes associated mortality including cardio-vascular death. Yet, the therapeutic effects of metformin vary considerably from patient to patient, and treatment is frequently associated with gastrointestinal side effects (20-30% of patients). This could be due to individual variations in the pharmacokinetic properties of the drug. Metformin is an organic cation and is therefore dependent on carrier mediated transport for oral absorption, hepatic uptake and renal excretion. Organic cation transporter 1 (OCT1) is essential for normal hepatic uptake of metformin and similarly, multidrug and toxin and extrusion 1 (MATE1) is involved in hepatic elimination of metformin. To investigate the effect of mRNA expression of OCT1 and MATE1 on hepatic volume of distribution (Vd) of metformin, we have labelled metformin with carbon-11 and used PET/CT to detect volume of distribution. We hypothesize that hepatic uptake of metformin is positively correlated to gene expression of OCT1 and negatively correlated to gene expression of MATE1.
Materials and methods: 12 subjects with biopsy proven non-alcoholic fatty liver disease (NAFLD) and 4 healthy controls were investigated after injection with a bolus of ~160MBq [11C]metformin (10 μg metformin) intravenously followed by 90 min scanning using PET/CT with field of view (FOV) over the liver. Liver biopsies from patients with NAFLD were analysed for OCT1 and MATE1 mRNA expression using microarray. Data demonstrated as mean ± SEM.
Results: Uptake of [11C]metformin in the liver and kidneys and lesser extent in the intestines was detected in all subjects. Vd was similar among NAFLD patients (2.43 ± 0.15) and healthy controls (2.45 ± 0.08) (Students t test). In subjects with NAFLD, there was no correlation between mRNA expression of neither OCT1 (r=0.29, p=0.35) nor MATE1(r=0.17, p=0.60) and Vd of metformin. Interestingly, data showed a positive correlation between mRNA expression of OCT1 and MATE1(r=0.68, p=0.014). No side effects were observed during or after the tests.

Conclusion: Our data demonstrate that the pharmacokinetic properties of metformin can be investigated in vivo using [11C]metformin PET in humans. Vd of metformin is not profoundly affected by gene-expression of OCT1 and MATE1, but future studies using this model can determine the factors regulating metformin distribution in human.
Supported by: NNF Excellence Project grant
Disclosure: **E.I.O. Sundelin:** None.

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Targeted metabolomics to predict the primary success of metformin monotherapy in type 2 diabetes

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Background and aims: Metformin is the preferred first-line medication for type 2 diabetes (T2D), but its primary success rate is only 40-70%. To this day, its effectiveness for an individual patient cannot be predicted precisely. We aimed to investigate whether targeted serum metabolomics can predict the primary treatment success of a metformin monotherapy in patients suffering from T2D.
Materials and methods: A non-interventional, multicenter study was conducted between June 2012 and July 2014 in 51 medical practices in Bavaria, Germany. The study cohort consisted of 99 adults with physician-diagnosed T2D started on a metformin monotherapy. We measured clinical parameters, 180 fasting serum metabolites before and after the first dose of metformin (Biocrates AbsolutIDQ p180 Kit), ataxia-telangiectasia-mutated and organic cation transporter 1 gene polymorphisms, and metformin serum concentrations. Primary treatment success was defined as an at least 10% improvement of the baseline HbA1c value within 80 to 180 days after starting metformin. We applied the Subwindow Permutation Analysis method for variable selection and logistic regression with bootstrap validation for prognostic models.
Results: Ten patients (10.1%) discontinued treatment due to adverse drug reactions. The primary success rate of metformin in the remaining 89 study participants was 44.9% (n=40). A model based on five metabolite (Serotonin, C5, SM_C22_3, lysoPC_a_C16_1, and C18_1_OH) concentration differences between before and after the first dose of metformin and five pre-metformin metabolite (H1, PC_aa_C32_3, C4_1, SM_C26_0, and C7_DC) concentrations predicted treatment success with an area under the receiver operating characteristic curve (ROC) of 0.86, a sensitivity of 0.78 (95% confidence interval: 0.61-0.89), and a specificity of 0.82 (95% CI: 0.67-0.91). Internal validation using the bootstrap method revealed a ROC of 0.79.
Conclusion: Targeted serum metabolomics before and after the first dose of metformin can improve prediction of the primary success of this drug for T2D patients. Further studies are required for application in clinical routine, but a similar approach may also be useful for other drug-disease combinations.
Supported by: Klinikum der Uni und HelmholtzZentrum München, German Cen. for Diabetes Research
Disclosure: **M. Rottenkolber:** None.

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Metformin increases small intestine glucose uptake in rodents

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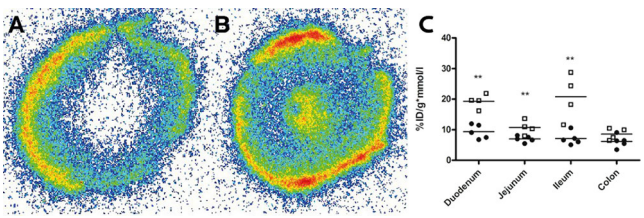
Background and aims: Despite decades of metformin use as a first line therapy for type 2 diabetes, its mechanisms of action are poorly understood. Therefore, we assessed the effects of metformin treatment on intestinal glucose metabolism in rodents using positron emission tomography and ex-vivo imaging.
Materials and methods: Euglycemic adult male BBDR (BioBreeding Diabetic Resistant) rats were treated either by metformin (n=4) or vehicle (n=5) administered subcutaneus via osmotic pumps for three months. In order to explore the intestinal glucose uptake (GU), dynamic (0-90 min) [18F]FDG PET/CT (Inveon Multimodality) acquisition was performed. After PET imaging pieces of intestine segments were emptied and sectioned for biodistribution measurements, autoradiography and for immunohistochemical studies.
Results: The metformin treated group showed higher uptake (SUV) in the small intestine compared to the placebo group (10.0 vs. 4.4; p<0,002). In line with this, relative distribution of [18F]FDG was significantly higher after metformin treatment. Autoradiography results showed radiotracer accumulation only in the mucosal layer of the intestinal sections (Figure A. vehicle and B. metformin). Metformin induced [18F]FDG uptake in the mucosal layer of rats duodenum, jejunum and ileum was observed to be higher than in the vehicle treated controls (Figure C, white symbols metformin, black symbols vehicle,** P<0.001). However, this metformin induced GU was not observed in colon. Glycemic control and weight was similar between the groups. Faecal biodistribution showed [18F]FDG flux from the blood circulation in to the intestinal lumen but no changes in faecal activity were shown between intervention groups.
Conclusion: Metformin amplifies basolateral glucose uptake by intestinal mucosa in rodents. This is in line with our recent findings in human data showing enhanced glucose uptake in the small intestine after metformin treatment. Further studies are ongoing to clarify the mechanism behind these actions.



Disclosure: **J.P. Koffert:** None.

OP 45 "Education, education, education" (Tony Blair, 1996)

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Structured type 1 diabetes education (DAFNE) reduces basal insulin and improves weight and glycaemia: 3-year follow-up study

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Background and aims: The 'Dose Adjustment For Normal Eating' (DAFNE) course is a 5-day structured group education programme to encourage adults with Type 1 diabetes (T1D) to improve self-management of their diabetes through carbohydrate counting and intensive insulin therapy. We began delivering the course in November 2009, and to date have 218 DAFNE 'graduates'. We audited how the DAFNE course affects glycaemic control, insulin needs and weight in adults (aged ≥16 years) with T1D over a 3-year follow-up period.

Materials and methods: Participants attended a 5-day training program in insulin dose adjustment and carbohydrate counting between November 2009 and February 2013. Baseline data was collected at pre-assessment clinics before the course and kept in an online database. Follow-up clinical information and prescription details were also collected at year 1, 2 and 3. Information included age at T1D diagnosis, age when completing the DAFNE course, weight (kg), body mass index (BMI), HbA1C and insulin dosage. $P < 0.05$ was considered to be statistically significant. Statistical analysis was completed using two-tailed paired t-test, one-way ANOVA and Tukey and Holm-Sidak's multiple comparison tests as post hoc.

Results: One hundred and one participants completed 3 years of DAFNE and were eligible for analysis. Significant weight loss was achieved at year 1 [2.3±0.7kg ($p<0.01$)], was maintained at year 2 [3.0±0.8kg ($p<0.01$)], however, lost significance at year 3 [1.3±0.9kg ($p=0.45$)]. Glycaemia as determined by HbA1C did not reduce significantly in the entire cohort. However, those with HbA1C in the highest quartile showed significant improvement [88.0±8.5mmol/mol to 77.0±14.1mmol/mol at year 1 and 80.0±10.7mmol/mol, at year 2 ($p<0.05$)]. Lower basal insulin doses (cf baseline) were maintained at year 1, 2 and 3 [-5.2±1.1IU/day, -5.9±1.2IU/d and -4.7±1.2IU/d respectively ($p<0.05$)] in the full cohort. Clinical variables over the follow-up period are highlighted in Table 1.

Conclusion: Our study demonstrates that structured T1D education such as the DAFNE course offers clinically important long-term benefits on weight and glycaemia. It is interesting to observe that these benefits occur despite significant reduction in basal insulin dose that was maintained over the 3-year follow-up period. From our study, it appears that patients in the highest HbA1C quartile seemed to have the most significant drop in HbA1C.

Table 1: Changes in clinical variables during follow-up.

	Baseline	Year 1	Year 2	Year 3
Weight (kg) Weight change (kg)	82.4	80.2 -2.3±0.7*	79.5 -3.0±0.8*	81.2 -1.3±0.9
$HbA1_C$ (mmol/mol)	69.0±13.7	69.0±13.4	70.0±14.2	67.0±14.3
$HbA1_C$ (mmol/mol) 1st / lowest quartile 2nd quartile 3rd quartile 4th quartile	 52.1±6.1 66.0±2.9 73.0±2.3 88.0±8.5	 56.0±8 68.0±11.4 71.0±10.2 77.0±14.1*	 55.0±8.8 69.0±8.7 70.0±13.1 80.0±10.7*	 54.0±8.8 66.0±6.6 70.0±13.3 81.0±13.6
Basal insulin (BI) requirements (IU/d) BI change (IU/d)	34.1	28.9 -5.2±1.1*	28.3 -5.9±1.2*	29.4 -4.7±1.2*

Results mean±SD
ns=no significance
statistical significance
* $p<0.05$, compared to baseline

Disclosure: **A.Y. Liu:** None.

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Evaluating the relationship between diabetes distress and biomedical parameters in type 1 diabetes using a novel assessment tool

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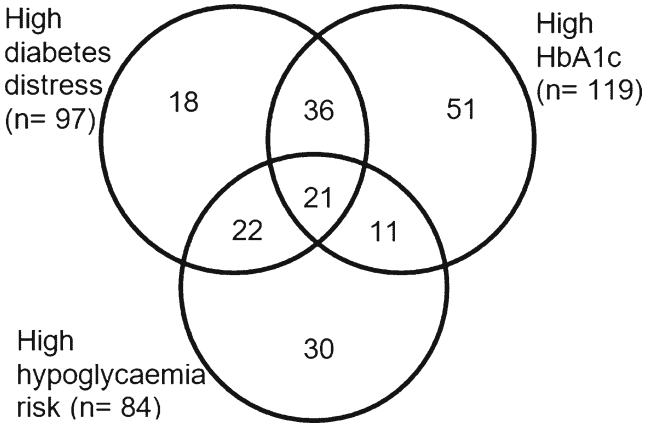
Background and aims: Diabetes distress is the specific psychological burden patients experience when living with diabetes and is often a barrier to effective self-management. This study aimed to pilot a novel assessment tool to measure diabetes distress, HbA1c and hypoglycaemia risk and evaluate relationships between these 3 parameters in adults with type 1 diabetes.

Materials and methods: Patients attending specialist type 1 clinics at two teaching hospitals used a novel tool to assess their status in three domains: glucose control [HbA1c], hypoglycaemia risk [Gold score/severe hypos within 1 year] and diabetes distress [Diabetes Distress Score 2 (DDS2)]. Data are presented from 280 patients. 57.5% were female, mean age was 43.3 ±15.6 years and 61.6% were insulin pump treated. Pearson correlations were used to evaluate relationships between DDS2 and HbA1c, and DDS2 and Gold scores. An HbA1c of 7.5-8.4% (58-68 mmol/mol) was reported as "moderately raised" and "high" if ≥8.5% (69 mmol/mol). Raised diabetes distress was reported as "moderate" with DDS2 score 3-3.5 and "high" if scoring ≥4. A Gold score of 3-4 was reported as "moderate" hypoglycaemia risk. "High" hypoglycaemia risk is a Gold score ≥4 or at least 1 severe hypoglycaemia episode in the past year.

Results: Mean HbA1c was 8.4% (68 mmol/mol), with 31.1% moderately raised and 42.5% high HbA1c. 21.8% of patients had at least 1 severe hypoglycaemia episode within 1 year and 15.4% had a Gold score >4, giving 30% of patients a high hypoglycaemia risk. 58.5% reported raised diabetes distress, including 34.6% having high diabetes distress. HbA1c was significantly associated with diabetes distress ($r=0.319$, $p=0.01$), as was Gold score ($r=0.257$, $p=0.01$). 50.4% of patients with high HbA1c had high diabetes distress, as did 51.2% of those with high hypoglycaemia risk. 65.6% patients with both high HbA1c and high hypoglycaemia risk reported high diabetes distress (see accompanying diagram). Of those who reached NICE targets of HbA1c <7.5% and low hypoglycaemia risk, 15.8% had moderate diabetes distress and 5.3% reported high diabetes distress. Only 2.1% of those with high diabetes distress achieved both of these NICE targets, compared to 25.9% of those without raised distress (DDS2 <3).

Conclusion: These results demonstrate that over half of patients with type 1 diabetes have significant diabetes distress. Sub-optimal glucose control and problematic hypoglycaemia are also both significantly associated with diabetes distress. Interestingly, 21.1% of those with "good control" do so at the expense of significant distress. Acknowledging and exploring the causes of distress in those with sub-optimal control may be key in achieving therapeutic success.



Disclosure: **P.J. Todd:** None.

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Adherence to different aspects of diabetes self-management and glycaemic control after two variants of patients' education in type 2 diabetes mellitus patients starting insulin

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Background and aims: Increasing adherence to recommendations is an important aim of patients' education. This study evaluates if additional focus on physical activity and structured SMBG can improve adherence and glycemic control after initiation of insulin treatment.

Materials and methods: Patients with T2DM starting insulin treatment were evaluated at baseline and after 6 months. At start of insulin treatment all patients were randomized to 5 sessions of structured education (Standard group, N=35) or same education with addition of physical exercise sessions and focus on structured self-monitoring of blood glucose (Active group, N=35). Assessment included clinical evaluation, HbA1c, creatinine and microalbuminuria. Adherence to recommendations in specific aspects of diabetes self-management was assessed by SDSCA (Summary of diabetes self-care activities) questionnaire. Data are presented as M(SD) format, Mann-Whitney U-test, Spearman correlation test were used for statistical analysis.

Results: SDSCA scores at baseline and 6 months are provided in Table 1. Only 1 smoker was among patients and this score wasn't analyzed. All patients reported 7 points on medication score. HbA1c was 8.7(1.1)% in Active group and 9.0(1.46) in Standard (p=0.7). No significant correlations were observed between HbA1c and any SDSCA score at baseline. At 6 months HbA1c decreased in both groups and was significantly lower in Active group (7.2(1.04) vs. 7.9(1.24)% p=0.045). SMBG adherence was significantly higher in Active group and there was a trend for better general diet and physical activity adherence, while no difference was observed for specific diet and foot care. In pooled analysis of all patients lower HbA1c at 6 months correlated with higher adherence to general diet recommendations (R=-0.49 p=0.0002) and SMBG recommendations (R=-0.48 p=0.0004) although not with adherence to physical activity.

Conclusion: Education with additional focus on structured SMBG and physical activity is associated with better adherence to diabetes self-management and better HbA1c at 6 months after initiation of insulin therapy in T2DM. HbA1c in insulin treated T2DM was significantly dependent on adherence on general diet and glucose monitoring. Medication adherence appears an unrecognized problem by patients as 100% compliance with drugs is unlikely.

Table 1. Adherence to recommendations on different aspects of diabetes self-management (SDSCA scores) at baseline and 6 months after initiation of insulin and education

	Standard group	Active group	p-level
Baseline			
General diet	4.2(1.79)	4.1(1.76)	0.8
Specific diet	3.6(1.52)	4.3(1.64)	0.05
Physical activity	4.4(1.18)	3.5(1.82)	0.04
Glucose monitoring	4.4(2.08)	3.6(1.79)	0.2
Foot care	4.0(2.31)	4.2(2.34)	0.6
Medication	7(0)	7(0)	
At 6 months			
General diet	4.5(1.79)	5.4(1.76)	0.06
Specific diet	3.7(1.52)	3.7(1.64)	0.78
Physical activity	4.4(1.18)	5.3(1.82)	0.08
Glucose monitoring	3.9(2.08)	5.6(1.79)	0.004
Foot care	5.3(2.31)	5.1(2.34)	0.73
Drug use	7(0)	7(0)	

Disclosure: **A.V. Petrov:** None.

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Impact of Ramadan focused education programme on hypoglycaemic risk and metabolic control for patients with type 2 diabetes

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Background and aims: Fasting the holy month of Ramadan could lead to acute complications and increased hypoglycemic risk for patients with type 2 diabetes (T2D). This study aimed to evaluate the impact of Ramadan focused educational program on hypoglycemia risk and metabolic markers.

Materials and methods: A prospective non-randomized interventional controlled design was run on three phases: before, during and after Ramadan on 262 T2D patients with HbA1c≥8. The intervention group (n=140) received focused individualized diabetic education sessions and anti-diabetic medications adjustment before and after Ramadan while the control group (n=122) received standard diabetic care. A validated hypoglycemic score was used in both groups to assess the change of the risk. Patients were also advised to adjust the dosage and timing of oral hypoglycemic agents and insulin according to 2010 Ramadan ADA position statement. Primary outcomes were post-intervention change of hypoglycemia score and HbA1c over 3 months of follow up. Data were presented as means±SD. HbA1c expressed in %.

Results: The hypoglycemic scores before, during and after Ramadan were 14.21±8.50, 6.36± 6.17, and 5.44±5.55 in the intervention group, respectively (P<0.001) and 14.01±5.10, 13.46± 5.30, and 9.27±4.65 in the control group, respectively (P<0.001). HbA1c levels were 9.79±1.89, 8.26±1.54 and 8.52±1.61 before, during and after Ramadan in the intervention group, respectively (P<0.001) and 10.04±1.47, 9.54±1.38, and 9.59±1.79 in the control group, respectively (P=0.021). Post-Ramadan reductions of HbA1c and hypoglycemic scores were significantly higher in the intervention group (-13.0% versus -4.5%, P=0.004 for HbA1c and -61.7% versus -33.8%, P<0.001 for hypoglycemic score). LDL cholesterol improved during Ramadan in the intervention group from 2.41 ±0.91mmol/l to 2.28 ±0.73mmol/l (p=0.009) while no change was observed in the control group. No statistically significant effects on blood pressure or body weight were observed. Multivariate linear regression analysis adjusted for age, gender, BMI, education, residence, and job showed 7.2% more post-Ramadan reduction in HbA1c (p=0.004) and 3.16 points more post-Ramadan reduction in hypoglycemia score (p<0.001) in intervention compared to the control group.

Conclusion: Ramadan educational program had a positive impact with reduction of hypoglycemic risk, HbA1c and LDL cholesterol. We would recommend this program for patients with increased risk of hypoglycemia during Ramadan fasting.

Disclosure: **A.M. Tourkmani:** None.

OP 46 Diabetes and the brain

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Both long-term and short-term glycaemic control are associated with micro- and macrovascular events - a DPV study on 152,121 patients with type 2 diabetes

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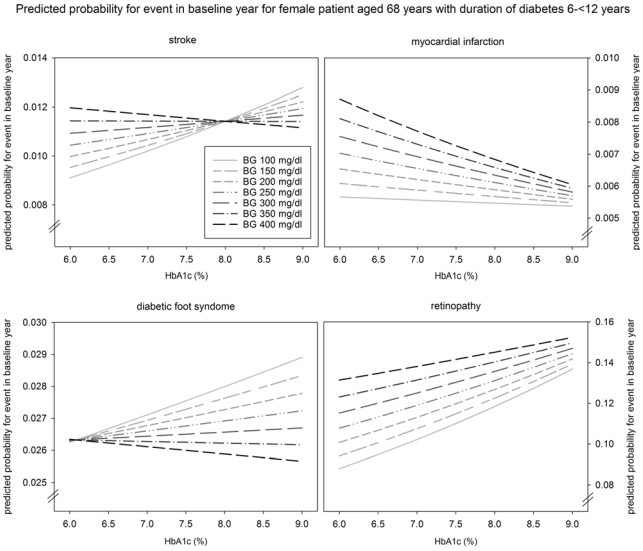
Background and aims: Poor glycaemic control is a risk factor for various comorbidities in patients with type 2 diabetes (T2D). However, many studies reported only weak associations between glycaemic control and macrovascular complications. Whereas most studies assessed either long-term or short-term glycaemic control, this study aimed to examine the association between micro- and macrovascular events and both HbA1c and blood glucose (BG) during office visits.
Materials and methods: A cohort of 152,121 patients with T2D from the German/Austrian Prospective DPV Registry was analysed. Data included gender, age, and duration of diabetes as well as HbA1c and BG at baseline visit (first documented visit). Multivariable logistic regression models were used to assess the association between both HbA1c and BG (main effects, interaction term) and the occurrence of stroke, myocardial infarction (MI), diabetic foot syndrome (DFS), and retinopathy. p-values <0.05 were considered significant (SAS 9.4).
Results: 52% of the patients were male. Median age (IQR) at baseline visit was 68 (60, 77) years, duration of diabetes was 8 (3, 15) years. Baseline HbA1c and BG were 7.1 (6.3, 8.5) % and 180 (137, 239) mg/dl, respectively. 8.4/8.8/9.2% of the patients had a history of stroke/MI/DFS. Retinopathy was diagnosed in 22.8% of those patients with eye examination (n=73,468). 1.2/0.8/4.0/12.3% of the patients had a diagnosis of stroke/MI/DFS/retinopathy within the baseline year. As expected, HbA1c significantly correlated with BG (Spearman correlation: r_{sp}=0.51, p<0.001). All four outcomes were significantly associated with HbA1c and BG (all p<0.001), but the patterns of association differed. The figure depicts estimated probabilities for the respective event in the year of the baseline visit by HbA1c as a function of BG. Patients with high glycaemic fluctuations (low HbA1c but high BG at office visit, or high HbA1c but low BG at office visit) had a higher risk for stroke than patients with lower glycaemic fluctuations. For MI and retinopathy, high glycaemic fluctuations seemed to play a role primarily at low HbA1c levels, whereas a higher risk for DFS was associated with high fluctuations at high HbA1c levels.
Conclusion: We found BG measured during office visits, in addition to HbA1c, to be associated with stroke, MI, DFS, and retinopathy in patients with T2D. Despite measuring the same construct, both long-term and short-term glycaemic control added to the prediction of micro- and macrovascular events. This finding should be further investigated using CGM data and taking into account other risk factors such as dyslipidaemia, hypertension, genetics, or smoking.



Supported by: German Center for Diabetes Research (DZD), Sanofi-Aventis
Disclosure: **J.M. Hermann:** None.

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Brain MRI abnormalities in patients with type 2 diabetes mellitus

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Background and aims: There is a growing body of evidence that brain damage represents another diabetic complication. Changes of cerebral structure and function could be attributed to the influence of the diabetic metabolic milieu. However, the exact brain abnormalities that are associated with type 2 diabetes mellitus (T2DM) need to be further elucidated. Therefore, the aim of this study was to investigate which abnormalities on brain MRI are associated with T2DM.
Materials and methods: We examined 93 patients with T2DM without history of prior cerebrovascular accidents (mean age 62.3±5.5 years, diabetes duration 9.7±6.7 years, BMI 32.5±10.4 kg/м2, HbA1c 8.1±1.3%) and 18 healthy subjects who served as the control group (mean age 59.5 ±5.7 years, BMI 29.1±4.0 kg/м2). All subjects were scanned on a 1.5T MRI scanner. Intracranial volume (ICV), total brain (TBV), total cerebrospinal fluid (CSF), white matter (WM), grey matter (GM), peripheral CSF, lateral ventricular (LV) and white matter hyperintensity (WMH) volume were determined on the MRI scans automatically by kNN-based probabilistic segmentation. Infarct volumes were manually segmented. Volumes were expressed as % of ICV and numbers represent percentages of ICV. Linear regression analyses adjusted for sex, age and education level were performed.
Results: We found a lower TBV (78.8±2.13 vs. 81.3±1.98; p<0.05), a lower WM volume (43.2±1.34 vs. 43.7±1.04%; p<0.05) and a lower GM volume (35.4±2.25 vs. 37.5±2.02%; p<0.05) in patients with T2DM compared to controls. Total CSF volume (21.2±2.13 vs. 18.7±1.98; p<0.05), peripheral CSF volume (19.2±1.78 vs. 17.0±1.93%; p<0.05) and LV volume (2.0±0.91 vs. 1.7±0.71%; p<0.05) were higher in patients with T2DM compared to controls. However, there were no statistically significant between group differences in WMH volume (0.16+0.18 vs. 0.12+0.13%) and infarct volume (0.2+0.56 vs. 0.03+0.13%). There was a statistically significant negative correlation between longer diabetes duration, on one side, and TBV and WM volume, on the other side. We found a positive correlation between disease duration and LV volume, WMH volume and total CSF volume.

Conclusion: In our study we revealed structural brain abnormalities that are associated with T2DM. These brain abnormalities could underlie the cognitive deficits frequently observed in patients with T2DM.
Supported by: EFSD Collaborative Program "New Horizons"
Disclosure: **B. Mankovsky:** None.

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Association between structural brain abnormalities and cognitive functioning in patients with type 2 diabetes mellitus

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Background and aims: Recent clinical and epidemiological studies revealed an association between cognitive impairment, dementia, on the one side, and type 2 diabetes mellitus (T2DM), on the other side. However, the exact association between structural brain abnormalities and cognitive functioning in patients with T2DM is still not entirely clear. Therefore, the aim of this study was to investigate the association between structural brain abnormalities and cognitive functioning in patients with T2DM.
Materials and methods: We examined 93 patients with T2DM (mean age 62.3±5.5 years, diabetes duration 9.7±6.7 years, BMI 32.5±10.4 kg/м2, HbA1c 8.1±1.3%). All subjects did not have a history of cerebrovascular accidents or depressive episodes. Cognitive functioning was assessed by means of a standardized psychometric test battery covering the domains Memory, Processing Speed and Executive functioning. All cognitive tests were performed in the morning. There were no episodes of hyperglycemia or hypoglycemia immediately before assessment of cognitive functioning. All subjects were scanned on a 1.5T MRI scanner. Intracranial volume (ICV), total brain (TBV), total cerebrospinal fluid (CSF), white matter (WM), grey matter, peripheral CSF, lateral ventricular and white matter hyperintensity (WMH) volume were determined on the MRI scans automatically by kNN-based probabilistic segmentation. Infarct volumes were manually segmented. Volumes were corrected for ICV. Pearson correlation tests were performed.
Results: We found statistically significant positive correlations between WM, on one side, and the Memory score ($r=0.214$, $p<0.05$) and Executive Functioning ($r=0.216$, $p<0.05$). Significant negative correlations were found between Processing Speed, on one hand, and WMH ($r=-0.22$, $p<0.05$), and total CSF ($r=-0.236$, $p<0.05$), on the other hand. Moreover, Processing Speed positively correlated with TBV ($r=0.236$, $p<0.05$). The correlations between domain scores and other brain volumes did not reach the level of statistical significance.
Conclusion: Our analysis indicates that WM volume positively correlates with the memory and executive functioning scores while the function of processing speed was negatively affected by WMH and total CSF but positively correlates with the total brain volume. These data could indicate the presence of an association between the structural brain abnormalities and cognitive impairments in patients with T2DM free of clinically significant cerebrovascular disease.
Supported by: EFSD Collaborative Program "New Horizons"
Disclosure: **N. Zherdova:** None.

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Effects of insulin degrading enzyme genetic variation on cognition and brain structure in patients with type 2 diabetes

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Background and aims: The dysfunctions of insulin degrading enzyme (IDE) represent key mechanism underlying the insulin resistance associated with Alzheimer's disease (AD). Exploring the roles of genetic variations of IED in brain structure and function will help to improve our understanding of AD pathophysiology.
Materials and methods: A total of 233 type 2 diabetic patients with mild cognitive impairment (MCI) were employed, and they all had evaluations cognitive function, structure and functional magnetic resonance imaging (MRI) scans. Whole exome sequencing of IDE gene was also performed and total of 11 common single nucleotide polymorphisms (SNPs) after quality control were enrolled in further analysis. We explored the associations with IDE variations and brain volume and function.
Results: For brain structure, interactions were detected with thickness of entorhinal cortex and both rs1887922 ($\beta = -0.58$, FDR-corrected $P = 0.035$) and rs4646954 ($\beta = -0.35$, FDR-corrected $P = 0.019$), and rs1887922 also associated with atrophy of parahippocampal ($\beta = -0.54$, FDR-corrected $P = 0.02$). Subsequent haplotype-based analysis further supported the association for the three mentioned SNPs and atrophy of entorhinal cortex ($P < 0.001$). In the analyses for neural activity, rs1832196 and rs1999764 showed negative connections with the activity in the right posterior cingulated (RPCC) ($\beta = -0.36$ and -0.47, FDR-corrected $P = 0.016$ and 0.038 respectively). Moreover, the more the atrophy of parahippocampal, the more worse the general cognition ($rho = 0.36$, $P = 0.001$) and memory ($rho = 0.45$, $P < 0.001$). The similar association was also detected between RPCC activity and memory ($rho = 0.57$, $P < 0.001$).
Conclusion: IDE play a role of in AD pathology partially by deteriorating the brain atrophy and activity, which may represent a clinical biomarker to predict the AD process.

SNPs	Minor allele	MAF	Location	Functional activity	β	FDR-corrected *P*
rs1887922	C	0.09	Intron	Atrophy of entorhinal cortex	-0.58	0.035
				Atrophy of parahippocampal	-0.54	0.02
rs4646954	C	0.05	5'UTR	Atrophy of entorhinal cortex	-0.35	0.019
rs1832196	G	0.06	Intron	Reduced activity in posterior cingulated	-0.36	0.016
rs1999764	C	0.05	Intron	Reduced activity in posterior cingulated	-0.47	0.038

Disclosure: **J. Huang:** None.

OP 47 Multihormonal control of islet cell function

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Impaired beta cell adaptation to reduced insulin sensitivity and increased insulin clearance explain glucose intolerance in mice with deletion of GLP-1 and GIP receptors

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Background and aims: It is known that the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) contribute to insulin secretion after mixed meal or oral glucose ingestion. However, the importance of the two incretin hormones for beta cell function or insulin clearance in the absence of food or glucose ingestion is not known. We therefore evaluated the glucose, insulin and C-peptide data after intravenous glucose administration in mice with double genetic deletion of both GLP-1 and GIP receptors.

Materials and methods: Mice heterozygous for the deletion of both the GIP receptor and GLP-1 receptor genes were generated from double homozygous deletion mutant mice and used to yield double incretin receptor knockout (DIRKO) and wild-type (Wt) mice (both groups had similar basal glucose 7.8±0.3 mmol/l; insulin 180±17 pmol/l and C-peptide 198±15 pmol/l). After fasting for five hours, mice were anesthetized and given a single injection of D-glucose solution (0.35 or 0.75 mg/kg). Blood was sampled from the intraorbital retrobulbar sinus plexus at 0, 1, 5, 10, 20, 30 and 50 minutes and assayed for glucose, insulin and C-peptide. The data were modeled to calculate glucose tolerance (KG), acute insulin response (AIR), beta-cell sensitivity to glucose (BCS), basal (QUICKI) and dynamic (SI) insulin sensitivity, glucose effectiveness (SG), insulin clearance and disposition index (SI x AIR).

Results: DIRKO mice had a severe glucose intolerance (see Table) having higher AUC glucose despite higher insulin, and impaired QUICKI and SI. Beta-cell response (AIR, BCS) tended to be higher in DIRKO, but not significantly, i.e., not enough to adapt for the lower insulin sensitivity, as mirrored by the lower disposition index. Also, insulin clearance was 21.4% higher in DIRKO (p=0.02). KG and SG were slightly lower in DIRKO (p<0.049).

Conclusion: We conclude that DIRKO mice have glucose intolerance, resulting from reduced insulin sensitivity, and also higher insulin clearance, as well as inappropriately low beta-cell adaptation to the insulin resistance. This shows that the incretin hormones may be of importance not only for the beta-cell function but also for insulin clearance and more generally for glucose tolerance.

Table: Parameters (mean±SEM) after administration of glucose; p indicates possible statistically significant difference between the groups (Mann-Whitney); p<0.05 is assumed as statistically significant.

	Wt (n=41)	DIRKO (n=27)	p-value
AUC glucose (mmol/l min)	531±16	611±22	0.003
AUC insulin (nmol/l min)	10.9±0.5	13.5±0.7	0.004
AUC C-peptide (nmol/l min)	10.3±0.5	13.4±0.8	0.0006
QUICKI (dimensionless)	0.125±0.001	0.120±0.001	0.006
SI [10^{-4} min^{-1}/(pmol/l)]	1.38±0.09	1.13±0.13	0.03
AUC insulin secretion (pmol)	79±6	98±10	NS
AIR (pmol/l)	16.3±0.6	17.9±0.8	NS
BCS ($mmol_{CP}/mol_{GLU}$)	0.021±0.001	0.022±0.001	NS
DI (10^{-4} min^{-1})	11.0±1.0	8.0±1.3	0.03

Disclosure: **A. Tura:** None.

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Islet derived peptide YY (PYY) is a key player in regulation of beta cell function and preservation of beta cell mass

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Background and aims: The physiological significance of classical islet peptide hormones including insulin, glucagon and somatostatin is established. However, the role of non-classical islet regulatory peptides, such as the gut hormone peptide YY (PYY) is unknown. Thus, although PYY is recognised as an important appetite regulator in control of body weight, possible involvement in beta-cell function and glucose homeostasis has been largely discounted. In the present study we have examined the effects of PYY(1-36) and PYY(3-36) on beta-cell function and preservation of beta-cell mass.

Materials and methods: Expression of PYY and NPY1, 2, 4 and 5 receptors were confirmed in rodent and human islet cells by real time RT-PCR. Isolated mouse islets, rodent BRIN-BD11 and human 1.1B4 beta-cells were used to evaluate insulinotropic activity (n=8) of PYY peptides (10^{-10} to 10^{-6} M). Further assessment of PYY mediated mechanisms of insulin secretory modulation was carried out in BRIN BD11 cells (n=8). Acute effects of PYY(1-36) and PYY(3-36) on food intake, glucose and insulin concentrations were examined in overnight fasted lean mice (25 nmol/kg bw, *ip*, n=8). The influence of PYY on islet adaptions to streptozotocin-induced (5 daily doses of 50 mg/kg bw, *ip*) insulin-deficiency and hydrocortisone-induced (10 daily doses of 70 mg/kg bw, *ip*) insulin resistance was assessed in C57BL/6 mice (n=6). Finally, direct effects of PYY(1-36) and PYY(3-36) (both at 10^{-6} M) on beta-cell proliferation and apoptosis was examined in BRIN BD11 and 1.1B4 beta-cells (n=8).

Results: As expected, in vivo administration of PYY(3-36), but not PYY(1-36), markedly (P<0.05) decreased food intake in overnight fasted mice. Neither form of PYY affected glucose disposal or insulin secretion following a bolus injection in combination with 18 mmol/kg glucose. However, both PYY(1-36) and PYY(3-36) inhibited (P<0.05 to P<0.001) glucose (16.7 mM), alanine (10 mM) and GLP-1 (10^{-6} M) stimulated insulin release from cultured rodent and human beta-cells, as well as isolated mouse islets. This effect was linked to inhibition (P<0.05 to P<0.001) of alanine-induced enhancement of BRIN BD11 cell membrane potential and $[Ca^{2+}]_i$, as well as GLP-1-induced elevations of cAMP. C57BL/6 mice treated with multiple low-dose streptozotocin presented with severe (P<0.01) loss of beta-cell mass accompanied by notable increases (P<0.001) in alpha and PP cell numbers. In contrast, hydrocortisone-induced insulin resistance substantially increased islet number (P<0.01) and beta-cell mass (P<0.001). Immunoflourescent staining co-localisation studies revealed that streptozotocin decreased islet

PYY co-localisation with PP (P<0.05) and somatostatin (P<0.001), whilst hydrocortisone increased (P<0.05) PYY co-localisation with glucagon. More detailed in vitro investigations demonstrated that both forms of PYY augmented (P<0.05 to P<0.01) human and rodent beta-cell proliferation and protected against streptozotocin induced cytotoxicity, to a similar extent as GLP-1.

Conclusion: Both type 1 and type 2 diabetes are associated with decreased beta-cell mass. Taken together, these data highlight the therapeutic potential offered by modulation of pancreatic islet PYY receptor signalling pathways for diabetes, by preserving or augmenting beta-cell mass and function.

Disclosure: **P.R. Flatt:** None.

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Functional and molecular adaptation of intestinal L cells in mice under high fat diet is associated to the preservation of alpha and beta cell function and to normoglycaemia

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Background and aims: Type 2 diabetes is characterized by insulin resistance as well as alterations of insulin (beta-cell), glucagon (alpha-cell) and GLP-1 (intestinal L-cell) secretions. The aim of the study is to identify molecular and functional alterations of proglucagon cells and their roles in diabetes.

Materials and methods: Transgenic mice (expressing the fluorescent protein Venus in proglugacon-expressing cells) were submitted to a 16 weeks control Low Fat Diet (LFD) or to a High Fat Diet (HFD). 3 groups were defined at the end of the study according to glycated hemoglobine: LFD (3,90±0,05%) ; HFD mice with impaired glucose tolerance (I-HFD) (4,00±0,04%) and HFD hyperglycemic mice (H-HFD) (4,9±0,10%). The molecular and functional aspects are studied in vivo and ex vivo on alpha and L-cells purified by FACS.

Results: I-HFD and H-HFD mice have the same weight gain, hyperinsulinemia, and insulin resistance. However, insulin secretion stimulation after an oral glucose load is maintained in I-HFD but strongly altered in H-HFD. I-HFD mice are principally characterized by intestinal L-cells molecular and functional adaptations. In response to glucose gavage, GLP-1 secretion is strongly increased (Fold=6.97+/-1.54 for LFD vs 44.86+/-7.93 for I-HFD) while the regulation of glucagon is preserved. Glucagon and PC1/3 genes are increased in L-cells as well as GLP-1 content. By contrast H-HFD mice exhibit dysfunctional alpha-cells, with an abolished response to glucose. Beta and L-cells numbers are increased without improvement of their function. Administration of the GLP-1R antagonist Exendin9-39 to I-HFD mice before a glucose gavage induces alterations of glucagon secretion without changes of insulin and reduces glucose tolerance (AUC=275.06+/-61,47 for I-HFD vs 699.38+/-112.03 mmol/l X min for I-HFD treated Ex9-39) leading to an increase of glycated hemoglobin when chronically administered (3.73+/-0.08 for I-HFD vs 3.95+/-0.06% for I-HFD treated Ex9-39).

Conclusion: These results highlight the crosstalk between endocrine L-cells and pancreatic islets and show that a compensatory adaptation of L-cells is implicated in the preservation of glucose homeostasis through the control of pancreatic alpha cell function.

Disclosure: **R. Dusaulcy:** None.

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The myokine irisin is released in response to saturated fatty acids and enhances pancreatic beta cell survival and insulin secretion

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Background and aims: Skeletal muscle releases mediators that adjust insulin secretion to the actual needs for appropriate peripheral glucose utilization. Saturated fatty acids (SFA) affect pancreatic beta-cell function and modulate expression of some cytokines in skeletal muscle. The aims of this study were to explore the potential role of irisin, a secreted skeletal muscle protein derived from cleavage of fibronectin type III domain-containing protein 5 (FNDC5), in the communication between skeletal muscle and beta-cells in response to excess SFA and its direct effects on beta-cells survival and function.

Materials and methods: Rat L6 myotubes were cultured for 4 or 24 h with 0.5 mM palmitate and the ability of conditioned media (respectively PM4h and PM24h) to affect apoptosis, glucose-stimulated insulin secretion (GSIS) and proliferation of INS-1E cells was explored. The effect of SFA on irisin secretion was analyzed both in vitro and in vivo. In human and rat beta-cell lines, as well as in human and murine islets, the effects of irisin on beta-cell apoptosis, insulin biosynthesis, GSIS and proliferation were studied. Cell apoptosis was detected by caspase-3 cleavage and cytosolic release of oligosomes. Insulin mRNA expression was evaluated by qRT-PCR. Insulin and irisin secretion were measured by ELISA. Proliferation was analyzed by BrdU incorporation assay.

Results: Conditioned medium from rat L6 myotubes exposed to palmitate for 24 h (PM24h) exerted detrimental effects on INS-1E beta-cells by increasing apoptosis, decreasing cell proliferation, and reducing GSIS (p<0.05). By contrast, when INS-1E cells were incubated with conditioned medium from myotubes exposed to palmitate for 4 h (PM4h), apoptosis markers were reduced (p<0.05). Treatment of rat myotubes with palmitate for 4 h resulted in increased FNDC5 mRNA and protein content and 3-fold higher irisin release in the culture medium (p<0.05). Short-term exposure to palmitate also promoted release of irisin from human myotubes. Mice fed a high fat diet showed a 2-fold increase in serum irisin levels within 24 h compared to standard diet (p<0.05). Importantly, the anti-apoptotic effect of the conditioned medium from PM4h was abrogated when INS-1E cells were cultured in the presence of an irisin neutralizing antibody (p<0.05). Finally, in human and rat pancreatic beta-cell lines, as well as in human and murine primary pancreatic islets, recombinant irisin prevented palmitate-induced apoptosis, by activating AKT/Bcl2 signaling, and directly stimulated insulin biosynthesis, GSIS and cell proliferation (p<0.05).

Conclusion: Myotubes-derived irisin emerges as a new player in the communication between skeletal muscle and beta-cells in response to short-term SFA challenge, since irisin can enhance GSIS, proliferation and promote beta-cell survival.

Disclosure: **N. Marrano:** None.

OP 48 Type 1 diabetes: from epidemiology to clinical conclusions

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Diabetic ketoacidosis among type 1 patients on insulin pump therapy: number of patients in the diabetes clinic modifies the association

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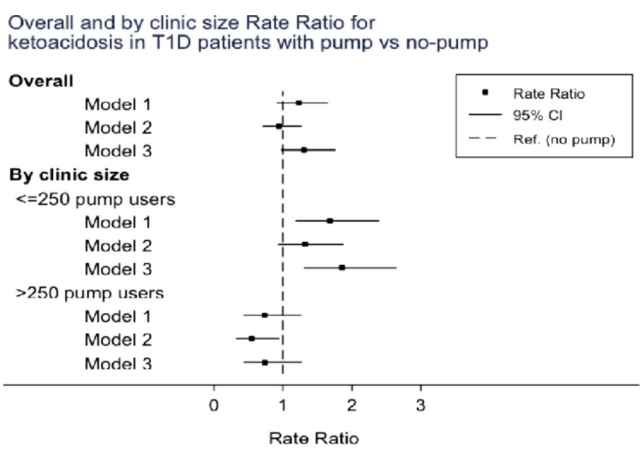
Background and aims: In patients treated with insulin pump therapy (continuous subcutaneous insulin infusion (CSII)), pump device malfunction or increased insulin demand may pose a risk of diabetic ketoacidosis (DKA) because of smaller insulin depot with CSII. Treatment with CSII should therefore be carried out in centres in which an experienced and coordinated multidisciplinary team is available. We hypothesized that high-volume CSII clinics might have lower rates of DKA compared to small clinics. Therefore, we compared the incidence rates of DKA among type 1 diabetes (T1D) patients on CSII vs. multiple daily injection (MDI), and examined how the number of CSII patients in the diabetes clinic affects the association between CSII use and DKA.
Materials and methods: A total of 20.902 T1D patients in the Danish Adult Diabetes Database were followed for an average of 5.4 years. Outcome was recurrent events of DKA and with CSII (yes/no) as the main exposure variable. Poisson regression analyses with risk time as offset were used to compare differences in rates of DKA between CSII vs MDI. Model 1 was unadjusted. Model 2, was adjusted for age, sex, diabetes duration and previous DKA events and model 3 was further adjusted for HbA1c level. A modifying effect of number of CSII patients in the clinic was tested.
Results: During 113.731 years of follow-up, 3.100 DKA events were registered (53 occurred among CSII patients). Patients on CSII were younger (42.3 vs 47.9 years), a larger proportion was female (59% vs 43%), had a slightly shorter diabetes duration (19 vs 21 y), and a lower HbA1c (61.9 vs 66.6 mmol/mol). There was no overall significant difference in the incidence rate of DKA between patients with CSII compared to patients with MDI (Model 3: RR 1.30, 95%CI: 0.97;1.76). However, the number of CSII patients in the diabetes clinic modified the association between CSII and DKA events (P=0.005). In clinics with more than 250 CSII patients, rates of DKA events were lower among CSII users, while the opposite was true for the smaller clinics (<250 pump users) (see figure).
Conclusion: Implementing a set-up equivalent to that seen in large diabetes clinics may ensure that CSII treatment does not lead to an increased risk of DKA compared to MDI.



Disclosure: **S. Hoshina:** None.

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Psychiatric medication use before and after the onset of type 1 diabetes in children and adolescents: a population-based cohort study

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[1]Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, [2]Department of Clinical Pharmacy, Division Laboratory and Pharmacy, University Medical Center Utrecht, Netherlands.

Background and aims: Several studies showed a bidirectional association between type 2 diabetes and psychiatric disorders in adults. There is limited information available about the association of type 1 diabetes (T1D) and psychiatric disorders in children and adolescents. The aim of this study is to assess the extent of psychiatric medication use before and after the onset of T1D in children and adolescents compared with a reference cohort without T1D.
Materials and methods: A population-based cohort study was conducted in the Dutch PHARMO Record Linkage System. All children and adolescents (<19 years) with at least two insulin dispensings between 1999 and 2009 were identified as a T1D cohort (N=925) and matched by age and sex with an up to four times larger reference cohort (N=3591). The period prevalences of psychiatric medication use (psycholeptics (ATC code: N05) and psychoanaleptics (ATC code: N06)) were calculated by dividing the number of patients with at least one dispensing for these medications by the number of patients available in the cohort during that time. Prevalences were calculated from 5 years before until 5 years after the onset of T1D (the index date in both cohorts) and stratified by age, sex, and medication subgroup.
Results: The mean age of the study participants was 10.1 years and 51% were boys. The 5-year prevalence of psychiatric medication use before the index date was significantly higher in the T1D cohort than in the reference cohort (7.2 vs. 4.7%, respectively, p=0.002). The same pattern was observed for the period after developing T1D (10.4 vs. 7.9% in the T1D and reference cohort respectively, p=0.015). In both cohorts adolescents (15-19 years) and boys had higher prevalences of psychiatric medication use. This increased prevalence of psychiatric medication use both before and after the index date in the T1D cohort was mainly driven by an increased use of psycholeptics (mainly anxiolytics).
Conclusion: Children with T1D were more likely to use psychiatric medication in the years before and after the onset of type 1 diabetes. This increased use was mainly driven by anxiolytics both before and after onset of T1D.
Disclosure: **S. Fazeli Farsani:** None.

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Falling all-cause mortality from the Yorkshire Register of type 1 diabetes in children and young adults

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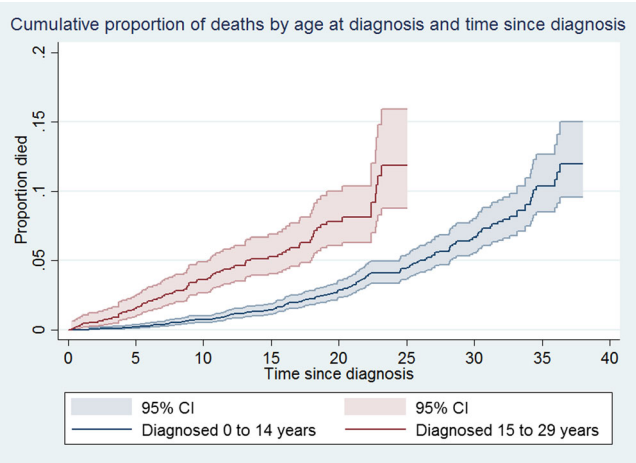
Background and aims: Data from the Yorkshire Register of Diabetes in Children and Young Adults previously showed excess all-cause mortality in individuals with type 1 diabetes (T1D) compared to the general population. This cohort includes updated death data and has been analysed to examine which factors increase mortality risk and if mortality has changed over time.
Materials and methods: The cohort included under 15s (early onset) diagnosed with T1D in Yorkshire from 1978 and individuals diagnosed between 15 to 29 years (late onset) in West Yorkshire from 1991. Person identifiers were linked to death certification data from the Office for National Statistics (ONS). Standardised mortality ratios (SMRs) and survival curves were produced by sex, age at death, age and year of diagnosis, ethnicity and deprivation. SMRs used England and Wales population death rates by 5-year age group and sex from 1978 to 2014. Deprivation was classified using 2001 Townsend score by residence at diagnosis. A classification algorithm derived ethnicity from full names.

Results: Out of 6,209 individuals with 107,492 person-years of follow-up, there were 233 deaths. The overall SMR was 4.3 (95% CI 3.8 - 4.9). SMRs for males (4.4 (95% CI 3.8 - 5.2)) and females (4.0 (95% CI 3.2 - 5.2)) were similar. For ethnicity, 171 individuals from a white ethnic origin had a non-significantly higher SMR compared to 11 South Asians (7.8 (95% CI 6.7 - 9.0) vs. 4.0 (95% CI 2.2 - 7.3)). There was a decreasing trend in SMR from the most to least deprived fifths (4.9 (95% CI 4.0 - 6.0) vs. 1.9 (95% CI 0.8 - 4.2)). There was no significant difference in survival between early and late onset groups when analysis time was set to age. However, the late onset group had a significant increased rate of death for time since diagnosis. The SMR for those diagnosed before 1980 with 20 years or more follow-up time (3.1 (95% CI 1.9 - 5.1)) was significantly higher than those diagnosed between 1990 to 1994 with 20 years or more follow-up time (0.7 (95% CI 0.3 - 1.4).
Conclusion: Our more recent data show lower mortality than previous studies. Early onset T1D remains a significant risk factor for mortality. However, age at death seems more important to risk of death than diabetes duration, suggesting that factors associated with later life are the key determinants driving risk of death. The decreasing trend in SMRs with later years of diagnosis provides some evidence to suggest that mortality has decreased over time.



Disclosure: **T.C. Evans-Cheung:** None.

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Classifying diabetes by type 1 genetic risk shows autoimmune diabetes cases are evenly distributed above and below 30 years of age

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Background and aims: There is considerable controversy about the extent to which autoimmune mediated Type 1 diabetes (T1D) presents in patients diagnosed older than 30 years. This is difficult to assess as autoantibodies are rarely measured in these individuals and clinical classification is imprecise. We recently developed T1D-GRS and have shown T1D is restricted to patients with a high T1D-GRS(>50th population TID-GRS centile). We used T1D-GRS measurement to evaluate the contribution of autoimmunity in diabetes diagnosed under 60 years in a cross-sectional study of UK adults.
Materials and methods: We analysed the development of diabetes using Kaplan-Meier estimates in 152,118 UK individuals from the UK Biobank(age 30-60 years) in different deciles of T1D-GRS.
Results: There is an excess of diabetes in the top five deciles(3436) compared to the bottom five deciles(2853)p<0.0001 consistent with 9.3% of the cohort having T1D. 48%(279/583) of T1D cases occur under 30 years where they contribute 75%(279/373) of all diabetes cases and 52%(304/583) T1D occurs aged 30-60 years contributing 5.1%(304/5916) of all cases. These T1D cases were predominantly in the top T1D-GRS decile(6.0% had diabetes vs 3.5% bottom T1D-GRS decile p<0.0001). Patients diagnosed 30-60yrs with a GRS suggesting T1D are diagnosed younger(41vs52 years p<0.0001), slimmer(body mass index(BMI) 27vs32kg/m^{2}p<0.0001) and progress more rapidly to insulin(72%vs7% within one year p<0.0001).
Conclusion: T1D-GRS is a novel tool to investigate diabetes aetiology in large cohorts without antibody measurement. T1D diabetes is evenly distributed within the first six decades of life but after 30 years the increase in Type 2 diabetes makes them harder to recognise and treat correctly.
Disclosure: **N.J.M. Thomas:** None.

PS 001 Mortality rate in diabetes around the world

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Premature deaths from ischaemic heart disease in childhood and young adult onset type 1 diabetes

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Background and aims: Ischaemic heart disease (IHD) is a major cause of death for individuals with type 1 diabetes (T1D). Age at onset of T1D and risk of IHD death was assessed.
Materials and methods: The Yorkshire Register of Diabetes in Children and Young Adults includes individuals diagnosed with T1D in Yorkshire under 15 years (early onset) from 1978. From 1991, the register also includes individuals diagnosed between 15 to 29 years (late onset) in West Yorkshire. Personal identifiers were linked to Office for National Statistics (ONS) death certification data. Underlying cause of death information using International Classification of Diseases (ICD-10) coding was validated by a specialist clinician. Standardised mortality ratios (SMRs) were calculated using England and Wales population and IHD death rates between 1978 to 2014 by 5-year age group and sex. Population IHD deaths were classed by ICD-9 codes 410 - 414 between 1979 and 2000 and ICD-10 codes I20 - I25 from 2001. SMRs were calculated by age at onset.
Results: The cohort included 6,209 individuals, with 107,492 person-years of follow-up. Out of 233 deaths, 16 deaths had an underlying cause due to T1D with IHD. Fourteen of these deaths (11 males, 3 females) were from the early onset group, where median age at death was 35.1. There were only 2 deaths (both male) from the late onset group, where median age of death was 43.2. The overall SMR for IHD deaths was 8.5 (95% CI 5.2 - 13.9). The SMR for the early onset group was 13.8 (95% CI 8.2 - 23.3) which is non-significantly higher than the SMR for the late onset group (2.3 (95% CI 0.6 - 9.3)). The SMRs for time since diagnosis was highest between 10 to 19 years for both the early and late onset groups at 116.1 (95% CI 48.3 - 278.9) and 3.2 (95% CI 0.4 - 22.5) respectively.
Conclusion: Death from IHD started to appear in patients from the age of 20 years in the early onset group, suggesting that childhood onset diabetes may result in premature death from this cause. Higher mortality in patients with early onset T1D compared with patients with later onset, suggests that childhood diabetes may result in unexpectedly early vascular death.

	Early onset			Late onset		
Age at death (years)	Observed	Expected	SMR (95% CI)	Observed	Expected	SMR (95% CI)
20 to 24	2	0.04	50 (12.5, 199.9)	0	0.01	0
25 to 29	2	0.09	22.6 (5.6, 90.2)	0	0.04	0
30 to 34	3	0.16	18.9 (6.1, 58.5)	0	0.11	0
35 to 39	2	0.25	8 (2, 31.8)	1	0.21	4.9 (0.7, 34.6)
40 to 44	3	0.28	10.6 (3.4, 33)	0	0.27	0
45 to 49	2	0.15	12.9 (3.2, 51.7)	0	0.18	0
50 and over	0	0.02	0	1	0.05	19.5 (2.7, 138.3)
Total	14	1.02	13.8 (8.2, 23.3)	2	0.86	2.3 (0.6, 9.3)

Supported by: YRDCYP is funded by the Yorkshire and Humber Paediatric Diabetes Network
Disclosure: **H.J. Bodansky:** None.

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Diabetes-related mortality in Cameroonian children

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Background and aims: Diabetes in children is associated with a very high mortality rate in sub-Saharan Africa due to lack of early diagnosis and limited access to quality health care for the majority of the children involved. The aim of this study was to determine the mortality rate and causes of death of childhood diabetes five years after the implementation of a project providing free health care to all children living with diabetes in Cameroon.
Materials and methods: During the period from October 2010 to October 2015 we identified all cases of diabetic-related child deaths in the project "Changing Diabetes in Children (CDIC)." This is a project that offers free diabetes treatment to all children up to 18 years on entry, in Cameroon. We analyzed the medical records of the children involved and interviewed doctors / nurses and parents on the circumstances and possible causes of death.
Results: Five hundred twenty-eight children (277 boys, 251 girls) were enrolled in the project over the study period. They had a mean age of 16 ± 4 years and mostly had type 1 diabetes (97%). Fifty-three deaths (10%) were recorded. The death rate ranged from 5 to 17.5% following the children's region. The main causes of death were diabetic ketoacidosis (10 cases), infections (6 cases), hypoglycemia (4 cases), renal failure (5 cases). Forty-four deaths occurred outside hospital setting. Some of the deaths due to ketoacidosis were because of reduction of participation in the project substituting it for indigenous medications or prayers.
Conclusion: Although in sharp decline with free care, the mortality rate of diabetes in children is still very high in Cameroon because of the environment, the behavior of the population but also the organization of the health care system.
Disclosure: **G. Lemdjo:** None.

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Mortality rates, causes of death and risk factors in a large cohort of childhood onset of type 1 diabetes

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Background and aims: The aim of this study was to examine mortality rates and causes of death among patients diagnosed with type 1 diabetes before their 15th birthday in Wales.
Materials and methods: The BRECON childhood onset type 1 diabetes registry (n=3288), with diagnosis from 1979 to 2013 (capturing 98% of all new cases from 1995), was used to investigate patterns in cause-specific mortality. 43141 patient-years of diabetes were analysed and 31 deaths were identified. Follow-up has been defined as time from diagnosis until date of death, time an individual has moved away from Wales or date of censoring (25th July 2015), whichever occurred first. 8.7% of our cohort was lost to follow-up due to emigration.The observed number of deaths was compared with number of deaths seen among different age groups (adjusted by gender and underlying cause when appropriate) in England and Wales, as reported by Office of National Statistics. Poisson regression was used to compare mortality by socio-economic status, family history of diabetes, age at diagnosis, size of centre involved in diabetic care and gender.
Results: The overall standardised mortality ratio (SMR) was 3.16 (95% CI 2.1-4.9), being highest in the age group 15-19 years, at 4.12 (95% CI 2.1-7.3). The largest number of deaths was attributed to diabetic ketoacidosis (n=8), followed by accidental deaths (n=4) and suicide (n=4). Increasing diabetes duration was associated with decreased mortality (16% decrease for each year holding all other variables constant, p-value <0.001).
Conclusion: Despite advances in diabetic treatment, type 1 diabetes is still associated with higher mortality rates, particularly in the age during transition from paediatric to adult care facilities. High mortality rates due to suicide are particularly worrying.
Disclosure: **D.R. Wasag:** None.

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Mortality 15 years after diagnosis of type 1 and type 2 diabetes in adults, with diabetes type defined by autoimmunity and C-peptide

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Background and aims: Most studies of adults classify diabetes by clinical criteria, with largest risk of misclassification of autoimmune, or type 1, patients. We report preliminary results from 15-year follow-up of the Diabetes Incidence in Kronoberg (DIK) 1998-2001 study where classification was the best available, a combination of islet cell antibodies and C-peptide. Guidelines have recommended more intensified treatment and control of risk factors the past decades, which may have influenced prognosis of diabetes.
Materials and methods: All adults, aged 18-100 years, with newly diagnosed diabetes during 3 years, in Kronoberg county (177 000 inhabitants), were included. Statistics Sweden provided death dates, and one population control each, matched for age, sex, and community of residence at baseline. Follow-up ended 20141231.
Results: At follow-up, 50.6% (814/1609) had died. Hazard ratio for death among all patients was 1.34 (1.21-1.49) compared to the matched population controls. In the type 2 group HR was 1.36 (1.23-1.51), and in the type 1 group HR was 1.08 (0.63-1.84). Of the type 2 diabetes patients 52.4% (785/1497) had died vs 25.9% (29/112) of the type 1 patients, ($p< 0.0001$). There were no gender differences, neither among type 2 (p 0.50) nor type 1 diabetes (p 0.33). Of the women 51.8% (404/780)had died; and 49.5% (410/829) of the men (p 0.35). Mean survival time was 10.9 (10.6-11.2) in the type 2 group, and 13.9 (13.1-14.7) years in the type 1 group($p <0.0001$). In type 2 diabetes the influence on mortality by BMI was about 5% ($p <0.0001$); for BMI and C-peptide together 16% ($p <0.0001$).
Conclusion: The risk of death within 15 years was increased with 30% in diabetes patients compared to matched population controls. Mortality rate among adults with type 2 diabetes was double that in type 1 diabetes, 50% vs 26%, 15 years after diagnosis, without significant gender differences, so a diagnosis especially of type 2 diabetes still carries a substantial risk regarding overall mortality. BMI influenced mortality risk in type 2, but not in type 1 diabetes.
Supported by: The Kamprad Family Foundation and Region Kronoberg
Disclosure: **M. Thunander:** None.

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Rates and causes of death in adult patients with diabetes

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Background and aims: To study the age and sex dependent rates and causes of death in a Romanian diabetes cohort as compared with the general population.
Materials and methods: All adult (20-64 years) patients receiving a free diabetes prescription in a major urban area during 2001-2008 were included and followed up for death until December 31st, 2011. Rates (per 1000 person-years) and standardized mortality rates (SMR) against general population (data from the National Institute of Statistics) were calculated based on age bands of 5 years for all-cause, and 15 years for 5, and then 18 specific causes of death. Years lost due to diabetes were computed assuming general population mortality rates for ages under 20 and above 65 years.
Results: During the 11 years study period, 49275 diabetes patients (49.1% women), mean age at screening 53±8.8 years contributed 297370 person-years of follow-up and 5053 deaths. Mortality rates for women by age groups were: 20-24 years - 1.79 (SMR 5.6 CI95% 0.8-39.9), 25-29 - 6.9 (SMR 14.8 CI95% 8.4-26.1), 30-34 - 5.1 (SMR 7.9 CI95% 4.7-13.3), 35-39 - 4.7 (SMR 4.3 CI95% 2.8-6.8), 40-44 - 5.7 (SMR 2.9 CI95% 2.1-4.0), 45-49 - 8.3 (SMR 2.4 CI95% 2.0-2.9), 50-54 - 8.8 (SMR 1.8 CI95% 1.6-2.0), 55-59 - 14.1 (SMR 1.9 CI95% 1.7-2.0), 60-64 - 20.1 (SMR 1.8 CI95% 1.6-1.9). Mortality rates for men by age groups were: 20-24 years - 4.9 (SMR 5.7 CI95% 1.8-17.6), 25-29 - 3.5 (SMR 3.2 CI95% 1.5-6.7), 30-34 - 7.1 (SMR 4.2 CI95% 2.9-6.1), 35-39 - 6.1 (SMR 2.3 CI95% 1.7-3.2), 40-44 - 9.1 (SMR 1.8 CI95% 1.5-2.2), 45-49 - 12.8 (SMR 1.5 CI95% 1.3-1.7), 50-54 - 17.2 (SMR 1.4 CI95% 1.3-1.5), 55-59 - 23.3 (SMR 1.3 CI95% 1.3-1.4), 60-64 - 32.5 (SMR 1.3 CI95% 1.2-1.3). Major (and detailed 18) causes of death modified rates and SMR and were represented by cardiovascular diseases 46.2% (vs. 36.1% general population, $p<0.001$), cancer 22.3% (vs. 28.0% general population, $p<0.001$), diabetes 8.2% (vs. 0.9% general population, $p<0.001$), renal failure 2.1% (vs. 0.7% general population, $p<0.001$) and others 21.2% (vs. 34.3% general population, $p<0.001$). Life years lost due to diabetes at age 20-25 years were 6.8 years for women (1.9 years at age 50-54 years) and 5.3 years for men (1.4 years at age 50-54 years).
Conclusion: Mortality rates increased, while mortality ratios (diabetes vs. general population) decreased with age. Men had higher mortality rates, but women had higher mortality ratios in gender analysis. Combined cardiovascular diseases and cancer were responsible for 68.5% of all causes of death (vs. 64.1% in general population, $p<0.001$). Each day, diabetes patients aged 20-25 years lost 197 minutes of life due to diabetes in women and 172 minutes in men.
Disclosure: **S. Ioacara:** None.

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Mortality rates in elderly patients with diabetes

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Background and aims: Many studies reported higher mortality rates in patients with diabetes compared with the general population. However, few data are available regarding mortality in elderly diabetes subjects, some suggesting that better care of diabetes subjects in this age group could represent a survival advantage. The aim of this study was to investigate mortality rates in a cohort of elderly patients with diabetes as compared with the general population.
Materials and methods: All diabetes patients aged 65 years or older residing in a major urban area were included and followed up for death until December 31st, 2011. Baseline was defined as the date of first available free diabetes prescription received between January 2001 and December 2008. Mortality rates (per 1000 person-years) and standardized mortality rates (SMR) were calculated in 5 years age bands, with ≥85 years as the last analysed age interval. For comparison, general population mortality data was available from the National Institute of Statistics.
Results: There were 29784 diabetes patients (58% women), mean age at screening 72.1±5.1 years, contributing 194828 person-years of follow-up and 13013 deaths. Mortality rates and SMR for women were: 65-69 yrs. - 28.88 (CI95% 26.41-31.59) / SMR 1.55 (CI95% 1.41-1.69), 70-74 yrs. - 44.31 (CI95% 42.27-46.45) / SMR 1.34 (CI95% 1.28-1.41), 75-79 yrs. - 65.47 (CI95% 62.86-68.21) / SMR 1.11 (CI95% 1.06-1.16), 80-84 yrs. - 98.41 (CI95% 93.86-103.17) / SMR 0.92 (CI95% 0.88-0.96), ≥85 yrs. - 133.13 (CI95% 124.72-142.11) / SMR 0.61 (CI95% 0.57-0.65). For men, the corresponding data were: 65-69 yrs. - 43.35 (CI95% 39.78-47.23) / SMR 1.16 (CI95% 1.06-1.26), 70-74 yrs. - 60.53 (CI95% 57.67-63.54) / SMR 1.12 (CI95% 1.06-1.17), 75-79 yrs. - 76.90

(CI95% 73.49-80.47) / SMR 0.94 (CI95% 0.90-0.99), 80-84 yrs. - 109.05 (CI95% 103.32-115.11) / SMR 0.86 (CI95% 0.81-0.91), ≥85 yrs. - 124.06 (CI95% 114.0-135.01) / SMR 0.54 (CI95% 0.50-0.59). Major causes of death were cardiovascular diseases 66.4%, cancer 15.4%, diabetes 6.0%, renal failure 1% and others 11.2%.

Conclusion: As expected, crude mortality rates are higher in males than females. However, the higher SMR in women for each age group suggest that diabetic women are exposed at a higher risk than men. After the age of 80 years in females and 75 years in males, diabetes seems to become a "protective" factor for mortality. Possible explanations could be a specific protective genetic background, more frequent medical monitoring and intensive multifactorial treatment of various cardiovascular risk factors in this population.

Disclosure: **C. Guja:** None.

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Mortality trends over time in patients with incident type 2 diabetes and controls

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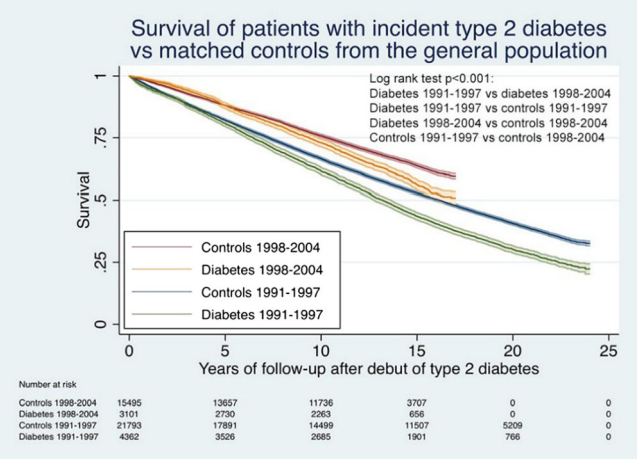
Background and aims: Mortality rates have decreased in the general population in Sweden and other Western countries during the last decades. Knowledge is limited about temporal trends in mortality of patients with incident type 2 diabetes compared with the general population. The aim of this study was to study long-term trends of mortality compared to the general population among patients with debut of incident type 2 diabetes in 1991-1997 and in 1998-2004.

Materials and methods: The Skaraborg Diabetes Registry (SDR) was active 1991-2004 and includes patients with prospectively clinically diagnosed diabetes after investigation mainly in primary care, of which 7 465 patients with incident type 2 diabetes. Using data from Statistics Sweden, 37 291 randomly selected controls from the general population were matched according to sex, age, calendar year and municipal (up to 5 controls per patient). The patients and controls were followed until emigration, death or 31 December 2014 using data from the Swedish National Cause of Death Register. Survival analysis was done using Kaplan-Meier curves and Cox regression.

Results: Patients in the 1991-1997 cohort (n=4 363) were older when diagnosed with type 2 diabetes than in the 1998-2004 cohort (n=3 102) (66.0 years vs 63.0 years, p<0.001), had higher first registered systolic BP (148.5 mmHg vs 143.9 mmHg, p<0.001), higher first registered diastolic BP (82.3 mmHg vs 79.9 mmHg, p<0.001), higher first registered HbA1c (6.6 vs 6.4, p<0.001), lower first registered BMI (29.1 vs 30.1, p<0.001) and lower proportion of smokers (16.2% vs 17.4%, p<0.001). The sex distribution was similar in the cohorts (male sex 53.2% vs 53.8%; p=0.59). In the 1991-1997 diabetes cohort 3 089 deaths (70.8%) occurred during 54 836 person-years of follow up, and in the control group 13 199 deaths (60.6%) in 297 840 person-years. In the 1998-2004 diabetes cohort 1 275 deaths (41.1%) occurred during 35 733 person-years, and in the control group 5 342 deaths (34.5%) in 181 781 person-years. See graph for Kaplan-Meier survival curves. HR for all-cause mortality among patients vs controls in the 1991-1997 cohort was 1.37 (95% CI 1.32-1.42, p<0.001) and 1.22 (95% CI 1.14-1.29, p<0.001) in the 1998-2004 cohort when adjusted for sex and age.

Conclusion: The risk estimates for all-cause mortality compared with the Swedish general population was lower for patients in the SDR with debut of type 2 diabetes in 1998-2004 than in 1991-1997. The difference could be influenced by dissimilarities in baseline characteristics.



Supported by: Närhälsan R&D Skaraborg, The Skaraborg Institute R&D, Skaraborg R&D Council

Disclosure: **T. Andersson:** None.

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The combined effect of adiponectin and resistin on all-cause mortality in patients with type 2 diabetes: evidence of synergism with abdominal adiposity

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Background and aims: The combined effect of serum adiponectin and resistin levels on all-cause mortality in patients with type 2 diabetes (T2D) has never been studied. We investigated such joint effect and its possible modulation by several demographic and clinical conditions.

Materials and methods: Patients with T2D from the Gargano Mortality Study (GMS; N = 895, follow-up = 10.5±3.7 years; 290 events) and the Foggia Mortality Study (FMS; N = 519, follow-up = 7.1±2.5 years; 140 events) were examined.

Results: In both studies, each SD increase of log-adiponectin [HRs; 95% CI = 1.38 (1.23-1.55); p = 4.30*10-8 and 1.59 (1.32-1.91); p = 1.22*10-6, in GMS and FMS, respectively] and log-resistin [(HRs; 95% CI = 1.42 (1.27-1.59); p = 6.81*10-10 and 1.37 (1.17-1.59); p = 8.28*10-5, in GMS and FMS, respectively], were associated with all-cause mortality. Since, no difference in effect sizes, across the two studies were observed (p values for within-study heterogeneity = 0.22 and 0.73 for adiponectin and resistin, respectively), GMS and FMS were pooled. In the whole sample, both adipokines were associated with death, independent of each other and of several additional covariates (p values ranging from 0.01 to 4.6*10-12). No adiponectin-by-resistin interaction was observed (p = 0.40), thus pointing to an additive effect of the two adipokines. To get deeper insights on such joint effect, the pooled sample was stratified according to relatively high and low adiponectin and resistin levels (i.e. above or below the median value). Four groups were obtained: low/low, high/low; low/high and high/high adiponectin/resistin levels named as group 1, 2, 3 and 4, respectively. As compared to group 1, the HRs (95% CI) for all-cause mortality were 1.81 (1.33-2.47), 1.97 (1.44-2.69) and 3.02 (2.26-4.03), in group 2, 3 and 4 (p < 0.0001 for all). These analyses confirm that the two adipokines exert an additive effect in shaping the risk of all-cause mortality. We then tested whether the highly

increased mortality risk in group 4 vs. group 1 was modulated by synergistic interaction with various demographic and clinical features (i.e. sex, age at recruitment, smoking habits, BMI, waist circumference, HbA1c, diabetes duration, eGFR, albuminuria and ongoing treatments). Among all these possible modifiers, only waist circumference turned out to act as such, with the increased risk in group 4 vs. group 1, being significantly more pronounced in individuals with relatively low waist circumference values (HR = 2.97 (1.80-4.91) vs. 1.58 (1.01-2.47), p for HR heterogeneity = 0.03).

Conclusion: This is the first study reporting an additive independent effect of increased adiponectin and resistin on all-cause mortality in patients with T2D. Such additive effect is modulated by abdominal adiposity. Further studies will tell if adding these two adipokines to already available prediction tools is useful in improving prediction ability of mortality rate in patients with T2D.

Supported by: EFSD/Pfizer

Disclosure: **L. Ortega Moreno:** None.

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Impact of type 2 diabetes on life expectancy and cause-specific mortality in white, South Asian and black patients

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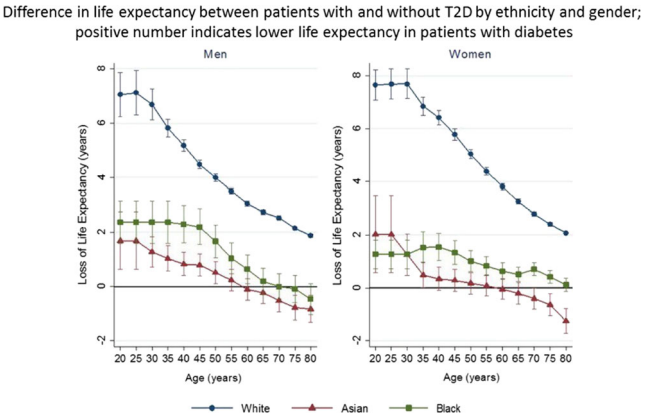
Background and aims: Little is known about the impact of diabetes on life-expectancy and cause-specific mortality in people with type 2 diabetes (T2D) across different ethnic groups. Therefore, we aimed to assess these outcomes in White, Black and South Asian people.

Materials and methods: We used linked electronic health records from the UK Clinical Practice Research Datalink (CPRD) to identify cohorts of 187,968 incident T2D patients from 1998-2015 and 908,016 age- and gender-matched controls without diabetes from the same general practices in England. We used abridged life tables to estimate life expectancy, and a flexible parametric survival model to quantify all-cause mortality associated with T2D. A competing risk model was used to calculate ethnic differences in cause-specific mortality rates in T2D patients adjusting for age, gender, patient-level deprivation and calendar year.

Results: We observed 40,286 deaths in T2D patients and 181,338 deaths in those without diabetes; resulting in crude mortality rates of 42.7/1,000 person years and 19.5/1,000 persons years, respectively. Type 2 diabetes was associated with a two-fold higher risk of death compared to patients without diabetes; adj HR 2.17 (95% CI:2.15-2.20). White patients with T2D observed the greatest difference in life expectancy compared to White patients without diabetes; a reduction of 7.1 years for men and 7.7 years for women (Figure). A loss of between 1.3-2.4 years was observed for South Asian and Black patients with T2D. Paradoxically, older South Asian men and women with T2D had up to 1 year longer life expectancy compared to South Asian people without T2D (Figure). The leading causes of death in patients with T2D were similar across ethnic groups: cardiovascular disease, cancer and respiratory disease. However, when compared to Whites with T2D, South Asians with T2D had lower adjusted risks for mortality from cardiovascular (HR 0.47 [95% CI:0.40-0.55]), cancer (HR 0.53 [95% CI:0.45-0.64]) and respiratory diseases (HR 0.58 [95% CI:0.46-0.73]). Lower risks for these outcomes were also seen in Blacks with T2D compared to Whites with T2D (adj HRs: 0.84 [95% CI:0.72-0.99]; 0.85 [95% CI:0.79-0.91] and 0.70 [95% CI:0.53-0.92] respectively).

Conclusion: The development of T2D has a greater impact on life expectancy in Whites than South Asian or Black patients. This may be partly explained by lower mortality risks from cardiovascular, cancer and respiratory diseases in these minority ethnic groups with T2D. The explanation for the apparent extended life expectancy associated with diagnosed T2D in older South Asians with T2D is uncertain and requires further investigation.



Supported by: Diabetes UK

Disclosure: **M.K. Rutter:** Employment/Consultancy; Consultant: Roche Diabetes Care GmbH, Consultant: Cell Catapult. Grants; Novo Nordisk. Stock/Shareholding; Modest stock holding: GSK. Other; Educational grants: Novo Nordisk and MSD.

PS 002 Does body shape and size matter?

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Muscle mass decline as a significant risk factor for type 2 diabetes development in middle-age subjects during the prospective 1000PLUS cohort study

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Background and aims: Muscle is the primary tissue contributing to whole-body insulin-mediated glucose disposal. Studies indicate that age-associated loss of muscle mass begins at the fifth decade of life and up to 50% of muscle may be lost by the age of 90. It is also known that sarcopenia may be an important factor in insulin resistance and basal metabolic rate decrease. Aim of our study was to investigate risk factors associated with type 2 development in middle-age (mean 47 yrs old) subjects during the 5-year prospective observational cohort study.
Materials and methods: For the 1000 PLUS cohort study we have recruited 1,119 subjects (763 overweight/obese, 356 with normal BMI, who underwent oral glucose tolerance test, anthropometric measurements and body composition analysis by bioimpedance method. Serum insulin and plasma glucose were measured from fasting blood samples to calculate HOMA-IR.
Results: During the follow up period type 2 diabetes was diagnosed in 7.4% subjects, impaired fasting glucose in 37.7% and impaired glucose tolerance in 9.3% in the studied group. Logistic regression models adjusted for age at the second visit were constructed in order to determine a set of features enabling prediction of type 2 diabetes development. Among others, changes in: glucose concentration, visceral fat tissue volume, HOMA-IR level, muscle mass were chosen as potential predictors and included in models. Features, which showed to have statically significant impact on DMt2 were taken for further investigation. Since, it was highly probable, that some of the selected features are somehow dependent, i.e. redundant, a set of independent variables was extracted. The constructed feature set comprised of: change in HOMA-IR level (OR=1.86, p-value<0.01), change in muscle mass (OR=0.60, p-value<0.03). Aiming at validation of prediction capability using selected attributes, the Support Vector Machines classifier and Leave-One-Out cross-validation procedure was applied yielding 91.92% classification accuracy.
Conclusion: The reduction of muscle mass is an independent (of change in HOMA-IR) risk factor for diabetes type 2 in the middle-age subjects. It would be reasonable to conduct studies to determine the effectiveness of exercise interventions required to improve muscle mass and glucose metabolism in terms of the risk of type 2 diabetes.
Disclosure: **K. Maliszewska:** None.

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Indicators of visceral adiposity as predictors of metabolic syndrome in population of Vojvodina Province in Serbia

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Background and aims: Metabolic syndrome (MS) represents a cluster of cardiovascular risk factors including central obesity, impaired fasting glucose, dyslipidemia and arterial hypertension. The basic feature responsible for the arise of disorders characteristic for MS is accumulation of visceral adipose tissue followed by development of insulin resistance. Over the last few years, much efforts have been given in search for simple and inexpensive indicator of body`s visceral adipose tissue quantity and various equations were made, serving up with simple and widespread used anthropometrical and laboratory parameters. Aim of our study is to test the value of some of these equations against traditional anthropometrical parameters, most frequently used for the assessment of visceral adiposity, in prediction of MS in outpatient clinic based study group.
Materials and methods: Cross-sectional study conducted at our clinic has included 1321 individuals. The sample was chosen from outpatient cohort of consecutively examined individuals, based on facts that participants were not self-reporting existence of any severe acute or chronic illness or use of any anti-diabetic, lipid-lowering or antihypertensive therapy. All participants underwent a measurement of waist circumference (WC), body height (BH), body weight, BP and sagittal abdominal diameter (SAD), bioelectrical impedance analysis for determination of total body`s fat percentage (fat%) and blood sampling for analysis of blood glucose and lipids. Based on the published formulas we have calculated BMI, visceral adiposity index (VAI) and lipid accumulation product (LAP). For diagnosis of MS we have used NCEP ATP III criteria. In testing of predictive value of different visceral adiposity indicators we have used Receiver Operating Characteristic (ROC) curves and comparison of derived areas under curve (AUC).
Results: 52.61% of participants were males. Median age was 47 (18-67) for males and 42 (18-65) for females. According to BMI calculation there was 0.14% underweight, 27.20% normal weight, 40.86% overweight and 31.80% obese males, and 0.32% underweight, 21.57% normal weight, 18.53% overweight and 59.58% obese females. MS was present in 55.11% males and 45.85% females. In both genders, we have tested the predictive value, in function of detecting MS, of the following visceral adiposity indexes: BMI, WC to BH ratio (WBHR), SAD to BH ratio (SADBHR), fat%, VAI and LAP. In males, predictive performances were ordered in the following way: LAP, WBHR, SADBHR, BMI, VAI, fat% (AUC: 0.802, 0.745, 0.730, 0.729, 0.647, 0.597, respectively). Direct comparison has shown that AUC for LAP was significantly greater than AUC for BMI, WBHR, SADBHR, fat% and VAI (p: 0.0003, 0.0023, 0.0004, <0.0001, <0.0001, respectively). In females, predictive value performances were ordered in this way: LAP, WBHR, BMI, VAI, SADBHR, fat% (AUC: 0.857, 0.758, 0.740, 0.712, 0.698, 0.671, respectively). Further analysis has shown significantly greater AUC of LAP in comparison to AUC for BMI, WBHR, SADBHR, fat% and VAI (p<0.0001 for all parameters).
Conclusion: LAP shows the best predictive performance in detecting MS, especially in women. These results appear unexpected, since LAP requires less inputs for calculation than VAI does. As expected, total body`s fat percentage shows the poorest predictive performance, which is somehow expected, since it gives no information on distribution of adipose tissue.
Supported by: **B. Vukovic:** None.

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Prevalences of different body composition phenotypes with aging and its changes after 4 years follow-up in Koreans

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Background and aims: Obesity is well known to be associated with metabolic disorders, but recently sarcopenia has attracted increasing attention. However, the complex interplay of changes in fat mass and muscle mass associated with aging are not well understood. We aimed to examine the prevalence and clinical significance of complex body composition phenotypes.
Materials and methods: The study population comprised 18,558 Korean subjects aged 21-60 years who received regular health checkups during

2007-2010 and followed during 2011-2014 in Health Screening and Promotion Center of Asan Medical Center. Participants were stratified by body composition checked by bioimpedence analysis. Body composition phenotypes were divided into 4 groups; low muscle mass with low fat mass (LM/LF), high muscle mass with low fat mass (HM/LF), low muscle mass with high fat mass (LM/HF), and high muscle mass with high fat mass (HM/HF).

Results: Prevalences of each phenotype were 32% for LM/LF, 18% for HM/LF, 18% for LM/HF, and 32% for HM/HF. With increasing age, the prevalences of HM/LF and HM/HF decreased, but that of LM/HF increased for men, and the prevalence of LM/LF was decreased, but those of LM/HF and HM/HF were increased for women. HM/LF groups had lower prevalences of diabetes and hypertension, higher HDL-cholesterol, lower LDL-cholesterol, triglyceride levels, and hsCRP than LM/HF groups in both sexes. After 4 years follow-up, one third of LM/LF group changed into LM/HF group and two third of LM/LF group remained in the same group. HM/LF group became to LM/LF (25%), HM/LF (30%), LM/HF (21%), and HA/HF (24%) groups. Most of the LM/HF group remained in the same group. One third of HM/HF group was changed to LM/HF group and two third of HM/HF group was stayed in the same group. When we compared the HM/HF-to-LM/HF changed group with HM/HF stayed group, prevalence of type 2 diabetes was higher in HM/HF-to-LM/HF group (22%) than in HM/HF stayed group (16%) in men although plasma fasting glucose and HbA1c were not different at baseline between the two groups.

Conclusion: With increasing age, fat mass tended to increase in both men and women, and loss of muscle mass was prominent in men. Loss of muscle mass was associated with higher prevalence of diabetes in men after 4 years follow-up.

Disclosure: **H. Kim:** None.

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Aging trajectories of obesity and body composition in south Asians and whites: a longitudinal analysis from the Whitehall II study

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Background and aims: South Asians have increased fat mass for a given body mass index (BMI), but there is limited information on ethnic differences in age-related changes of body composition, a factor that may explain the higher risk of type 2 diabetes in south Asians compared to whites with the same BMI. To address this limitation, we investigated ethnic differences in aging trajectories of BMI, waist circumference (waist), fat mass, and fat free mass.

Materials and methods: In this longitudinal cohort study of British adults with 5-yearly clinical examinations (n=159/4939 south Asian/white, age 50-74 years at baseline), the 11-year (between 2002-2013) age-related trajectories of BMI, waist circumference, fat mass, and fat free mass (based on bioimpedance using the Tanita TBF-300 body composition analyser) were fitted for south Asians and whites who remained free of diabetes.

Results: South Asians had similar BMI and fat mass as whites, but their waist circumference and fat-free mass were lower (mean±SD; 90.3±11.9 vs. 87.7±10.7 cm, 56.8±9.9 vs. 48.3±8.2 kg, p<0.01) at baseline. According to age and sex-adjusted mixed-effects models, south Asians had a non-significantly decreasing BMI trajectory from 26.3[SE 0.4] to 25.7[0.4] kg/m2 between ages 50 and 80, while in whites BMI increased from 26.2[0.08] to 26.6[0.08] kg/m2 (p for slope difference 0.026). Waist circumference was 1.7[0.9] cm smaller among south Asians and increased with age both in south Asians and whites. Fat mass followed parallel quadratic trajectories in both ethnicities with 1.7[0.6] kg lower values among south Asians. Fat free mass was 6.0[0.5] kg lower among south Asians at age 65 and decreased 0.4[0.2] kg faster per decade. Adjustment for physical activity, dietary habits, and social grade had no major effects on these ethnic differences.

Conclusion: In contrast to whites, no age-related increase was observed in BMI trajectories in south Asians. However, they had a faster decline in fat-free mass (including muscle and bone tissues) that may contribute to adverse metabolic outcomes and increased risk of diabetes.

Supported by: MRC, BHF, NHLBI, and NIA

Disclosure: **A.G. Tabák:** None.

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An inducer of glyoxalase 1 improves insulin resistance and glycaemic control in overweight and obese non-diabetic subjects

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Background and aims: Risk of insulin resistance and impaired glycaemic control is excessive in overweight and obese populations. Glyoxalase 1 (Glo1) is linked genetically body weight in mice and to clinical obesity. Recent studies found that high fat diet-induced insulin resistance and increased body weight in mice was suppressed by overexpression of Glo1. Similar studies have shown microvascular complications of diabetes were prevented by Glo1 overexpression. Increasing Glo1 expression is currently unaddressed by therapeutic agents Glo1 is part of the glyoxalase metabolic pathway which catalyses the metabolism of the reactive metabolite and glycating agent, methylglyoxal (MG), and thereby prevents formation of advanced glycation endproducts (AGEs). There is a regulatory antioxidant response element in the GLO1 gene which, when bound by transcription factor Nrf2, increases basal and inducible expression of Glo1. We screened dietary bioactive compounds for Glo1 inducer activity, confirmed hits and improvement of cell function in human cell primary cultures. The aim of this study was to validate target pharmacology of an optimised Glo1 inducer formulation in Phase 1 clinical trial in overweight and obese subjects and assess effect on insulin resistance and glycaemic control.

Materials and methods: The Glo1 inducer formulation was evaluated in a randomised, placebo-controlled, double-blinded crossover clinical trial in overweight and obese subjects. Treatment was with Glo1 inducer or placebo once daily for 8 weeks with 6 week washout period before crossover. Insulin resistance was assessed by 2-h Oral Glucose Insulin Sensitivity (OGIS) index in an oral glucose tolerance test (oGTT). Glycaemic control was assessed by fasting plasma glucose (FPG) and postprandial glucose as area-under-the-curve glucose in the oGTT (gAUC). Thirty-two subjects were recruited and 29 completed the study. Data are analysed per protocol.

Results: Subject characteristics at baseline were: age 45 ± 13 years, gender (M/F) 8/21, body mass index (BMI; kg/m2) 30.0 ± 3.8 (18 overweight and 11 obese subjects), A1C 36.2 ± 4.3 mmol/mol Hb (5.5 ± 0.7%), prediabetes (Y/N) 9/20, hypertension (Y/N) 11/18, and estimated glomerular filtration rate (eGFR, ml/min) 97 ± 17. In highly overweight subjects (BMI >27.5 kg/m2, n = 20), the Glo1 inducer formulation increased activity of Glo1 in peripheral blood mononuclear cells (+27%, P<0.05). It decreased FPG (- 5%, P<0.01) and gAUC (- 6%, P<0.03). The Glo1 inducer increased 2-h OGIS index (+42 mlmin-1m-2, P<0.02). It also produced a minor decrease in body weight and improvement in eGFR. There was no effect of the placebo control. The Glo1 inducer was highly tolerated and there were no adverse effects.

Conclusion: Improvement of insulin resistance and glycaemic control by the Glo1 inducer matches or exceeds that of current alternative treatments

for overweight and obese subject groups. There are also suggestions that it may be beneficial in evaluation for diabetic nephropathy. The Glo1 inducer could be a suitable treatment for improved metabolic health in overweight and obese populations.

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Disclosure: **P.J. Thornalley:** Grants; Yes.

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Discovering carbohydrate metabolism alterations in normoglycaemic obese patients (the DICAMANO study)

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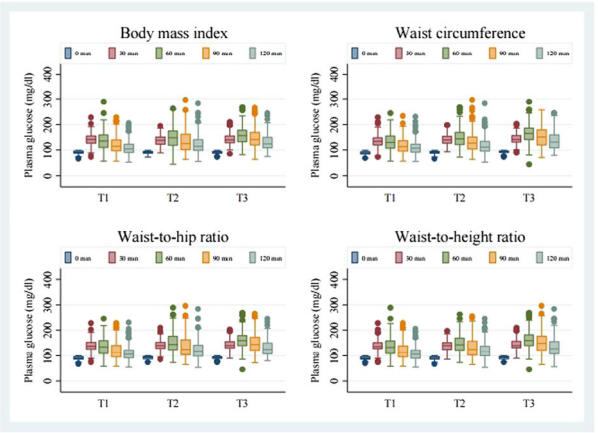
Background and aims: There is a clear relationship between obesity and carbohydrate metabolism alterations (CMA), such as impaired glucose tolerance (IGT) and type 2 diabetes (T2D). Oral glucose tolerance test (OGTT) is used for its diagnosis yet is not usually performed in case of normal fasting glucose (NFG) values. Moreover, body mass index (BMI) underestimates the diagnosis of obesity, and body fat distribution can be crucial to the development of CMA. Hence, we aimed to examine the association between several anthropometric surrogates of body and visceral fat and CMA among patients with NFG. This could help us to identify those patients for CMA, so performing an OGTT could be worthy and cost-effective even though NFG values.
Materials and methods: Cross-sectional study of 522 nondiabetic patients >18-years with NFG (≤100 mg/dl) who underwent a 75g OGTT and anthropometric study [BMI, waist circumference (WC), neck circumference (NC), waist-to-hip ratio (WHR), waist-to height ratio (WHtR)] between 2000-2014. Regarding anthropometric measurements (AM) patients were classified into three tertiles (T1-T3). Clinical and cardiometabolic characteristics were also studied.
Results: 66.9%women; 30,54±14.7 years; BMI 34.5±7.6kg/m2; WC 108.5±17.5cm; NC 39.2±43cm; WHR 0.94±0.1; WHtR 0.65±0.1). Presence of cardiovascular risk factors such as hypertension, obstructive sleep apnoea and dyslipidaemia was higher in T3vsT1 ($p<0,05$). Global prevalence of CMA was 24.3% (21.6% IGT;2.7% T2D). However, it increased in those patients in T3, being more evident in WHR (T1 14.5%; T3 39.5%), specifically in men (T1 18,6%; T3 50%). A statistically significant association was observed between BMI, WC, WHR, WHtR and CMA, after multivariable adjustment. Being in T3 of WHR, multiplied by more than 3 (OR=3 (1.7-5.2) $p<0.001$) the risk of CMA compared with T1, being this risk even greater in men (OR=3.4 (1.3-9.0) $p<0.014$). Plasma glucose behavior during OGTT differed among AM tertiles also. Peak plasma glucose was earlier in T1 (30-min) than in T3 (60-min), probably reflecting an early β-cell dysfunction.
Conclusion: NFG does not rule out the existence of CMA as we found a high prevalence of this disorder in this population especially in T3 of AM. WHR may be helpful in diagnosing CMA in patients with NFG, in particular in male subjects. Moreover, it could be a useful tool in order to select those patients in which an OGTT should be performed.

Figure 1. Variation of plasma glucose during a 2-hour 75-g OGTT stratified by tertiles of anthropometric measurements.



Disclosure: **B. Pérez-Pevida:** None.

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A metabolite ratio associates with measures of insulin secretion and altered risk of type 2 diabetes; a DIRECT study

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Background and aims: Metabolomics might be useful for further elucidating the biology and early stratification of those at increased risk of type 2 diabetes. Ratios between two metabolites may specifically reveal perturbations in relevant pathways. OGTT derived measures have been used in metabolomics studies but do not allow a further detailed analysis of insulin secretion in response to various insulin secretagogues. In this study, we have used the hyperglycaemic glucose clamp with glucose, GLP-1 and arginine stimulation to study the association between metabolite levels, their ratios and insulin response. In addition, we tested whether identified metabolites and ratios are also associated with prevalent and incident type 2 diabetes.
Materials and methods: For the hyperglycaemic clamp study 130 healthy Dutch volunteers were investigated. Association with OGTT derived measures (n=438), prevalent (n=4911) and incident (n=3958) type 2 diabetes was tested in four independent Dutch and German cohorts (LLS, POGO, KORA and EPIC-Potsdam). Targeted metabolomics was measured using the Biocrates technology. Data were analysed using regression analysis with adjustment for potential risk factors and confounders and corrected for multiple testing.
Results: We found three single metabolites and eighteen metabolite ratios that were associated with insulin secretion (all $p<5.8*10^{-5}$). Especially the ratio between valine and phosphatidylcholine acyl-alkyl C32.2 (PC.ae.C322) was significantly and reproducibly associated with glucose

stimulated insulin secretion as measured by hyperglycaemic clamp ($p=2.0*10^{-7}$) or OGTT ($p_{meta}=7.8*10^{-6}$). Furthermore, this ratio was associated with both increased prevalent ($p_{meta}=1.0*10^{-27}$) and incident diabetes ($p_{meta}=1.0*10^{-14}$). Notably, the association was stronger than the individual metabolites (both $P\leq1.8*10^{-8}$). Adding the metabolite ratio to a model including all established risk factors (incl. glucose) improved the area under the receiver operating characteristic curve from 0.75 to 0.77 ($p<0.05$) and 0.83 to 0.84 in KORA and EPIC-Potsdam, respectively.
Conclusion: We show that the ratio between valine and PC.ae.C322 is significantly associated with insulin secretion, prevalent and incident type 2 diabetes. This suggests that it could be useful as an early biomarker for beta cell dysfunction and stratification of those at increased risk for type 2 diabetes.
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Disclosure: **L.M. 't Hart:** Grants; The work is supported by the Innovative Medicines Initiative Joint Undertaking (115317, DIRECT).

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Treatment patterns and glycaemic control of type 2 diabetes affected by body mass index
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Background and aims: Obesity is a major risk factor for the development of Type 2 Diabetes (T2D). The aim of this study was to examine treatment patterns and glycemic control in the context of Body Mass Index (BMI) in a T2D population.
Materials and methods: Using the GE Centricity electronic medical records (EMR) database, a cross-sectional analysis was conducted in adults (age ≥20 years) with documented BMI measurements between 10/1/2013 - 9/30/2014. Index BMI was defined as the average of all BMI results during the study period. T2D was identified by ICD-9 codes. Individuals with T1D ICD-9 codes were excluded. Patients were grouped according to BMI (kg/m^2) <30, 30 to <35, 35 to <40, and ≥40. T2D related treatments were classified by group: OADs, GLP-1 receptor agonists (RA), and Insulins. Glycemic control was assessed as each patient's latest available A1c measurement during the study. 66% of patients in the cohort had A1c data available. A1c ≥8% is considered a lower limit of poor glycemic control. Thus, the distribution of patients with A1c ≥8% was examined as a function of treatment and BMI to ascertain if a relation between glycemic control, BMI and treatment modality could be observed.
Results: 626,386 patients were included in this analysis. Mean age was 63.8, with 51.3% female, and 78.5% white. Overall BMI distributions were: 234,236 subjects (37.4%) with BMI <30, 177,476 (28.3%) with BMI 30 to <35, 113,223 (18.1%) with BMI 35 to <40 and 101,451 (16.2%) with BMI ≥40. Patients in these 4 BMI categories prescribed with OADs-only constituted 55.2%, 53.8%, 50.5%, and 47.2%, and those prescribed insulins, with or without OADs, constituted 24.4%, 26.5%, 29.4%, and 32.1% of each BMI group, respectively. Prescriptions with a GLP-1RA were almost 3 times more prevalent in the BMI ≥40 group compared with the BMI <30 group (4.4% vs. 1.5%). The percentage of patients with A1c ≥8% was 21.0%, 25.0%, 28.0%, and 30.2% in the respective BMI groups, while among patients prescribed insulins, with or without OADs, the percentage with A1c ≥8% was 47.3%, 49.8%, 51.6%, and 51.4%, respectively.
Conclusion: Increased BMI was observed to occur in a higher percentage of patients being prescribed insulin or GLP-1RAs, and with A1c ≥8%.
Disclosure: **W. Weng:** Employment/Consultancy; Employee of Novo Nordisk Inc.

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Metabolic outcomes after an 8-weeks low-calorie-diet in overweight, pre-diabetic individuals: the role of gender in the PREVIEW study
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Background and aims: The PREVIEW intervention study is to date the largest, multinational study aiming to prevent T2D among pre-diabetic individuals with a combination of diet, physical activity and behavior modification. Initially, all participants followed a low-calorie diet (LCD). The aim was to compare the effect of 8 weeks' LCD on weight loss (WL) and metabolic outcomes between pre-diabetic men and women.
Materials and methods: The participants received LCD [810 kcal daily] for 8 weeks. Data from participants who achieved 8% WL were included in the analysis. Two-sided t-tests were used throughout to test for differences between men and women.
Results: Of 2,326 individuals eligible for the LCD period, 1,842 (79%) participants (1,225 women and 617 men) completed the WL phase successfully. At baseline, mean (±SD) age was 51.6±11.6 years, BMI 35.3 ±6.5 kg/m2, systolic blood pressure (SBP) 129.1±15.9 mmHg, diastolic blood pressure (DBP) 78.1±11.1 mmHg, HbA1c 36.7±4.0 mmol/mol, and fasting serum insulin (FSI) 13.4±7.8 mU/L. Average WL was 10.6 ±4.0 kg, with men losing 12.7±4.2 kg and women 9.6±3.4 kg (difference between gender, P<0.001). Men lost 11.7±3.5% of initial body weight while women lost 10.2±3.1% (P<0.001). SBP dropped by 8.6±0.5 mmHg in men and by 6.0± 0.4 mmHg in women (P<0.0001). DBP dropped by 5.2±0.3 mmHg in men and by 2.3±0.2 mmHg in women (P<0.0001). HbA1c decreased by 2.2±0.09 mmol/mol in men, and by 1.8±0.06 mmol/mol in women (P<0.001). FSI decreased by 5.8±7.4 mU/L in men and by 3.8±5.4 mU/L in women (P<0.001).
Conclusion: An 8 weeks' LCD intervention resulted in a marked decrease in body weight, blood pressure, HbA1c and FSI among pre-diabetic subjects. Significantly larger decreases were seen in men versus women.
Clinical Trial Registration Number: NCT01777893
Supported by: EU FP7,GA 312057; HNMRC AUS; NZ HRC (14/191), UoA FRDF; CWP donated the LCD
Disclosure: **P. Christensen:** None.

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Determinants of lifestyle behaviour change to prevent type 2 diabetes in high-risk subjects
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Background and aims: Although effective lifestyle interventions to prevent type 2 diabetes mellitus (T2DM) are available, little is known on which behaviors and determinants mediate intervention effectiveness. SLIMMER is one of the first effective diabetes prevention interventions

in a Dutch 'real-life' setting. The present study aims to provide insight into the effective intervention pathways of SLIMMER, using formal mediation analyses.
Materials and methods: SLIMMER was a randomized, controlled intervention study, consisting of a combined lifestyle intervention in participants with increased risk of T2DM. In total 239 participants were included in the analyses over 18 months. Firstly, we investigated whether significant changes in fasting insulin (pmol/L) and body weight (kg), were mediated by changes in dietary and PA behaviour, in a multiple mediator model. Secondly, we investigated whether significant changes in dietary and PA behaviour were mediated by changes in behavioural determinants and the participant's psychological profile, in multiple single mediator models. The mediation analyses used linear regression models, where significance of indirect effects was calculated with bootstrapping. The effect measure was the percentage of the effect mediated .
Results: The association between the intervention and decreased fasting insulin was mediated for 40% by change in dietary and PA behaviour, where dietary behaviour was an independent mediator of the association (34%). The association between the intervention and decreased body weight was mediated for 20% by change in dietary and PA behaviour, where PA behaviour was an independent mediator (17%). The intervention significantly changed intake of fruit, fat from bread spread, and fibre from bread. Change in fruit intake was mediated by change in action control (combination of consciousness, self-control and effort), motivation, self-efficacy, intention and skills. Change in fat intake was mediated by change in action control and the psychological profile. No mediators could be identified for change in fibre intake. The change in PA behaviour was mediated by change in action control, motivation and the psychological profile.
Conclusion: The effect of the SLIMMER intervention on fasting insulin and body weight was mediated by changes in dietary and PA behaviour. Change in action control was an important mediator in the change of dietary and PA behaviour. These findings provide valuable insight for development and implementation of future interventions.
Disclosure: **N.R. den Braver:** None.

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Understanding how access to fast food outlets is associated with obesity. The SPOTLIGHT project

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Background and aims: Dietary habits are key behavioural determinants of successful self-management and prevention of obesity and type 2 diabetes. Dietary behaviours are likely to be influenced by more upstream determinants, such as the social or physical environment individuals live in. Understanding how the food environment is associated with adult weight status is thus important for the prevention of obesity and type 2 diabetes. We linked objectively measured access to fast food outlets to overweight and obesity and explored whether perceived presence of fast food outlets and fast food consumption could explain such an association.
Materials and methods: Cross-sectional population-based sample of 5,205 adults participating in the SPOTLIGHT survey, conducted in 60 neighbourhoods in urban regions of five different countries across Europe. Participants reported on socio-demographics, fast food consumption, weight and height and neighbourhood perceptions. A virtual neighbourhood audit was conducted to geolocalize and categorize fast food outlets. Direct associations and mediating effects of access to fast food outlets, perceived presence and use of fast food outlets, fast food consumption and obesity were explored using multilevel logistic and multinomial regression analyses, adjusted for individual socio-demographics variables, density of other food outlets and residential self-selection.
Results: After full covariate adjustment, access to fast food outlets was not associated with fast food consumption (OR=1.31, 95%I=0.82; 2.11), or obesity (OR=0.81, 95%CI=0.51; 1.26). Access to fast food outlets was associated with perceived presence and use of fast food outlets (OR=4.04, 95%CI=1.94; 8.42), and perceived presence and use of fast food outlets was associated with consuming fast food at least once a week (OR=1.61, 95%CI=1.11; 2.35).
Conclusion: In areas where fast food outlets are ubiquitous and thus highly accessible, individual perceptions of availability of fast food outlets in the residential neighbourhood may be more important than objective access to such outlets. These result emphasize the complexity of individual and environmental influences on lifestyle behaviours and weight status. Perceptions of the food environment should be included in approaches to prevent obesity and type 2 diabetes.
Supported by: Seventh Framework Programme (CORDIS FP7) of the European Commission
Disclosure: **J.D. Mackenbach:** Grants; This work was supported by the Seventh Framework Programme (CORDIS FP7) of the European Commission, HEALTH (FP7-HEALTH-2011-two-stage) [278186].

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Dietary pattern analysis of maternal pregnancy diet reveals combinations of nutrients associated with islet autoimmunity in the offspring

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Background and aims: Studies investigating maternal diet during pregnancy and risk of islet autoimmunity (IA) in offspring have been inconsistent, perhaps due in part to synergistic or antagonistic interactions between nutrients. Dietary pattern (DP) analysis may identify novel combinations of nutrients and foods in the maternal diet that affect the offspring's risk of IA.
Materials and methods: From 1993-2004, The Diabetes Autoimmunity Study in the Young (DAISY) enrolled newborns at increased risk for type 1 diabetes (T1D) and prospectively followed them for IA (defined as persistent presence of autoantibodies to insulin, GAD65, IA-2, or ZnT8). Of 640 DAISY children whose mothers completed a food frequency questionnaire (FFQ) shortly after delivery regarding intake during the third trimester of pregnancy, 66 children developed IA by January 2016. Nutrient intake from food only and frequencies of food group consumption were obtained from the FFQ. We used reduced rank regression (RRR) to identify DPs explaining the maximum variation in a set of response variables hypothesized to be on the pathway between food intake and T1D. The RRR model included 39 food groups as predictor variables and the 4 nutrient response variables selected based on previously reported associations with IA: vitamin D, omega-3 fatty acids, cow's milk protein, and sugars. Nutrients were log-transformed and energy adjusted using the residual method. Cox proportional hazards analysis was used to test DP scores and individual nutrients on IA risk.
Results: Four empirically derived DPs explained 63.7% of the variation in the response nutrients. DP 1 was characterized by food intake high in low-fat milk products and low in deep-fried potatoes and meats; DP 2 was characterized by intake high in low-fat milk products, fish, and creamy dressings, and low in sugar-sweetened beverages; DP 3 was characterized by intakes high in sugar-sweetened beverages, creamy dressings and margarine, and low in meat; whereas DP 4 was characterized by a diet high in high-fat milk products, and low in legumes, fish, and cereal intake. DPs 1 and 4 were significantly associated with increased risk of IA, adjusting for offspring's high-risk HLA genotype, ethnicity, first-degree relative with T1D, maternal age, and maternal education, while DPs 2 and 3 were not (Table 1). When tested separately in survival analyses, neither vitamin D (HR: 0.97, CI: 0.75-1.26), omega-3 fatty acids (HR: 1.04, CI: 0.82-1.31),

cow's milk protein (HR: 1.30, CI: 0.88-1.90), nor sugars (HR: 1.07, CI: 0.85-1.36) intakes were associated with IA.
Conclusion: While three late-pregnancy DPs contain high intake of cow's milk protein, only two are associated with IA risk. Combinations of other nutrient intakes distinguish which DPs are associated with IA. The examination of complex dietary exposure via DP analysis may offer new insights into the mechanism by which diet can influence disease risk.

Table 1: Summary of dietary pattern relationships

Dietary Pattern (DP)	Association of Dietary Pattern Score with IA[a] HR (95% CI)	Nutrient Weight[b] in the Dietary Pattern Score: Vitamin D	Omega-3 Fatty Acids	Cow's Milk Protein	Sugars
DP 1	1.33 (1.04-1.70)	0.52	-0.32	0.51	0.61
DP 2	1.12 (0.86-1.46)	0.48	0.68	0.40	-0.39
DP 3	0.98 (0.75-1.29)	-0.21	0.66	-0.20	0.69
DP 4	1.46 (1.08-1.98)	-0.68	0.03	0.73	-0.02

[a]*Adjusted for offspring's HLA, ethnicity, first degree relative with T1D, maternal education, and maternal age*
[b]*Weights are generated from the RRR procedure, and can be thought of as correlations between nutrients and each dietary pattern*

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Disclosure: **R.K. Johnson:** None.

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Fatty acid consumption and incident type 2 diabetes: evidence from the E3N cohort study

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Background and aims: Fatty acids are vital sources of energy and important components of our diets, yet evidence on their association with the risk of type 2 diabetes is lacking and controversial. Therefore, the objective of this study was to evaluate the association between dietary estimates of fatty acid consumption and type 2 diabetes risk over 14 years of follow-up in the French prospective E3N cohort study.
Materials and methods: 71 334 women who were non-diabetic at baseline were followed from 1993 to 2011. Diabetes was identified using questionnaires and drug reimbursement claims, and incident cases were subsequently validated. Fatty acid consumption in 1993 was estimated from a validated dietary questionnaire. Cox regression was used to estimate hazard ratios (HR) and 95% confidence intervals (CI) of diabetes risk.
Results: A positive association was observed between high omega-3 polyunsaturated fatty acid consumption and the risk of type 2 diabetes; this persisted after adjustment for confounders, including other fatty acid groups and body mass index (BMI) (HR=1.26 [1.13-1.41]). Upon stratification by overweight (BMI≥25kg/m2)/non-overweight, the total polyunsaturated fatty acid consumption also demonstrated a positive association with diabetes, but only in non-overweight women (HR=1.22 [1.05-1.42]), and omega-3 consumption was associated with an increased risk of diabetes in both BMI strata (HR=1.19 [1.01-1.40], BMI <25kg/m2; HR=1.38 [1.20-1.59], BMI ≥ 25kg/m2). Closer examination of the omega-3 polyunsaturated fatty acid group found docosapentaenoic acid (DPA) and high α-linoleic acid (ALA) consumption (in overweight women only), associated to an increased risk of diabetes (HR=1.41 [1.23-1.63], HR=1.17 [1.01-1.36], respectively). Within the omega-6 fatty acid group, only arachidonic acid (AA) was associated with diabetes (HR=1.49 [1.33-1.66]). The associations of docosapentaenoic acid and arachidonic acid persisted even after sensibility analyses were carried out, with the adjustment on their principal source in this cohort, the consumption of meat.
Conclusion: The effects of polyunsaturated fatty acids are heterogeneous within the fatty acid group. The consumption of docosapentaenoic acid and arachidonic acid may contribute to the development of type 2 diabetes.

Cox proportional hazards ratios (95% CI) of the risk of incident type 2 diabetes by fatty acid consumption tertile groups (g/day) in the E3N cohort (N=71 334)

Model	Tertile group of fatty acid consumption (g/day)	SFA	TFA	MUFA	n-3 PUFA	ALA (n-3)	DPA (n-3)	n-6 PUFA	AA (n-6)
Model 1	T1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
	T2	1.11 [0.99-1.25]	**0.88 [0.78-0.98]**	1.10 [0.99-1.22]	**1.18 [1.06-1.31]**	1.04 [0.94-1.16]	**1.36 [1.20-1.53]**	1.07 [0.96-1.18]	**1.29 [1.15-1.44]**
	T3	1.13 [0.97-1.31]	0.93 [0.81-1.07]	**1.23 [1.10-1.38]**	**1.47 [1.32-1.64]**	1.11 [0.99-1.25]	**1.96 [1.71-2.25]**	**1.15 [1.04-1.27]**	**2.04 [1.83-2.28]**
	P for trend	0.14	0.25	**<0.001**	**<0.0001**	0.06	**<0.0001**	**<0.01**	**<0.0001**
Model 1 + BMI	T1	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
	T2	1.06 [0.95-1.19]	0.90 [0.80-1.00]	1.01 [0.91-1.12]	1.10 [0.99-1.22]	1.00 [0.90-1.12]	**1.15 [1.02-1.30]**	1.01 [0.91-1.12]	1.11 [0.99-1.24]
	T3	1.07 [0.92-1.25]	1.02 [0.89-1.17]	1.06 [0.95-1.18]	**1.26 [1.13-1.41]**	1.03 [0.92-1.15]	**1.41 [1.23-1.63]**	1.00 [0.90-1.10]	**1.49 [1.33-1.66]**
	P for trend	0.40	0.85	0.25	**<0.0001**	0.62	**<0.0001**	0.94	**<0.0001**

Model 1: adjusted for consumption of other fatty acids, daily energy intake, alcohol consumption, and non-dietary factors: level of education, family history of diabetes, physical activity, hypertension, hypercholesterolaemia, and smoking status
SFA (saturated fatty acids), TFA (trans fatty acids) MUFA (monounsaturated fatty acids), n-3 PUFA (omega-3 polyunsaturated fatty acids), n-6 PUFA (omega-6 polyunsaturated fatty acids), ALA (alpha-linolenic acid), DPA (docosapentaenoic acid), AA (arachidonic acid)

Disclosure: **C. Dow:** None.

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Effect of a lifestyle intervention program in women at risk for type 2 diabetes with a history of GDM

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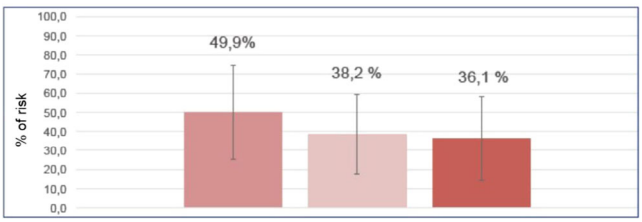
Background and aims: Healthy lifestyle habits, such as good diet and physical activity, are modifiable factors to prevent or delay the development of type 2 diabetes (T2DM) and other obesity-related morbidities. The FINDRISC is a simple, fast, inexpensive tool to identify individuals at high risk for T2DM. Reduce the risk of the development of diabetes with a 6-month lifestyle intervention program in women with a history of GDM. The risk of developing T2DM was evaluate using the validated FINDRISC questionnaire.
Materials and methods: We recalled 88 non-pregnant Caucasian women, aged 39.3 ± 5 years (mean±SD), with a history of GDM. At baseline, their current glycaemic status was revisited by a 75 gr OGTT. Every three months, they received an individual counselling session about healthy diet, physical activity, diabetes and risk factors, maintenance of a healthy lifestyle. Every visit, height, weight, waist circumference were assessed and questions of the FINDRISC questionnaire (use of blood pressure medications, family history of T2DM, physical activity <4 h/week, daily consumption of vegetables, fruits or berries) were addressed.
Results: 71,5% of women had family history of DMT2 in first- or second-degree relative, 5,7% used blood pressure medications. All the modifiable factors improved after educational intervention. BMI (baseline vs 3-months vs 6-months: 26,7±5 vs 26±5 vs 25,8±6 Kg/m^2) and waist circumference (87±12 vs 85±12 vs 84,5 ±12 cm) decreased. Whereas, physical activity (16% vs 36,2% vs 49%) and daily consumption of vegetables, fruits or berries (16% vs 36,2% vs 49%) improved. During the study, the risk of the development of diabetes, by FINDRISC, was significantly reduced (Fig. 1). The mean change of FINDRISC was a reduction of - 2,18 points from baseline.
Conclusion: Lifestyle intervention programs may have a significant effect in people with high risk to develop diabetes. Action should be taken to encourage women with GDM in order to allow the prevention and/or early detection of T2DM in Italy.

Fig.1 Score and percentage of risk obtained through the FINDRISC algorithm



Risk	Baseline	3-months	6-months
Score	15,40 (±3,43)	13,67 (±3,69)*	13,22 (±3,65)**

*p<0,05 vs Baseline; **p<0,01 vs Baseline (χ^2)

Disclosure: **G. Meregalli:** None.

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Coffee but not green tea consumption is inversely associated with metabolic syndrome: an epidemiological study of Korean adults

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Background and aims: Cumulative evidnece suggests that intake of caffeinated beverages, such as coffee and green tea, may have beneficial effects on metabolic syndrome (Mets). The aim of this study was to evaluate whether or not comsumption of coffee or green tea was associated with the prevalance of Mets in a Korean population.
Materials and methods: We analyzed 15,568 Korean adults, aged 19-65 years, using cross-sectional data from the The Sixth Korea National Health and Nutrition Examination Survey (KNHANES VI-2, 2013-2015). Coffee consumption level was assessed based on food frequency questionnaire and 24-h recall. Demographic and lifestyle factors were assessed using self-administered questionnaires. Data on metabolic biomarkers were obtained from a health examination. Multivariate analyses were performed to clarify the association between coffee or green tea consumption and the components of metabolic syndrome.
Results: The smaller was the number of components of metabolic syndrome, the higher was the level of coffee consumption, especially those who consumed coffee >3 times/day ($p=0.005$) and the larger dose of coffee per cup ($p<0.001$). Among all components of metabolic syndrome, high blood pressure were inversely associated with the dose of coffee per cup (SBP; $p=0.003$, DBP; $p=0.045$), after adjusting for sex, age, body mass index, smoking status, drinking status. However, the consumption of green tea was not significantly associated with the prevalence of metabolic syndrome or its components.
Conclusion: Coffee but not green tea consumption was inversely associated with metabolic syndrome.
Disclosure: **E. Kim:** None.

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Adherence to a mediterranean diet and autofluorescence of skin advanced glycation end products in older persons: the Three-City study

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Background and aims: AGEs toxic products resulting from the glycation of proteins, accumulate slowly with aging, diabetes and chronic kidney disease. One of the most important external source of AGEs is food particularly with a high content in fat and protein or when cooked at high temperature. Futhermore, AGEs have been considered as a marker of metabolic memory, and may reflect long-term, low-grade hyperglycemia before established diabetes. Higher adherence to Mediterranean diet (MeDi) has been related to a lower risk of diabetes, and may thus be associated with a lower accumulation of AGEs reflected by skin autofluorescence (sAF) in elderly people. We aimed to analyze the relationship between adherence to the MeDi and sAF 8 years later in French elderly community dwellers.
Materials and methods: All elderly community-dwellers, participants of the Three-City-Bordeaux cohort (France), free of diabetes at the time of MeDi assessment and among whom sAF has been assessed were included. Adherence to the MeDi was evaluated at baseline by a MeDi score, on a 10-point scale based on a food frequency questionnaire and a 24h recall. sAF of AGE (mean of 3 different measures) was measured 8 years later with the AGE READER. Association between adherence to the MeDi and sAF 8 years later was analyzed by multivariate linear regression.
Results: The study sample consisted of 305 participants, aged 74.4 years on average and with a moderate MeDi adherence, the baseline median MeDi score was 5 (from 1 to 8). Eight years later, the mean sAF of those with a Medi score was 2.8 (SD = 0.7) Arbitrary Units (AU). At the end of the follow-up, the duration of chronic kidney disease was recent for 95 (31.2%) participants while 35 (11.5%) participants had a longstanding chronic kidney disease. After adjusting for age, sex, chronic kidney disease duration, smoking, calories intake, body mass index, hypercholesterolemia, hypertension, renin-angiotensin system medication, physical activity and education, the baseline MeDi score was inversely associated with sAF 8 years later (+1 point in MeDi was associated with -0.06 AU , 95% CI : -0.11 ; -0.01 , $p = 0.02$).
Conclusion: This study suggests that a higher adherence to the MeDi contribute to prevent the accumulation of AGEs with aging.
Disclosure: **K.A. Rajaobelina:** None.

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Exposure to endocrine disruptors in the general Dutch population: a pilot study in the LifeLines cohort

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Background and aims: Exposure to Endocrine Disrupting Chemicals (EDC) may play an important role in the current epidemic of obesity and diabetes. While the majority of epidemiological studies focused on more persistent EDC compounds, the effects of common EDC to which we are widely exposed in our daily life are largely unknown. Here we present the results of a pilot study aiming to simultaneously assess the exposure to multiple non-persistent EDC including bisphenol A (BPA) and other environmental phenols, parabens and phthalates and to set-up an analytical pipeline for large-scale biomonitoring.
Materials and methods: Using a validated online TurboFlow-liquid chromatography-tandem mass spectrometry methodology (LC-MS/MS) and developed customized panel of analytes, we determined the excretion of seven parabens, nine phenols and the metabolites of 14 different phthalates in 24hr urine samples obtained from forty participants of the LifeLines cohort study (non-diabetic 18 men and 22 women, age 27-73yrs). The correlation between EDC levels and different clinical and biochemical phenotypes was examined using Spearman's rho test. The Mann-Whitney U-test was used to compare EDC concentrations between genders.
Results: In all samples, BPA, two parabens (MeP, EtP), and the metabolites of six common phthalates (BBzP, DEP, DEHP, DiBP, DiNP, DnBP) were detected. The levels of the detected compounds were in the ranges similar to the levels reported in other European studies. We found significant correlations between some EDC levels, and age, body composition, and metabolic traits such as blood glucose, lipids, and HbA1c (Table 1). The concentration of the phthalate metabolite MCPP was significantly higher in men than in women (mean±SD: 21.8±13.2 in men; 13.0±8.3 in women, p-value=0.02).
Conclusion: For the first time, we successfully measured the exposure to multiple common EDC in participants from the Dutch general population. Our data are comparable to other European EDC exposures and indicate the associations between concentrations of some EDC in urine and metabolic profile, including glucose and lipid levels, as well as suggest gender-specific differences in exposure to some phthalates. Currently, the follow-up study in a larger sample (n>600) from the LifeLine cohort is ongoing.

Table 1. Correlations between urinary excretion of EDC in 24hr urine samples (n=40) and traits#

	Analyte	n > LOD	Age	WHR	Glucose	HbA1c	Triglyceride	LDL-Chol	Cholesterol
Parabens	EtP	40	0.12	0.03	0.06	-0.07	-0.13	-0,33*	-0.20
	iBuP	20	-0.36	-0.27	0.37	0,50*	-0.34	-0,54*	-0.40
	BzP	15	0,52*	0.09	0.33	0,67**	-0.14	-0.09	-0.14
Phenols	BPA	40	0,39*	0.15	-0.09	-0.14	-0.11	0.18	0.25
	TCS	9	0.05	0.18	-0.20	0.11	0,77*	0.55	0,78*
Phthalates (Urine metabolite)	DnBP (MnBP)	40	0.13	0.16	-0,33*	0.04	-0.05	-0.10	0.05
	DEHP (MCMHP)	40	0.25	0,31*	-0.05	0.02	-0.01	0.08	0.10
	DOP (MCPP)	37	0,36*	0,39*	0.03	0.18	0.04	0.16	0.18
	DHpP (MCHxP)	15	0,61*	-0.03	-0.19	0.48	-0.19	0.50	0,56*
	DiNP (MCiOP)	40	0.13	0,32*	0.10	-0.02	0.05	0.14	0.07

Results are shown for EDC compound/traits if at least one EDC compound—trait correlation reached level of significance $p<0.05$. # Spearman rank correlation coefficient: * p-value < 0.05; ** p-value <0.01
Abbreviations: EDC: Endocrine Disrupting Chemicals; LOD: level of detection; WHR: waist-hip-ratio. EDC analytes: Parabens: ethyl paraben *(EtP)*; isobutyl paraben *(iBuP)*; benzyl paraben *(BzP)*. Phenols: bisphenol A (BPA); 2,5-DCP 2,5-dichlorophenol (2,5-DCP); triclosan (TCS). Phthalates and metabolites: Di-n-butyl phthalate (DnBP)/Mono-n-butyl phthalate (MnBP); Di-(2-ethyl-hexyl) phthalate (DEHP)/mono(2-carboxymethylhexyl) phthalate (MCMHP); Di-octyl phthalate (DOP)/Mono-3-carboxypropyl phthalate (MCPP); Di-n-heptyl phthalate (DHpP)/(MCHxP); Di-iso-nonyl phthalate (DiNP)/Mono-iso-nonyl phthalate (MiNP) and mono(carboxyisooctyl) phthalate (MCiOP)

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Disclosure: **J.V. van Vliet-Ostaptchouk:** None.

PS 004 Focusing on the liver

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Nonalcoholic fatty liver disease is associated with incident type 2 diabetes in combined hyperlipidaemic pedigrees: a 10-years follow-up study

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Background and aims: Familial combined hyperlipidaemia (FCHL) is the most prevalent genetic dyslipidaemia in Western society. We have previously demonstrated that nonalcoholic fatty liver disease (NAFLD) plays a central role in the development of dyslipidaemia, independent of the degree of (abdominal) obesity. Given the association of NAFLD with insulin resistance it is anticipated that FCHL patients are also prone to develop type 2 diabetes mellitus (T2DM), independent of obesity.
Materials and methods: This study is part of a larger longitudinal study in which our original FCHL cohort - consisting of FCHL patients, their normolipidaemic (NL) relatives and spouses (n=600) - are re-invited to address the incidence of cardiometabolic disease. For this study, all individuals who underwent ultrasound of the liver at baseline (to determine the presence/absence of NAFLD) were included (n=275). The clinical diagnosis of T2DM was established by questionnaires and verified by contacting the individual's clinician. Cox proportional hazards models were constructed to adjust for potential confounders (age and sex) and to assess baseline determinants of new-onset T2DM.
Results: Of the 275 individuals who underwent ultrasound of the liver at baseline, 212 (77%) agreed to participate in this follow-up study (average follow-up duration: 10.5 ± 0.4 years). The incidence of T2DM was 2%, 6% and 17% in spouses, NL relatives and FCHL patients, respectively (HR: 9.2 [95% CI 1.1-76.1], FCHL vs spouses; age- and sex-adjusted). Baseline BMI was significantly associated with incident T2DM and partly accounted for the greater incidence of T2DM in FCHL patients (HR: 6.3 [0.8-52.5], FCHL vs spouses; age-, sex- and BMI-adjusted). Of interest, when baseline NAFLD was added to the model, BMI was no longer significantly associated (p=0.32) and the hazard ratio for T2DM decreased further (HR: 4.1 [0.5-35.1], FCHL vs spouses; age-, sex-, BMI- and NAFLD-adjusted). In fact, in this model NAFLD was the only statistically significant baseline determinant of incident T2DM (HR: 7.0 [1.3-38.1).
Conclusion: During a 10-years follow-up period, FCHL patients had a substantially increased susceptibility to develop T2DM. This greater susceptibility may be related to the presence of NAFLD, rather than the degree of obesity at baseline. This study positions the liver at the heart of the metabolic abnormalities that are commonly observed in FCHL. It encourages the pursue of the chromosomal loci (1q42.3 , 17p12-21 and 22p13-q11) that we have previously linked to NAFLD in FCHL. Moreover, it favours the development of novel compounds that specifically target the liver to reduce cardiometabolic risk.
Supported by: Biomolecular Resources Research Infrastructure (BBMRI-NL CP2013-80)
Disclosure: **M.C.G. Brouwers:** None.

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NAFLD deteriorated fasting insulin, urinary albumin excretion, cystatin C and high-sensitivity C-reactive protein independent of glycaemic markers

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Background and aims: We examined whether non-alcoholic fatty liver disease (NAFLD) deteriorated fasting insulin (FIRI), urinary albumin

excretion (UAE), cystatin C (cysC) and high-sensitivity C-reactive protein (hs-CRP) independent of glycemic markers.

Materials and methods: This study comprised periods I and II from January 2007 to May 2009 and from June 2009 to December 2011, respectively. After excluding people with diabetes in the Period I and those with ethanol intake >140g/week, a total of 962 people (591 men, 371 women; mean age: 43 years) who underwent an annual medical check-up through the health care system in the Periods I and II were included. The analysis of people without diabetes (n=942) during the observation period was separate from the analysis of incident diabetes in the Period II (n=20). We used only 2 independent variables in regression analysis at the same time for the 20 cases. We used Japanese Diabetes Risk Score (JPDRISC) and fatty liver using ultrasonography score (FLUS).

Results: The overall mean observation period was 26 months. In non-diabetic people, regression analysis with FIRI in the Period II as the dependent variable showed that ALT (adjusted β [Aβ]=0.214, p<0.001) and FLUS (Aβ=0.172, p<0.001) in the Period I were independent determinants of FIRI, as were BMI (Aβ=0.310, p<0.001), age (Aβ=−0.114, p=0.003) and JPDRISC (Aβ=0.088, p=0.032). Interestingly, fasting plasma glucose (FPG), diastolic BP, cholinesterase, gamma-GTP and triglyceride were not significant determinants. UAE in the Period II was independently determined by ALT (Aβ=0.102, p=0.038) in the Period I as well as by BMI (Aβ=0.404, p<0.001), age (Aβ=0.098, p=0.034) and JPDRISC (Aβ=0.118, p=0.017). FPG, FLUS, cholinesterase, gamma-GTP, triglyceride and diastolic BP were not significant determinants. The above results held when we used HbA1c as an independent variable instead of FPG. CysC in the Period II was independently determined by ALT (Aβ=0.220, p=0.002) in the Period I as well as by age (Aβ=0.418, p <0.001) and BMI (Aβ=0.203, p =0.027). As to hs-CRP in the Period II, FLUS in the Period I was the only significant determinant when we used FPG or HbA1c as an independent variable (FLUS: Aβ=0.215, p=0.016 or Aβ=0.216, p=0.016, respectively) while other variables were not. In the 20 people with incident diabetes, ALT in the Period I was a significant independent determinant of FIRI in the Period II when analyzed with BMI, age, JPDRISC, FPG or HbA1c (Aβ=0.754, Aβ=0.827, Aβ=0.814, Aβ=0.833 or Aβ=0.819, respectively, p<0.001 for each), which were not determinants. ALT in the Period I was also a significant independent determinant of UAE in the Period II when analyzed with BMI, age, JPDRISC, FPG or HbA1c (Aβ=0.720, Aβ=0.654, Aβ=0.626, Aβ=0.647 or Aβ=0.718, respectively, p<0.01 for each), which were not determinants.

Conclusion: ALT was a determinant of FIRI, UAE in people without diabetes and those with incident diabetes within 26 months independent of glycemic markers such as FPG or HbA1c. CysC, FIRI and hs-CRP were independently determined by ALT (CysC) and FLUS (FIRI and hs-CRP) in non-diabetic people.

Clinical Trial Registration Number: 20-130 5420

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Disclosure: **S. Katoh:** Grants; KAKENHI22590609.

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Non-alcoholic fatty liver disease strongly predicts incident diabetes in patients with coronary artery disease

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Background and aims: Both coronary artery disease (CAD) and non-alcoholic fatty liver disease (NAFLD) are associated with type 2 diabetes. Whether NAFLD predicts future diabetes in CAD patients who do not have diabetes yet is unknown.

Materials and methods: We therefore prospectively recorded diabetes incidence in a large cohort of 1018 consecutive non-diabetic patients with angiographically proven CAD; for the diagnosis of NAFLD we used the validated fatty liver index (FLI); diabetes was diagnosed according to ADA criteria.

Results: At baseline, 44.3% of our patients had impaired fasting glucose (IFG) and 55.2% had an HbA1c of 5.7-6.4% and thus were at risk of diabetes according to ADA categories. The prevalence of NAFLD was significantly higher in patients with IFG than in those with normal fasting glucose (46.8 vs. 34.0%; p<0.001) but not between patients with an HbA1c of 5.7-6.4% and those with an HbA1c <5.7% (40.8% vs. 38.5%; p=0.478). Prospectively, 11.2% of our patients newly developed diabetes during a follow-up period of 6.3 ±3.7 years; both IFG (OR 3.24 [2.03-3.32]; p=0.001) and an Hba1c of 5.7-6.4% (OR 2.90 [1.50-5.61]; p=0.002) significantly predicted incident diabetes. Importantly, diabetes incidence was significantly higher in patients with NAFLD than in those who did not have NAFLD (18.4 vs. 8.5%; p<0.001), and NAFLD strongly predicted incident diabetes both univariately (OR 2.41 [1.56-3.73]; p<0.001) and after multivariate adjustment including both baseline fasting glucose and HbA1c (OR 1.76 [1.11-2.79]; p=0.017).

Conclusion: We conclude that NAFLD in patients with CAD strongly predicts incident diabetes independently from the baseline glycemic state.

Disclosure: **A. Mader:** None.

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Impact of liver transplantation on glucose homeostasis in cirrhotic patients

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Background and aims: Abnormalities of glucose regulation are common in cirrhotic patients and may be modified by liver transplantation (LT). This study aimed at assessing the impact of LT on glucose homeostasis in cirrhotic patients.

Materials and methods: 89 cirrhotic patients underwent an anthropometric and metabolic evaluation, including an OGTT, prior and 3, 6 and 12 months after LT.

Results: Before LT, 18 patients had normal glucose tolerance (NGT), 30 had impaired glucose tolerance (IGT), and 41 had diabetes mellitus (DM). HOMA-IR increased from NGT to DM. Moreover, DM was associated with lower total and LDL cholesterol, albumin, pseudocholinesterase (CHE) and platelet count than IGT and NGT. After LT, 24 patients recovered from DM (regressors, R), 17 did not (non-regressors, NR), 3 developed DM, and 45 remained non-diabetic. After LT, DM prevalence gradually decreased from 46.0% to 34.8% at 3 months, 33.7% at 6 months and 22.4% at 1 year, when 35 patients had NGT and 34 had IGT. At 1 year, patients who had DM at baseline showed a significant improvement in insulin, cholesterol, triglycerides, total proteins, albumin, bilirubin, AST, ALT, CHE and platelet count, whereas HOMA-IR improved significantly only in R subjects (2.36±1.8 vs. 4.22±3.0, P<0.05), though all these parameters remained significantly worse than in IGT and NGT subjects. At multiple regression analysis, independent predictors on NR vs. R were male gender (β=0.7269; P=0.015), baseline HbA1c (β=0.727; P= 0.015) and γ-GT (β=0.0038; P=0.05) levels and inversely CHE (β=-0.0003; P=0.008) and platelet count (β=-0.0089; P=0.05).

Conclusion: LT is associated with regression of DM in ~50% of patients, with failure to regress being predicted by male sex and baseline HbA1c and liver function.

Clinical Trial Registration Number: NCT02038517

Disclosure: **V. Grancini:** None.

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The effect of new anti-HCV infection combination therapy of ombitasvir, paritaprevir and dasabuvir on glucose metabolism: preliminary study

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Background and aims: Hepatitis C virus (HCV) infection is an established risk factor for developing diabetes, mostly due to significant decrease of insulin sensitivity. Traditional antiviral therapies, i.e. interferon, are known to severely deteriorate glucose control. New antiviral combination therapy of NS5A inhibitor ombitasvir (OBV), NS3/4A protease inhibitor paritaprevir (PTV) boosted with ritonavir, and NS5B polymerase inhibitor dasabuvir (DSV), used with or without ribavirin (RBV) was recently introduced. This treatment is the first all oral interferon-free regimen containing three distinct direct acting antivirals approved for therapy of patients infected with HCV genotype 1 and 4, both naive and treatment-experienced. We conducted a preliminary study assessing the effect of this combined treatment on insulin sensitivity, beta cell function and GLP-1 secretion.
Materials and methods: 6 non-diabetes patients (mean age 41.3±11.7 years, HCV infection duration 8.2±7.5 years, BMI 27.7±6.3 kg/m2) were treated for 12 weeks with the dose of co-formulated OBV/PTV/r (Viekirax 12.5 mg/75 mg/50 mg) was 25 mg/150 mg/100 mg daily and the dose of DSV (Exviera 250 mg) 500 mg daily divided in two doses. Daily dose of RBV was 1000 mg in patients weighing less than 75 kg or 1200 mg if the body weight was 75 kg or more. Standard intravenous 1 mg glucagon challenge test was conducted three times in all patients: at baseline, after completing the treatment and after another 12 weeks of regimen-free follow-up. Plasma glucose, insulin and GLP-1 were assessed at 0, 10, 20 and 30 minutes after glucagon administration.
Results: OBV/PTV/DSV treatment did not affect mean plasma glucose, insulin and GLP-1 levels assessed at baseline, immediately and 12 weeks after the treatment completion. However, there was a significant decrease in HOMA-IR index over the course of the study in all patients; i.e. mean HOMA-IR at baseline was 5.31±6.37, immediately after the treatment 3.30±3.28 ($p<0.05$ vs baseline) and at follow-up 2.46±1.20 ($p<0.05$ vs baseline). Of note, all patients were found to have undetectable HCV RNA already at 4 weeks of the treatment, and that effect was maintained until the end of follow-up. No serious adverse effects of the combined therapy were noted during the study.
Conclusion: New combined treatment of HCV infection with NS5A inhibitor ombitasvir, NS3/4A protease inhibitor paritaprevir boosted with ritonavir and NS5B polymerase inhibitor dasabuvir resulted in improvement of insulin sensitivity which might have been related to the complete elimination of hepatitis C virus. As this type of therapy does not compromise glucose metabolism, and may even improve it, it might be recommended to be used in patients with diabetes and HCV infection.
Disclosure: **L. Czupryniak:** None.

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Expression of hepatic DPP-4 is regulated by DNA methylation and participates in glucose metabolism

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Background and aims: Dipeptidyl peptidase 4 (DPP4) is a protease that cleaves and inactivates a variety of substrates including incretin hormones, growth factors, neuropeptides and cytokines. Its expression in adipose tissue as well as its circulating level is dysregulated in obesity. Moreover, it was shown that hepatic DPP4 is increased in patients with non-alcoholic fatty liver disease. However, it is not known whether this is a cause or a consequence of the disease. Here, we investigated the role of DPP4 in hepatic glucose and lipid metabolism and tested if inter-individual differences in DPP4 expression are mediated by epigenetic mechanisms.
Materials and methods: Expression and DNA methylation of the Dpp4 gene was investigated in livers of C57BL/6J mice which were either prone or resistant to diet-induced obesity. In addition, primary murine hepatocytes were transduced with an adenovirus coding for Dpp4 and effects on hepatic glucose and lipid metabolism were assessed.
Results: Already at 6 weeks of age, Dpp4 expression was significantly elevated in livers of obesity-prone mice when compared to obesity-resistant animals. DNA methylation of four intronic CpG sites were reduced, of which positions 877, 1253 and 1255 correlated with elevated Dpp4 expression. In vitro methylation of this region resulted in a substantially reduced promotor activity, whereas high glucose treatment led to an elevated promoter activity. Overexpression of Dpp4 in primary hepatocytes did not impair lipid accumulation but resulted in an elevated glucose production, which was associated with an increased glycogen content.
Conclusion: In summary, our results indicate that individual differences in hepatic Dpp4 expression are associated to changes in DNA methylation early in life. Furthermore, we showed that elevated Dpp4 expression affects hepatic glucose homeostasis and therefore might participate in the progress of obesity and insulin resistance.
Supported by: BMBF-DZD
Disclosure: **C. Baumeier:** None.

PS 005 Get up and go: exercise in diabetes

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The long-term effect of exercise intervention on improving fatty liver and cardiovascular risk factors in obese adults: a one-year follow-up study

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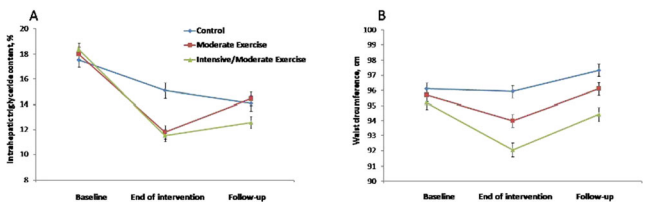
Background and aims: Exercise training can reduce hepatic fat accumulation and cardiovascular risk among patients with nonalcoholic fatty liver disease, but how long these benefits extend beyond the period of active intervention is unclear. We aimed to investigate the persistence of these effects in the long term.
Materials and methods: We analyzed 220 obese subjects aged 40-65 years with nonalcoholic fatty liver disease who had participated in a 12-month exercise intervention program and were randomly assigned to intensive/moderate exercise, moderate exercise, or no-exercise control. Intrahepatic triglyceride content measured by proton magnetic resonance spectroscopy, waist circumference, body fat, and metabolic risk factors were assessed at the 12-month follow-up.
Results: Intrahepatic triglyceride content was significantly reduced in the two exercise groups compared to control over the 12-month active intervention. It remained significantly reduced by -2.39% in intensive/moderate exercise compared to control at the 1-year follow-up (95% confidence interval -4.72 to -0.05%, p=0.045). Changes in intrahepatic triglyceride content were not significantly different between the two exercise groups or moderate exercise and control groups at the 1-year follow-up. Waist circumference and diastolic blood pressure remained significantly reduced in intensive/moderate exercise and moderate exercise compared to control over the subsequent 1 year after intervention, and systolic blood pressure was only significantly reduced in the intensive/moderate exercise group. Body fat lost with intensive exercise was regained over the 1-year follow-up, and visceral adipose fat remained significantly reduced but with no differences among the three groups.
Conclusion: A 12-month exercise intervention induced reductions in hepatic fat accumulation, abdominal obesity, and blood pressure for up to 1 year after the active intervention, with some attenuation of the benefits.

Figure. Changes in liver fat and abdominal obesity after intervention and at follow-up.



Clinical Trial Registration Number: NCT01418027
Supported by: Xiamen Systems Biology Research Program for Metabolic Disease (3502Z201)
Disclosure: **H. Zhang:** None.

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Bicycling to work and primordial prevention of cardiovascular and type 2 diabetes risk: a cohort study from Northern Sweden

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Background and aims: An active lifestyle is known to reduce risk for cardiovascular disease and type 2 diabetes, but is also frequently reported to be difficult to fit into daily life. Here, we investigate the relationship of active commuting by cycling to work with incidence of clinical cardiovascular and type 2 diabetes risk factors across a decade of follow-up in middle-aged men and women.
Materials and methods: Participants from the Västerbotten County in Northern Sweden were invited to a health examination on their 40th, 50th, and 60th birthday within the framework of the Västerbotten Intervention Program. The sample for this analysis consists of 23,712 men and women with a mean age of 43.5 years (SD 6.8) at baseline, who attended a health examination twice during a 10-year period from 1990 to 2011. The outcome measures of this analysis were 10-year incidence of obesity, hypertension, hypertriglyceridemia and impaired glucose tolerance.
Results: Cycling to work at baseline was associated with lower odds of incident obesity (OR=0.85, 95%CI=0.73-0.99), hypertension (OR=0.87, 95%CI=0.79-0.95), hypertriglyceridemia (OR=0.85, 95%CI=0.76-0.94), and impaired glucose tolerance (OR=0.88, 95%CI=0.80-0.96) compared with passive travel, adjusting for putative confounding factors. Dose-response relationship of cycling according to seasonal frequency and distance to work were evident for all incident cardiovascular risk factors ($p<0.05$ for linear trend). Participants, who maintained or began cycling to work during follow-up, had lower odds of obesity (OR=0.61, 95%CI=0.50-0.73), hypertension (OR=0.89, 95%CI=0.80-0.98), hypertriglyceridemia (OR=0.80, 95%CI=0.70-0.90), and impaired glucose tolerance (OR=0.82, 95%CI=0.74-0.91). This is compared with participants not cycling to work at both time points, or who switched from cycling to other modes of transport during follow-up, having also adjusted for baseline- and follow-up confounding factors. Based upon these results, if all participants maintained or switched to cycling to work during the 10-year follow-up period, an estimated 24% (95%CI=16-32), 6% (95%CI=1-11), 13% (95%CI=6-20), and 11% (95%CI=5-16) of new occurrences of obesity, hypertension, hypertriglyceridemia, and impaired glucose tolerance (respectively) could have been prevented in this population.
Conclusion: Our results suggest that commuting to work by bicycle has a dose-response level reduction in the incidence of clinical cardiovascular and diabetes risk factors among middle-aged men and women. Furthermore, our results indicate that this beneficial effect can be observed even when the mode of transport was switched to cycling from another form. We conclude that cycling to work is an important strategy for primordial prevention of clinical cardiovascular and diabetes risk factors in middle-aged men and women.
Supported by: AG: Lundbeck Foundation (R151-2013-14641), DCIR (DFF-4004-00111)
Disclosure: **R.W. Koivula:** None.

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Does a new diabetes diagnosis influence lifestyle behaviors within the household? The INfluence of Families On Modification of Diabetes Risk (INFORMED) study

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Background and aims: When a person is diagnosed with diabetes, changes in their health behaviors, particularly around diet and exercise, may influence the behavior of their spouses. The diabetes diagnosis may also affect other household members' perceptions of their own health risks, which could trigger behavioral change as well. However, whether spouses of people with newly-diagnosed diabetes change their health behaviors is unknown.
Materials and methods: The study population consisted of members of the Kaiser Permanente Northern California (KPNC) health plan from 2007-2011. Among co-residing spouses of persons with newly-diagnosed diabetes (n=30,755) and a 5:1 matched sample of persons without diabetes and their respective spouses (n=150,775), we applied multivariate models to assess the adjusted difference-in-differences in the one-year change pre- and post-diabetes diagnosis among spouses for the following eight outcomes: blood glucose screening; lipid testing; blood pressure screening; influenza vaccination; smoking cessation medication use; tobacco use; participation in weight management-related wellness classes; and clinically meaningful weight loss (>=5% of body weight). Models were adjusted for gender, age, race/ethnicity, and baseline characteristics: body mass index, yearly primary care visits, and Census block average education. All data were obtained from the KPNC electronic health record.
Results: After adjusting for baseline characteristics and behaviors, the spouses of patients with newly-diagnosed diabetes had statistically-significantly higher rates of glucose screening (OR=1.12, 95% CI=1.09,1.15); lipid level testing (OR=1.10, 95%CI=1.07,1.13); blood pressure screening (OR=1.11, 95% CI=1.08,1.15); influenza vaccination (OR=1.07, 95% CI=1.03,1.10); smoking cessation medication use (OR=1.27, 95% CI=1.05,1.54); and participation in weight management-related wellness classes (OR=1.53, 95% CI=1.41, 1.67) compared with spouses of people without diabetes. There was no statistically significant difference in clinically meaningful weight loss or tobacco cessation between the two spouse groups.
Conclusion: We found small but statistically significant improvements in health-related behavioral change among spouses of people with newly-diagnosed diabetes compared to spouses of people without diabetes, even when no intervention was present. This suggests a diabetes diagnosis within a family may be a 'teachable moment' that can be leveraged by new interventions to improve health care behaviors, and potentially improve outcomes and decrease diabetes risk, at the household level.
Supported by: NIDDK
Disclosure: **J.A. Schmittdiel:** None.

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Dimensions of physical activity and sedentary time in identification of risk groups for future type 2 diabetes: a decision tree analysis within the addition-pro cohort

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Background and aims: While the protective effects of physical activity on impaired glucose metabolism (IGM) is well established, little is known about the role of specific dimensions of physical activity and their interaction with other IGM risk factors. We aimed to apply a Decision Tree algorithm to assess which dimensions of physical activity and sedentary time are associated with IGM and how the associated physical activity dimensions differed by age, sex and obesity.
Materials and methods: 1512 Danish middle aged men and women with different diabetes risk profiles were included in the analysis. An oral glucose tolerance test was performed and 7 days of physical activity energy expenditure was measured using a combined accelerometer and heart rate monitor (ActiHeart®), individually calibrated by a step test. A Decision Tree methodology was applied to identify sub-groups at risk for IGM, allowing different dimensions of physical activity and sedentary time to have different effects as predictors of IGM (fasting plasma glucose >=6.1 and/or 2h plasma glucose >=7.8). Age, sex and BMI were also included in the model.
Results: The decision tree was found to distinguish and delineate a wide range of risk profiles (Figure 1). BMI, age and sex were most predictive of IGM. Among overweight males, moderate-to-vigorous physical activity was the dominant physical activity predictor of IGM, while for overweight women time spent sedentary while awake was the main predictor. Among individuals with normal weight and aged >53 years, time spent in light physical activity was the main predictor. Only sex was predictive among individuals with normal weight aged ≤53 years.
Conclusion: Applying a Decision Tree algorithm to physical activity data identifies several important interactions and can provide insight to development of personalised physical activity intervention strategies for the prevention of type 2 diabetes.

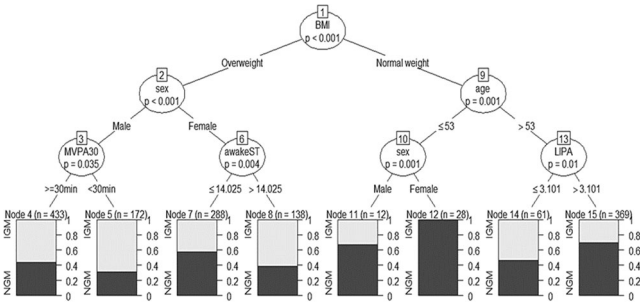

Figure1.The decision tree depicts 8 risk groups (nodes) with corresponding prevalence of normal glucose metabolism (NGM) vs. impaired glucose metabolism (IGM).
LIPA: hours spent in light intensity physical activity (1.5-3.0METs), MVPA30: moderate-to-vigorous physical activity of ≥ or <30min (≥3.0 METs), awakeST: hours spent sedentary while awake (≤1.5 MET, subtracting sleep).

Supported by: DHA, IFD, UA
Disclosure: **H. Amadid:** Grants; The Danish Heart Association, Innovation Fund Denmark, University of Aarhus.

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Physical activity, dietary intake and eating behaviour in genetic risk of obesity

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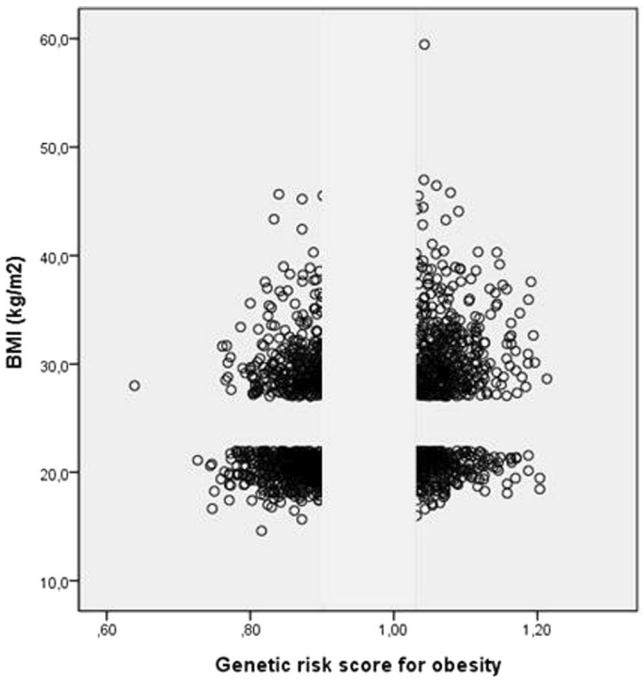
Background and aims: Observational studies cannot determine whether unfavourable changes in physical activity, dietary intake and eating behaviour are a cause of obesity or, rather, reflect a result of increased body mass index (BMI) itself. We aimed to separate cause and effect by using a special design of four corners identifying people as being either at low or high genetic risk for obesity and having either a low or high measured BMI.
Materials and methods: Sixty females (mean age 45.8 ± 6.9 years, BMI range 17.4 - 43.9 kg/m2) were selected out of 11495 people registered in the large longitudinal Netherlands Twin Register database with available data on BMI and a genetic risk score (GRS) for BMI based on 77 single

nucleotide polymorphisms (SNPs) identified in a recent genome-wide association meta-analysis. Selection was based on four corners from a scatter diagram created to identify people as having either a low or high GRS and having either a low or high measured BMI (Figure). We used seven-day accelerometry, three-day 24-hour recalls and questionnaires to assess physical activity, dietary intake and eating behaviour. The expectation was that variables that are associated with BMI irrespective of GRS are secondary to high BMI itself. In contrast, variables that are associated with BMI only in the individuals with high GRS may be causal factors contributing to the genetic predisposition to obesity.

Results: Participants with high BMI had significantly less daily step counts, more time spent in sedentary behaviour and more emotional and restrained eating than participants with low BMI, irrespective of GRS for obesity. Intake of daily energy or macronutrients did not differ between the low and high BMI groups when GRS was ignored. However, participants in the corner of high GRS and high BMI had a higher intake of protein, in specific animal protein, than participants in the corner of high GRS and low BMI. This BMI-related difference was absent when GRS for obesity was low.

Conclusion: Our data suggest that unfavourable changes in overall physical activity, sedentary behaviour, emotional and restrained eating are likely secondary consequences of high BMI, rather than a cause. In contrast, higher intake of (animal) protein may be an aetiological component of the genetic predisposition to obesity. This study provides ground to call for further interventional studies characterizing the effects of (animal) protein intake on BMI.



Clinical Trial Registration Number: NCT02025595
Supported by: NWO ZonMw
Disclosure: **S. Doornweerd:** Grants; Netherlands Organisation Scientific Research.

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Newly diagnosed and prevalent type 2 diabetes patients are socially isolated: the Maastricht study

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Background and aims: Social network characteristics are associated with health, but their relation with type 2 diabetes mellitus (T2DM) is unclear. We compared structural and functional social network characteristics in individuals with normal glucose metabolism (NGM), pre-diabetes, newly diagnosed T2DM and prevalent T2DM.

Materials and methods: Participants (n=2967) originate from The Maastricht Study, a population-based cohort study (mean age 60.0±8.2 years, 49% female, 28.8% T2DM (oversampled)). Social network characteristics were collected through a questionnaire by use of a name generator method. To determine diabetes status, participants underwent an oral glucose tolerance test. Cross-sectional multinomial regression analyses were performed to examine the association between social network characteristics and glucose metabolism groups (NGM as reference), adjusted for age, educational level and body mass index, and stratified for sex.

Results: Compared to NGM, individuals with newly diagnosed and prevalent T2DM had a smaller structural network and lower contact frequency. The mean number of network members declined from 11.0 in NGM to 10.0 in pre-diabetes to 7.7 in newly diagnosed T2DM to 7.5 in prevalent T2DM. Adjusted odds ratios (ORs) and 95% confidence intervals for newly diagnosed and prevalent T2DM were 1.12 (1.04-1.21) and 1.12 (1.07-1.17) in women, and 1.11 (1.04-1.19) and 1.08 (1.04-1.11) in men, compared to NGM. Newly diagnosed and prevalent T2DM reported less functional social support at all levels (informational, emotional and practical support): adjusted ORs for emotional support in newly diagnosed and prevalent T2DM were 1.33 (1.07-1.66), 1.24 (1.11-1.40) in women and 1.20 (1.01-1.42), 1.15 (1.06-1.26) in men. These associations were not observed for pre-diabetes. However, low social participation was associated with pre-diabetes and T2DM in women and T2DM in men: adjusted ORs for pre-diabetes, newly diagnosed and prevalent T2DM were 1.67 (1.18-2.34), 1.76 (0.88-3.55), 2.66 (1.86-3.80) in women, and 1.29 (0.92-1.80), 1.52 (0.90-2.58) and 1.54 (1.17-2.03) in men, respectively. Further, living alone was associated with a higher odds for T2DM in men, but not in women (adjusted ORs 2.07 (1.41-3.04) and 1.09 (0.71-1.65), respectively).

Conclusion: This study is the first measuring a broad range of structural and functional characteristics of the social networks and their associations with pre-diabetes, newly diagnosed T2DM and T2DM in a large sample of adults. Results of the present study may identify useful targets in social network characteristics that may prove promising in tailoring prevention strategies for T2DM.

Disclosure: **S. Brinkhues:** None.

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Reversion from prediabetes to normal metabolism is positively correlated with a loss of body weight in a nationwide observational population study

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Background and aims: Control of body weight is widely recognized as a major factor for dealing with cases characterized by increased risk to develop diabetes, especially those already on the prediabetes range. However, as with all behavioral changes, the attainment of these goals in real-life interventions is often put into question. With the present study, we aimed to use a nationwide observational study on progression to diabetes that spanned 5 years to uncover the frequency of "regressors" (individuals that revert from prediabetes to normal glucose metabolism) in a general real-life population, and to assess to what extent weight loss is related to this process in the absence of an intervention.
Materials and methods: Individuals characterized by "normal" or "prediabetes", through an oral glucose tolerance test (OGTT) and WHO criteria, in the initial PREVADIAB observational study were recalled for a follow-up after 5 years. These individuals were representative of the nationwide non-diabetic distribution observed on the first study. An OGTT was again performed to evaluate glycemic control.
Results: The considered cohort consisted of 1024 participants, 300 of which had initially prediabetes. In relation to the evolution of glycemic control with time, of these, 21.0% developed diabetes, while 37.3% remained with prediabetes. Additionally, 41.7% presented normal glucose metabolism at follow-up, i.e. can be classified as "regressors" during this time period. Furthermore, while these "regressors" where associated with a mean loss of 1.7% in body weight, those that remained with a diagnosis of prediabetes at follow-up were associated with a mean gain of 0.8% in body weight (p=0.02; CI at 95%: -4.5 to -0.4%).
Conclusion: Control of body weight arises in this observational population study as closely related with the natural regression from prediabetes to a normal glucose metabolism. This supports the inclusion of this particular variable in community diabetes prevention programs, not only as an advice but as a monitored factor to be followed closely.
Supported by: DGS - Portuguses General Directorate of Health
Disclosure: **R.T. Ribeiro:** None.

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The association of resting heart rate with the presence of diabetes in the Korean adults: the 2010-2013 Korea national health and nutrition examination survey

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Background and aims: Previous epidemiologic studies have shown that elevated resting heart rate is associated with higher cardiovascular disease (CVD) morbidity and mortality. Although the relationship between elevated heart rate and CVD is well established, the association between resting heart rate and diabetes has been relatively understudied, particulary in non-Western populations.
Materials and methods: We assesssed the association between the presence of type 2 diabetes and resting heart rate in the Korean adult population using the data from the 2010-2013 Korea National Health and Nutrition Examination Survey (KNHANES). The fasting plasma glucose (FPG) level was categorized into the following five groups: normal fasting glucose (NFG) 1 (<90 mg/dl), NFG 2 (90-99 mg/dl), impaired fasting glucose (IFG) 1 (100-110 mg/dl), IFG 2 (111-125 mg/dl) and diabetes (≥126 mg/dl. Among 25,712 adults (≥19 years old) who participated in the 2010-2013 KNHANES, a total of 22,512 subjects completed laboratory examinations and were included in this analysis.
Results: The unadjusted weighted resting heart rate were 69.6, 69.4, 69.8, 70.1, and 72 bpm in the NFG 1, NFG 2, IFG 1, IFG 2, and diabetes groups, respectively (P<0.001) . We assessed the adjusted weighted resting heart rate according to the FPG level, after adjusting for age, sex, smoking history, heavy alcohol drinking, daily energy intake, waist circumference, serum total cholesterol level, serum TG level, serum WBC, serum hemoglobin, and the presence of hypertension. The adjusted weighted resting heart rate significantly increased across the FPG groups. (P<0.001) The weighted prevalences of diabetes were 6.8 (6.2-7.5), 7.6(6.7-8.5), 8.0 (7.0-9.1), and 11.7 (10.7-12.7) % in subjects with HR ≤64, 65-69, 70-75, ≥76 bpm, respectively (P<0.001) after adjustment for above- mentioned confounding factors. Using resting HR ≤64 bpm as the control, resting HR ≥76 bpm was correlated with the presence of diabetes (adjusted OR 1.84, 95% CI 1.56-2.17, P<0.001). Each 10 bpm increment of HR increased the risk of the presence of diabetes by 36% (P<0.001). This association of high resting heart rate with the presence of diabetes was not influenced by the status of BP medication.
Conclusion: In conclusion, we demonstrated that higher heart rate was associated with daibetes in a representative sample of Korean adults. These positive associations were independent of age, sex, current smoking, heavy alcohol drinking, daily energy intake, waist circumference, and the presence of hypertension and other potential confounders. This study suggests that individuals with higher heart rate are at risk of diabetes and may benefit from diabetes screening.
Disclosure: **D. Kim:** None.

PS 006 Diabetes prevalence and prevention around the world

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Prevalence of diabetes and pre-diabetes in a cohort of adults with William-Syndrome: a 5 year follow-up

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Background and aims: Williams syndrome (WS) is a multisystem genetic condition caused by submicroscopic deletion at 7q11.23. Impaired glucose metabolism (DM, IGT and IFG) is a well-known complication among patients with WS, and hemizygosity for syntaxin-1A (STX-1A) and MLXIPL, which are implicated in insulin secretion and glucose metabolism, is likely the major factor responsible. However, a previous study of our WS population showed a crucial role of reduced insulin sensitivity in the onset of glucose metabolism alteration in WS patients, without significant differences in insulin secretion compared to normal adults, matched for age, sex and BMI.
Materials and methods: Twenty Italian young adults with WS (13 females, 7 males, mean age at baseline 29.8±5.5 yr) were evaluated during a 5 year follow-up with glycometabolic and anthropometric parameters and a 75 g oral glucose tolerance test (OGTT). Subjects positive at baseline for IGT or DM were treated accordingly to guidelines and overweight/obese patients started a dietetic treatment.
Results: At baseline, DM and IGT were diagnosed in 5% (1/20) and 55% (11/20) of subjects, respectively, and 5 patients with IGT also had impaired fasting glucose (IFG). After 5 years, the prevalence of IGT decreased (40%, 8/20), while new DM occured only in one patient. IFG was present in 6 subjects with IGT, whereas none of the patients with NGT had FG≥100 mg/dl. Obesity (BMI≥30 Kg/m2), at baseline, affected 10% (2/20) of patients and after dietetic treatment it was present in only 1 patient (5%). After 5-years, IGT patients showed lower β-cell function indices compared to euglycemic patients (HOMA-B%: 76.3±34.5 vs 113±29.8, P=0.06; Disposition Index: 3.15±1.22 vs 5.71±1.14, P=0.01), and insulin resistance was higher in these patients (HOMA-IR: 2.02±0.5 vs 1.7±1.06, P=0.46). Comparing insulin secretion indices in IGT patients at baseline and during follow-up, HOMA-B% and Insulinogenic Index showed a reduction (baseline: 108.2±43.6, 5-yr: 73.6 ±36.3, P=0.106; baseline: 1.03±0.46, 5-yrs: 0.89±0.68, P=0.637, respectively) over time, while HOMA-IR values remained unchanged.
Conclusion: After 5 years of lifestyle modification, the whole prevalence of glucose alterations appeared reduced in WS patients. However, these datas seem to suggest that glycometabolic alterations in WS are associated primarily with reduced insulin sensitivity, but afterwards also occurs impaired beta-cell function, probably caused by deletion of a gene such as STX-1A within the WS chromosome region. Therefore, it's necessary a strict follow-up and the correction of associated risk factors, in order to avoid the onset of DM in this population.
Disclosure: **M.E. Lunati:** None.

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No increased incidence of type 1 diabetes under 40 years 2009-2014 in Norwegians and immigrants

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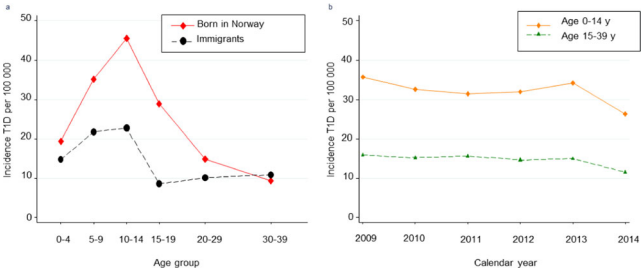
Background and aims: The incidence of type 1 diabetes in children has increased in most countries over the past decades. While the incidence of type 1 diabetes in age-groups <15 years have been widely studied, fewer have assessed age-groups up to 40 years. We aimed to study the incidence of type 1 diabetes before age 40 years in Norway 2009-2014.
Materials and methods: In this nationwide population-based cohort study we used the central population registry as the population frame (complete Norwegian population from January 1st 2009; age under 40 years, n= 3,057,221), and identified type 1 diabetes by linking data from several national registries using the personal identification number assigned to all residents in Norway. The Norwegian Prescription Database (NorPD) has registered drugs redeemed by individual patients from all pharmacies in Norway since Jan 1st 2004. Diagnoses in primary care (KUHR) have been registered at the individual level since Jan 1st 2006. The Norwegian Patient Register (NPR) has registered hospital and outpatient clinic diagnoses at the individual level since Jan 1st 2008. Type 1 diabetes was defined as registration of at least one type 1 diabetes diagnosis in primary or secondary health care, and start of insulin within 6 months of diagnosis and use for at least 6 months thereafter, and no use of oral glucose lowering medication 12 months after the diagnosis. We estimated the incidence from Jan 1st 2009 to Jun 30th 2014, which was the end of the study period. Immigrants were defined as subjects not born in Norway or having two parents born abroad.
Results: Overall incidence <40 year was 23.1 per 100,000 persons/year for Norwegians (95% CI: 22.2-24.0) and the highest incidence rates were at age 10-15 years (Fig panel a). The incidence rates were stable across the calendar years (Fig panel b). Immigrants from Europe represented 11.5% of the population and non-European immigrants 11.4%.Incidence in different regions of origin were: Africa 28.8 per 100,000 persons/year (95% CI: 23.4-35.5), Europe (non-Norway) 12.0 per 100,000 persons/year (95% CI: 10.0-14.2), Asia 8.0 per 100,000 persons/year (95% CI: 6.2-10.2) and South America 5.3 per 100,000 persons/year (95% CI: 2.0-14.2).
Conclusion: The incidence of type 1 diabetes in Norway seems to be stable over time in both children and young adults. Immigrants in total had an incidence that was nearly half of that in people born in Norway, but there were large differences between regions.



Supported by: Norwegian Research Council and Institute of Public Health, Health SouthEast
Disclosure: **P.L.D. Ruiz:** None.

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Decreasing incidence of type 2 diabetes in Norway 2009-2014

H.L. Gulseth[1,2], P.L.D. Ruiz[1,2], I.J. Bakken[1], H. Strøm[1], K.I. Birkeland[2,3], S.E. Håberg[1], L.C. Stene[1];
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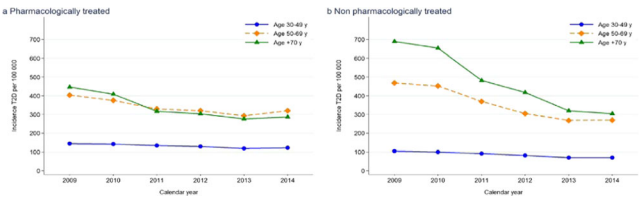
Background and aims: The prevalence of type 2 diabetes (T2D) is increasing in many countries, but few have assessed trends in incidence. We aimed to study incidence of diagnosed T2D age ≥30 years in Norway 2009-2014, in both Norwegians and immigrants.
Materials and methods: In this nationwide population-based cohort study we used the central population registry as the population frame (complete Norwegian population from January 1st 2009; age ≥30 years

, n=3,258,708), and identified T2D by linking data from several national registries using the personal identification number assigned to all residents in Norway. The Norwegian Prescription Database (NorPD) has registered drugs redeemed by individual patients from all pharmacies in Norway since Jan 1st 2004. Diagnoses in primary care (KUHR) have been registered at the individual level since Jan 1st 2006. The Norwegian Patient Register (NPR) has registered hospital and outpatient clinic diagnoses at the individual level since Jan 1st 2008. Incident T2D was classified as pharmacological treated if there was at least one prescription of glucose lowering medication within the first six months of diagnosis, and non-pharmacological if there were two or more T2D diagnosis codes registered without any anti-diabetic drug prescription within the first six months. We estimated the incidence from Jan 1st 2009 to Jun 30th 2014. Immigrant was defined as subject not born in Norway or with two parents born abroad.

Results: The overall incidence of T2D was 494 per 100,000 persons/year for Norwegians (95% CI: 491-498), and 49% were pharmacologically treated. The highest incidence rates were seen at age 70-79 years. The total incidence rate decreased across calendar years (p for trend <0.001). Average decrease per year for non-pharmacological treated diabetes was 13% (95% CI: 12.8-13.9, p<0.001), and the decrease for pharmacologically treated diabetes was 6.5% (95% CI: 6.0-7.1, p<0.001). Subjects ≥70 years had the largest absolute decrease in incidence (Fig), but incidence rates were significantly decreased across all age-groups, and in both Norwegians and immigrants (all p for trend<0.001). Non-European immigrants represented 6.3% of the Norwegian population aged ≥ 30 years, incidence rate 756 per 100,000 persons/year (95% CI: 738-775). Incidence rates by region of birth were: Africa 759 per 100,000 persons/year (95% CI: 719-802), Asia 870 per 100,000 persons/year (95% CI: 845-897) and South America 550 per 100,000 persons/year (95% CI 493-615). Treatment the first 6 months varied according to immigrant status; 47% of Norwegians and 57% of the non-European immigrants received glucose lowering medication.

Conclusion: There was an apparent decrease in incidence of diagnosed T2D 2009-2014 in Norway in all age groups and in both Norwegian and immigrants.



Supported by: Norwegian Research Council and Institute of Public Health, Helse SørØst

Disclosure: **H.L. Gulseth:** None.

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Prevalence and trends of obesity and overweight in Chinese adults, 2004-2013

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Background and aims: Large-scale epidemiologic studies to assess the increased prevalence of obesity in China, and relate this increase to economic development, are lacking. In 2001, the China Marrow Donor Program (CMDP) began collecting anthropometric data on volunteers and information on >1.8 million individuals from 31 branch registries are currently available.

Materials and methods: CMDP data from 1,163,094 healthy adults examined in years 2004-2013 were included in a cross-sectional analysis to estimate the yearly prevalence of obesity and overweight. The annual per capita Gross Domestic Product (GDP) was used to evaluate the relationship between economic growth and obesity.All statistical analyses were conducted with the use of SAS for windows version 9.2 software (Research Triangle Institute). Approximate power calculations were performed using StatCalc (Sample Size and Power for Population Survey) in Epi Info version 7 (National Cancer Institute, USA), assuming a survey design effect of 1.5. The sample sizes for the groups with the smallest sample size were sufficient to estimate the prevalence with confidence limit of 0.05 with more than 80% power; most of the estimates can achieve more than 90% power.

Results: In 2013, the age and sex-standardized prevalence of obesity and overweight among Chinese adults was 10.16% and 32.40%, affecting 86.10 and 257.38 million individuals, respectively. The prevalence of obesity and overweight increased with increasing age, and were higher in males (13.08% and 38.66%) as compared to females (7.48% and 22.81%). From 2004 until 2013, the prevalence of obesity increased from 6.38% to 10.16% where the increase was greater from 2011-2014 as compared to 2004-2010. Females had a greater increase in recent years as compared to males, suggesting that differences by gender are becoming smaller. Among poorer provinces, the prevalence of obesity rose sharply with increasing GDP, whereas among developed regions, there was a nominal increase in obesity with increasing GDP.

Conclusion: The prevalence of obesity and overweight are dramatically increasing in recent years in China, especially among women and in regions with previously low GDP. Strategies aimed at preventing and treating obesity are needed.

Supported by: China Marrow Donor Program (CMDP)

Disclosure: **J. Shen:** None.

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Dynamics in prevalence of diabetes, diabetic complications and quality of diabetes care in Russian Federation in 2014-15 by data of national diabetes register

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Background and aims: Due to worldwide increase in prevalence of diabetes mellitus (DM) the data of registers are important tool for evaluation the trends in DM epidemiology and efficiency of healthcare system in certain population. The aim of the study was to analyze the prevalence of DM, complications and HbA1c level in Russian Federation (RF) in 2014-15 years.

Materials and methods: The study summarized the data of the National diabetes register, included databases of 63 regions of RF with more than 3750 clinics of primary care. The data exported from online register, powered by Microsoft Dynamics CRM platform. Statistics performed by SPSS, 19.

Results: At the end of 2015 yr the register included 3310517 DM patients: 99.3% adults (≥18yr), 0.5% children (0-14yr), 0.2% adolescents (15-17yr). Distribution by DM type in age groups: adults 93.1% type 2 (T2)/ 5.2% type 1 (T1)/ 1.6% other DM types, children 89.1/9.7/1.2%, adolescents 93.9/4.6/1.5%, respectively; distribution by gender (M/F) in T1 52.7/47.3%, T2 28.3/71.7%, other types 22.7/77.3%. The average T1 prevalence was 1.66 cases/per 1000 population, T2 26.62 cases/per 1000 population with the wide range of variability among regions from 0.28 to 41.2 and 4.33 to 37.71/1000 population, respectively; the dynamics in prevalence +1% for T1, +2.9% for T2. The most frequent complications in T1/T2 were diabetic neuropathy 40.0/20.6%, retinopathy 33.6%/15.1%, hypertension 17.4/39.2%, nephropathy 19.2/5.2%, macrovascular disease 16.1/13.4%; without significant dynamics compared 2014 yr. In 2015 registered 66093 DM-related deaths (+1% compared 2014); the

leading cause was MVD: cumulatively heart failure, stroke and myocardial infarction - 46.2% of deaths in T2 and 30.3% in T1, the third leading cause in T1 - kidney failure (7.1%), in T2 - oncology (8.4%). Distribution by HbA1c level of T1 patients: less 7% - 30.9% of patients; 7-7.9% - 29.1%; 8-9% - 19.2%; > 9% - 20.8%; T2 patients: 45.4%; 31.9%; 13.5%; 9.2%, respectively; dynamics in the patients proportion by HbA1c range compared with 2014 yr: +6.0/-0.4/-2.8/-2.8% and +7.9/-3.1/-2.9/-2.0 for T1 and T2, respectively.

Conclusion: The data of National diabetes register have shown increased DM prevalence in RF over 2014-15 years, predominantly with T2 DM, that is in concordance with the global world trends; stable frequency of the registered diabetic complications and MVD as the main cause of DM-related death both in T1 and T2. Based on HbA1c dynamics with increasing proportion of patients within target in about 45% of T2 and 30% of T1 patients let to consider the better quality of diabetes care during the last year.

Supported by: Aston Consulting Group for technical support of register

Disclosure: **M.V. Shestakova:** None.

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Screening strategies for early diagnosis of undiagnosed diabetes and other disorders of glucose metabolism. The DSP (Diabetes Screening Palermo) study

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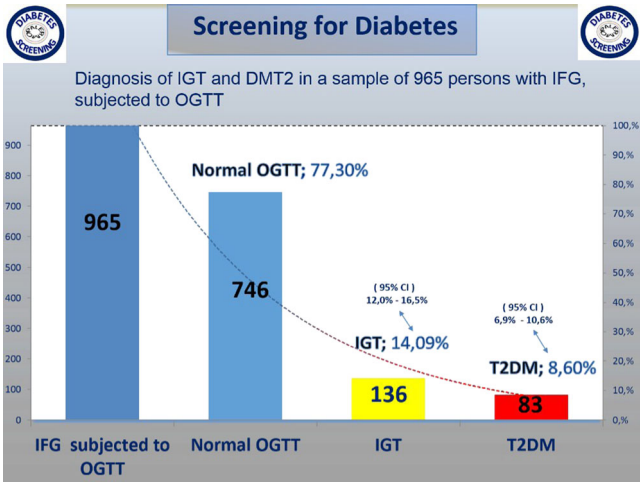
Background and aims: The Diabetes Screening Palermo Study evaluated, in the general practice setting, the effectiveness of a screening strategy for type 2 diabetes mellitus (T2DM). This study used electronic instruments to identify individuals at a high risk of diabetes and to provide early detection of undiagnosed T2DM and prediabetes.

Materials and methods: This is an observational study, lasting 24 months, in primary care from Italy, conducted by 20 GPs.The study partecipants were drawn from the general patient population of these physicians. Included were men and women, aged > 14 years, after the exclusion of individuals previously diagnosed with diabetes. The screening programme was divided into two phases. Phase 1: identification of patients at high risk of diabetes through the analysis of databases of general practitioners. To identify those at high risk for T2DM, the criteria adopted were from the Italian Standard of Medical Care in Diabetes. Testing to detect type 2 diabetes in asymptomatic people, have been considered in adults of any age, who were overweight or obese (BMI ≥25 kg/m2) and who had one or more additional risk factors for diabetes. For all patients, without these risk factors,testing began at age 45 years. Phase 2: screening to test for diabetes or prediabetes, using fasting plasma glucose (FPG) and the 2-h plasma glucose value after a 75-g oral glucose tolerance test (OGTT). The OGTT was a central component of the screening programme and a significant proportion of individuals at high risk for diabetes, which is defined as those with impaired fasting glucose (IFG), had blood glucose levels, after the glucose load, that were compatible with either a diagnosis of T2DM or impaired glucose tolerance (IGT).

Results: the total study was composed of 26 410 subjects, of which 13319 (50.43%) were at high risk of T2DM. A total of 40.75% of these high-risk individuals had impaired fasting glucose (IFG). A total of 965 subjects with IFG were than, at random, subjected to an OGTT, on the basis of which 136 subjects (14.09%) were identified with IGT and 83 subjects (8.60%) gave a response compatible with the diagnosis of T2DM

Conclusion: In a primary care setting, a proactive approach towards diabetes screening, especially performing OGTTs in subjects with IFG, facilitated the early diagnosis of T2DM. This reduced the percentage of cases of undiagnosed diabetes and allowed the identification of individuals with prediabetes, who required preventive interventions. Through a process of a clinical audit, the computerised systems used by general practitioners,facilitated the identification of people at high risk for diabetes and improved the management of the screening programme.



Disclosure: **T. Iraci:** None.

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Statin use and the risk of type 2 diabetes in subjects with impaired fasting glucose: results from the prospective study IT-DIAB

S. Smati[1], M. Hanf[2], M. Pichelin[1], A. Richard[1], B. Bressolette[1], V. Jacquin[1], M. You[1], C. Authier[3], G. Breton[1], B. Cariou[1]; [1]Endocrinology, CHU NANTES, [2]INSERM CIC004, [3]CPAM Loire-Atlantique, Nantes, France.

Background and aims: Meta-analyses of randomized clinical trials have demonstrated that statins are associated with an increased risk of type 2 diabetes (T2D). Here, we assessed the impact of statin use on the occurrence of new onset T2D in a population of subjects with pre-diabetes.

Materials and methods: IT-DIAB is an observational study in which subjects with impaired fasting glucose (IFG) are prospectively followed annually for five years in order to understand the pathophysiological mechanisms involved in the switch from pre-diabetes to T2D. 452 subjects with IFG were included in this interim analysis with a mean follow-up of 3.0 years. Conversion to T2D was defined by a fasting plasma glucose (FPG) ≥ 7 mmol/L or HbA1C ≥ 6.5%. Univariate and multivariate cox analyses were performed to analyse the effect of statin use on conversion to T2D.

Results: Amongst the 452 patients included, 122 were under statins at baseline. Subjects under statins were older (62 vs 58 years, p<0,0001) and displayed higher HbA1C (5,9 vs 5,8%, p<0,0001) and BMI (29,8 vs 28,7 kg/m2, p=0,07) compared to non-statin users. FPG was similar between the two groups (114 mg/dL). After 3 years, 117 subjects (25.9%) progressed to T2D, including 30.3% (n=37/122) in statin users vs 24.2% (n=80/330) in non-statin users. Univariate analysis showed a tendency for a correlation between statin use and T2D risk defined either by FPG ≥ 7 mmol/L (HR [95% CI] : 1.36 [0.92-2.01] ; p=0.13) or HbA1C ≥ 6.5% (HR [95% CI] : 1.74 [0.94-3.25] ; p=0.08). In multivariate analysis, statin use was significantly associated with T2D risk defined by HbA1C ≥ 6.5% only (HR [95% CI] : 1.91 [1.00-3.66] ; p=0.05). In the same multivariate analysis, BMI was more associated with T2D risk than statin-use (HR [95% CI] : 3.26 [1.23-8.64] ; p=0.017). In addition the reversion from IFG to normoglycaemia (i.e. FPG < 5.5 mmol/L) was significantly less frequent in statin (13.9%) vs non-statin users (23.9%) (OR [95% CI] : 0.51 [0.29-0,91] ; p=0.021).

Conclusion: Statin use was associated with an increased risk of T2D in patients with prediabetes in a real-life setting. This effect seemed to occur independently of BMI, at least when conversion to T2D was defined by HbA1C ≥ 6.5% .

Clinical Trial Registration Number: NCT01218061
Supported by: OSEO

Disclosure: **S. Smati:** None.

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Determinants of glucagon secretion in prediabetes and recently diagnosed type 2 diabetes: an IMI-DIRECT study

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Background and aims: Patients with type 2 diabetes exhibit abnormal regulation of glucagon secretion. Compared to healthy volunteers, individuals with T2D have shown to have unaltered, reduced or upregulated glucagon response following OGTT/MMT indicating variation among subsets of T2D populations. Part of the inconsistency in study findings could be due to differences in study populations that cannot be sufficiently accounted for in small studies. Therefore large studies are required. We aimed to investigate determinants of glucagon response in a large cross-sectional study consisting those high risk for diabetes and recently diagnosed T2D individuals from the IMI-DIRECT project.
Materials and methods: Fasting and 60 minute glucagon concentrations following an oral glucose or mixed meal tests were assessed in individuals with normal glucose tolerance (NGT, n=1551), isolated impaired fasting glucose tolerance (i_IFG = 339), isolated impaired 2hour glucose tolerance (i_IGT = 179), impaired fasting and 2hour glucose tolerance (IFG&IGT = 110), screen-detected type 2 diabetes (sd_T2D, 89), and clinically diagnosed type 2 diabetes (DM, 836). Multiple linear regression model was carried out using log-transformed glucagon measures, with adjustments for age, sex, BMI, glycaemic status, oral glucose insulin sensitivity (OGIS) and center. Post OGTT or MMT, the 60 minute glucagon is also adjusted for baseline glucagon. All results represent the beta for the covariate adjusted for all other covariates.
Results: Compared to individuals with NGT there was higher fasting glucagon with worsening glycaemia (i_IGT (+5.5%, p=0.03), sd_T2D (+8.5%, p=0.02), DM (+8.9%, p<0.001). In those high risk for diabetes, IFG&IGT (+6.4%, p=0.01) and sd_T2D (+6.4%, p=0.03) had higher 60 minute glucagon concentration (adjusted for baseline glucagon) post OGTT than NGT. Older age was associated with lower fasting glucagon (-0.31% per year, p<0.001). Being male (+5.8%, p<0.001) and higher BMI (+0.9%, p<0.001) were associated with higher fasting glucagon concentration in both high risk and DM. In the high risk group, men had lower 60 minute glucagon concentration post OGTT than women (-5.4%, p < 0.009). Fasting glucagon is inversely correlated with insulin sensitivity (OGIS) in both high risk for diabetes ($r^2 = 0.23$, $p < 0.001$) and clinically diagnosed type 2 diabetes groups ($r^2 = 0.13$, $p < 0.001$). In patients with diabetes, metformin use was not significantly associated with either fasting or 60 minute glucagon concentration.
Conclusion: This finding indicates that dysregulated glucagon secretion could occur already before the development of diabetes and is most pronounced in men. Age, sex, BMI and insulin sensitivity are strong determinants of glucagon secretion in prediabetes and clinically diagnosed T2D.
Supported by: IMI-JU Grant 115317 (DIRECT) funded by EU FP7 and EFPIA
Disclosure: **A.Y. Dawed:** None.

PS 007 It's all about HbA_{1c}

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Survival as a function of HbA_{1c} in people with type 2 diabetes using differing glucose-lowering regimens

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Background and aims: Observational studies have demonstrated a U- or J-shaped association between glucose control and all-cause mortality. Hypoglycaemia represents one possible explanation for this pattern of association. Our purpose was to characterise survival as a function of HbA1c in those treated with six glucose-lowering regimens that are differentially associated with hypoglycaemia risk.
Materials and methods: Retrospective data were available from ≈7% of the UK population. Patients with type 2 diabetes treated with one of the following glucose-lowering regimens for >90 days were selected: metformin monotherapy (n=93,915); sulfonylurea monotherapy (n=23,075); insulin monotherapy (n=10,891); a composite group of regimens with low hypoglycaemia risk (metformin monotherapy or acarbose, DPP-4 inhibitors, GLP-1 agonists, SGLT-2 inhibitors or thiazolidinediones as monotherapy or in combination with metformin, n=129,198); a composite of regimens with higher hypoglycaemia risk but excluding insulin (meglitinides or sulfonylureas as monotherapy or in combination with metformin, n=65,985) and a composite of regimens with higher hypoglycaemia risk including insulin (meglitinides, sulfonylureas or insulin as monotherapy or in combination with metformin, n=88,743). Risk of all-cause mortality was evaluated using a multivariable Cox proportional hazards model, introducing mean HbA1c as a quarterly updated, time-dependent variable. Findings were determined by prespecified HbA1c categories and by deciles of HbA1c in within-group comparisons.
Results: There were 6,646 deaths, with total follow-up of 374,591 years. Lower (<7%), high (≥8·5%<9.5%) and very high (≥9.5%) HbA1c were associated with increased mortality, compared with moderate HbA1c (≥7%≤8·5%): adjusted hazard ratios (aHR) 1·12 (95%CI 1·06−1·18), 1·18 (1·07−1·32) and 1·16 (1·03−1·32), respectively. However, survival for lower versus moderate HbA1c differed by glucose-lowering regimen: metformin, aHR=1·03 (0·95−1·12); sulfonylurea, 1·11 (0·99−1·25); insulin, 1·47 (1·25−1·72); low-hypoglycaemia-risk regimens, 1·02 (0·94−1·10); higher-risk regimens excluding insulin, 1·24 (1·13−1·35); and higher-risk regimens including insulin, 1·28 (1·18−1·37). When analyzed by HbA1c decile, there existed further revealing structure in these data. In all six regimens, there was an increased association with all-cause mortality in the lowest decile (6.04%-6.25%) compared with the nadir (7.15-7.27%), where the aHR ranged from 1·50 (95%CI 1·19-1·88) to 2·30 (1·63-3·24). By comparison with higher deciles of HbA1c, lower deciles of HbA1c were associated with a higher risk of mortality in regimens with a higher risk of hypoglycaemia.
Conclusion: Lower HbA1c levels were associated with elevated mortality risk versus moderate control; however, the pattern of association differed by regimen. Only insulin monotherapy and composite regimens that included drugs with a higher risk of hypoglycaemia were associated with elevated risk in the pre-specified lower versus moderate HbA1c categories. However, *post-hoc* analysis using deciles of HbA1c revealed elevated risk of all-cause mortality in the lowest decile of HbA1c for all regimens investigated. The possibility of residual confounding should be considered when interpreting these results.
Supported by: Merck Sharpe & Dohme
Disclosure: **C.J. Currie:** Employment/Consultancy; CC is an employee of Cardiff University; SEH, DW, EB, SJ-J are employees of Pharmatelligence; BV, SR, BA and SSE are all employees of Merck & Co. Grants; Merck Sharpe & Dohme (payment to Pharmatelligence). Stock/Shareholding; CC is the director of Pharmatelligence; BV, SR, BA and SSE are stock/shareholders of Merck & Co.

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HbA_{1c} and second line glucose lowering drug initiation in Denmark, Norway and Sweden: an observational study comparing type 2 diabetes management in primary care

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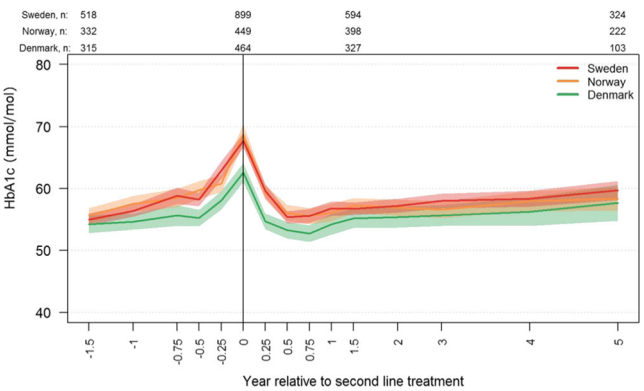
Background and aims: Second line treatment with glucose lowering drugs (GLD) is an important part of type 2 diabetes (T2DM) management and guidelines argue for early intervention if HbA1c is unsatisfactory. Real world data on HbA1c, choice of second line drug and follow up is scarce. The aim of this study was to describe HbA1c and initiation of second-line treatment in primary care using electronic medical records (EMR) data from Denmark (DK), Norway (NO) and Sweden (SWE).
Materials and methods: EMR data were extracted from 60 primary care clinics in DK, NO and SWE including all patients having a T2DM diagnosis and/or prescription of any GLDs between 2005 and 2015. Patient characteristics, laboratory data, and prescribed drugs were used in the analyses. First line mono-metformin patients were included at second line initiation (index date), defined as either an add-on or switch. Baseline data is defined as last available data prior to index date within a 3 month period. Data at year one was the minimum value during the year following index. The figure displays the mean value of HbA1c with 95% confidence intervals for the last available value in each time period. Correlation of repeated measurements within participants not accounted for.
Results: Of the 3,427 patients; 690, 660 and 2077 were identified in DK, NO and SWE respectively, with mean age 61.0, 61.0 and 61.8 years, and proportion of males 60.3, 60.5 and 59.4%. Baseline HbA1c was 63.0, 68.7 and 68.0 mmol/mol (p<0.01), followed by a decrease during the first year of -11.1, -15.3 and -14.6 mmol/mol (p<0.01) respectively (Figure). HbA1c maintained at similar levels during the following 4 years. Corresponding changes for systolic blood pressure and LDL were -6.8, -11.6 and -9.2 mmHg (p=0.27), and -0.34, -0.41, and -0.22 mmol/l (p=0.09), respectively. In 2015, the most common second-line drugs (mono- or in combination) were insulin (13, 10, 26%), SU (18, 18, 29%), DPP-4i (54, 58, 33%), GLP1-RA (8, 5 and 5%) and SGLT2i (6, 10 and 3%) for DK, NO and SWE, respectively (p<0.01).
Conclusion: Patients initiated on second line treatment had similar characteristics in DK, NO and SWE. However, in DK patients were initiated at lower HbA1c, which may indicate a more proactive disease management. Newer classes of GLDs were more commonly used in DK and NO compared to SWE that also had a substantially higher use of insulin and SU.



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Effect of diabetes and of therapy with statins on the hospitalisation risk for infections: a population study

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Background and aims: Diabetic patients are at increased risk of infections and whether use of statins can reduce such a risk is still open to discussion. To answer this question we carried out an observational cohort study referring to the whole population living in Tuscany, a region of centre of Italy retrospectively followed up along a five years' period (from December 31, 2010 to December 31, 2015) comparing the effect of prevailing statins' use between diabetic and non diabetic people, as to the risk of first hospitalisation for pneumonia (PNEU), or, separately, due to any bacterial infections including pneumonia (BI).
Materials and methods: The database used for this investigation was obtained from linking four administrative datasets: a) the registry of all recipients of regional health system resident in Tuscany and alive on December 31, 2010 (n=3,663,415); b) the regional hospital admissions' dataset containing first ever hospital discharges with main ICD-CM code of PNEU, or of any BI: (PNEU, or urosepsis, osteomyelitis, cellulitis, bacterial meningitis, sinusitis, otitis media/externa, septicemia/bacteremia, abscess; c): the dataset of diabetic population identified by the regional diabetes registry or by hospital discharge with ICD-9-CM code: 250.xx (n=192,375). Finally, the baseline exposure to statin therapy was determined by d): the regional dataset of drug prescriptions. Effect of diabetes and or of statin use on hospitalization for PNEU or BI were measured according to a Cox regression analysis model, after adjusting for age, diabetes therapy, Charlson index, and hospitalisations for cardiovascular diseases (CVD) prior to December 31, 2010.
Results: During the 5yr period we observed 30,597 hospitalisations for PNEU with 1.43(1.42-1.45)x1000p-yr in non diabetics and 6.02(5.86-6.18)x1000p-yr in diabetics, and 44,751 for BI with 2.08(2.06-2.10)x1000p-yr in non diabetics and 9.13(8.94-9.32)x1000p-yr in diabetics. Presence of diabetes conferred a greater risk for both PNEU and BI hospitalisations: [HR (95%CI): 1.48 (1.43-1.53) and respectively 1.64 (1.60-1.68); p<0.0001 for both]. Prevailing statin therapy had a protective effect on PNEU or BI hospitalisations either in non diabetic [(HR: 0.76(0.73-0.80) and 0.77(0.74-080); p<0.0001 for both)] or diabetic subjects [(0.77(0.72-0.81) and 0.75(0.71-0.78); p<0.0001 for both)]. Previous hospitalisation for CVD significantly increased the protective effect associated to statins in non diabetic population [(HR: 0.69 (0.65-0.74), in CVD+ vs. 0.93 (0.89-0.97) in CVD-; p<0.05 for difference between groups)],while the protective effect was independent of prior hospitalisation for CVD in diabetic subjects [(0.74 (0.69-0.78) in CVD+ vs. 0.76 (0.70-0.83) in CVD-; p=NS for difference between groups)]. Data were confirmed after matching exposure to statins in CVD- and CVD+ subjects by use of a propensity score.
Conclusion: In this cohort of Tuscan population diabetes increased the 5-yr-adjusted-risk of PNEU and BI by about 50%. Prevailing statin therapy was equally risk protective in diabetic subjects with or without prior hospitalisation for CVD, contrarily to non diabetic population in which statin-associated protection was significantly more evident in subjects with prior hospitalisation for CVD.
Disclosure: **G. Seghieri:** None.

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Hypoglycaemia and glycaemic variability are associated with survival rate after admission in hospitalised terminal cancer patients with diabetes

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Background and aims: Diabetes has been reported to be associated with various cancer risks and mortality. Low HbA1c levels have extended survival time significantly in terminal cancer patients with diabetes mellitus. Hypoglycaemia and glycaemic variability (GV) are associated with mortality in critically ill hospitalized patients; hence, we speculate that less GV is desirable in terminal cancer patients. White blood cell (WBC), C-reactive protein (CRP), Albumin (Alb), hemoglobin (Hb), lactate dehydrogenase (LDH), and blood urea nitrogen (BUN) have been generally reported as prognostic factors for cancer. In addition to these factors, GV may be associated with prognosis in terminal cancer patients. In this study, we investigated the relationship of survival rate after admission (survival rate) with prognostic factors for cancer and glycaemic control in hospitalized terminal cancer patients with diabetes mellitus who underwent interventions for glycaemic control.
Materials and methods: We retrospectively analysed 496 hospitalized terminal cancer patients with diabetes mellitus who underwent glucose monitoring >3 times per day from 2009 to 2015. We evaluated the survival rate within 90 days after admission. We extracted data regarding age, sex, BMI, HbA1c, prognostic factors for cancer on day 1 of hospital stay, and change in prognostic factors for cancer (day of discharge from hospital − day 1 of hospital stay). We evaluated the presence of underlying etiology of cancer besides diabetes (underlying etiology). Hypoglycaemia was defined as a blood glucose level <70 mg/dL on any test performed in the hospital. Hypoglycaemia, GV (standard deviation [SD], coefficient of variation [CV] and average daily risk range [ADRR]), and mean glucose concentrations were determined from all the glycaemic data collected during the entire hospital stay. We analysed the association of these factors with survival rate by using a Cox proportional hazards model.
Results: We found a mortality rate of 38.3%. In the univariate analysis, neither age, sex, BMI, HbA1c, underlying etiology, nor any of the prognostic factors for cancer were associated with survival rate. However, hypoglycaemia, ADRR, CV, SD, and mean glucose concentrations showed a significant association. After stepwise multivariate adjustment, hypoglycaemia, ADRR, CV, SD, and mean glucose concentrations were independently significantly associated with survival rate (hazard ratio [HR] 1.51 (95% confidence interval [CI] 1.06-2.14) p=0.02, HR 1.04 (95% CI 1.00-1.07) p=0.04, HR 1.11 (95% CI 1.04-1.19) p=0.002, HR 1.04 (95% CI 1.01-1.07) p=0.01, and HR 1.03 (95% CI 1.02-1.04) p<0.0001, respectively).
Conclusion: Glycaemic control (especially hypoglycaemia and GV), rather than prognostic factors for cancer, is associated with survival rate in hospitalized terminal cancer patients with diabetes mellitus.

Univariate / Stepwise multivariate	Survival rate after admission		Univariate	Survival rate after admission		Univariate	Survival rate after admission	
Variable	HR (95% CI)	p	Variable	HR (95% CI)	p	Variable	HR (95% CI)	p
Mean glucose level, mg/dL	1.01 (1.01–1.01) / 1.03 (1.02–1.04)	<0.0001 / <0.0001	WBC, mg/dL	1.00 (1.00–1.00)	0.0501	Change in WBC, mg/dL	1.00 (1.00–1.00)	0.2
SD, mg/dL	1.02 (1.01–1.02) / 1.04 (1.01–1.07)	<0.0001 / 0.01	CRP, mg/dL	0.99 (0.96–1.03)	0.7	Change in CRP, mg/dL	1.01 (0.97–1.05)	0.58
CV, %	1.04 (1.03–1.06) / 1.11 (1.04–1.19)	<0.0001 / 0.002	Alb, g/dl	0.97 (0.78–1.21)	0.8	Change in Alb, g/dl	1.23 (0.88–1.74)	0.23
ADRR	1.04 (1.03–1.05) / 1.04 (1.00–1.07)	<0.0001 / 0.04	Hb, g/dl	0.95 (0.89–1.02)	0.15	Change in Hb, g/dl	1.00 (0.98–1.02)	0.9
Hypoglycemia, n (%)	1.56 (1.17–2.09) / 1.51 (1.06–2.14)	0.003 / 0.02	LDH, IU/L	1.00 (1.00–1.00)	0.74	Change in LDH, IU/L	1.00 (1.00–1.00)	0.5
			BUN, mg/dL	1.00 (0.99–1.01)	0.88	Change in BUN, mg/dL	1.01 (0.99–1.02)	0.25
			BT, ° C	1.32 (0.99–1.75)	0.06	Change in BT, ° C	0.91 (0.75–1.11)	0.35

Clinical Trial Registration Number: UMIN000020729
Disclosure: **S. Takeishi:** None.

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Rates of hospital admission for hypoglycaemia by glucose-lowering regimen and by glycated haemoglobin status in people with type 2 diabetes

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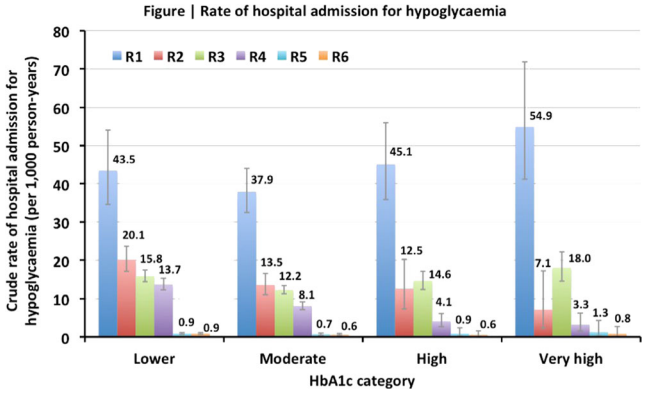
Background and aims: Different glucose-lowering regimens are associated with differing rates of hypoglycaemia. The purpose of this study was to select, *a priori*, common glucose-lowering regimens and determine the rate of admission to hospital for hypoglycaemia by regimen and, within regimens, by differing levels of HbA1c.
Materials and methods: This was a retrospective study using primary-care data from the UK Clinical Practice Research Datalink linked to Hospital Episode Statistics (HES). We selected HES-eligible patients with type 2 diabetes treated with one of the following glucose-lowering regimens for more than 90 days: insulin monotherapy (R1, n=4,107); sulfonylurea monotherapy (R2, n=8,738); a composite of regimens with higher hypoglycaemia risk including insulin (meglitinides, sulfonylureas and insulin as monotherapy or in combination with metformin, R3, n=39,361); a composite of regimens with higher hypoglycaemia risk but excluding insulin (meglitinides or sulfonylureas as monotherapy or in combination with metformin, R4, n=30,036); metformin monotherapy (R5, n=46,064); and a composite of regimens with low hypoglycaemia risk (metformin monotherapy or acarbose, DPP-4 inhibitors, GLP-1 agonists, SGLT-2 inhibitors and thiazolidinediones as monotherapy or in combination with metformin, R6, n=60,131). Index date was defined as the start of the regimen of interest plus 91 days. Inpatient admissions for hypoglycaemia were identified from relevant primary- or secondary ICD-10 codes. The crude incidence rate of hospital admission for hypoglycaemia was calculated by regimen and mean HbA1c over the follow-up. HbA1c categories were defined as: lower (less than 7%), moderate (greater than or = 7% and less than or = 8·5%), high (greater than 8·5% and less than or = 9.5%) and very high (greater than 9.5%).
Results: There were 1,195 admissions relating to hypoglycaemia. The total numbers of events for each regimen were as follows: R1, 368; R2, 253; R3, 1,101; R4, 559; R5, 84; and R6, 94. The corresponding total follow-up times in years were: R1, 8,718; R2, 15,541; R3, 78,454; R4, 57,871; R5, 100,146; and R6, 124,870. These translated into the following unadjusted rates of hypoglycaemia per 1,000 person-years: R1, 42.2 (95%CI 38.1-46.7); R2, 16.3 (14.4-18.4); R3, 14.0 (13.2-14.9); R4, 9.7 (8.9-10.5); R5, 0.8 (0.7-1.0); and R6, 0.8 (0.6-0.9). Within the glucose-lowering regimens, rates of hypoglycaemia varied by HbA1c category (Figure).
Conclusion: Admission rates for hypoglycaemia varied by both regimen and by glucose control. The empiric pattern of hypoglycaemia rates for the various regimens matched *a priori* expectations. In people exposed to insulin resulting in high, very high and lower HbA1c were all associated with increased risk of hypoglycaemia (versus moderate HbA1c). In people exposed to any secretagogue, only those with lower HbA1c had an increased risk of hypoglycaemia.



Disclosure: **S.E. Holden:** Employment/Consultancy; SEH and SJ-J are employed by Pharmatelligence. Stock/Shareholding; CJC is a Director of Pharmatelligence.

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Effect of beta thalassaemia trait on haemoglobin A_{1c} levels in individuals without diabetes

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Background and aims: Glycosylated Hemoglobin A1c (HbA1c) has been used for monitoring individuals with diabetes mellitus for decades and, since 2010, it has been introduced as a novel criterion for the diagnosis of the disease. HbA1c levels, however, are influenced by a number of factors other than glycaemia, predominantly those affecting red blood cell (RBC) production and/or lifespan, rendering its value unreliable as a means of diagnosing and monitoring diabetes mellitus under certain circumstances. Beta thalassemia trait is a benign hereditary hemoglobinopathy characterized by mild chronic hemolysis, with a global distribution and a high prevalence among certain Mediterranean areas, including Greece (mean frequency approximately 7.4%). Affected individuals have mild to moderate anemia owing to ineffective erythropoiesis and slightly diminished RBC lifespan. The association between beta thalassemia trait and HbA1c levels has not been previously investigated in detail. The aim of the present study is to determine whether the presence of beta thalassemia trait affects HbA1c levels in a population without diabetes.
Materials and methods: We examined two groups of individuals matched for age and gender, one consisting of carriers of the beta thalassemia trait and the other of normal controls. Heterozygosity for beta thalassemia in the first group was confirmed via hemoglobin electrophoresis. Diabetes mellitus, hemoglobinopathies other than beta thalassemia trait, pregnancy of any age, major surgery or blood donation during the previous 3 months, renal disease and any other condition potentially affecting hemopoiesis were considered as exclusion criteria. In all participants fasting plasma glucose (FPG), fructosamine, ferritin, hsCRP and HbA1c levels were measured, the latter using turbidimetric inhibition immunoassay. Statistical analysis was carried out using SPSS statistics version 20.
Results: A total of 162 individuals were examined, 92 carrying the beta thalassemia trait and 70 controls with normal erythrocyte indices. The two groups were comparable in terms of age, gender, BMI and first degree family history of diabetes. Mean FPG and fructosamine did not differ between the two groups (85.8 vs. 86.0 mg/dl, p=0.91 and 220.7 vs. 222.9mg/dl, p=0.45, respectively). Mean HbA1c value was also similar between the two groups (5.22% vs. 5.16%, p=0.13), even after adjusting for possible confounders. However, within the beta thalassemia group, there was a statistically significant positive correlation between HbA1c and hemoglobin levels (r=0,382, p<0,001), which was independent of glucose and fructosamine levels or other possible confounders, including ferritin levels. In the same group, individuals in the lower quartile of descending hemoglobin levels, had significantly lower HbA1c than those in the highest one (5.08% vs. 5.37%, p=0.002). The correlation between HbA1c and hemoglobin was not found within the control group.
Conclusion: Individuals with beta thalassemia trait have similar fasting plasma glucose, fructosamine and HbA1c levels to controls. Within the thalassemic group, however, lower hemoglobin concentration is associated with lower HbA1c. This should be taken into account when using HbA1c as a diagnostic criterion for diabetes in individuals with the beta thalassemia trait.
Disclosure: **K. Makrilakis:** None.

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Is the association between glycaemia and incident cardiovascular disease modified by haemoglobin levels?

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Background and aims: Recent evidence indicating that the association between fasting blood glucose and HbA_{1c} is weaker at lower haemoglobin (Hb) levels has been used to question the validity of HbA_{1c} as a diagnostic tool for diabetes, especially in people with altered Hb metabolism. However, the interaction between Hb and HbA_{1c} has not been studied in relation to cardiovascular disease (CVD), despite indications that iron metabolism is implicated in the pathogenesis of both diabetes and CVD. Our aim was to examine whether the association between baseline HbA_{1c} levels and the risk of incident CVD is modified by Hb levels in the English Longitudinal Study of Ageing (ELSA).
Materials and methods: We studied 2,133 women and 1,636 men who participated in wave 0 of the ELSA cohort (1998-2001), had no history of CVD and had valid data for Hb, HbA_{1c} and BMI at baseline and at least one follow-up visit. We fitted Cox proportional hazard models for the association between baseline HbA_{1c}, Hb, their multiplicative interaction term and the risk of incident CVD, stratifying by sex and adjusting for age and BMI.
Results: The female and male participants were aged 64.9 years (SD: 9.2) / 64.2 years (8.6) and had a BMI of 27.7 kg/m^2 (5.1) / 27.5 kg/m^2 (4.0) respectively. During a median follow-up duration of 7.7 years, 352 and 307 CVD events were observed in women and men respectively (crude incidence rates: 26.0 and 30.4 per 1,000 person years respectively). Hb levels were not associated with the risk of incident CVD at a statistically significant level in either sex (HR: 1.05 (95%CI: 0.95;1.15) per g/dL Hb in women and 1.02 (0.93;1.11) in men in age and BMI adjusted models). HbA_{1c} was associated with elevated CVD risk in both sexes (HR: 2.93 (2.37;3.63) per % HbA_{1c} in women and 1.82 (1.64;2.02) in men in age and BMI adjusted models). In unadjusted models, there was an indication that for men the association between HbA_{1c} and CVD risk was progressively less pronounced with higher Hb levels (p_{int}: 0.017). However, this interaction term lost statistical significance on adjustment for age and BMI. There was no indication of interaction between Hb and HbA_{1c} in women.
Conclusion: We have shown that in a large and ageing cohort, Hb levels do not modify the association between glycaemia and incident CVD beyond the confounding impact of age and BMI. We confirm a strong association between HbA_{1c} levels and CVD risk in both men and women and show that this association holds at all Hb levels in a general, ageing, European population.
Disclosure: **D.R. Witte:** None.

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Longitudinal changes of insulin resistance and beta cell function in relation to glucose regulation status in Koreans with prediabetes

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Background and aims: Both insulin resistance and beta-cell dysfunction are known to be associated with development of type 2 diabetes.

Prediabetes is a high-risk state for diabetes development, but the relative contributions of these two factors in the progression to type 2 diabetes are not fully understood yet and may differ by ethnicity or degree of obesity. The aim of this study was to investigate the changes of insulin resistance and insulin secretion over time and their association with the changes in glucose regulation status in Korean individuals with prediabetes.

Materials and methods: We screened clinical and laboratory data of 17,971 Korean adults (age 20-79 years, 39% women) who underwent routine medical check-ups in 2007-08 (baseline) and again in 2012-13 (follow up) with a mean 5-year (range 4.1-5.9 years) interval. Prediabetes was defined as fasting plasma glucose (FPG) 5.6-6.9 mmol/l or HbA1c 5.7-6.4% (39-46 mmol/mol). Among them, 7208 (40.1%) individuals diagnosed as prediabetes (3544 by FPG criteria only, 1713 by HbA1c criteria only, and 1951 by both criteria) at baseline were included for the analysis. Insulin resistance (HOMA-IR) and beta-cell function (HOMA-%B) indices at baseline and at follow up were assessed by homeostasis model assessment. Incident diabetes was defined as FPG ≥7.0 mmol/l, HbA1c ≥6.5% (48 mmol/mol), or initiation of antidiabetic medications.

Results: Among the 7208 (5056 men and 2152 [30%] women, age 50.6 ±8.2 years) participants who had prediabetes at baseline, 4410 (61.2%) remained as prediabetes, 2123 (29.5%) reverted to normal glucose regulation, and 675 (9.4%) progressed to type 2 diabetes after 5 years. Compared with participants remained as prediabetes, those who progressed to diabetes had significantly higher baseline HOMA-IR (2.48±1.45 vs. 2.06±1.20, P <0.001), but similar baseline HOMA-%B (74.6±47.6 vs. 73.1±41.4, P >0.05). By contrast, those who reverted to normal glucose regulation had significantly lower HOMA-IR (1.98±1.14 vs. 2.06±1.20, P=0.035), but higher HOMA-%B (77.4±43.1 vs. 73.1 ±41.4, P=0.001) at baseline compared with those who remained as prediabetes. After 5 years, participants who progressed to diabetes showed a mean 31% increase in HOMA-IR (2.48±1.45 vs. 3.24±2.10, P<0.001) and a mean 15% decrease in HOMA-%B (74.6±47.6 vs. 63.8±40.4, P<0.001) compared to baseline. By contrast, those who reverted to normal glucose regulation showed a mean 29% decrease in HOMA-IR (1.98 ±1.14 vs. 1.41±0.78, P<0.001) and a mean 4% increase in HOMA-%B (77.4±43.1 vs. 80.2±47.9, P=0.010). Participants who remained as prediabetes showed no significant change in HOMA-IR (2.06±1.20 vs. 2.02 ±1.17, P>0.05) and HOMA-%B (73.1±41.4 vs. 72.7±42.3, P>0.05) during the 5-year period.

Conclusion: These results showed that both increase of insulin resistance and decrease of beta-cell function contribute to progression to type 2 diabetes from prediabetes in Koreans. However, longitudinal changes in insulin resistance were the predominant factor associated with progression to type 2 diabetes or regression to normal glucose regulation from prediabetes. Our findings suggest that development of effective measures improving insulin resistance would be important for prevention of type 2 diabetes.

Disclosure: **C. Kim:** None.

PS 008 Biomarkers: from blood to P value

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Novel biomarkers predicting a microvascular composite outcome in people with dysglycaemia in the ORIGIN trial

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Background and aims: Novel biomarkers may identify people at high risk for microvascular outcomes.

Materials and methods: One ml of stored serum from 8401 ORIGIN participants was blindly assayed for 237 novel biomarkers. During a median follow-up of 6.2 years, 1794 of these individuals developed a composite microvascular outcome defined as the first occurrence of either progression of albuminuria (from normoalbuminuria to either microalbuminuria or clinical proteinuria, or from microalbuminuria to clinical proteinuria), a doubling (or greater increase) of serum creatinine from baseline, renal replacement therapy, or diabetic retinopathy requiring photocoagulation or vitrectomy. A Cox regression model using a forward selection approach was used to identify the subset of assayed biomarkers that independently predicted the MV outcome after accounting for 8 clinical risk factors for MV outcomes (sex, age group, prior CV event, albuminuria, smoking, established diabetes, LDL/HDL ratio, and hypertension).

Results: A Bonferroni-corrected P value for inclusion in the model of <0.00021 (i.e. 0.05/237) identified 6 significant biomarkers that included: a) MMP-7 (HR 1.22); b) IGF-BP2 (HR 1.14); c) alpha-1-microglobulin (HR1.15); d) gelsolin (HR 0.91); e) angiopoietin-2 (HR 1.11); and f) interleukin 12 subunit p40 (HR 0.94). Compared to the clinical model, the C statistic (95%CI) improved from 0.619 (0.605, 0.634) to 0.660 (0.646, 0.674), and the net reclassification index (95%CI) was 0.177 (0.114, 0.243). When the model was rerun after also accounting for baseline serum creatinine as a 9th clinical risk factor for microvascular outcomes, gelsolin was no longer significant and a new biomarker, growth/differentiation factor 15 (HR 1.13) became significant. This biomarker and angiopoietin-2 were also independent predictors of cardiovascular outcomes.

Conclusion: Novel biomarkers can therefore help identify dysglycemic people who are at risk for future MV outcomes and are potential therapeutic targets.

Clinical Trial Registration Number: NCT00069784
Supported by: Sanofi-Aventis

Disclosure: **S. Hess:** Employment/Consultancy; Sanofi-Aventis Deutschland GmbH.

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Profiling of circulating microRNAs in children with recent onset of type 1 diabetes

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Background and aims: Type 1 Diabetes (T1D) is an autoimmune disease that is largely silent in its initial stages until the majority of beta cells are destroyed. There is an unmet clinical need for cost-effective, reliable biomarkers to predict and diagnose early diabetes-onset. Micro-RNAs (miRNAs) are found at high concentrations in body fluids and the potential of circulating miRNAs to serve as biomarkers of various disease conditions is currently being explored given their amenability for rapid,

sensitive and specific detection and quantification. We sought to identify a miRNA signature unique to recent-onset of T1D that could potentially serve as a biomarker to diagnose early diabetes onset and monitor disease progression.

Materials and methods: We used a Locked-Nucleic-Acid (LNA) based qPCR profiling platform to screen 745 miRNAs in the sera of ten children with recent-onset of diabetes (31.30±2.44 days after diagnosis) and seven age-matched controls. Levels of miRNAs with greatest fold differences ($p \leq 0.05$) were measured by qPCR using LNA-enhanced primers from children with different duration of T1D.

Results: Thirty-five miRNAs were significantly different ($p \leq 0.05$) between the controls and children with recent-onset of T1D, 27 of which were elevated. Multivariate analysis using partial least squares supervised classification method revealed 4 specific miRNAs stratifying recent-onset of T1D. miR-454, miR-222, miR-144* and miR-345 were the top four miRNAs with the highest variable of importance scores. The area under the Receiver Operating Characteristic (ROC) curves for miR-454, miR-222, miR-144* and miR-345 were 1, 0.97, 0.99 and 0.99, respectively. Interestingly, levels of these miRNAs were comparable to controls in the later stages of diabetes (6-12 months, 12-24 months, >24 months). To identify potential biological functions impacted by these miRNAs, we performed in silico pathway analysis based on the inferred miRNA target genes, using the DIANA-miRPath v2.0 web server. Interestingly, glycosaminoglycan biosynthesis was the statistically most targeted pathway among others, such as PI3K-Akt, MAPK and Wnt signaling pathways. We also analyzed the correlation between HbA1c and miRNA levels using Pearson`s r. Among the 35 dysregulated miRNAs, 28 miRNAs were not correlated with HbA1c levels, indicating specificity of these miRNAs to T1D rather than hyperglycemia. There were 134 other miRNAs that significantly correlated ($p \leq 0.05$) with HbA1c levels; 18% negatively and 72% positively correlated.

Conclusion: In conclusion, our study identifies a serum miRNA pattern of recent-onset diabetes which might lead to a biomarker test to monitor miRNA levels throughout disease progression. Also, signaling pathways identified in this study may provide insight into the mechanisms underlying pathogenesis of T1D.

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Disclosure: **S. Erener:** None.

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Elevated glutamate and branched chain amino acid levels in overweight/obese individuals: links to insulin resistance and visceral adiposity

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Background and aims: Several studies have examined the plasma amino acid profile of overweight/obesity in humans to date. In these analyses, branched chain amino acids (BCAAs) and glutamate have repeatedly been found to be elevated but it remains unclear what is really driving these alterations. The three options discussed are associations of elevated amino acid levels with higher quantity of adipose tissue in overweight/obese individuals, specifically with visceral adiposity, or primarily with insulin resistance (IR). The aim of this study was to distinguish between these concepts by analysing a deeply phenotyped, homogenous cohort of human subjects.

Materials and methods: In a cross-sectional analysis, anthropometric and clinical chemistry data, medical history, a 5-point oGTT and fasted plasma samples were collected from 151 young women from the PPSDiab-Study (age 35.6±3.9 years; BMI 25.4±5.7 kg/m2; recruited 2011-2014). Plasma amino acids (Ala, Arg, Asn, Asp, Cit, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Orn, Phe, Pro, Ser, Thr, Trp, Tyr, Val) and related biogenic amines (acetylornithin, asymmetric dimethylarginine, α-aminoadipic acid, kynurenine, putrescine, serotonin, taurine) were quantified using targeted metabolomics (Biocrates AbsolutIDQ p180 Kit). Subcutaneous and visceral fat was measured in a subgroup of 86 women using MRI. Statistical analysis was conducted using Mann-Whitney U tests with Bonferroni correction for multiple testing and multivariate linear regression models. BMI and ISI (the Matsuda index of insulin sensitivity) were logarithmized for the linear models.

Results: The plasma levels of glutamate ($p<0.00001$) and the BCAAs leucine, isoleucine and valine ($p=0.00034$, $p=0.00040$ and $p=0.00057$, respectively) were significantly elevated in overweight/obese women (BMI ≥ 25 kg/m2), confirming previous studies. Glycine ($p=0.00075$) was reduced and α-aminoadipic acid ($p=0.00006$) as well as tyrosine ($p=0.00010$) were elevated. Multivariate linear regression analyses with BMI and ISI as independent variables showed that glutamate was associated with a low ISI (standardized regression coefficient (β)= -0.4; $p=0.0003$) and a high BMI (β=0.2; $p=0.02$), whereas the BCAAs were positively associated only with the BMI (β=0.3/$p=0.02$, β=0.2/$p=0.03$, β=0.3/$p=0.008$ for Leu, Ile and Val, respectively), but not with the ISI. In further multivariate regression analyses, the BCAAs were more strongly linked to visceral obesity (waist circumference in the whole study cohort and abdominal visceral fat mass in the MRI subcohort) than to overall adiposity. Glutamate and the BMI predicted ISI with an adjusted R2 of 0.52.

Conclusion: We confirmed that plasma glutamate and BCAAs are elevated in overweight/obese subjects but also found that the primary drivers of these changes probably differ. Whereas IR and overweight/obesity were both independently associated with elevated Glutamate levels, the BCAAs were mainly linked to visceral obesity without a significant contribution of IR. Our findings suggest that these two amino acid changes in overweight/obese individuals are representative of alterations in different metabolic pathways and that fasting plasma glutamate can serve as an additional biomarker for IR.

Disclosure: **C. Hawlitschek:** None.

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Remnant cholesterol predicts the development of type 2 diabetes in patients with established coronary artery disease

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Background and aims: Remnant cholesterol recently has attracted interest as a marker of cardiovascular event risk and is associated with the metabolic syndrome as well as with type 2 diabetes (T2DM). However, it is unknown whether remnant cholesterol also predicts the development of diabetes in patients who do not have diabetes yet.

Materials and methods: We prospectively recorded incident diabetes over 6.1±3.7 years in 861 consecutive non-diabetic Caucasian patients with angiographically proven coronary artery disease (CAD). Diabetes was diagnosed according to ADA criteria.

Results: At baseline, 41.3% of our non-diabetic CAD patients had impaired fasting glucose (IFG); remnant cholesterol was significantly higher in IFG than in NFG patients (23±21 vs. 19±22 mg/dl; $p<0.001$). During follow-up, diabetes was newly diagnosed in 111 patients, i.e. in 12.9% of the study population. Remnant cholesterol strongly predicted diabetes both univariately (OR 1.88 [1.56-2.27]; $p<0.001$) and after multivariate adjustment including both fasting glucose and HbA1c values (OR 1.40 [1.40-2.11]; $p<0.001$).

Conclusion: We conclude that the incidence of diabetes is high in patients with established CAD and that remnant cholesterol strongly and independently predicts the development of diabetes in this population.

Disclosure: **H. Drexel:** None.

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Serum uromodulin is associated with impaired glucose metabolism

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Background and aims: Uromodulin is the most abundant urine protein under physiological conditions. It has recently been described as a serum marker of kidney disease. Whether uromodulin also is associated with impaired glucose metabolism is unknown and addressed in the present study.
Materials and methods: We measured serum uromodulin in a series of 529 patients who were undergoing coronary angiography for the evaluation of established or suspected stable coronary artery disease (CAD); in patients without established diabetes oral glucose tolerance tests were performed. Prospectively, diabetes incidence was recorded over 4 years.
Results: Serum uromodulin was significantly and inversely correlated with fasting plasma glucose (r=-0.158; p<0.001), with plasma glucose 2 hours after an oral 75g glucose challenge (r=-0.144; p=0.002), and with HbA1c (r=-0.103; p=0.018). From our patients 146 (27.6%) had type 2 diabetes. Uromodulin was significantly lower in patients with T2DM han among non-diabetic patients (147.7±69.9 vs.171.4±78.9 ng/ml, p=0.001). Analysis of covariance confirmed that T2DM was an independent determinant of serum uromodulin (F=5.6, p=0.019) after multivariate adjustment including both the glomerular filtration rate and urinary albumin excretion. Prospectively, 21 patients of the initially non-diabetic subjects developed diabetes. Their uromodulin was intermediate (164±67ng/ml) between those who did not develop diabetes and those who already at baseline had diabetes (p for trend over these three categories <0.001).
Conclusion: We conclude that serum uromodulin is significantly associated with impaired glucose metabolism; patients with T2DM have significantly higher levels of uromodulin than non-diabetic subjects.
Disclosure: **A. Muendlein:** None.

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Circulating microvesicles correlate with body composition, plasma lipids and markers for ectopic fat in testosterone deficient type 2 diabetic men

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Background and aims: Low testosterone in men has been associated with the metabolic syndrome (MetSy) and type 2 diabetes (T2D), and testosterone replacement therapy (TRT) shown to improve several components associated with higher T2D risk. The multifunctional receptor CD36 may be involved in dyslipidaemia, insulin resistance and ectopic fat storage in MetSy. We aimed to investigate how plasma levels of specific microvesicle (MV) phenotypes are associated with insulin sensitivity, body composition, plasma lipids and ectopic fat accumulation in the liver and hypothesized that the changes elicited by TRT are reflected in levels of circulating MVs.
Materials and methods: Thirty-nine Caucasian males with T2D and low testosterone levels were assigned to either TRT or placebo (CTRL) groups, subjected to a 24-week treatment regime, and evaluated at baseline and after 24 weeks. MVs were analysed by flow cytometry and defined as lactadherin binding particles within the 0.1-1.0μm gate. MVs of platelet (PMV), monocyte (MMV) and endothelial cell (EMV) origin were identified by cell-specific markers and their expression of CD36 was investigated. Data were analysed by Wilcoxon's s-r test and Spearman's ranked correlation analysis (rho).
Results: Triglycerides correlated with PMVs, CD36+PMVs, EMVs, CD36+EMVs, MMVs and CD36+MMVs (rho=0.37-0.58, p<0.05). Furthermore, indicators of ectopic liver fat, alanine aminotransferase (ALT) and gamma glutamyltransferase (GGT) correlated with PMVs, EMVs, CD36+PMVs and CD36+MMVs (rho=0.33-0.49, p<0.05). Body composition measures were associated with CD36+MMV (rho=0.33-0.35, p<0.05), while insulin sensitivity was not correlated with any of the studied MV phenotypes. No differences in any MV levels were identified between TRT and CTRL at the end of the trial period.
Conclusion: MetSy components were associated with MV phenotypes, in particular CD36+MVs, which may support the involvement of CD36 in MetSy pathogenesis. Although TRT improved body composition measures, levels of MV phenotypes were unaffected, thus refuting our hypothesis.
Disclosure: **J. Botha:** None.

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Betatrophin concentration in first degree relatives of patients with autoimmune diabetes

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Background and aims: Betatrophin was found as a novel stimulator of beta cell proliferation in mice but its role in humans remains to be established. Moreover, there are no data about its potential role in first degree relatives of patients with autoimmune diabetes. The aim of the study was the evaluation of betatrophin concentration in first degree relatives of patients with autoimmune diabetes in relation with anti- islet antibodies concentrations and beta cell function.
Materials and methods: The study was performed in a group of 90 first degree relatives of patients with autoimmune diabetes and 60 healthy individuals. Antibodies against beta cells (GADA, IAA, IA-2A) and serum betatrophin concentrations were measured by ELISA method. HOMAIR and HOMA%B indices were calculated using fasting insulin concentration (EASIA method) and fasting glucose concentration (hexokinase method).
Results: Significantly higher betatrophin concentration (4.8 ± 1.8 ng/ml vs 1.9 ± 0.8 ng/ml,p<0.001) and HOMAIR index (1.16 ± 0.63 vs 0.79 ± 0.34, p<0.002) were found in the first degree relatives in comparison to the controls. HOMA%B was significantly lower in the group of relatives as compared to healthy individuals (92.8 ± 29.1 vs 114.1 ± 47.1, p<0.001). In 31.1% of relatives a positive titer of at least one of anti-islet antibodies was observed. A trend towards higher betatrophin concentrations was found in a subgroup of relatives with positive anti-islet antibodies (Ab[+]) in comparison to the subgroup of relatives without antibodies (Ab[-]). HOMAIR (1.2±0.6 vs 0.8±0.5, p=0.02) was significantly higher and HOMA%B (90.7±31.2 vs 108.8±47.1, p=0.04) was significantly lower in the relatives with Ab(+) as compared to the relatives Ab(-). In the whole group of relatives, betatrophin concentration positively correlated with HOMAIR (r=0.732, p<0.0001) and negatively with HOMA%B (r=-0.431, p<0.0001).
Conclusion: Significantly higher betatrophin concentrations in first degree relatives of patients with autoimmune diabetes can be attributed to the potential role of this cytokine in the pathogenesis of autoimmune diabetes. However, no significant differences in betatrophin concentrations between the subgroups of relatives with and without anti- islet antibodies may suggest no direct role for betatrophin in autoimmune process in beta cells. On the other hand, betatrophin may be used as an indirect marker of insulin resistance or/and impaired beta cell function in first degree relatives of patients with autoimmune diabetes.
Disclosure: **M. Szelachowska:** None.

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Fibroblast Growth Factor-21 (FGF-21) predicts development of diabetes and pioglitazone attenuates the increase in FGF-21 in subjects with IGT: results from the ACTNOW study

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Background and aims: Although Fibroblast Growth Factor-21 (FGF-21) has been shown to reduce glucose and lipid levels in animal models of diabetes, in humans FGF-21 is elevated in obesity and type 2 diabetes suggestive of FGF-21 resistance. We examined the effect of pioglitazone on FGF-21 and the relationship between change in serum FGF-21 and glucose tolerance in subjects with IGT.

Materials and methods: ACT NOW is a randomized double-blind, placebo-controlled study to examine whether pioglitazone (PIO) can prevent/delay development of type 2 diabetes mellitus (T2DM). 602 IGT subjects were randomized to PIO (45 mg/day) or placebo (PLAC) and followed for 2.4 years. From the original cohort we randomly chose 80 IGT subjects; PIO (n=41, 29F/12M, age 53 ± 1.7yrs, BMI 34 ± 0.8kg/m2) and PLAC (n=39, 26F/13M age 49± 1.9yrs, BMI 33±0.8 kg/m2). Indices of insulin secretion and insulin sensitivity (Matsuda index) were derived from the plasma glucose, insulin, and C peptide concentrations during the OGTT before and at study end. Serum FGF 21 was measured by ELISA.

Results: After 2.4 years 13 subjects converted to T2DM; 11 in the PLAC group and 2 treated with PIO ($p<0.005$). There was no difference in serum FGF-21 levels between the placebo and PIO groups at baseline (324±31 vs 357±30 pg/ml, p=ns). However, after 2.4 years, FGF-21 levels in PLAC treated subjects significantly increased (324±31 to 460±52 pg/ml, $p<0.05$) while that in the PIO treated subjects no change was observed (357±30 to 377±30 pg/ml, p=ns). There was no difference in age, BMI, and gender distribution between the groups at baseline. FGF-21 increased from 328±59 to 609±89 pg/ml ($p<0.05$) in individuals who converted to T2DM. However in those who remained IGT (343±52 vs 316± 64 pg/ml, p=ns) or reverted to NGT (366±39 vs 376±49 pg/ml, p=ns) no significant change was observed. At study end plasma FGF-21 progressively increased from NGT to IGT and T2DM ($p <0.05$ for trend). FGF-21 inversely correlated with Matsuda index of insulin sensitivity at baseline ($r=-0.232$, $p=0.02$) and at study end ($r=-0.402$, $p=0.005$).

Conclusion: Increase in FGF-21 predicts development of T2DM and Pioglitazone attenuates this effect. These results suggest the insulin sensitizing of pioglitazone is, in part, mediated by the drug's effect to reduce FGF-21 resistance.

Clinical Trial Registration Number: NCT00220961

Disclosure: **D. Tripathy:** None.

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Assessing the likely function of HNF1A missense variants using CRP and allele frequency data

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Background and aims: HNF1A-MODY is the most common form of monogenic diabetes. We have previously reported that C-reactive protein (CRP) is lower in those with HNF1A-MODY compared to other forms of diabetes. Increasingly large-scale sequencing studies have shown that some of *HNF1A* variants previously reported as causal for MODY are also seen in those with type 2 diabetes (T2D) and in non-diabetic individuals. We hypothesised that the *HNF1A* variant alleles seen only in those with a clinical diagnosis of MODY will have a more deleterious impact on protein function than the *HNF1A* variant alleles associated with milder phenotypes and that CRP levels may be used as a marker of *HNF1A* variant allele functional severity.

Materials and methods: Two hundred and eighty European individuals with a clinical diagnosis of MODY and a missense variant in *HNF1A* were studied and CRP measured using a high-sensitivity assay. *HNF1A* variants were categorised according to whether they were only seen in MODY cases or reported in 17,000 exomes from the T2D-GENES study comprising participants with T2D and non-diabetic controls (http://www.type2diabetesgenetics.org). We also examined whether CRP varied according to the predicted functional effect of the variant allele using Polyphen 2.

Results: There were 70 different missense *HNF1A* variants found in 280 individuals. Most participants (246/280) had variants reported only in MODY cases, 9/280 had variants reported in subjects with T2D only, 19/280 in both T2D and non-diabetic controls and 6/280 in controls only. The median [IQR] CRP was lower if the variants were reported only in MODY cases (0.10 [0.03-0.30] mg/L) compared with the variants found in T2D (0.39 [0.03-1.63] mg/L), T2D and controls (2.20 [0.76-11.54] mg/L) or controls only (0.95 [0.69-1.29] mg/L) ($p=1.34 \times 10^{-9}$). CRP had an AUC of 0.84 for differentiating the group with *HNF1A* variants present only in MODY cases from the groups with variants found in T2D and non-diabetic individuals. CRP was lower if Polyphen 2 predicted that a variant was *probably damaging* (0.11 [0.03-0.30] mg/L, n=252) rather than *possibly damaging* (0.86 [0.30-1.10] mg/L, n=6) or *benign* (1.0 [0.53-2.65] mg/L, n=22) ($p=1.61 \times 10^{-8}$).

Conclusion: In individuals presenting with MODY phenotype and found to have an *HNF1A* missense variant, a low CRP and absence of the variant from the exomes of normoglycaemic individuals and those with T2D may be a useful adjunct when interpreting the disease causality of an *HNF1A* variant allele, especially if the variant is novel or has been assigned an uncertain significance.

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Disclosure: **A. Juszczak:** Grants; Diabetes UK.

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Quality of life assessment in patients with genetic diagnosis of HNF1A-MODY and GCK-MODY

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Background and aims: Individuals with GCK-MODY are characterized by stable mild hyperglycemia that does not require pharmacological treatment. In contrast, HNF1A-MODY patients require pharmacotherapy in order to prevent chronic diabetic complications. We aimed to examine QoL (Quality of Life) in patients with a genetic diagnosis of either HNF1A-MODY or GCK-MODY.
Materials and methods: The study included 80 patients with HNF1A-MODY and 89 GCK gene mutation carriers. Diabetes-specific QoL was assessed using the Polish edition of the Audit of Diabetes Dependent Quality of Life (ADDQoL) questionnaire.
Results: Both HNF1A-MODY and GCK-MODY (45 diabetic and 44 prediabetic) study groups had similar mean age (41.7±14.8 vs. 38.0 ±14.2 years, in that respective order, p=0,098), BMI (24.1±-15.4 kg/m2 vs. 24.3±3.7 kg/m2, p=0,587). Less than a third of GCK mutation carriers were on pharmacotherapy (31%), while the majority of HNF1A mutation carriers used oral drugs or insulin (82.5%). The mean QoL scores (AWI) were lower in HNF1A-MODY patients than in GCK-MODY (-1.52 vs. -0.95, respectively, p=0.01). The largest difference in the impact of diabetes between the MODY groups were observed for 'local or long distance journeys', 'social life', and 'dependence' ($p<0.005$ for each comparison). Pharmacotherapy (insulin and/or OHA) had a significant impact on QoL in HNF1A-MODY. The 'present QoL', 'impact of DM on QoL' and AWI scores were significantly lower (p=0,02; p=0,000; p=0,003 respectively) for patients on insulin (n=30) versus non-insulin dependent (n=50) subjects. All differences remained significant when patients on diet only were compared with patients on pharmacotherapy. In GCK-MODY no significant difference were found in 'present QoL', 'impact of DM on QoL' and AWI when comparing prediabetic against diabetic group or patients on diet only against those on pharmacotherapy.
Conclusion: Diagnosis of most frequent subtypes of MODY are associated with diminished QoL in afflicted individuals. Mode of treatment seems to influence QoL for these subjects, which highlight the importance of utilizing genetic results to tailor treatment.
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Disclosure: **M. Szopa:** Grants; Polish National Research Centre "New directions for clinical characteristics of patients with MODY" (ODW-5224/B/P01/2011/40) grant.

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Differential diagnostics of inherited lipodystrophies in patients with diabetes, prediabetes or insulin resistance in Russia

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Background and aims: Lipodystrophies (LDs) are heterogeneous disorders characterized by selective loss of body fat, which can be generalized (GL) or partial (PL), inherited or acquired. Inherited LDs are usually associated with different metabolic disorders, like diabetes with marked insulin resistance, dyslipidemia, arterial hypertension, hepatic steatosis and hepatosplenomegaly, and so often remain not diagnosed, especially familial partial lipodystrophy. GL may be a sign of progeroid syndromes. Genetic diagnostics may be challenging because of many candidate genes and similar phenotypes. The aim of the study was to diagnose different forms of lipodystrophies in Russian population and study their clinical and molecular-genetic characteristics.
Materials and methods: 52 patients (40 adults and 12 children) from 46 families with diabetes, prediabetes or insulin resistance and different lipodystrophic fat loss pattern. 14 congenital lipodystrophies candidate genes (LMNA, PPARG, PLIN1, AKT2, LMNB2, POLD1, CIDEC, WRN, PPP1R3A, ZMPSTE24, BSCL2, CAV1, PTRF, AGPAT2) were sequenced using a Custom Ion Ampliseq panel and PGM semiconductor sequencer (Ion Torrent).
Results: Mean age of the patients was 33.8 years [2; 72], 13 patients (25%) were male and 39 (75%) were female. Prevalence of men in GL (N=14) was 36%, in PL (N=38) - 16%, what corresponds with the previously published data of women being more affected with PL. Mean HbA1c level was 7.6% [4.2; 13.6], basal insulin - 30.9 mcU/ml [0.9; 69], C-peptide - 9.7 [0.5; 21.57], HOMA-IR 8,9 [0.3; 44.8]. This data confirms marked insulin resistance in patients with LDs. In 57% of GL (N = 8) mutations in the following genes were found: 5 in WRN, 1 in AGPAT2, 1 in POLD1, 1 in LMNB2, 1 in LMNA. In 42% of PL patients (N = 16) mutations in the following genes were found: 2 in LMNA, 1 in PPARG, 1 in AKT2, 2 in PP1R3A, 1 in PTRF, 2 in BSCL2. The most common mutation was a heterozygous R482W mutation in the 8 exon (hot-spot) of the LMNA gene found in 3 families (7 patients) with PL.
Conclusion: We recommend a candidate genes panel as an effective diagnostic tool for diagnostics of the different forms of lipodystrophies.
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Disclosure: **E. Sorkina:** Grants; supported by the grant of Russian Scientific Foundation (project № 14-35-00026.

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To diet or not to diet in neonatal diabetes responding to sulphonylurea treatment

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Background and aims: Diabetes diagnosed within six months of life is almost never type 1, but neonatal diabetes. Most cases affected by a mutation in KCNJ11 or ABCC8 gene encoding for the potassium ATP channel respond to sulphonylurea treatment with a glucose dependent insulin secretion. As this food derived glucose dependence arise from GLP1 secretion and not hyperglycemia per se, we hypothesized that diabetes diet might no longer be necessary to achieve metabolic control in sulphonylurea responders.
Materials and methods: A 9 years old girl was admitted on December 4th, 2015 in our emergency university hospital, for neurological assessment and treatment of absence epilepsy. Absence seizures lasting 2-4 minutes started in February 2015, and gradually increased in frequency, severely interfering with patient normal life. She was diagnosed with neonatal diabetes at age 3 months, treated with insulin until age 7 months when she was switched to sulphonylurea, without genetic testing. She received glibenclamide treatment until January 2015, when her doctor decided to switch back to insulin (basal bolus) due to perceived high dose of glibenclamide (only 0.21 mg/Kg body weight), high blood glucose values and HbA1c=10.6% (92 mmol/mol). She also had moderate mental delay, with an IQ of 66 in a neuropsychiatric investigation (Raven test) performed in October 2015, suggesting the presence of a DEND syndrome. Patient had severely impaired performance in simple 2 digit addition or subtraction math exercises.
Results: Blood samples were sent abroad for genetic analysis and patient was successfully switched to glibenclamide (0.42 mg/kg body weight). Special diabetes diet (carb counting) was stopped and then liberalized. Genetic analysis confirmed a newly acquired missense mutation (p.R201C). Reevaluation at three months showed complete remission of absence seizures, very good blood glucose values and HbA1c = 7.3% (56 mmol/mol) on a complete liberalized diet. Glibenclamide dose was

slightly increased to 0.5 g/kg body weight, in observation to further future dose increase to help alleviate the neurologic delay.
Conclusion: Pediatric doctors responsible for neonatal diabetes patients should be carefully trained to understand the correct dose requirements of these patients. This is one of the few cases of DEND syndrome associated with p.R201C mutation, with persistent moderate neuropsychiatric delay. Liberalized diet might add a significant increase in the quality of life for neonatal diabetes patients responding to sulphonylurea treatment.
Disclosure: **S. Fica:** None.

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In vitro analysis of suspected GCK-MODY causative mutations reveals high share of those causing only mild kinase activity inhibition
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Background and aims: Inhibitory mutations in glucokinase (GCK) gene cause MODY. GCK catalyzes the first reaction of glycolysis by converting glucose into glucose 6-phosphate, is not inhibited by the product, and displays a cooperativity, the feature characteristic otherwise for regulatory oligomeric proteins.
Materials and methods: We identified a series of GCK mutations among Czech diabetics and their family members. Based on this cohort and based on other mutations known from Central Europe, we prepared a series of constructs carrying GCK mutations. We tested the enzymatic activity of such recombinant proteins under optimal and suboptimal conditions, such as various glucose and ATP concentrations, thus mimicking the function of the enzyme in affected patients.
Results: The mutations that cause clinical phenotypes of varying severity showed also differences in the intrinsic activity, conformation and stability of the tested enzymes. Some mutations completely suppressed the kinase activity, whereas the others had not a significant influence, or they affected only some of the kinetic parameters of the enzyme under suboptimal conditions. Such proteins were inactive under suboptimal glucose or ATP concentrations, but displayed moderate to strong activity in a presence of high concentration of the substrate or ATP. Although all the mutations tested were originally identified in GCK-MODY patients, they displayed the whole gradient of suppression of the kinase activity, represented, e.g., by the following mutations: WT > R250C > C434Y > T209K > F419L > L314P > F150L.
Conclusion: The GCK-MODY associated mutations were expected to cause severe suppression of kinase activity of the affected enzyme. However, we found that there was rather a continuum of observed effects. Although there were some similarities between the disease severity and observed kinase inhibition, the absence of any threshold was surprising and calls for more detailed testing of the effects of the most benign mutations and for the re-evaluation of causes of diabetes in affected patients.
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Disclosure: **P. Heneberg:** Grants; Czech Science Foundation 15-03834Y.

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Nuclear localisation of glucokinase in pancreatic beta cells is mediated by a nuclear localisation signal and is regulated by SUMOylation
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Background and aims: Glucokinase is the predominant hexokinase expressed in hepatocytes and pancreatic beta-cells. It plays a pivotal role in regulating hepatic glucose clearance and glucose-stimulated insulin secretion, illustrated by glucokinase gene mutations causing monogenic diabetes and congenital hypoglycemic hyperinsulinism. The localization of glucokinase in pancreatic beta-cell nuclei represents a controversial issue. Although previous reports suggest such localization, the mechanism for import has so far not been identified. We previously reported that conjugation of Small Ubiquitin-like MOdifyer-1 (SUMO-1) affects the stability and activity of human pancreatic glucokinase, in vitro and in insulin-secreting model cells. Since the level of SUMOylated glucokinase was found increased in the presence of the nuclear pore SUMO E3 ligase RanBP2, we here aimed to investigate a role of SUMO in the nuclear transport/localization of glucokinase, as well as additional signals necessary for glucokinase transport.
Materials and methods: Immunofluorescence microscopy of human islets and mouse MIN6/rat INS-1 beta-cells, subcellular fractionation, immunoblotting, and mass spectrometry analyses have been used in this study.
Results: We here present the first evidence supporting that glucokinase is present in the nuclei of primary human pancreatic beta-cells, as well as in rat INS-1 and mouse MIN6 cells. We further identified a conserved, seven-residue nuclear localization sequence (^{30}LKKVMRR^{36}) in the human enzyme. Substituting KK^{31,32} and RR^{35,36} with AA led to a loss of glucokinase nuclear localization in transfected cells. Co-transfection with SUMO-1 and RanBP2 increased the number of cells demonstrating nuclear accumulation of glucokinase. Glucose concentrations did not influence the cytosolic versus nuclear distribution of glucokinase. Moreover, co-localization of glucokinase, SUMO-1 and Ubc9 (E2) was observed in the nuclear membrane.
Conclusion: Glucokinase is transported from the cytosol to the nucleus by a redundant mechanism involving a nuclear localization signal and protein SUMOylation. This adds new knowledge to the function of glucokinase in the human pancreatic beta-cell. The functional implications of glucokinase in the nucleus represent a future challenge.
Supported by: University of Bergen, Helse Vest, The Novo Nordisk Foundation
Disclosure: **I. Aukrust:** None.

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Identification of molecular dysfunction induced by GDH S445L mutation associated with hyperinsulinism/hyperammonaemia syndrome
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Background and aims: Hyperinsulinism/Hyperammonemia (HI/HA) syndrome is a common form of congenital hyperinsulinism. Affected children display unregulated protein-induced insulin secretion from pancreatic beta-cells, fasting hypoglycemia and elevated plasma ammonia levels. Mutations associated with HI/HA were identified in the *Glud1* gene, encoding for mitochondrial glutamate dehydrogenase (GDH). The most frequent mutation is the Ser445Leu and is associated with both HI/HA and epilepsy. We aimed at identifying the molecular causes of dysregulation in insulin secretion and ammonia production conferred by this mutation.
Materials and methods: All the investigated cell types were transduced with adenoviruses carrying the human GDH-wild type or GDH-S445L-mutant gene, respectively. The GDH enzymatic activity was measured by NADH autofluorescence under varying concentrations of substrates, co-substrates, allosteric modulators in both anaplerotic and cataplerotic directions in transduced INS-1E beta-cells. OCR (oxygen consumption rate), stimulated with glucose versus glutamine, was measured using Seahorse apparatus. The ammonia production was measured in isolated and virus-transduced mouse hepatocytes after stimulation with neoglucogenic substrates (alanine versus glutamine). Insulin secretion was tested in INS-1E cells, mouse Bet-*Glud1*$^{-/-}$ (GDH ko) and human

islets following adenoviral transduction under different stimulating conditions (low and high glucose concentrations, 5mM glutamine). For each experiment, GDH expression levels were assessed by western blot analysis.

Results: Western blots showed efficient expression of both wild type and mutant GDH in the different cell preparations. Enzymatic activity tested in INS-1E cells revealed that, in the cataplerotic direction, GTP was less effective as an allosteric inhibitor on GDH-mutant compared to the wild type. Importantly, the mutant was much more sensitive to the allosteric activator ADP, rendering it highly active at any substrate concentrations. INS-1E cells expressing either wild type or mutant GDH responded similarly to glucose stimulation regarding OCR and insulin secretion. However, at basal glucose glutamine stimulation increased OCR and insulin release only in mutant cells. In mouse and human islets, expression of mutant GDH resulted in robust elevation of insulin secretion upon glutamine stimulation, not observed in control islets. Hepatocytes expressing either wild type or mutant GDH produced similar levels of ammonia when exposed to glutamine, although alanine response was strongly elevated with the mutant form.

Conclusion: The GDH S445L mutation confers hyperactivity to this enzyme due to much higher sensitivity to ADP allosteric activation. This renders beta-cells responsive to amino acid stimulation, explaining protein-induced hypoglycemia secondary to non-physiological insulin release. Hepatocytes carrying mutant GDH produce more ammonia upon alanine exposure, which underscores hyperammonemia developed by the patients. Current work focuses on identification of inhibitors targeting the S445L residue.

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Disclosure: **M. Grimaldi:** None.

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Deficiency in the tRNA methyltransferase TRMT10A activates the intrinsic pathway of apoptosis in pancreatic beta cells

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Background and aims: Loss-of-function mutations in TRMT10A, a putative transfer RNA (tRNA) methyltransferase, cause young-onset diabetes and microcephaly. Intronic variants in CDKAL1, a tRNA methylthiotransferase, are associated with an insulin secretory defect and type 2 diabetes. These data suggest that abnormalities in tRNA modification might cause β-cell failure. Against this background, our aim was to study the function of TRMT10A in β-cells.

Materials and methods: RNA methylation was examined by HPLC-MS/MS and TRMT10A substrates were identified by primer extension assays in lymphoblasts from TRMT10A-deficient patients and controls. TRMT10A was silenced using two different siRNAs in insulin-producing rat INS-1E and human EndoC-βH1 cells (≥70% knockdown, $p<0.001$). Apoptosis was examined by staining with nuclear dyes and Western blot. Oxidative stress was measured using dichlorofluorescin diacetate (DCF).

Results: RNA methylation analyses revealed reduced guanosine methylation in lymphoblasts from TRMT10A-deficient patients (≥40% reduction compared to controls). Primer extension assays showed that TRMT10A methylates tRNA(Gln) and tRNA(iMeth) in guanosine at position 9. TRMT10A knockdown increased DCF oxidation (≥60% increase, $p<0.05$, n=5) and activated the intrinsic pathway of apoptosis in rat and human β-cells, as assessed by caspase-9 cleavage and cytochrome c release ($p<0.05$, n=3-12). In line with this, TRMT10A silencing induced mRNA expression of the pro-apoptotic Bcl-2 mediators Bim and Bad (≥50% increase, $p<0.05$, n=9-10). At the protein level, TRMT10A silencing induced the most pro-apoptotic Bim splice variant, namely BimS (≥50% increase, $p<0.05$, n=8-9), but it did not affect Bad phosphorylation. Bim, but not Bad, silencing protected TRMT10A-deficient β-cells against apoptosis (13±2% apoptosis in TRMT10A-deficient cells vs 7 ±1% when Bim was silenced in parallel, $p<0.05$, n=4-5). The cyclic AMP inducer forskolin (20 μM) protected TRMT10A-deficient β-cells from apoptosis (16±1% apoptosis in TRMT10A-deficient cells vs 7±2% in the presence of forskolin, $p<0.05$, n=5). The incretin analog exendin-4 (50 nM) was also β-cell protective (16±1% apoptosis in TRMT10A-deficient cells vs 11±1% in the presence of exendin-4, $p<0.05$, n=3).

Conclusion: We confirmed that TRMT10A is a tRNA methyltransferase, and identified tRNA(Gln) and tRNA(iMeth) as its substrates. Deficient tRNA methylation may affect tRNA stability, amino-acylation and cleavage. TRMT10A deficiency in β-cells triggers the intrinsic pathway of apoptosis with activation of the pro-apoptotic protein Bim. This is the first evidence that defective tRNA methylation causes β-cell demise. Forskolin and exendin-4, known to attenuate oxidative stress and up-regulate anti-apoptotic Bcl-2 proteins, protected TRMT10A-deficient β-cells against apoptosis. This suggests that incretin analogs may be β-cell protective in TRMT10A deficient patients.

Disclosure: **C. Cosentino:** None.

PS 010 Drilling down on genetic mechanisms

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Identification and functional characterisation of diabetes genes in mice

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Background and aims: Type 2 diabetes is a polygenic disease characterized by beta-cell apoptosis and failure to induce regeneration in mice and human. New Zealand Obese (NZO) mice show beta-cell failure, whereas obese mice on C57BL/6-background (B6-ob/ob) are protected from diabetes due to a compensative beta-cell proliferation in response to an in-vivo glucose challenge. Our aim was to identify and characterize responsible genes mediating either beta-cell failure in NZO or proliferation in B6-ob/ob mice.
Materials and methods: RNA-sequencing based transcriptomics were performed on RNA of islets from NZO and B6-ob/ob mice that received a short carbohydrate challenge after a long-term carbohydrate deprivation. Pathway enrichment studies in combination with QTL-mapping were conducted in order to crystallize novel candidate genes. The effects of the candidate genes on beta-cell division and function were analyzed upon adenoviral overexpression in primary islet cells and the cell line INS1 832/13.
Results: Islets of B6-ob/ob mice responded to carbohydrate feeding with an induction of 22 cell-cycle associated genes, whereas NZO islets exhibited a modulation of cell adhesion transcripts that might result in beta-cell loss. Within two diabetes-QTL we identified 8 hypomorphic genes in NZO (chr.1: Lefty1, Apoa2, Pcp4l1, Mndal, Slamf7, Pydc3; chr.13: Crhbp and S100z) and one in B6-ob/ob (chr.1: Ifi202b). Adenovirus-mediated overexpression of Lefty1, Apoa2, Pcp4l and Crhbp in dispersed primary islet cells increased proliferation, whereas overexpression of Ifi202b suppressed it. We highlight Lefty1 as a key diabetes suppressor because the pathway enrichment analysis indicated its action on the inhibition of the Smad3 signaling leading to an induction of beta-cell division. Moreover, 2 SNPs in human LEFTY1 show a nominal association with insulin and C-peptide release. Glucose-stimulated insulin secretion of INS1-cells was improved upon overexpression of Pcp4l1 and Mndal but impaired by Ifi202b.
Conclusion: The differentially expressed islet genes Lefty1, Apoa2, Pcp4l1, Mndal and Crhbp participate in adaptive islet hyperplasia and insulin secretion during obesity and insulin resistance and might be novel therapeutical targets.
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Disclosure: **O. Kluth:** None.

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FTO silencing inhibits insulin secretion in GRINCH cells: a new pancreatic beta cell line tool for beta cell function

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Background and aims: FTO (Fat mass and obesity-associated) has been identified as an obesity-susceptibility gene, which is strongly associated with increased risk of obesity. A recent study showed that the FTO gene product has a rapid turnover in pancreatic β cells and affects the regulation of insulin secretion under glucose stimulation. However, the functional role and molecular mechanisms of FTO in pancreatic β cells is still unclear. In this study, we aim to investigate the role of FTO in the pancreatic β cells using a new pancreatic β cell line tool for β cell function called GRINCH cells (Glucose-Responsive Insulin-secreting C-peptide-modified Human-proinsulin).
Materials and methods: The GRINCH cell line was established from regular rat INS-1 cells with inserted superfolder GFP in human proinsulin C-peptide. GRINCH cells exhibit excellent glucose-stimulated insulin secretion. The secreted fluorescent C-peptide is recoverable by monitoring the GFP signal, which reflects the degree of insulin secretion. Gene silencing of FTO was done using siRNA and transfection efficiency was assesses by qRT-PCR. Secreted insulin was measured in the incubation medium (1 h) with an ELISA Insulin kit and GFP fluorescence intensity with a TECAN plate-reader (Infinite M200) at excitation wavelength 489 nm and emission at 527 nm.
Results: Sonicated lysate preparation from GRINCH cells showed high GFP intensity compared to INS-1 (831-13) which had no GFP intensity. qRT-PCR demonstrated that GRINCH cells express the rat and human insulin genes. Secreted insulin measured by ELISA showed 3 fold increase between the basal level (2.8 mM Glucose) and stimulated release (16.7 mM Glucose) and 7 fold increase between the 16.7 mM glucose and 16.7 mM glucose with 1mM IBMX and Forskolin. Using GFP signal as a measurement assay for insulin secretion we observed 2 fold increase between the basal and stimulated level and 5 fold increase between 16.7 mM glucose and 16 mM glucose + 1 mM IBMX and Forskolin. siRNA silencing of FTO in GRINCH cells showed high knockdown efficiency (90%). Insulin secretion (measured by ELISA) in response to 2.8 mM and 16.7 mM glucose 72 hr after siRNA transfection showed 50% reduction ($p < 0.5$) and 40% reduction ($p<0.5$) measured by GFP intensity compared to negative control. Stimulation of insulin exocytosis with depolarizing (30 mM) KCL concentrations showed no difference between transfected cells and controls.
Conclusion: GRINCH cells were characterized as a new cell line model to study pancreatic beta cell function. The cells retain insulin secretion responses to glucose and exhibit similar physiologic characteristics comparable to those of regular INS-1 cells. Silencing of FTO expression significantly inhibits glucose-stimulated insulin secretion of GRINCH cells without affecting the exocytosis machinery. Further molecular studies are needed to explore the role of FTO in metabolism-secretion coupling in the beta cell and islet function.
Disclosure: **J. Taneera:** None.

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Pax6 is required for the functional identity of the adult pancreatic beta cell

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Background and aims: Mutations in the gene encoding the transcription factor paired box protein 6 (PAX6) in man are associated with defective glucose homeostasis. Single nucleotide polymorphisms in the human PAX6 gene are also associated with reduced insulin secretion from isolated islets. Global inactivation of Pax6 in mice is lethal, as mutants fail to develop beta, and most other islet cell types. Here, we have assessed whether PAX6 stabilizes the functional maturity of the adult beta cell by deleting the murine Pax6 gene in eight week old mice. We deploy in vivo metabolic phenotyping alongside immunocytochemical analysis and high speed confocal imaging of beta cell ATP and Ca2+ dynamics in situ in isolated islets.
Materials and methods: Mice bearing LoxP sites flanking exons 5, 5[a] and 6, which encode the paired box domain, of the Pax6 gene were bred to Pdx1CreERT mice. Deletion in 8 week-old Pax6f/f:PdxCreERT mice was

achieved by five daily intraperitoneal tamoxifen(Tx) injections. Islet isolation and loading with fluo-2 allowed rapid confocal Ca2+ imaging on an Olympus IX microscope fitted with a Nipkow spinning disc confocal head and 10x air objective. ATP:ADP ratio was monitored using the fluorescent probe Perceval, and gene expression by qRT-PCR analysis. Immunocytochemistry was performed on a Zeiss AxioObserver microscope with a 40x air objective.

Results: Floxed mice carrying the PdxCreERT transgene (Pax6f/f::PdxCreERT+/-) became glucose intolerant compared to litter mate controls (Pax6f/f::PdxCreERT-/-) eight days post Tx treatment (9.45±0.35 mmol/L vs. 26.15 ± 1.13 mmol/L; Cre- vs. Cre+ respectively; $p<0.001$; n=2/4), with glycemia reaching 50.0 mmol/L by 14 days in Pax6f/f::PdxCreERT+/- animals. Consistent with an overtly diabetic phenotype, the glycaemic profiles seen in Pax6f/f::PdxCreERT mice were accompanied by reduced body weight (20.5±0.25 g vs. 18.9±0.61 g; Cre- vs. Cre+ respectively; ns; n=2/4). Examined eight days post Tx, islets derived from Pax6f/f::PdxCreERT-/- mice responded to 11 mmol/L glucose with a rapid and largely monophasic increase in beta cell free cytosolic Ca2+ which was synchronised across the islet. In contrast, Pax6f/f::PdxCreERT+/- islets displayed a delayed, and oscillatory response and the peak amplitude of Ca2+ responses was sharply reduced (0.90 ± 0.13 AU vs. 0.33±0.04% AU; Cre- vs. Cre+ respectively; $p<0.05$; n=15/17). Furthermore, the amplitude of glucose-evoked ATP:ADP rises in Pax6f/f::PdxCreERT+/- was also significantly reduced (0.15±0.01 AU vs. 0.11± 0.01 AU; Cre- vs. Cre+ respectively; $p<0.05$; n=9/13). A significant reduction in the ratio of insulin:chromogranin A staining was observed after Pax6 deletion (0.89±0.02 vs. 0.38±0.02; Cre- vs. Cre+ respectively; $p<0.001$; n=50/75 islets).

Conclusion: Pax6 plays an essential role in the preservation of adult beta cell identity whose loss leads to interference with gene expression, glucose-induced ATP synthesis and Ca2+ signalling. These changes may contribute to defective insulin secretion in human carriers of PAX6 mutations.

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Disclosure: **G.A. Rutter:** None.

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System biology of the IMIDIA biobank from organ donors and pancreatectomised patients defines the transcriptomic signature of type 2 diabetes islets

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Background and aims: Pancreatic islet beta cell failure causes human type 2 diabetes (T2D). To identify gene expression changes in T2D islets the IMIDIA consortium (www.imidia.org) established a comprehensive, unique multicenter biobank of human islets and pancreas tissue from organ donors (OD) and from partially pancreatectomized patients (PPP).

Materials and methods: Pancreatic islets were isolated either by enzymatic digestion from 103 OD, including 84 non-diabetic (ND) and 19 T2D subjects, or by laser capture microdissection (LCM) from surgical specimens of metabolically phenotyped 103 PPP, including 32 ND, 36 with T2D, 15 with impaired glucose tolerance (IGT) and 20 with T3cD. A diagnosis of T3cD was made for PPP with recent onset diabetes (<1 year), conceivably secondary to the pancreatic disorder leading to surgery. The islet transcriptome was assessed on Affymetrix microarrays. Bioinformatic tools were used to i) compare the islet transcriptome of T2D vs. ND OD and PPP as well as vs. IGT and T3cD within the PPP group; ii) identify transcription factors driving gene co-expression modules correlated with insulin secretion in vitro and glucose tolerance in vivo. Selected genes of interest were validated for expression and function in beta cells.

Results: Comparative transcriptomic analysis identified 19 genes differentially expressed (fdr ≤0.05, fold change ≥1.5) in T2D islets vs. ND islets of both OD and PPP. Nine out of these 19 dysregulated genes were never previously reported to be differentially expressed in T2D islets. Systems biology approaches identified HNF1A, PDX1 and REST as drivers of gene co-expression modules correlated with impaired insulin secretion ex vivo or glucose tolerance in vivo, and enriched in 14/19 differentially expressed genes of T2D islets. Notably, none of these genes was significantly dysregulated in islets of IGT or T3cD PPP.

Conclusion: These studies enabled the stringent definition of a transcriptomic signature of T2D islets. Lack of dysregulation of T2D islet signature genes in IGT and T3cD islets suggests that the transcriptomic changes in T2D islets capture but do not precede beta cell failure.

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Role of the type 2 diabetes GWAS gene TCF7L2 in LKB1-mediated regulation of insulin secretion in the murine beta cell

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Background and aims: Deletion of the tumour suppressor liver kinase B1 (LKB1/STK11) from the pancreatic beta cell leads to cellular hyperplasia, hypertrophy and enhanced glucose-stimulated insulin secretion. The transcription factor-7-like 2 (TCF7L2/TCF4), associated with type 2 diabetes (T2D) in man through genome-wide association studies, encodes a member of the high mobility group (HMG) box family of factors regulated by wingless (Wnt) signalling. Tcf7l2 is required in rodents for normal β cell expansion and insulin secretion under metabolic stress. LKB1 and Wnt signalling have previously been shown to interact in more primitive systems. Thus, deletion of XEEK1, the Xenopus laevis homologue of LKB1, leads to developmental abnormalities similar to those observed in Wnt signalling mutants. These data suggest that TCF7L2 may lie downstream of LKB1 in a pathway contributing to cell growth and/or function. Here, we used a genetic epistasis approach to explore the possibility that Tcf7l2 may be required for the effects of Lkb1 deletion on insulin secretion in the mouse beta cell.

Materials and methods: C57BL6 mice bearing floxed Lkb1 and/or Tcf7l2 alleles were bred with knock-in mice bearing Cre recombinase inserted at the Ins1 locus(Ins1Cre), thus allowing highly β cell selective deletion of either or both genes. Breeding pairs were established to produce (1) Lkb1 homozygous null, $Tcf7l2^{+/+}$ mice, (2) Lkb1 null and Tcf7l2 heterozygous or (3) homozygous animals ($Tcf7l2^{+/-}$ or $Tcf7l2^{-/-}$). Intraperitoneal glucose (1 g/kg) tolerance was measured every four weeks from eight weeks of age, and insulin sensitivity (0.75U/kg) was determined at nine weeks of age.

Results: Male mice lacking both Lkb1 alleles, but retaining wild type Tcf7l2 alleles ($Lkb1^{f/f}$:$Tcf7l2^{+/+}$:$Ins1Cre^{+/-}$), displayed significantly improved glucose tolerance compared to animals deleted for either or both Tcf7l2 alleles up to 12 weeks of age (AUC= 625 vs 750 and 782 mmol/L*min respectively, $p<0.05$, n=3-9). This difference normalised from 16 weeks of age and no genotype-dependence differences in glucose tolerance or insulin sensitivity were observed in female mice. Body weight, fed and fasting glycemia and insulin sensitivity did not differ significantly between genotypes in male and female mice.

Conclusion: We provide evidence that TCF7L2 lies on a pathway through which LKB1 acts in the beta cell to restrict insulin secretion. These data thus provide a new example of how GWAS-identified variants, in this case in the TCF7L2 gene, may influence beta cell biology to affect diabetes risk. LKB1 may thus provide an interesting target for personalized therapy based on TCF7L2 genotype.

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Disclosure: **M. Nguyen-Tu:** None.

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CAR isoform expression in the human pancreas

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Background and aims: Coxsackie-adenovirus receptor (CAR), a putative transmembrane cell-adhesion protein, is utilised as a receptor to mediate the entry of enteroviruses into cells and may be essential for their ability to generate a productive infection. Human pancreatic beta cells can be readily infected with enteroviruses in vitro suggesting that these cells are permissive for viral entry and replication. Moreover, enteroviral infection has been detected in the beta cells of patients with type 1 diabetes and this has been postulated to contribute to the development of autoimmunity in such patients. Despite this evidence, very little is known of the factors that control viral entry into human beta cells and it is important that CAR expression is analysed in greater detail. This is because CAR exists as at least 5 different isoforms, (which are generated via alternative mRNA splicing of multiple exons) but their distribution has not been mapped in the pancreas. Therefore, we have studied CAR isoform expression using both proteomic and PCR approaches in human pancreas sections and in isolated human islets to establish the expression profile.
Materials and methods: Formalin-fixed paraffin embedded pancreatic sections from 7 non-diabetic controls ranging from 4 months to 47 years of age and 5 type 1 diabetes patient (age range:6-47 years) were investigated using 3 different CAR antisera. These were chosen because of their selective immunoreactivity against the N-terminal region, the C-terminus or the extracellular domain which allowed specific isoforms to be distinguished. Immunohistochemistry and confocal immunofluorescence microscopy were employed to monitor CAR isoform expression. RNA was extracted from isolated human islets and amplified using isoform specific RT-PCR. PCR products were sequenced to confirm the specific splicing of exons to facilitate isoform identification.
Results: Isoform specific antisera revealed that specific isoforms of CAR are expressed in both the exocrine and endocrine cells of the pancreas in humans. However, there were clear differences in the distribution of each isoform within the endocrine and exocrine compartments. Importantly, a selective antiserum directed against an isoform containing a unique PDZ binding domain at the C-terminus (known as "SIV") labelled only islet beta cells. Expression of the SIV isoform was confirmed at both the protein and RNA level in human islets. Two further isoforms of CAR having different C-terminal domains (and known as TVV and CAR 4/7) were expressed in the exocrine compartment of the pancreas and confirmed at both the protein and RNA level.
Conclusion: The predominant CAR isoform present in human pancreatic beta cells is the SIV isoform which harbours a unique PDZ-binding domain at its C-terminus. This protein is not present in the other islet endocrine cells or in the exocrine pancreas, in humans. The restricted distribution of this isoform of CAR may contribute to the selective infection of beta cells by enteroviruses during the development of type 1 diabetes
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Disclosure: **S.J. Richardson:** None.

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Grandpaternal high-fat diet transgenerationally impacts the skeletal muscle unfolded protein response in F2-female offspring

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Background and aims: During the last decades the number of persons diagnosed with type 2 diabetes has increased, with obesity as one driving cause. Parental overweight and obesity disturbs glucose and lipid metabolism in the offspring in a transgenerational manner. We have previously shown that grandpaternal high-fat diet (HFD) impairs whole-body glucose metabolism in F2-female offspring. However, if grandpaternal diet directly affects glucose and lipid metabolism in skeletal muscle is still unknown.
Materials and methods: Male Sprague-Dawley rats were fed either HFD or control (chow) diet for 12 weeks, and bred with control fed females to create the F1 generation. Male F1 rats on control diet bred with a new set of control fed females to create the F2 generation. Adult F1 and F2 female offspring were also fed control or HFD for 12 weeks. Thereafter, EDL muscle was dissected, processed and subjected to a transcriptomic analysis using an Affymetrix rat gene 1.1 ST Array. Gene array data was analyzed using a gene set enrichment analysis tool.
Results: When comparing F2 offspring fed a control diet derived from either a chow- or HFD-fed grandfather, no differences were observed in the skeletal muscle transcriptomics profile after correcting for multiple testing. However, offspring HFD consumption affected the unfolded protein response pathway only in offspring from HFD-fed grandfathers (normalized enrichment score = -1.6, FDR = 0.01), when compared to control fed grandfathers. Among the core enriched genes selected for RT-qPCR validation, five of eight genes were significantly changed ($p > 0.05$) in F2 skeletal muscle, but none were altered in F1. To investigate whether DNA methylation plays a role in altered gene expression, we analyzed target-specific promoter DNA methylation by MBD-captured followed by qPCR. No changes were observed in promoter DNA methylation.
Conclusion: Our results indicate that grandpaternal HFD consumption transgenerationally impacts the response of F2-female offspring to HFD by altering the skeletal muscle transcriptomic profile, independently of changes in target-specific promoter DNA methylation. Furthermore, our results also provide evidence that an additional stressor is required from the grandpaternal legacy to impact glucose and lipid metabolism. This study highlights the importance of lifestyle choices over parental heritage on whole body glucose and energy homeostasis.
Supported by: ERC, VR, Swedish Diabetes Foundation, SRP
Disclosure: **P.S. Alm:** None.

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Detection of beta cell virus infection in type 1 diabetes by short fluorescently labelled oligonucleotide probes

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Background and aims: Type 1 diabetes (T1D) results from a complex interplay between genetic polymorphisms, immune system and environment. Viruses, mainly enteroviruses such as members of the Coxsackie B virus (CVB) family, have been suspected since long time to trigger diabetes and epidemiological studies support a causative role of viral infections during diabetes onset. Nonetheless, the presence of the enterovirus in diabetic tissue has not been fully confirmed, since recent RNA-based approaches failed to detect the viruses in many autopsy pancreases. Our aim was to detect the presence of low copies of the virus genome in tissue samples from T1D patients by using highly sensitive RNA probes.
Materials and methods: Short fluorescently labeled oligonucleotide probes consisting of 40 short oligonucleotides with 17-22 nucleotides in length were designed to bind to the whole viral genome and target single RNA molecules. Our current set of virus RNA probes has been intentionally designed to identify a wide range of CVB serotypes, as well as other members of the picornavirus family.
Results: The specificity of the probes was tested against several viruses with a different degree of similarity towards the consensus sequence used

to generate the probe set. We confirmed that only viruses belonging to the picornaviridae family with a degree of sequence similarity above 60% could be detected by the assay. We were able to successfully detect viral RNA in both cell culture and formalin fixed paraffin embedded (FFPE) cell and tissue sections, cell microarrays as well as in sections from patients with type 1 diabetes with no limitations on time of sample processing and storage conditions, bypassing the potential limitation of fragmented RNA. When compared to other in situ slide-based RNA detection approaches in a continuous dilution series, our assay showed at least 4 log-fold greater sensitivity towards RNA viruses at identical experimental conditions without loss of specificity. Comparison of RNA probes staining to classical immunohistochemistry using an antibody against viral protein 1 (VP1; at a dilution which allows specificity for VP1) showed at least a 7 log-fold greater sensitivity in a dilution series. Sensitivity and specificity of detection was confirmed without the cross-reactivity with cellular proteins shown by the VP1 antibody under certain conditions. The use of RNA probes allowed the discrimination between single RNA molecules and virus replication loci. Co-staining of small RNA probes with β-cell markers enabled us to further identify the presence of the virus in a cell-specific fashion.

Conclusion: Our probes sets enable specific and sensitive detection of enterovirus presence from both new and old collections of various FFPE and frozen tissue samples. Even latent infections with hardly any viral proteins could be identified by the oligonucleotide probes. Further tailoring of the probes sets will allow a more serotype specific detection of enteroviruses.

Supported by: nPOD & JDRF

Disclosure: **N. Busse:** None.

PS 011 Metabolic pillow talk between tissues

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Liver fat content and insulin secretion failure are associated with the long-term success of a lifestyle intervention to prevent type 2 diabetes: results of the TULIP study

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Background and aims: There is a large variability in the effectiveness of a lifestyle intervention to improve glucose metabolism. In the TUebinger Lifestyle Intervention Program (TULIP) study we could recently show that insulin resistant nonalcoholic fatty liver disease (NAFLD) and insulin secretion failure, both measured prior to the start of the intervention, are associated with a lower chance to revert from pre-diabetes to normal glucose regulation during a lifestyle intervention that is based on the recommendations of the DPP and DPS studies. It is not known whether such relationships of risk phenotypes with glucose metabolism are sustained after a longer time of follow-up.

Materials and methods: Out of 400 participants of the TULIP study, a group of 190 subjects could be re-examined 9±1.6 years after enrolment. All individuals underwent a 5-point 75g OGTT with calculation of glycemia ($AUCglucose_{0\text{-}120min}$), insulin sensitivity and insulin secretion relative to insulin sensitivity (Disposition Index, DI). Liver fat content was measured by ^{1}H-MR spectroscopy.

Results: Of the 190 subjects 56% had normal glucose regulation, 13% had isolated IFG, 16% had isolated IGT and 15% had IFG+IGT at baseline. During follow-up, 11% of the participants developed type 2 diabetes and 43% had pre-diabetes (IFG and/or IGT). The cumulative probability of developing type 2 diabetes was higher in subjects with a low DI at baseline (Cox regression $p<0.0001$). Multivariate regression analysis showed that the DI, liver fat content and the BMI at baseline, as well as the change of the DI and of the BMI during the first 9 months of the lifestyle intervention (all $p<0.05$), predicted the long-term effect of the lifestyle intervention for $AUCglucose_{0\text{-}120min}$ levels. Furthermore, a risk assessment approach containing liver fat content and the DI, both measured at baseline and the change of the DI during 9 month of the lifestyle intervention predicted glucose metabolism (glycemia/glycemic status) after follow-up ($ROC_{AUC}=0.83$).

Conclusion: Our results show that the short-term improvement of relative insulin secretion during a lifestyle intervention, as well as a high baseline insulin secretion and a low liver fat content, predict the long-term success of a lifestyle intervention to reduce hyperglycemia, independently of baseline BMI and the change of BMI during the intervention.

Disclosure: **V. Schmid:** None.

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Decorin: a new positive component of skeletal muscle to beta cell communication which prevent TNF-alpha and insulin resistance condition medium effect on beta cells

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Background and aims: Inter-organ crosstalk including possible communication between skeletal muscle and beta cells is an important component of normal physiology that may contribute towards the

pathophysiology of type 2 diabetes. Indeed, skeletal muscle cells secrete different sets of soluble proteins (myokines) which could act positively or negatively (in health and disease respectively) on the function of distant tissues including pancreatic islets. We have observed that conditioned medium from skeletal muscle of healthy individuals has positive effects on beta cell function. Decorin, was identified as a myokine regulated by muscle fiber type. The major aim of this study was to explore the impact of decorin on primary sorted beta cells under control conditions and after exposure to TNF-α or insulin resistance condition medium from skeletal muscle cells.

Materials and methods: All studies were performed using sorted rat primary beta cells and human islets exposed to decorin for 24h. Glucose-stimulated insulin secretion (GSIS) was measured by radioimmunoassay after 1h at 2.8 mmol/l (basal) and 1h at 16.7mmol/l (stimulated) glucose. Cell death and proliferation were measured by TUNEL assay and BrdU incorporation respectively. To study the potential beneficial/protective effects of decorin, beta cells were exposed to decorin for 24h alone or with addition 20 ng/ml TNF-α alone or condition medium from insulin resistance soleus skeletal muscle cells (S-SKMC-IR-CM). Data are mean ± SE for 5-8 independent experiments.

Results: Decorin treatment of rat primary beta cells and human islets for 24h increased GSIS (control: 12.4 ± 3.2% content/h; 1μg/ml decorin: 15.08 ± 3.8%*; 10 μg/ml decorin: 14.1 ± 1.8%*; *p<0.05 vs. control for rat beta cells and control: 11.2 ± 2.5% content/h; 1μg/ml decorin: 13.45 ± 1.2%*; 10 μg/ml decorin: 15.9 ± 0.9%*; *p<0.05 vs. control for human islets) and decreased cell death (TUNEL-positive beta cells control: 0.7 ± 0.2%; 1μg/ml decorin: 0.38 ± 0.1%*; 10 μg/ml decorin: 0.4 ± 0.1%*; *p<0.05 vs. control for rat beta cells and TUNEL-positive insulin positive cells control: 1.4 ± 0.3%; 1μg/ml decorin: 0.17 ± 0.2%*; 10 μg/ml decorin: 0.25 ± 0.1%*; *p<0.05 vs. control for human islets). Decorin had no effect on beta cell proliferation. Decorin protected against the adverse effects of TNF-α and S-SKMC-IR-CM on GSIS (control: 10.20 ± 0.8% content/h; TNF-α: 8.6 ± 0.8%*; TNF-α + 1 μg/ml decorin: 11.03 ± 0.9%**; *p< 0.05 vs. control; **p<0.05 vs. TNF-α alone for rat beta cells and control: 9.20 ± 0.7% content/h; S-SKMC-IR-CM: 4.2 ± 0.7%*; S-SKMC-IR-CM + 1 μg/ml decorin: 11.5 ± 0.4%**; *p< 0.05 vs. control; **p<0.05 vs. S-SKMC-IR-CM alone) and blocked the effects of S-SKMC-IR-CM on apoptosis (control: 0.75 ± 0.1% TUNEL-positive beta cells; cytomix: 5.9 ± 0.12%; cytomix + 1μg/ml decorin: 0.95 ± 0.35% for rat beta cells and control: 1.5 ± 0.2% TUNEL-positive beta cells; S-SKMC-IR-CM: 4.7 ± 0.22%; S-SKMC-IR-CM + 1μg/ml decorin: 1.85 ± 0.65% for human islets).

Conclusion: We demonstrate for the first time that decorin has a positive effect on beta cells and human islets, increasing glucose-stimulated insulin secretion and decreasing cell death in the basal state. Moreover, decorin protects beta cells and human isles from the adverse effects of TNF-α and S-SKMC-IR-CM, indicating the potential for a specific impact of decorin on cytokine signaling pathways in beta cells.

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Disclosure: **K. Bouzakri:** None.

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The interaction of insulin resistance and genetically-determined pancreatic response shapes the time trajectories of insulin secretion and glucose tolerance

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Background and aims: The age-related deterioration of the insulin secretory capacity of the pancreas is a fundamental mechanism in the aetiology of type 2 diabetes, but little is known about its determinants.

Materials and methods: We conducted a study in 635 non-diabetic participants of the MRC Ely population-based cohort study who were followed-up for an average of 10 years. We investigated the relationships of the following risk factors with change in insulin secretion (estimated on the basis of frequently-sampled oral glucose tolerance tests at baseline and follow-up): (a) a genetic risk score associated with impaired pancreatic beta-cell function, (b) overall and regional adiposity measured by body mass index and waist circumference and (c) insulin resistance measured by the inverse of the Matsuda Index.

Results: Genetic predisposition to impaired beta-cell function was negatively associated with insulin secretion at baseline (beta per standard deviation [SD] of genetic score, -0.18; 95% confidence interval, -0.26 to -0.11 SD of outcome; p = 2.7 x 10E-06) and with change in insulin secretion at 10 years (-0.10, -0.18 to -0.03; p = 0.009). Change in insulin resistance was a major determinant of change in insulin secretion, even after adjusting for adiposity (0.35, 0.27 to 0.41; p = 3.8 x 10E-18). In individuals with worsening insulin resistance, genetic predisposition to a poor beta-cell function was simultaneously associated with weaker insulin secretory response and higher 120 minutes glucose (p-values for interaction, 0.001 and 0.027 respectively).

Conclusion: Insulin resistance and genetically-determined pancreatic response interact to shape the change in insulin secretion over time. Interventions that improve insulin sensitivity have the potential to preserve pancreatic secretory capacity and glucose tolerance, in particular in individuals who are genetically predisposed to a poor beta-cell function.

Supported by: Medical Research Council

Disclosure: **L.A. Luca:** None.

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Faecal microbiota transfer from donors post bariatric surgery does not improve insulin sensitivity in metabolic syndrome subjects

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Background and aims: Metabolic improvements after Roux-en-Y gastric bypass surgery (RYGB) are only partly understood. Studies in mice have underscored the role of the intestinal microbiota by showing that the metabolic improvements occurring after RYGB can be transferred by Fecal Microbial Transfer (FMT). We thus aimed to validate these findings in humans by infusing post-RYGB donor feces into treatment naive male metabolic syndrome subjects.

Materials and methods: Twenty-two subjects with metabolic syndrome were randomized to either FMT from a post RYGB donor (n=11) or FMT from an allogenic donor with metabolic syndrome (n=11). Glucose and glycerol metabolism were assessed by hyperinsulinemic euglycemic clamp with 2H2-glucose and 2H5-glycerol tracers before and 2 weeks post treatment. Duodenal and fecal microbiota were studied. Intestinal transit time was monitored using SITZMARK-capsules. Analyses on morning fecal samples (short-chain fatty acids and calprotectin), 24h feces (bile acids), plasma (lipids, inflammation markers) and urine were done. All parameters were also studied in all donors.

Results: We found no improvement on peripheral (Rd), hepatic insulin sensitivity (EGP suppression) or lipolysis upon post RYGB donor FMT. However, a significant decline in peripheral insulin sensitivity (Rd) was seen in the metabolic syndrome allogenic fecal microbiota transplantation control group (median Rd decreased from 29.4 to 25.5.6, p=0.021) with donor insulin sensitivity being highly predictive for the metabolic effects in the acceptor (r=0.67, p=0.001). Intesteringly, intestinal transit time only improved in the post RYGB donor group from 39+/-18 to 56+/-12 excreted markers/72h (p=0.050). Donor transit time was highly predictive for this effect (p=0.000).

Conclusion: Allogenic obese to obese FMT worsened peripheral insulin resistance in metabolic syndrome subjects, whereas post bariatric fecal microbiota transplantation did not affect insulin sensitivity, The latter group however showed an increased intestinal transit time upon post RYGB FMT suggesting a microbiota dependent transmissible trait. Our data suggest that regulation of insulin sensitivity and intestinal transit time

can be affected in humans by FMT. and we are currently investigating which specific bacterial strains are driving these effects.
Clinical Trial Registration Number: NL43964.018.13
Disclosure: **P.F. de Groot:** None.

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Mitochondrial stress and altered mitophagy in type 2 diabetes

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Background and aims: T2DM is characterized by chronic persistent hyperglycemia and hyperglycemia-induced oxidative stress may impede the mitochondrial morphology and function, as mitochondria are the primary sites for endogenous reactive oxygen species (ROS) production. Impaired mitophagy associated with increased ROS levels has been proposed to contribute to insulin resistance and β-cell dysfunction. Mitophagy markers like NIX, PARKIN, PINK-1 are essential for flagging the damaged mitochondria and subsequent degradation by autolysosomes. The present study aims to elucidate the status of mitochondrial stress and mitophagy markers at transcriptional and translational levels in the diabetic subjects.
Materials and methods: Patients with newly diagnosed T2DM (NDT2DM), advanced duration of T2DM (ADT2DM, duration of diabetes 5-10 years) and healthy controls were enrolled in the study (n=10 each). Mitochondrial ROS content (mtROS) and mitochondrial membrane potential (MMP) in PBMCs were assessed by FACS analysis, followed by transcriptional and translational expression of mitophagy markers by Real time PCR and Western Blot.
Results: Baseline clinical parameters were comparable in the study subjects. The mean HbA1C levels in NDT2DM, ADT2DM and controls were 7.7%, 10.5% and 5.3%, respectively. mtROS levels were significantly elevated ($p< 0.001$) in patients with diabetes and it was significantly greater in ADT2DM ($p <0.05$) as compared to NDT2DM. MMP was significantly reduced ($p <0.001$) in diabetic patients and it was significantly reduced in ADT2DM ($p <0.05$) relative to NDT2DM. mRNA and protein expression of NIX, PARKIN, PINK-1 were significantly down-regulated ($p<0.05$, $p<0.01$, $p<0.001$, respectively) in patients with diabetes, though, a greater decline in ADT2DM relative to NDT2DM subjects was observed.
Conclusion: Increasing hyperglycemia is associated with progressive rise in oxidative stress and attenuated expression of mitophagy-related genes. Our observations indicate HbA1C > 7% is associated with progressive decline in mitophagy irrespective of duration of diabetes, strengthening the rationale of achieving HbA1C < 7% for good glycemic control.
Disclosure: **S. Bhansali:** None.

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Renal hyperfiltration is associated with the risk of sarcopenia independent of obesity or metabolic syndrome

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Background and aims: The loss of muscle mass can increase mortality and disability in the elderly. Renal hyperfiltration is also closely linked to diabetes, hypertension, obesity and even could increase mortality in general population. Although the association between these cardiometabolic diseases and sarcopenia has been well-established, the relationship between renal hyperfiltration and sarcopenia has not been reported yet. We investigated the association between renal hyperfiltration and sarcopenia in the general population.
Materials and methods: This was a population-based, cross-sectional study using nationally representative samples of 13,800 subjects from the Korea National Health and Nutrition Examination Survey 2008-2011. Renal hyperfiltration was defined more than 90th percentile of age-, and sex-specific glomerular filtration rate (GFR) in subjects with normal kidney function (estimated GFR >60ml/min/1.73m2). The skeletal muscle index (SMI) [SMI(%)=total appendicular skeletal muscle mass (kg) / weight (kg)x100] measured by dual-energy X-ray absorptiometry was used to diagnose sarcopenia with cut points of 32.3% for men and 25.5% for women.
Results: Renal hyperfiltration was negatively correlated with SMI, body mass index, and, waist circumference (All Ps<0.001). Subjects with renal hyperfiltration had an increased risk of sarcopenia (odds ratio[OR]=1.48, 95% confidential interval[CI]=1.29-1.71, P<0.001). After stratification, subjects with renal hyperfiltration had a higher risk of sarcopenia regardless of obesity (ORs=1.32 to 1.37, Ps<0.05) or metabolic syndrome (ORs=1.29 to 1.28, Ps<0.05). Multiple logistic regression analysis also demonstrated this independent association between hyperfiltration and sarcopenia after adjustment for confounding factors (ORs=1.77 to 1.85, Ps<0.05).
Conclusion: Renal hyperfiltration is associated with increased risks of sarcopenia independent of obesity or metabolic syndrome.
Supported by: Korea Healthcare Technology R&D Project and NRF by ICT & Future Planning
Disclosure: **Y. Lee:** None.

PS 012 Exploring novel associations

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UMOD locus and glomerular filtration rate in individuals with type 2 diabetes: evidence of heterogeneity across two different European populations

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Background and aims: Reduced glomerular filtration rate (GFR) is a main risk factor for major cardiovascular events, end stage renal disease and premature death, in the general population and diabetic patients as well. GFR recognizes both genetic and environmental backgrounds. Among the single nucleotide polymorphisms (SNPs) so far genome-wide (GW) associated with GFR levels in the general population, is rs12917707 at the UMOD locus. It is of note that rs13333226, which is perfect linkage disequilibrium with rs12917707 (r2=1 in HapMap CEU), has been GW-associated with GFR in 4,888 Swedish individuals with type 2 diabetes (T2D), thus pointing UMOD as a strong genetic determinant of kidney function also in this subset of patients. Whether such finding is extensible also to diabetic patients from other populations deserves further studies. Our aim was to investigate the relationship between UMOD variability and GFR in patients with T2D from Italy.
Materials and methods: Four independent samples of patients from Italy with T2D (defined according to ADA 2003 criteria, n=3,087) were studied. The four samples were recruited in outpatient diabetic clinics at four different research/academic hospitals from Central-Southern Italy. All patients underwent physical examination and measurements of glycated haemoglobin, lipid levels, blood pressure and BMI. Urinary albumin and creatinine concentration were determined on an early morning first void sterile urine sample. Estimated GFR (eGFR) was calculated by using the CDK-EPI. Microalbuminuria was diagnosed if the ACR was 2.5 or 3.5 mg/mmol in men or women, respectively. Macroalbuminuria was defined as an ACR≥30 mg/mmol. Presence of hypertension was defined as a systolic blood pressure >140 mmHg or diastolic blood pressure >90 mmHg, or both, or the presence of antihypertensive treatment. Genotyping of the rs12917707 SNP at UMOD locus has been carried out by TaqMan allele discrimination.
Results: In none of the four Italian study cohorts, rs12917707 was significantly associated with GFR (p>0.05 for all). Similar results were obtained when the four samples were pooled and analysed together (β=0.83, p=0.19). Such effect size was strikingly smaller than that previously reported in Swedish patients (p for heterogeneity=1.2x10-7).
Conclusion: The previously reported strong association between rs12917707 and GFR in diabetic patients from Sweden is not observed in Italian diabetic patients, thus clearly pointing to a heterogeneous effect across the two different samples. This suggests that UMOD is a strong genetic determinant of kidney function in patients with T2D from some, but not all, populations.
Supported by: Italian Ministry of Health RC2015
Disclosure: **S. Prudente:** None.

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GHRL gene polymorphisms are related to type 2 diabetes and interact with olive oil intake in a Spanish population

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Background and aims: Ghrelin plays an important role in glucose metabolism and homeostasis. Several studies have reported common variants in GHRL gene related to Obesity, Metabolic Syndrome, and Type 2 Diabetes, becoming in a potential candidate gene. The aim of this study was to investigate single nucleotide polymorphisms (SNPs) and the susceptibility to develop Type 2 Diabetes, and look for possible interactions with diet.
Materials and methods: A cross-sectional population based study with 892 individuals was performance in two andalucian towns; Cabra and Pizarra, aged between 40-65 years, 43,2%men y 56,8%women, in risk to develop metabolic diseases (inclusion criteria: BMI>25 and/or some Metabolic Syndrome component by ATPIII). Followed by a prospective study after one year in a 277 individuals selection who presented alterations in glucose metabolism after the Oral Glucose Toletance Test (OGTT)(75mg/dL), with dietary/exercise advices. All participants filled nutritional surveys and anthropometric measures were taken, as long as, blood pressures, biochemical parameters, OGTT in order to detect carbohydrate metabolism alterations. Mediterranean diet adherence was calculated by "Score Predimed", specially olive oil intake. DNA was isolated and representative tagSNPs of the whole gene variability were selected for genotyping using TaqManOpenArray technology (rs10490815-rs10490816-rs1629816-rs2619507-rs26802-rs27647-rs35679-rs35683-rs4684677-rs696217). We calculated Hardy-Weinberg equilibriums and SNPs not in equilibrium were excluded from the analysis. Logistic regresion and lineal models adjusting by age, sex, BMI and Type 2 Diabetes, were calculated to detect associations.
Results: 16.1% presented Type 2 Diabetes, 45.9% presented impaired glucose metabolism. In the cross-sectional study, variants rs10490815,rs10490816,rs2619507 were associated to Type 2 Diabetes presence (p-value=0.001, p-value=0.001 and p-value=0.01, respectively), presenting heterozygous individuals lower prevalence compared to homozygotes, overdominant model. rs10490815 and rs35683 variants were associated to impaired glucose metabolism(IFG-IGT) by the same model (p-value=0.05, p-value=0.002). Also, rs10490815 was associated with insulin-resistance by HOMA-IR (p-value=0.05). In the follow-up study, we found associations with risk to develop Type 2 Diabetes for the same variants described in the cross-sectional study. Related to interactions with olive oil intake, individuals presenting the risk genotypes for rs10490815 and rs35683 variants, if they exclusively consume olive oil, presented lower Type 2 Diabetes prevalence compared to individuals who consume other oil types (p-value=0.01 and p-value=0.0004, respectively).
Conclusion: GHRL gene variants are associated with T2D presence and a protective interaction with olive oil intake exists in this individuals.
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Disclosure: **A.M. Lago-Sampedro:** None.

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Clinical and metabolomic study of carriers of NOS1AP variant rs12742393 in type 2 diabetes patients

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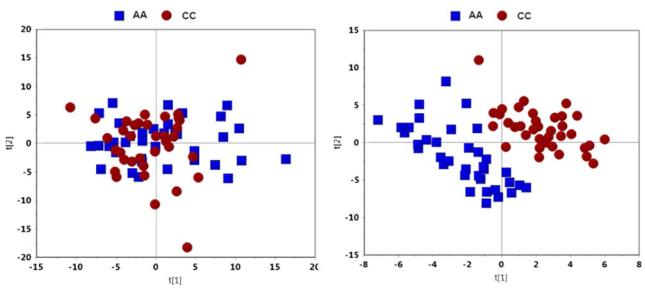
Background and aims: Nitric oxide synthase 1 adaptor protein (NOS1AP), also named as CAPON, regulates the neuronal nitric oxide synthase (nNOS)

activity and has an effect on nitric oxide (NO) release by binding N-methyl-d-aspartate receptors. Our previous study showed that rs12742393 in NOS1AP was involved in type 2 diabetes susceptibility in the Chinese population, with C allele as the risk allele (OR 1.17, 95% CI 1.07-1.26; P =0.0005) and there were significant different in the metabolic profiles of AA and CC genotypes in normal glucose tolerance subjects. In this study, we report a comprehensive metabonomic study of different genotypes (AA and CC) of rs12742393 using two complementary analytical platforms, gas chromatography time-of-flight mass spectrometry (GC-TOFMS) and ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-QTOFMS) in type 2 diabetes patients.

Materials and methods: Seventy-four type 2 diabetes patients were selected for metabolomic investigation, including thirty-seven CC homozygote and thirty-seven AA homozygote individuals. All the individuals for the metabolomic analysis were matched strictly with age, sex, BMI. For all the subjects, venous blood samples were obtained after overnight fasting for at least 10 h and subjected to GC-TOFMS and UPLC-QTOFMS. The GC-TOFMS data was analyzed by ChromaTOF software, the UPLC-QTOF-MS ES+ and ES- raw data were analyzed by the MarkerLynx Applications Manager version 4.1 and then metabolites were identified by comparing the accurate mass, mass fragments, characteristic ions with the available reference standards and published reports, in addition to the web-based resources such as the Human Metabolome Database. The data sets were then analyzed and validated by uni- and multi-variate statistical methods, separately.

Results: There were no significant differences in glucose and insulin levels across all groups. Similarilly, there were no significant differences in Insulin release and insulin sensitivity as surrogated by IGI and Modified Matsuda index methods and β-cell function as assessed by DIo among various genotypes studied. A total 262 metabolites were identified and ten significantly altered serum metabolites identified in AA carriers relative to CC carriers were selected according to the VIP threshold (VIP>1) in OPLS-DA model coupled with the Mann-Whitney U test ($p<0.05$). These including four cholic aicds: apocholic acid, lithocholic acid, ursodeoxycholic acid, isolithocholic acid, four amino acids: isoleucine, norvaline, phenylalanine, homoserine and two fattyp acids: nervonic acid and arachidonic acid.

Conclusion: We detected some metabolic difference between CC and AA carriers in type 2 diabetes patients. These metabolites might associate with the development of type 2 diabetes in subgroup of patients through the crosstalk with NOS1AP protein, which might provide us a new perspective to the mechanism of type 2 diabetes.



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Disclosure: **Y. Zhang:** None.

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Investigating the lipodystrophy genetic score associations with insulin sensitivity, ectopic and visceral fat in people at risk or with diabetes: a DIRECT study

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Background and aims: The lipodystrophy score is a genetic risk score composed of 11 common variants associated with fasting insulin. It was previously shown to be associated with a metabolic profile consistent with a common subtle form of lipodystrophy. The lipodystrophy score was associated with different traits measured by different consortia, i.e. higher fasting insulin, higher hepatic steatosis, and higher visceral-to-subcutaneous adipose tissue ratio, but lower BMI. It was also associated with an increased risk of T2D, CAD and hypertension. In this study we aimed to investigate the association of the lipodystrophy score with anthropometric measurements, insulin sensitivity modeled from OGTT, and with MRI measurements of ectopic and visceral fat of pre- and diabetic subjects from the IMI-DIRECT (Diabetes Research on Patient Stratification) consortium.

Materials and methods: Participants (1448 high risk for diabetes and 759 with diabetes) were recruited into a longitudinal study of glycaemic deterioration. Linear regression was performed (using sex, age, center, BMI and ancestry principal components as covariates) to assess the association between the lipodystrophy score and the following outcomes: abdominal, liver, pancreatic and subcutaneous fat measures calculated from the MRI; oral glucose insulin sensitivity (OGIS) from OGTT; and anthropometric measures (waist, hip, waist-hip ratio (WHR), thigh, calf). We performed subgroup analyses stratified by sex.

Results: In both groups combined the lipodystrophy gene score was associated with anthropometric measures in men (n=1529) but not in women (n=678). In men higher gene score was associated with lower BMI (males beta -4.14 kg/m^2, p=0.004; females beta -1.07 kg/m^2, p=0.69), narrower waist (males beta -13.29 cm, p=0.001; females beta -1.53 cm, p=0.82), and lower WHR (males beta -0.09, p=0.0002; females beta 0.01, p=0.78). In an analysis adjusted for BMI the lipodystrophy gene score was associated with higher liver fat in women (beta 1.83 percentage, p=0.007) but not men (beta 0.29 percentage, p=0.43). There was no association with MRI derived measures of intraabdominal, abdominal subcutaneous and truncal adiposity. In those with a high risk for diabetes the higher lipodystrophy score was associated with lower insulin sensitivity (beta -74.59 ml min^{-1} m^{-2}, p=0.0002) after adjusting for BMI.

Conclusion: The genetic lipodystrophy score is associated with lower BMI, waist and WHR measures only in men, and lower insulin sensitivity after adjusting for BMI. We do observe an increase in hepatic fat in females but this effect is not large. There is a need to increase the power, but our findings suggest that the effect of genetically driven lipodystrophy is sex specific.

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Circulating levels of eight miRNAs had a disease-related effect on diabetes duration 1, 3, 6, 12 and 60 months after diagnosis in children with type 1 diabetes

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Background and aims: During the past two decades there has been an increased interest in disease related variations in microRNA (miRNA) expression in circulation as biomarkers for several diseases. The leading hypothesis has been that differential miRNA expression profiles may provide insight to pathological processes related to disease origin and thereby potentially serve as non-invasive predictive biomarkers for diagnosis and disease progression. The objective of this study was to identify circulating microRNAs (miRNAs) expression that associate with disease over time in newly diagnosed children with type 1 diabetes mellitus.
Materials and methods: A subset of 40 children and adolescents with newly diagnosed type 1 diabetes mellitus from The Danish Remission Cohort (n=129) were intensively followed with blood samples the first 60 months after diagnosis. In this subgroup RNA was extracted from plasma samples obtained at 1, 3, 6, 12 and 60 months. miRNA profiling was performed using a predefined panel of 179 human miRNAs. To investigate the effect of duration on miRNAs expression across time-points (1, 3, 6, 12 and 60 months) data were analyzed by a Mixed Model for Repeated Measurement. This is a joint model that accounts for potential dependence between the values being made for each patient. The model has a linear effect of duration (time-points).
Results: Eight miRNAs: hsa-miR-99a-5p, hsa-miR-30e-5p, hsa-miR-497-5p, hsa-miR-10b-5p, hsa-miR-423-3p hsa-miR-125b-5p, hsa-miR-17-5p and hsa-miR-93-5p were found to be differentially expressed (p-value ≤ 0.01) after Hochberg correction for multiple testing. All had a disease-related effect of duration analyzed by a mixed model for repeated measurement with a linear effect on duration and adjusted for the effects of gender and age. The targets of these eight miRNAs were found to be associated with metabolic, MAPK signaling, chemokine and insulin signaling pathways based on KEGG pathway annotations.
Conclusion: The analysis demonstrated that only a limited number of miRNAs showed differential expression over time. Linear modeling demonstrated that eight of these miRNAs were significantly associated with disease duration, suggesting a possible effect on disease progression. Interestingly, all eight miRNAs were involved in various metabolic and signaling pathways, such as insulin, chemokine and MAPK signaling.
Supported by: EFSD/JDRF/Novo Nordisk, Beckett Foundation
Disclosure: **N. Samandari:** None.

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Is HLA the cause of the high incidence of type 1 diabetes in the Canary Islands? Results from the Type 1 Diabetes Genetics Consortium (T1DGC)

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Background and aims: The incidence of childhood-onset type 1 diabetes in the Canary Islands is the highest described so far in Spain, and one of the highest worldwide. Our aim was to assess high-risk and protective HLA haplotype distribution in the Canarian families included in the T1DGC, compared with the rest of Spain
Materials and methods: The T1DGC is an international effort to study the genetics and pathogenesis of type 1 diabetes. It included more than 3300 families with type 1 diabetes worldwide. Spain provided 149 of these families, of whom 42 were from the Canary Islands Tenerife and Gran Canaria. HLA was genotyped centrally in Malmö, Sweden, using a PCR-based, sequence-specific oligonucleotide probe system. A deterministic algorithm (alleHap) was developed in the environment R, to impute HLA haplotypes. Based on previous T1DGC results in Caucasian population, haplotypes DRB1*0405-DQA1*0301-DQB1*0302, DRB1*0401-DQA1*0301-DQB1*0302, DRB1*0301-DQA1*0501-DQB1*0201 and DRB1*0402-DQA1*0301-DQB1*0302 and DRB1*0404-DQA1*0301-DQB1*0302 were considered high-risk. DRB1*0701-DQA1*0201-DQB1*0303, DRB1*1401-DQA1*0101-DQB1*0503, DRB1*1501-DQA1*0102-DQB1*0602, DRB1*1104-DQA1*0501-DQB1*0301, DRB1*1303-DQA1*0501-DQB1*0301, DRB1*1301-DQA1*0103-DQB1*0603 and DRB1*0403-DQA1*0301-DQB1*0302 were considered protective. The distribution of protective, high-risk and other haplotypes were compared in the (first two) affected siblings and unaffected parents in the Canarian and non-Canarian Spanish families (chi-squared).
Results: Complete unambiguous haplotypes were obtained and compared in Canarian (72 siblings with type 1 diabetes and 70 non-diabetic parents) and non-Canarian subjects (162 siblings with type 1 diabetes and 139 non-diabetic parents).
Conclusion: Based on this family-based study, the high incidence of childhood-onset type 1 diabetes in the Canarian population does not seem to be explained by higher-risk class II HLA haplotypes.

Diabetic siblings*	Canary Islands	Rest of Spain
Protective haplotypes (%)	2.4	0.9
Risk haplotypes (%)	58.4	55.2
Other (%)	39.2	43.9
Non-diabetic parents**		
Protective haplotypes (%)	7.8	9.2
Risk haplotypes (%)	39.8	37.6
Other (%)	52.4	53.2

*p=0.27 **p=0.81.

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Disclosure: **A.M. Wägner:** None.

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Genome wide meta-analysis identifies novel regulators of circulating serum progranulin

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Background and aims: Progranulin is a secreted protein with important functions in processes including immune and inflammatory response and embryonic development. Genetic factors determining progranulin concentrations are yet unknown.
Materials and methods: We conducted a meta-analysis of genome-wide association studies (GWAS) for serum progranulin in three independent cohorts: Sorbs and KORA (Cooperative Health Research in the Region of Augsburg) from Germany and PPP-Botnia from Finland (total N=2,791). All single nucleotide polymorphisms (SNPs) associated with progranulin levels were genotyped for replication in 2 additional cohorts: LIFE Heart Study (N=967) and Berlin cohort (Metabolic Syndrome Berlin Potsdam; N=794). In addition, we measured expression levels of genes within the associated loci in peripheral blood mononuclear cells (PBMC) by microarrays and performed mRNA expression quantitative trait and expression-progranulin association studies to functionally substantiate identified loci. Finally, we conducted siRNA silencing experiments in vitro to elucidate the role of associated genes on progranulin secretion.
Results: The heritability estimate of circulating progranulin levels was 31.8% in the Sorbs cohort. Nine SNPs at chromosome 1 within the CELSR2-PSRC1-MYBPHL locus reached genome-wide significance ($p<5.0x10-8$) in the meta-analysis with the strongest evidence for association at rs660240 ($p=3.98x10-15$, beta=-0.161). All other SNPs within the cluster were in linkage disequilibrium with rs660240 ($r2=0.8$ in the Sorbs cohort). Rs660240 was associated with expression levels of PSRC) in

PBMCs (p=1.51x10-21). Psrc1 knock-down in murine 3T3-L1 preadipocytes led to a consecutive 30% reduction in progranulin secretion.
Conclusion: Our findings highlight the role of PSRC1 in the regulation of circulating progranulin.
Supported by: CRC1052-C1
Disclosure: **J. Krüger:** None.

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A genome-wide association study identifies common genetic loci associated with lipid levels in Chinese patients with type 2 diabetes
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Background and aims: A large body of literatures indicated that levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) are important and modifiable risk factors for cardiovascular disease. These associations have been attributed to both genetic and environmental factors. Here we performed a genome-wide association study for these lipids measured at baseline from the Hong Kong Diabetes Registry (HKDR), which includes more than 8,000 patients with type 2 diabetes (T2D) and prospective follow-up.
Materials and methods: After excluding individuals known to be on lipids-lowering medication (i.e. statins and fibrates) at baseline, total 4,680 Chinese-ancestry individuals (45.1% male, mean age 56.6 ± 13.3 years and BMI 25.0 ± 3.9 kg/m^2) were included in analysis. Fasting blood samples were collected for the measurements of TC, TG and HDL-C. LDL-C was calculated using Friedewald's formula. Inverse normalized residuals transformation was applied on all lipid traits, adjusting for sex, age, body mass index and principal components. We genotyped the DNA samples using the Illumina Omni 2.5+ exome array and imputed the genotypes using minimac 3 with the 1000 Genomes Project phase 3 v5 as reference panel . After quality control, ~8 million common SNPs were included in the linear regression analysis.
Results: We identified 1362 SNPs within 36 independent loci associated with lipid levels at $P < 10^{-5}$, including 13 previously reported loci (*DOCK7*, *GCKR*, *LPL*, *TRIB1*, *ABCA1*, *ABO*, *FADS1-2-3*, *APOA1*, *LIPC*, *CETP*, *LIPG*, *LDLR*, *APOE*) and 23 novel suggestive loci. Of these, one TC-related locus (*APOE*), 5 TG-related loci (*DOCK7*, *GCKR*, *LPL*, *APOA1* and *APOE*), 5 HDL-related loci (*LPL*, *FADS1-2-3*, *APOA1*, *LIPC*, *CETP*) and one LDL-related locus (*APOE*) exhibited a genome-wide significant association ($P < 5\times10^{-8}$). Moreover, we found nominal evidence of association for coronary heart disease (CHD) with *LPL* ($0.011 < P < 0.049$) and *APOE* ($8.8\times10^{-6} < P < 0.038$) loci.
Conclusion: Our findings support the important contribution of these genetic variants for lipid levels in T2D patients compared with general population. We have also demonstrated the evidence of genetic determinants shared among lipid traits and CHD in a Chinese population. Genotyping on additional samples is now ongoing for the confirmation of the novel suggestive loci.
Supported by: Theme-based Research Scheme and the Focused Innovations Scheme
Disclosure: **C.H.T. Tam:** None.

PS 013 Complications: patterns, treatments and detection

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Clustering of microvascular complications in patients with type 1 diabetes
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Background and aims: Diabetic complications have traditionally been studied in isolation, and although it is well known that their occurrence is related, insight into the complex patterns of complication clustering is limited. Examination of the pattern of co-occurrence of microvascular diabetic complications may enhance our understanding of the mechanisms driving complication development.
Materials and methods: We performed a cross-sectional analysis in a clinical cohort of patients with type 1 diabetes (T1DM), who were treated at any time between 1 January 2001 and the index date of 31 December 2007 at a Danish diabetes research hospital. Information from yearly structured clinical check-ups of complication status was used to create a cross-sectional dataset characterizing the presence of all microvascular diabetic complications at the index date. We calculated the prevalence of neuropathy (biothesiometry >25 mV), retinopathy (retinopathy ≥ grade two) and nephropathy (micro- or macro albuminuria) and each combination of complications in patients who had had at least one valid retinopathy assessment as well as two valid neuropathy and nephropathy assessments prior to the index date. The expected prevalence for each complication state was calculated by using log-linear analysis under the hypothesis of no association
Results: 2391 patients (1248 male, mean age: 52 years (SD=14.9), mean HbA1c: 65.3 mmol/mol (12.8), mean duration of diabetes: 27.2 years (13.8) fulfilled the inclusion criteria. The prevalence of nephropathy, neuropathy and retinopathy was 37.3% 95%CI: (35.4 ; 39.3), 38.6% (36.6 ; 40.5) and 62.7% (60.7 ; 64.6)), respectively. Table 1 shows the prevalence of each combination of complications. 25.6% of patients had no complications, 29.1% had one complication, 26.7% had two complications and 18.4% had all three complications. The observed prevalence of no complications and of the combination of all three complications was higher than expected, while all other complication states seemed to occur less frequently than expected. Log-linear models showed two-way interactions for all three complication pairs, but no evidence for three-way interaction.
Conclusion: Microvascular complications affect almost 3/4 of adult patients with T1DM and clustering seems to occur at both extremes of the three-dimensional distribution of complications. Our findings are in accordance with a strong shared aetiology between the processes leading to each of the three microvascular complications.

Complications 31.12.2007	Observations N=2,391	Observed Prev. (95%CI)	Expected Prev. (95%CI)	Ratio
No complications	613	25.6 % (23.9 ; 27.4)	14.3% (13.0 ; 15.8)	1.79
Neph	96	4.0% (3.3 ; 4.9)	8.6% (7.5 ; 9.8)	0.47
Neur	127	5.4% (4.5% ; 6.3%)	9.0% (7.9 ; 10.2)	0.60
Ret	469	19.7% (18.1 ; 21.3)	24.2% (22.5 ; 26.0)	0.81
Neur + Neph	57	2.4% (1.8 ; 3.1)	5.4% (4.6 ; 6.4)	0.44
Ret + Neph	291	12.2% (10.9 ; 13.5)	14.3% (13.0 ; 15.8)	0.85
Ret + Neur	290	12.1% (10.9 ; 13.5)	15.2% (13.8 ; 17.0)	0.80
Neur + Ret + Neph	448	18.7% (17.2 ; 20.3)	9.0% (7.9 ; 10.2)	2.08

Table 1. Observed and expected prevalence of microvascular complications and their combination in patients with T1D. Neph: nephropathy, Neur: neuropathy, Ret: retinopathy.

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Disclosure: **L. Bjerg:** Grants; Danish Diabetes Academy, Research and Innovation foundation, Denmark.

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Can interleukin-2 receptor antagonists reduce the incidence of new-onset diabetes after renal transplantation?

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Background and aims: To examine the association between interleukin-2 receptor antagonists (IL-2Ra) and new-onset diabetes after transplantation (NODAT) among renal transplantation recipients.
Materials and methods: We conducted a retrospective cohort study of 915 renal transplantation recipients from January 1993 to March 2014 in our university hospital. Patients with uncomplete data, death or graft loss in first year, multiple organ transplantation, a second transplantation and pre-transplant diabetes were excluded from the present retrospective cohort study. A total of 557 renal transplantation recipients were included in final analysis. Prevalence rate of NODAT after renal transplantation were evaluated. Meanwhile, univariate and multivariable regression analysis were fitted to evaluate risk factors for NODAT and show the association between IL-2Ra and NODAT among renal transplantation recipients.
Results: All renal transplantation recipients involved in this study were followed at a mean time of (6.76±4.00) years after transplantation. Prevalence rate of NODAT at 1, 3, 10 and 15 years among patients were 10.23%, 15.15%, 33.06% and 43.59%, respectively. Univariate analysis indicated that IL-2Ra has an association with the development of NODAT among renal transplantation recipients ($P < 0.05$). After adjusting for potential confounders including age, BMI, history of smoking, family history of diabetes, donor renal type, hepatitis virus infection, cytomegalovirus infection, preoperative fasting plasma glucose, preoperative blood lipid levels, type and concentration of immunosuppressant regimen, multivariable regression analysis showed IL-2Ra can still reduce the risk of NODAT (OR = 0.22; 95%CI = 0.13-0.36; $P < 0.001$).
Conclusion: There was a high prevalence rate of NODAT among renal transplantation recipients and IL-2Ra was a protective factor for the development of NODAT.
Disclosure: **M. Yu:** None.

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The inter-relationship among the proteinuria, eGFR and duration of disease on type 2 diabetes in usual physician care-based on DCMP2001, Taiwan

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Background and aims: In order to evaluate the interrelationship among proteinuria, eGFR and diabetes duration in T2DM for the ensuing development of personalized prevention program.
Materials and methods: From 2008 to 2013, 4096 cases of T2DM without macro-albuminuria were cumulatively enrolled in DCMP 2001. All patients were having the general anthropometric data collected; metabolic variables, sequential urine examination, albumin creatinine ratio (ACR) and eGFR (simplified MDRD) measured in 3-6 months interval. The highest ACR was classified into 3 groups, <30 (n=2994), ≧30 & <300 (n=801), and ≧300 (n=301) and the lowest eGFR (ml/min/1.73m^2) was divided into 4 different ranges, <30, 30-59, 60-89, ≧90 in each ACR group respectively. The ANOVA and Post-hoc comparison were used for data analysis.
Results: The case number and percentage distribution of 4 different ranges of eGFR in each ACR group demonstrated respectively in Table 1. There were almost 10% of patients with T2DM in ACR≧300 had eGFR<30, whereas 2-4% diabetes with ACR<300 already had eGFR<30. The duration (year, Mean± SD) from diabetes onset to the time of different ranges of eGFR in these 3 groups of ACR were shown in Table 2. The time of onset of 4 different ranges of eGFR in each ACR group was statistically demonstrated that there was significantly earlier decline of eGFR in group with ACR<30 rather than other 2 groups.
Conclusion: The results showed that there were significant number of T2DM has early declined their kidney function before ACR measure becoming clinically irreversible, therefore the assessment of diabetes nephropathy with its functional status in T2DM, combination of both ACR and eGFR measures periodically would be highly recommended. Further longitudinal follow-up study would be required.
Disclosure: **M.M. Fuh:** None.

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Diabetes predicting albuminuria incidence in Australian indigenous adults from rural and remote communities: 7 year follow up study

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Background and aims: Australian Indigenous people have excess prevalence of both diabetes and albuminuria, particularly in remote and rural communities. We aimed to study the association of albuminuria incidence with diabetes in Australian Indigenous adults from 19 communities in North Queensland during 1998-2007.
Materials and methods: All Indigenous people were invited during 1998-2000 to participant a population based wellness screening program. Baseline anthropometric measures (weight, height, waist circumference), BP, fasting blood glucose and lipid, urine albumin creatinine ratio were recorded. Demographic, tobacco and alcohol consumption, and physical activity level were assessed using questionnaires. Follow up was conducted in 2004-2007 among a total of 434 consented adults without albuminuria. Diabetes was defined as either clinical diagnosis verified by the participants' medical records or a 2 hour glucose tolerance test of glucose >=11.1 mmol/L, or fasting blood glucose level > 7.0 mmol/L. Hypertension was defined as BP >= 140/90 mmHg, or an established hypertension diagnosis in clinic files. Albuminuria was defined as urine albumin to creatinine ratio >=2.5 g/mol in males and >=3.5 g/mol in females. Incidence was computed and compared using Survival analysis. The association between diabetes and albuminuria was assessed using Cox proportional model adjusted for demographic and biomedical factors.
Results: The mean age of the cohort was 37 years and 54% were females. At baseline, 39% were obese, 31% had hypertension and 13.0% had diabetes. During a median follow up of 7 years, 75 new cases of albuminuria were ascertained and the overall incidence was 26.0/1000 pys (95% CI: 20.7-32.6). 38 were females (incidence rate 24.2, 95% CI 17.6-33.2/1000 pys) and incidence in males was 28.2 (95% CI 20.5-39.0/1000 pys). The incidence was significantly higher in adults with diabetes (51.2, 95% CI 31.8-82.3/1000 pys) compared to those without (22.7, 95% CI 17.6-29.4/1000 pys) with an HR of 2.6 (95% CI 1.5-4.7) adjusted for age, sex, ethnicity, BMI, hypertension, GGT, and lifestyle factors. In the non-diabetes strata, albuminuria incidence increased by glucose tertiles from 4.2 for the 1st tertile (glucose < 4.5 mmol/L) to 7.2 for the 2nd and 12.4/1000 pys for the 3rd tertile (glucose > =5.3 mmol/L) (trend P<0.001).
Conclusion: Albuminuria incidence in Australian Indigenous adults from rural and remote communities in North Queensland was twice the rate of non-Indigenous adults in Australia. High background prevalence of diabetes and increased fasting glucose predicted it. Early screening and effective management of diabetes and albuminuria should be implemented at the primary health care level in order to prevent the complications.
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Disclosure: **M. Li:** None.

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Diabetes complications and alcohol are important causes of death in individuals diagnosed with type 1 diabetes in late adolecence and young adulthood: long-term follow-up

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Background and aims: The aim of this study was to determine long-term mortality and causes of death in type 1 diabetes (T1D), aged 15-29 years at diagnosis.
Materials and methods: The cohort was nationwide and population-based, collected retrospectively by contacting medical and paediatric departments in Norway, and through the nationwide National Insurance Institution. Year of diagnosis was 1978-1982. We classified individuals treated with insulin from diagnosis, as T1D. The degree of ascertainment has been estimated to almost 90%. Survival and emigration status was obtained by linking to the National Population Registry and causes of death by linking to the nationwide Cause of Death Registry. Individuals were followed from diagnosis until death, emigration or to September 30, 2013. In addition a clinical committee reviewed the causes of death by evaluating medical records, autopsy reports and death certificates. We calculated standardized mortality ratios (SMRs) to compare mortality and causes of death with the background population. Alcohol-related death was defined by ICD-codes in accordance with the definition used by Norwegian Institute of Public Health.
Results: Among the 719 individuals, representing 21,272 person-years, 148 (20.6%) died, during a mean follow-up of 29.6 years (range 0.1-35.8), 106 males and 42 females. Mean age at diagnosis was 22.4 years (15.0-29.9). Cumulative mortality according to diabetes duration was 6.0% (95% CI 4.5-8.0) at 10 years, 12.2% (95% CI 10.0-14.8) at 20 years and 18.4% (95% CI 15.8-21.5) at 30 years. Mortality was between four and five times higher in individuals with T1D compared with the background population (4.4, 95% CI 3.7-5.1). Diabetes was mentioned on the death certificate in 68.2% of the cases. Death was caused by acute diabetic complications in 20.5%, chronic complications in 32.2%, violent death in 19.9% and any other cause in 27.4%. According to the death certificate, death was related to alcohol in 15.1% (22/148) of the cases. Among those with alcohol-related death 86.4% (19/22) was men and mean age at death was 36.2 years (23.0-55.0). Alcohol caused or contributed to death in 22% of all deaths at 20 years duration of diabetes. SMR was significantly elevated for alcohol-related death: 6.8 (95% CI 4.5-10.3), cardiovascular death: 7.3 (95% CI 5.4-10.0) and violent death (suicide, intoxications and accidents): 2.8 (95% CI 1.9-3.9), but not for cancer: 1.7 (95% CI 0.9-2.6). An autopsy was performed in 51% of the deceased.
Conclusion: Mortality was almost five times higher in people diagnosed with T1D in late adolescence and young adulthood, compared with the background population. The high mortality at 20 years duration can partly be explained by alcohol abuse, mainly occurring in men.
Supported by: The South-Eastern Norway Regional Health Authority
Disclosure: **V. Gagnum:** None.

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Cardiovascular risk in persons with prediabetes

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Background and aims: To estimate 3-year risk of cardiovascular events, overall and acute cardiovascular mortality in persons with impaired fasting glucose (IFG), impaired glucose tolerance (IGT) and screen detected type 2 diabetes (T2DM).
Materials and methods: According to population based study among 1280 adults, 3-year risk of overall and acute cardiovascular mortality and cardiovascular events (myocardial infarction and stroke, coronary heart disease) was estimated in people with IFG, IGT, IFG+IGT and T2DM in comparison with normal glucose tolerance. Abnormal glucose tolerance was diagnosed using standard OGTT according to WHO 1999/2006 criteria. Relative risk (RR) and B unstandardized regression coefficient was calculated using Cox-regressive analysis. RR was adjusted for age, sex, BMI, systolic blood pressure, smoking.
Results: Prevalence of glucose metabolism abnormalities was 24.9%, including IFG (8.8%), IGT (5.1%), IFG+IGT (3.8%) and first time diagnosed T2DM (7.2%). In 3 years, percent of cardiovascular events was almost identical in people with T2DM (16.4%) and IFG+IGT (16.0%), with normoglicemia was 6.1%. RR of cardiovascular events was significantly increased among people with IFG (2.2, 95%CI:1.02-4.53, p=0.044) and in persons with newly diagnosed T2DM (2.7, 95%CI:1.28-5.68, p=0.009) in comparison with normal glucose tolerance. Highest percent of cardiovascular mortality was seen among people with IFG (5.2%), in people with IGT and IFG+IGT was 3.1% and 2.0% consequently, the lowest percent of cardiovascular mortality was seen in people with T2DM (1.1%) and normoglicemia (0.9%). Adjusted RR of cardiovascular mortality was significantly higher among people with IFG (3.5, 95%CI:1.17-10.62, p=0.024) compaired with normoglycemia. People with IGT had significantly greater adjusted risk of overall mortality (2.2, 95%CI:1.03-5.12, p=0.041). There was not linear association between fasting blood glucose levels and cardiovascular mortality risk (B=0.145, p=0.311) in contrast to the continuous linear relationship observed between fasting blood glucose levels and coronary heart disease risk (B=0.244, p=0.001).
Conclusion: IFG increased 3-year risk of acute cardiovascular mortality. IGT increased 3-year risk of overall mortality. There was not linear association between blood glucose levels and cardiovascular mortality risk. These may provide insights, that hyperinsulinemia influence on acute cardiovascular mortality risk.
Disclosure: **T.G. Dzebisashvili:** None.

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High burden of cardiovascular risk factors and poor glycaemic control in type 2 diabetes patients diagnosed before the age of 45 years in Denmark: results from the DD2 study

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Background and aims: The rising prevalence of type 2 diabetes (T2D) globally is accompanied by an earlier onset age. We aimed to describe clinical characteristics, health behaviour, and treatment in young patients ≤ 45 years of age newly diagnosed with T2D, compared with older newly diagnosed T2D patients > 45 years of age.
Materials and methods: We conducted a cross-sectional study of 4,214 T2D patients enrolled between 2010 and 2014 in the cohort of the Danish Centre for Strategic Research in Type 2 Diabetes (DD2). For the comparison of characteristics, gender-adjusted prevalence ratios (PRs) with 95% confidence intervals (CI) were calculated using Poisson regression analysis.
Results: Younger T2D patients constituted 10.2% (n=429) of the cohort. Compared with older T2D patients, younger patients had higher levels of HbA1c (HbA1c ≥75 mmol/mol: 12% vs. 5%, PR=2.49 (95% CI 1.86-3.33)), were more obese (BMI >40 kg/m^2: 19% vs. 7%, PR=2.62 (95% CI

2.03-3.37)), more dyslipidemic (LDL >3 mmol/L: 27% vs. 21%, PR=1.27 (95% CI 1.07-1.51)), and had higher rates of elevated C-reactive protein (CRP >3 mg/L: 54% vs. 36%, PR=1.50 (95% CI 1.22-1.84)). Younger patients had higher rates of diastolic hypertension (diastolic blood pressure >90 mmHg: 17% vs. 11%, PR=1.51 (95% CI 1.20-1.91)), but lower rates of systolic hypertension (systolic blood pressure >140 mmHg: 18% vs. 24%, PR=0.77 (95% CI 0.62-0.95)) than older patients. Daily smoking was more frequent in younger than in older patients (24% vs. 18%, PR=1.36 (95% CI 1.13-1.64)), and fewer younger than older patients reported at least 30 minutes of physical activity 6-7 days/week (22% vs. 32%, PR=0.67 (95% CI 0.56-0.81)). Hospital-diagnosed retinopathy was more frequent in younger than older patients (8% vs. 3%), whereas macrovascular complication rates were higher in older patients (5% vs. 25%). Combination-therapy with insulin and a non-insulin drug was more frequent in younger than in older patients (9% vs. 5%, PR=1.95 (95% CI 1.39-2.73)), but treatment with antihypertensive drugs, hypolipidemic drugs, and anticoagulation drugs was less frequent in younger than in older patients.

Conclusion: Young patients with early-onset T2D are a high-risk group characterized by poor glucose control and a high burden of cardiovascular risk factors, such as obesity, low physical activity levels, and dyslipidemia, compared with T2D patients diagnosed at a later age. Thus, future health services should ensure early intensive treatment, close follow-up, and evidence based interventions for improved self-management for young T2D patients.

Supported by: Research grants from The Danish Diabetes Academy supported by the NNF

Disclosure: **A. Bo:** None.

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Early detection of type 2 diabetes and intensive treatment may reduce social inequalities in cardiovascular morbidity and mortality, ADDITION-Denmark

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Background and aims: Individuals with type 2 diabetes and low socio-economic status (SES) have high rates of cardiovascular morbidity and mortality. Screening and early treatment of diabetes have been proposed in a number of countries. Socioeconomic disadvantaged people have been found to be diagnosed later in the disease trajectory which is why early detection of diabetes and early treatment may reduce socioeconomic inequalities in health outcomes. Among a cohort of individuals with screen-detected diabetes, we examined the association between socioeconomic status and mortality and cardiovascular disease (CVD).

Materials and methods: The ADDITION-Denmark trial cohort includes 1533 individuals aged 40-69 years with type 2 diabetes detected by screening between 2001 and2006. Information on baseline education, income and cohabitation status was obtained from national registers. CVD was defined as first event of non-fatal MI, non-fatal stroke or CVD death. Using Cox regression, we calculated the long-term risk of CVD and all-cause mortality by socio-economic status, adjusting for age, gender and prevalent CVD.

Results: After five years of follow-up, individuals with a low educational level had a higher risk of CVD (HR 2.3, 95% CI 1.2 to 4.3) compared to those with higher educational level. Those with a moderate income also had a higher risk of CVD (HR 2,3, 95% CI 1.4 to 4.3) compared to individuals with the highest income. There was no association between education and risk of mortality (HR 1.6, 95% CI 0.9 to 2.6), nor between income and mortality (HR 1.6, 95% CI 0.9 to 2.8). People with type 2 diabetes living alone had a higher risk of mortality compared to those cohabiting (HR 1.7, 95% CI 1.3 to2.4).

Conclusion: In this screen-detected type 2 diabetes population we found a higher risk of CVD among people with low educational levels and low income levels. There was no association between these SES indicators and all-cause mortality. Compared to other studies of individuals with clinically diagnosed individuals, there was less evidence of social inequality in risk of mortality. However, there remained a SES gradient with regards to risk of CVD.

Association between baseline socio-economic status and long-term risk of cardiovascular disease and death among the ADDITION-Denmark trial cohort (n=1533)

	Composite Cardiovascular event (HR, 95%CI)*	All-cause mortality (HR, 95% CI)**
Educational level		
≥10 years	2.3 (1.2 to 4.3)	1.6 (1.0 to 2.6)
10-15 years	1.6 (0.9 to 3.0)	1.4 (0.8 to 2.2)
>15 years	1	1
Income		
20% percentile	1.8 (0.9 to 3.7)	1.6 (0.9 to 2.8)
20-80% percentile	2.3 (1.4 to 4.3)	1.8 (1.0 to 3.1)
80% percentile	1	1
Cohabiting status		
Living alone	0.8 (0.5 to 1.3)	1.7 (1.3 to 2.4)
Cohabiting	1	1

*Composite cardiovascular event (first of non-fatal MI, non-fatal stroke or CVD death) with follow-up until 31.12.2009

**Follow-up until 31.12.2011

All analyses adjusted for age, sex and prevalent CVD

Clinical Trial Registration Number: NCT00237549

Disclosure: **E. Dalsgaard:** None.

PS 014 Understanding differences and similarities in diabetes around the world

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Type 1 diabetes phenotype is similar in UK white and south Asian people with young-onset diabetes: results from the MY DIABETES study

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Background and aims: Differentiating type 1 diabetes (T1D) from other subtypes in ethnic groups is challenging. Non-white ethnicity in young adults is often assumed to favour type 2 diabetes, however the characteristics of T1D have not been studied in non-white ethnic groups. Data suggest second generation migrant populations adopt the local T1D risk, but the phenotype in these groups remains unexplored. We used fasting C-peptide (fCP) to classify T1D in a UK multiethnic population with young-onset diabetes and aimed to explore their characteristics by ethnicity.
Materials and methods: The MY DIABETES study is a multi-centre cross-sectional study, systematically phenotyping people diagnosed with any type of diabetes under 30 years of age from white (WE), south Asian (SA) or African-Caribbean ancestry. In this preliminary analysis we report the clinical and biochemical characteristics of people with T1D (fCP <200 pmol/L) by ethnicity and generation of migration. The study continues to recruit ~30 participants per month across 21 sites.
Results: Of 420 people recruited, 73% and 18% were of WE and SA ethnicity respectively. The criteria for T1D was met in 89% of WE and 63% of SA recruits (p<0.001). Clinical phenotype: In those classified with T1D, SA and WE people had similar ages at diagnosis (23.7 vs 21.6 years, p 0.260), BMI (26.0 vs 26.5 kg/m2, p 0.864) & HbA1c (62 WE and 62 mmol/mol SA, p=0.649), however duration of diabetes was significantly lower in the SA group (21.6 years vs 30.0 years, p=0.003). Antibody status: No difference in the proportion with detectable antibodies was observed between ethnic groups (55% WE vs 58% SA, p=0.765), even when adjusted for duration. The proportions of GAD65-antibody positivity were similar between ethnic groups (45% WE vs 48%SA p=0.764), as were the proportions who were IA-2 antibody positive alone (9.6% WE vs 9.7% SA, p=0.238). To assess the potential impact of antibody positivity on phenotype, we compared parameters in WE and SA people with T1D by antibody status, however no additional effects were observed between ethnic groups. Migration status: We finally assessed the impact of migration generation on phenotype in SA people with T1D; 2nd generation SA people (UK born) were diagnosed younger than 1st generation (11.4 vs 23.3 years, p=0.039) but had similar durations of diabetes (19.8 years 1st generation vs 22.8 years 2nd generation). Despite similar durations of diabetes, 68.0% of 2nd generation SA people were antibody positive vs 46.7% of 1st generation (p=0.213). Other parameters were similar.
Conclusion: Young onset T1D in UK SA and WE individuals is phenotypically very similar. These data suggest that in young people presenting with diabetes, ethnicity should not impact on clinical diagnosis, even in those without detectable antibodies. Migration generation may affect phenotype and additional investigations are needed to study differences between native and migrant ethnic groups. Further work is required to characterise additional antibodies, T1D and type 2 diabetes genetic risk scores and other classification groups in the MY DIABETES cohort.
Clinical Trial Registration Number: NCT02082132
Supported by: DRWF
Disclosure: **S. Misra:** None.

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Age-related differences in ketoacidosis prevalence at type 1 diabetes diagnosis in chidren from Wielkopolska Province, Poland

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Background and aims: Ketoacidosis is a life-threatening complication of type 1 diabetes frequently present at its diagnosis. Younger children are at greater risk of developing ketoacidosis. The prevalence of ketoacidosis at diagnosis in children aged <5 years varies between 17.3-54.5%. This trend is alarming due to worldwide rise in type 1 diabetes incidence with the greatest increase in children aged <5 years. We studied the prevalence of diabetic ketoacidosis at type 1 diabetes diagnosis and factors associated with its occurrence in youth from Wielkopolska province, Poland.
Materials and methods: The study cohort comprised 735 children (girls: 329; boys: 406) aged 0-18 years with new onset type 1 diabetes admitted to our Diabetes Centre between 2009 and 2014. Diabetes was diagnosed based on WHO criteria. The mean age at diagnosis was 9.2 years. Standard laboratory methods were used to measure plasma glucose and blood pH. Diabetic ketoacidosis was defined as blood pH < 7.30 and considered mild, moderate, severe if pH was < 7.3, <7.2 and <7.1, respectively. To confirm autoimmune diabetes origin typical autoantibodies were tested (IAA, GAD-ab, IA2-ab, ZnT8). A questionnaire on diabetes from first symptom(s) to diagnosis was completed by children's caregivers.
Results: Ketoacidosis was diagnosed in 36,0% of patients of whom 12,9% developed mild form, while 14,5% and 8,7% moderate and severe, respectively. In children aged 0-4, 5-9, 10-14 and 15-18 years ketoacidosis was present in 48.5, 34.7, 31.4 and 28.2%, respectively. In individuals aged <4 years it occurred significantly often (p=0.001). Mild ketoacidosis was present mainly in children aged 5-9 years, while severe in children aged 28 days) (p=0.014) and diabetes misdiagnosis (p=0.001). ZnT8 autoantibody was detected significantly more often in children with ketoacidosis compared to children without ketoacidosis (p=0.44). There was no relationship between ketoacidosis presence or its severity and other typical autoantibodies. Blood ketones, blood glucose and HbA1c levels were significantly higher in children with ketoacidosis compared to children without it at diagnosis (p=0.0001, p=0.00001, p=0.0004, respectively), while insulin and c-peptide levels were lower (p=0.0001 and p=0.0001, respectively).
Conclusion: The prevalence of diabetic ketoacidosis is high and its severity is substantial in children from Wielkopolska. Children aged < 4 years have the highest risk of developing ketoacidosis which usually presents as severe one. The highest frequency of severe ketoacidosis is related to symptoms' duration and diabetes misdiagnosis. It indicates that medicine training and postgraduate programs should pay more attention to childhood diabetes. ZnT8 autoantibodies are associated with the worst general condition at the time of diabetes diagnosis.
Disclosure: **E. Niechcial:** None.

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Relationship between ancestry and self-reported ethnicity in patients with type 1 diabetes: a nationwide survey in Brazil

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Background and aims: Type 1 diabetes (T1D) is a chronic disease with an increasing incidence in developed as well as in developing countries, including Brazil. Brazilian population is multi-ethnic with a great miscegenation among European and Middle East Caucasians, Africans, Asians and Native Indians that has been occurring for 5 centuries. So far the

majority of studies have used self-reported ethnicity instead of genetic ancestry to evaluate clinical and laboratorial data in patients with T1D. The aim of our study was to determine the relationship between ancestry, self-reported ethnicity in T1D patients under routine clinical care in Brazil.

Materials and methods: This was a cross-sectional, nationwide survey conducted between August 2011 and August 2014 in 14 public clinics from 10 Brazilian cities. Data were obtained from 1,760 patients, aged 30.02 ± 11.91 years (55.8% females, 54.4% self-reported as Caucasians, 34.9% as Mullatos, as 7.7% African-Brazilians, as 1.1% Asiatic descendants and as 0.9% native Indians).The mean time since diabetes diagnosis was 15.5 ± 9.3 years. The global and individual genetic ancestry was inferred using a panel of 46 AIM-INDEL (Ancestry-Informative Markers). These markers have different allele frequencies among European, African and Amerindian Native populations. Genotyping of 46 AIM-indels was performed by multiplex PCR followed by capillary electrophoresis. We used the StructureV.2.3.3 software to estimate ancestry and the HGDP-CEHP diversity panel (Sub-Set H95) as reference date of ancestral Populations. The cut-off point to define each ancestry was a percentage ≥ 0.60. The ancestry percentage based on the 46 AIM-indels of studied T1D population were compared with published data of the Brazilian population for the same markers, containing 936 unrelated individuals, control (C) from different metropolitan areas.

Results: 1,627 patients with T1D were included in this study. A higher European ancestry percentage was observed in patients with T1D in comparison to C, respectively 0.5868 ± 0.1742 vs 0.5527 ±, p<0.001. Patients with T1D had and lower African percentage ancestry compared to C, respectively (0.2169 ± 0.1422 vs 0.2580 ± 0.1673, p<0.001. No difference was observed in Native Indian percentage ancestry, (0.1957 ± 0.1152 vs 0.1892 ± 0.1144, p=0.17), respectively, The European percentage ancestry contribution was 0.6468 ± 0.1557 in T1D patients who self-reported them as Caucasians ; 0.3941 ± 0.17 in those who self-reported as African-Brazilians; 0.5386 ±0.1536 in those who self-reported as Mullatos and 0.4073 ±0.1409 in those who self-reported as Native Indians.

Conclusion: Patients with T1D in Brazil had a predominant European ancestry. Even in those patients who self- reported as Native Indians, African-Brazilian and mullatos, a significant percentage of European genome ancestry contribution was found.

Supported by: cnpq,faperj

Disclosure: **M.B. Gomes:** None.

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Addressing the diabetes mellitus (DM) and tubercolosis (TB) co-epidemic among TB patients in Luanda, Angola

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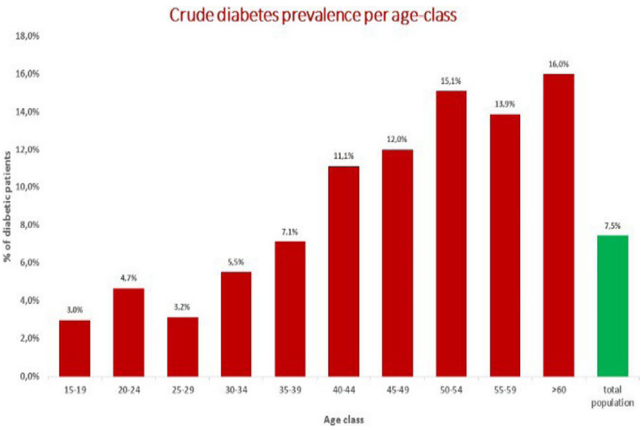
Background and aims: Diabetes mellitus (DM) increases the risk of developing active tuberculosis and is associated with worse outcomes. Globally, 15% of tuberculosis cases are estimated to be attributable to diabetes. Few epidemiological data about the co-epidemic are available and none in a 22 million country like Angola. The goal of this project is to improve diabetes detection in TB patients through the integration of type 2 DM surveillance activities in TB national programs. We aimed to determine DM prevalence among TB patients accessing to 6 directly observed treatment (DOT) centers in Luanda (Czariango, Luanda and Cacuaco Municipality, Kilamba Kiaxi and Maianga District), the capital of Angola.

Materials and methods: All patient that accessed the 6 DOT centers have been screened for diabetes after being diagnosed of TB. TB diagnosis was preferentially carried out through microscopy, otherwise clinically and radiologically. DM was tested with either Fasting Blood Sugar (considered positive if ≥ 7.0mmol/l) or Random Blood Glucose Test (considered positive if ≥ 11.1mmol/l). The project aims to enroll 7,000 TB positive patients.

Results: According to preliminary data, out of 3,686 TB diagnosed patients, 51% were female and 54% had a positive microscopy test. Concerning BMI, 53% of the population resulted underweight with a BMI lower than 18.5, 28% had a BMI comprised between 20 and 24, while 8% of the population showed a BMI higher than 25. The crude prevalence of DM among TB patients resulted 7,5%, more than two-fold the prevalence among the general population (3,3%). DM prevalence increased with age, being 4.9% in subjects aged 20-39 and 12.7% in those aged 40-59 years.

Conclusion: Although data collection still needs to be completed, preliminary findings confirm the high double disease burden. Although challenging, TB and DM services need to be integrated.



Supported by: WDF

Disclosure: **G. Segafredo:** None.

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Danish women see more progress than men in years of life lost to diabetes

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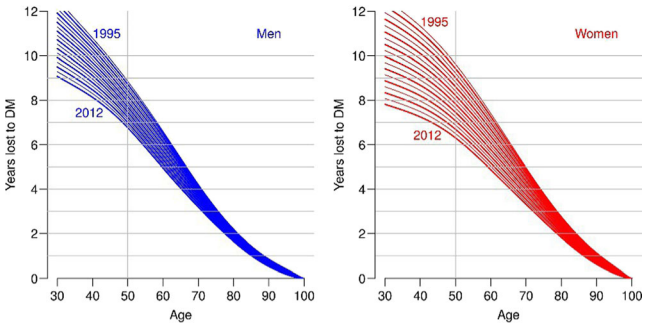
Background and aims: A summary measure of the population burden of diabetes is the mortality relative to the population mortality summarized as the years of life lost to diabetes. The aim of this study was to assess how the years of life lost to diabetes has developed since 1995 in Denmark, specifically for each sex.

Materials and methods: We used the Danish National Diabetes register for the years 1995-2012. We followed patients with diabetes for occurrence of death, and modeled mortality separately for men and women by age, calendar time and birth cohort. We subtracted deaths and person years in diabetes patients from that of the total population (obtained from Statistics Denmark) and used the resulting data to model the mortality in the non-diabetic part of the population in a similar way. The mortality rates were used to compute survival and expected lifetime from different ages in persons with and without diabetes for different calendar years; the difference being the years of life lost to diabetes.

Results: The years of life lost to diabetes decreased by age, broadly from about 10 years for a 40-year old person with diabetes to about 4 years for a person aged 70, but differently between sexes and over time. A 50-year

old man lost 8.9 year 10 diabetes in 1995, but only 6.8 years in 2012, but for women the decrease was from 9.6 years in 1995 to 6.3 years in 2012. For 70-year old persons the loss decreased from 4.2 to 3.2 years for men and from 5.0 to 3.3 years for women. The changes are illustrated in the figure.

Conclusion: The life expectancy for diabetes patients as measured by years of life lost to diabetes has substantially improved over the last 20 years. The gain has been larger in women than in men in Denmark; some 2 years for 50 year old men and some 3 years for 50 year old women. These gains presumably reflect both earlier detection of diabetes and better care and treatment of patients. It remains for future studies to elucidate why the improvement has been larger for men than women.



Disclosure: **B. Carstensen:** Employment/Consultancy; NovoNordisk. Stock/Shareholding; NovoNordisk.

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Type 2 diabetes: a family history associated with incident diabetes but only in women. The D.E.S.I.R. cohort

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Background and aims: While type 2 diabetes shows a familial aggregation, the association according to sex and with different family members has been little studied.

Materials and methods: Participants in the D.E.S.I.R. cohort (Data from an Epidemiological Study on the Insulin Resistance syndrome), recruited in 1994-1996, completed a detailed baseline questionnaire on the presence of diabetes in their family: mothers, fathers, siblings, children (1st degree family) and in maternal and paternal: grand-parents, uncles and aunts (2nd degree family). The incidence study included the 1,864 men and 1,965 women who provided some information about the presence or not of diabetes in their family and whose diabetic status was known at year 9.

Results: 7.3% (n=136) of the men and 3.2% (n=63) of the women had incident diabetes (P<.0001). While 28% of the men and 31% of the women (P=.04) had a 1st /2nd degree member of their family with diabetes, 18% and 20% had a 1st degree family member (P=.2), respectively. In women, the odds ratio for incident diabetes for each additional 1st /2nd degree family member with diabetes was 1.7 (95%CI: 1.3, 2.1) (P<.0001); in contrast for men, the odds ratio was 1.1 (0.8, 1.4) (P=.4). In women the risk was increased when either the father or the mother was diabetic with a slightly higher odds ratio for the mother (P=.3) (table). Adjusting for existing hypertension, waist circumference and smoking, factors associated with incident diabetes in this population, did not substantially change these results. A genetic risk score (65 SNPs associated with type 2 diabetes: Vaxillaire, Diabetologia 2014) was predictive of type 2 diabetes in this population but it was not associated with a family history of diabetes. In women, 1st /2nd degree heredity and the genetic score were associated with incident type 2 diabetes in a multivariable model, with odds ratios 2.5 (1.4, 4.4) and 1.1 (1.0, 1.2), P=.0006 and P=.01 respectively. In men, only the genetic risk score was predictive.

Conclusion: In women but not in men, the risk of diabetes was higher in the presence of diabetes in the family, independently of the genetic risk score. There was some evidence for maternal transmission. Epigenetic factors could play a role.

Member of family with diabetes	Men in D.E.S.I.R. (n=1864)		Women in D.E.S.I.R. (n=1965)		Differences men/women
	OR (95% CI)	P	OR (95% CI)	P	$P_{interaction}$
≥ one 1st degree	1.2 (0.7, 1.9)	0.4	3.1 (1.8, 5.3)	<.0001	0.004
≥ one 1st /2nd degree	1.1 (0.7, 1.7)	0.5	2.7 (1.6, 4.6)	<.0001	0.006
Per 1st /2nd degree	1.1 (0.8, 1.4)	0.4	1.7 (1.3, 2.1)	<.0001	0.007
Father	1.3 (0.6, 2.3)	0.4	2.3 (1.1, 4.7)	0.02	0.2
Mother	1.0 (0.5, 2.1)	0.9	4.2 (2.2, 7.6)	<.0001	0.002
Genetic risk score	1.1 (1.0, 1.2)	<.0001	1.1 (1.0, 1.2)	0.008	0.4

Table. Odds ratios (95% confidence intervals) for incident diabetes in men and women, according to their family history of diabetes

Disclosure: **B.J. Balkau:** None.

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The association between social jetlag, the metabolic syndrome and type 2 diabetes in a 40+year old population: the New Hoorn study

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Background and aims: Social jetlag is the discrepancy between our internal circadian clock and social clock and is a measure of circadian misalignment. Previous studies have shown that up to two thirds of the general population, aged 18-35y, suffer from social jetlag and its negative effects on metabolic parameters. As data from the general population over 40+y is missing, the aim of this study is to examine the prevalence of social jetlag and the association between social jetlag, the metabolic syndrome and type 2 diabetes in a 40+year-old, population-based cohort.

Materials and methods: We used cross-sectional data from the New Hoorn study cohort, n=1734 , 48% male, aged 45-73y. Social jetlag was measured using a questionnaire, calculated as the difference in mid-point sleep on week and weekend days and defined as 0-1h, 1-2h or >2h social jetlag. Metabolic syndrome was defined according to the Adult Treatment Panel III, including waist circumference, hypertension and levels of fasting plasma glucose, HDL-C and triglycerides. Pre-diabetes and Type 2 diabetes were defined according to the WHO guidelines; glucose levels ≥ 6.1 mmol/l, HbA1c ≥ 6% or use of diabetes medication.

Results: In our 40+year-old population-based cohort, we observed that only 15% of the unemployed/retired participants had social jetlag of >1h and 65% of the employed participants. In the unemployed/retired group no significant associations were observed between social jetlag status, (parameters of) metabolic syndrome and (pre-)diabetes. However, in the employed group, logistic regression adjusted for age, sex and sleep duration showed a positive association between social jetlag, metabolic syndrome and (pre-)diabetes, with respectively OR 1.1 (95%CI 0.7-1.7) and OR 1.7 (95%CI 1.2-2.4) for participants with 1-2h social jetlag, as well as OR 2.1 (95%CI 1.2-3.6) and OR 2.3 (95%CI 1.5-3.8) for >2h of social jetlag, when compared to participants with 0-1h social jetlag.

Conclusion: Social jetlag is associated with metabolic syndrome and (pre-)diabetes in working, 40+year-old participants.

Disclosure: **F. Rutters:** None.

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Type 2 diabetes and mortality in hypogonadal men improve upon long-term treatment with injectable Testosterone Undecanoate (TU): a controlled registry study

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Background and aims: Short studies using testosterone therapy (TTh) in hypogonadal men with T2DM have yielded inconsistent results, long-term TTh has shown beneficial effects. This study assesses long-term effectiveness and safety of TU in a urological setting in comparison to an untreated hypogonadal control group.
Materials and methods: Registry in 656 hypogonadal men. 225 men (34%) had T2DM. All men with T2DM had been diagnosed and are treated by their family physician. In the urology office, 113 men received TU 1000 mg/12 weeks (T-group), 112 had opted against TTh and served as controls (CTRL). Measurements were performed at least twice a year for 8 years. Mean changes over time between groups were compared by mixed effects model for repeated measures with random effect for intercept and fixed effects for time, group and their interaction. Changes were adjusted for age, weight, waist circumference, fasting glucose, blood pressure and lipids to account for baseline differences between groups.
Results: Mean age: 63.4±4.7 years. Fasting glucose decreased from 6.2 ±0.8 to 5.2±0.05 mmol/L ($p<0.0001$) in the T-group and remained stable at 5.8±0.3 in CTRL. The estimated model-adjusted difference between groups was -0.9 mmol/L ($p<0.0001$). HbA1c decreased from 8.03±0.83 to 5.77±0.43% in the T-group and increased from 7.44±0.66 to 8.01 ±0.78% in CTRL, difference between groups: -2.52% ($p<0.0001$ for all). The triglyceride:HDL ratio, a surrogate parameter for insulin resistance, decreased from 5.4±2.4 to 2.3±0.5 ($p<0.0001$) in the T group and from 7.8±4.4 to 7.0±4.3 ($p<0.05$) in CTRL, difference between groups: -3.8 ($p<0.0001$). The product of fasting glucose and triglycerides (TyG Index), another surrogate for insulin resistance, decreased from 4.2±0.1 to 4.0±0.0 ($p<0.0001$) in the T group and remained stable at 4.2±0.1 in CTRL, difference between groups: -0.3 ($p<0.0001$). In the T-group, 42 (37%) had achieved HbA1c <5.7% and 60 (53%) HbA1c of 5.7-6.4% (prediabetes range) at the last observation. A total of 104 men (92%) had achieved HbA1c ≤ 6.5%, 110 men (97%) an HbA1c target ≤ 7%. Only three men still had HbA1c >7%. These three men had a treatment duration of less than 3 years, and one of them was taken off TTh as he had been diagnosed with prostate cancer. In CTRL, 103 (92%) experienced an increase in HbA1c, 4 (4%) had no change, and 5 (4%) had a slightly improved HbA1c. Only 2 men achieved the HbA1c target of ≤ 7%. Weight change from baseline was -19±6% ($p<0.0001$) in the T-group and +1±3% ($p<0.01$) in CTRL. Difference between groups: -21% ($p<0.0001$). Since injections were administered in the doctor's office and no patient dropped out, there was a 100% adherence to TTh. 1 patient (0.9%) in the T-group died. In CTRL, 14 myocardial infarctions (12%), 16 strokes (13.7%), and 6 deaths (5.4%) occurred. 4 men in the T-group and 5 men in CTRL were diagnosed with prostate cancer.
Conclusion: Long-term TTh with TU in hypogonadal men with T2DM improved diabetic and anthropometric measures and reduced mortality and major adverse cardiovascular events compared to untreated controls.
Supported by: Partial funding for data entry and statistical analyses by Bayer Pharma
Disclosure: **F. Saad:** Employment/Consultancy; Farid Saad is an employee of Bayer Pharma, Berlin Germany. Stock/Shareholding; Farid Saad owns stock of Bayer Pharma, Berlin, Germany.

PS 015 The search for novel biomarkers

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Microarray analysis in insulin receptor knockout mice identified novel biomarkers to prevent type 2 diabetes

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Background and aims: Type 2 Diabetes Mellitus (T2DM) is characterized by several degrees of insulin resistance and relative deficiency in its secretion. In this form of diabetes, the impairment of insulin action is a consequence of a decrease on insulin receptor numbers or failure in insulin-receptor binding. Moreover, the altered pancreatic β-cells action to compensate the insulin resistance leads to chronic hyperglycemia and pre-diabetes state. Recently, several studies are seeking for biomarkers through proteomic and microRNA (miRNA) approaches. However, the multifactorial nature of this disease did not allow finding specific miRNA used as predicted marker and/or molecular target. Therefore, in the present study, we sought to discover new miRNA with the final objective to provide novel diagnostic, prognostic, and treatment alternatives for T2DM and/or its relative complications.
Materials and methods: miRNA Microarray was performed for miRNAs obtained by male mice liver tissues and hepatic cell line lacking of Insulin Receptor (IR). Expression levels of specific miRNAs were validated by qRT-PCR using TaqMan assay. We used miR-Walk algorithm to determine miRNA targets and mRNAs expression was validated by qRT-PCR. Different protein profiles of murine liver tissues mutated in IR was analyzed by 2D PAGE and nLC MS/MS mass spectrometry. HMGB1 acetylation and SIRT1 expression were analyzed by Immunoprecipitation and Western Blot. While HMGB1 secretion was studied by ELISA kit (Shino Test), and immunofluorence microscopy.
Results: Proteomic analysis in IR knockout (IR-/-), heterozygous (IR+/-) and wild type (IR+/+) mice as a diabetic murine model, identified 28 proteins by using 2-DE MALDI-TOF/TOF and peptic nLC-MS/MS shotgun profiling, while identified 24 proteins by nLC-MS/MS shotgun, differentially expressed among the 3 genotypes. Bioinformatic analysis revealed a central role of High Mobility Group Box 1/2 and huntigtin (HTT) never reported before to be associated with metabolic and related liver disease. MiRNAs array detected only 4 miRNAs (miR-376b, miR-154, miR-543, and miR-199b) differently expressed among IR+/+, IR+/- and IR-/-. qR TPCR confirmed these results in liver tissues, while we observed any differences in hepatic cell lines lacking IR. Bioinformatic analysis revealed interesting mRNA targets involved in metabolic pathways linked with proteomic analysis. In fact we found the involvement of SIRT1, a novel deacytetilase of HMGB1, in the translocation and in the activation of inflammatory state mediated by HMGB1. We confirmed these data observing by fluorescence microscopy HMGB1 traslocation and HMGB1 secretion by ELISA assay in vitro and in vivo models.
Conclusion: The "omics" study add new data in the pathophysiology of T2DM: the results identified new possible targets, which must be integrated with clinical data in order to obtain the insights necessary to delay the onset of T2DM.
Supported by: ASI n 2013-084-RO COREA,PON03PE_00146_1/10 BIBIOFAR
Disclosure: **B. Capuani:** None.

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Identification of circulating metabolic biomarkers for early beta cell death in pre-diabetic mice

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Background and aims: Pre-diabetic individuals presenting asymptomatic beta-cell mass decline should be targeted for disease prevention. Identification of early biomarkers for beta-cell death is a prerequisite for developing preventative strategies for type 2 diabetes. In a mouse model where spontaneous loss of beta-cells ultimately leads to diabetes, we aimed at establishing a strong correlation between the beta-cell mass and putative changes in the liver and plasma metabolites, which will serve as biomarkers reflecting early decline of beta-cell mass prior to the appearance of hyperglycemia.
Materials and methods: We used the beta-cell specific prohibitin-2 knockout mouse model, where mitochondrial dysfunction leads to gradual loss of beta-cells, resulting in development of diabetes over a short timeframe of 3 weeks (at 5 to 7 weeks of age). We applied non-targeted metabolomics to identify candidate biomarkers in the liver and plasma of knockout and control mice (n = 27 and 32, respectively). Principal component analysis was used to unravel differences between the metabolites of knockout and control mice. Several machine learning algorithms, including linear discriminant analysis, L1-classifier and random forests, were used to calculate cross-validation scores.
Results: 48 and 102 metabolite clusters discriminating control and upcoming diabetic mice were identified in liver and plasma, respectively (q-value < 0.01). Metabolite clusters in fructose and mannose metabolic pathway showed early changes in pre-diabetic stage, with gradual depletion of deoxy sugars over time. Branched-chain amino acids, which have been associated with type 2 diabetes, decreased in liver but increased in plasma during pre-diabetic stage. These early changes were reinforced upon the appearance of hyperglycemia. Machine learning algorithms were able to distinguish between control and pre-diabetic subjects with high prediction power (cross validation score = 97%).
Conclusion: Asymptomatic decline of beta-cell mass (up to 50%) can be reflected by changes in some liver and plasma metabolites. Biomarkers in fructose and mannose metabolic pathways were identified as fingerprint for early beta-cell death, and predictive for the upcoming diabetic state.
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Disclosure: **L. Li:** None.

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Predictive utility of European derived genetic variants associated with type 2 diabetes in a black South African population

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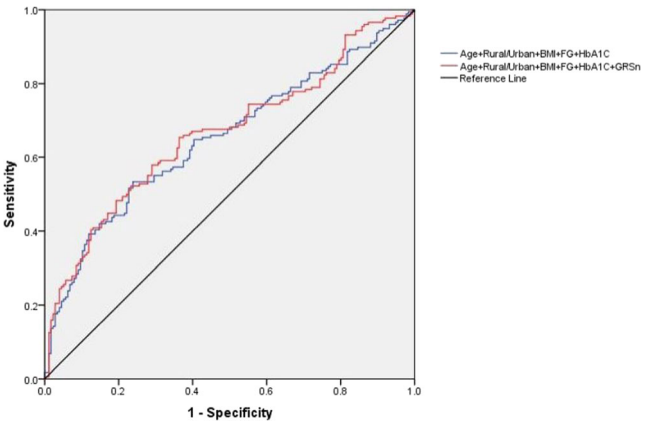
Background and aims: Type 2 diabetes (T2D) is postulated to have a polygenic etiology. Recently, polygenic risk scores of variants associated with T2D have been noted to be of useful in predicting risk in multiple ethnicities as opposed to single variants that have been reported to be population specific. However, the evidence of this phenomenon is limited among the black South Africans. This study was done to determine polygenic scores associated with T2D and their usefulness in the black South African Population.
Materials and methods: Sixty six variants associated with T2D were successfully genotyped using the BeadXpress platform among 178 cases and 178 controls nested in the Prospective Urban and Rural Epidemological (PURE) Study. Four types of polygenic risk scores were computed, which consisted of all the 66 variants which was termed GRSt, significant variants (GRSn), beta related variants (GRSbeta) and trans ethnic shared variants associated with T2D (GRStrans).
Results: GRSn which consisted of 4 variants, was the only polygenic risk score associated with increased T2D risk indicated by OR (95CI) of 1.21 (1.02-1.43) p-value = 0.015. Stratified analysis indicated the GRSn to be significantly associated with T2D among the non-obese and people less than 50years old .Receiver operating curves (ROC) were use to assesses the ability of the convectional risk factors (age, sex, urbanisation, fasting glucose, glycated hemoglobin and BMI) alone or with GRSn to identify a case. The area under the ROC of the T2D risk factors (age, BMI, urbanisation,fasting glucose and glycated hemoglobin) alone was 0.652 (p value < 0.001) and with the addition of GRSn it was 0.665 (p value < 0.001)as indicated in Figure 1 attached.
Conclusion: The polygenic risk scores of variants associated with T2D, derived from European and Asian ethnicities are less predictive in the black South African population. However, the improved predictiveness among non-obese and people less than 50 years, make the polygenic risk score approach an attractive tool which might be more effective in the future with the inclusion of rare and population specific for early identification of high risk T2D patients.



Supported by: NRF SA
Disclosure: **T. Chikowore:** None.

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Imbalance of bacteriome profiles in children with islet autoimmunity: mass sequencing of 16S DNA and viromes in stool samples from Finnish DIPP study

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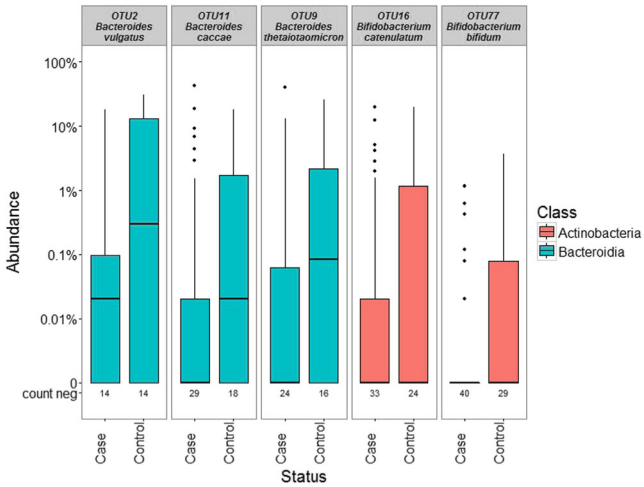
Background and aims: The study explored associations of the stool bacteriome profiles with early-onset islet autoimmunity subsequently leading to type 1 diabetes, taking into account the interactions with the virus component of the microbiome.
Materials and methods: Stool samples were longitudinally collected from 18 infants and toddlers with early onset of islet autoimmunity (median age 17.4 months) followed by development of type 1 diabetes (median age 4.2 years), and 18 tightly matched controls from the Finnish Diabetes Prediction and Prevention (DIPP) birth cohort. Three stool samples taken 3, 6 and 9 months before the first detection of autoantibodies in serum of the case child were analysed. The bacteriome profiles were

assessed using 16S ribosomal DNA sequencing and virome composition using metagenomic sequencing of both RNA and DNA viruses. The risk of islet autoimmunity was evaluated in relation to bacteriome diversity, composition of the bacteriome profiles at various taxonomic levels, correlations between abundances of bacteriophages and bacteria, and prominent unknown motifs in the virome.

Results: Five bacterial operational taxonomic units were significantly less abundant in children with islet autoimmunity as compared to controls - most markedly the species of *Bacteroides vulgatus, Bacteroides thetaiotaomicron* (both corrected P values <0.001) and *Bifidobacterium bifidum* (p=0.0014). The alpha or beta diversity, or the taxonomic levels of bacterial phyla, classes or genera, showed no differences between cases and controls. The *CrAssphage* bacteriophage correlated with *Bacteroides dorei* and *B. thetaiotaomicron*. No apparent associations were seen between islet autoimmunity and sequences of yet unknown origin.

Conclusion: The results confirm previous findings that an imbalance within the prevalent *Bacteroides* genus is associated with islet autoimmunity. The detected quantitative relation of the novel "orphan" bacteriophage *CrAssphage* with two prevalent species of the *Bacteroides* genus may exemplify possible modifiers of the bacteriome and herald the complex nature of the gut microbiome comprised of not only bacteria, but also numerous viruses.



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Disclosure: **O. Cinek:** None.

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Electrical patterns on the surface of the skin as a predictor for diabetes onset

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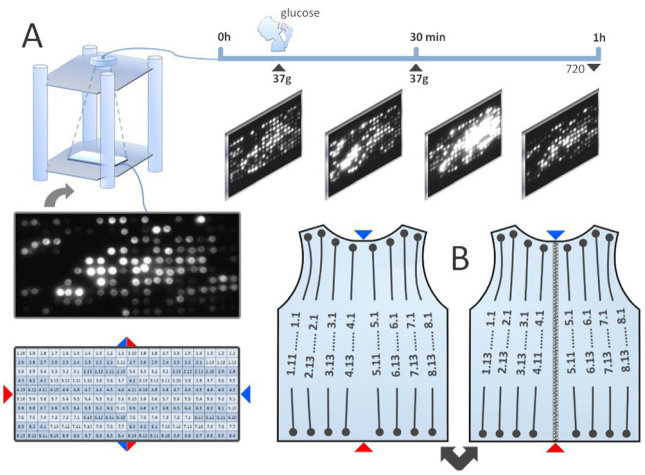
Background and aims: The skin has been often brought over the years to the forefront of diabetes as a noninvasive method for diagnosis. The most used approaches have considered glycosylated proteins in the skin or individual electrodes for measuring the electrodermal activity (EDA). EDA represents a property of the human body which consists of continuous variation in the electrical characteristics of the skin. In most studies the EDA has been determined by the use of a small number of electrodes which led to unclear answers regarding the metabolic response of the patient. In our study, the electrodermal activity has been derived from a large collection of 200 sensors located across the entire thorax. The aim of the study was the early detection of diabetes. Thus, the relationship between electrodermal activity and diabetes has been considered by analyzing the patterns derived from the sensors.

Materials and methods: Our study embedded 4 groups of 20 individuals each: the first group - 20 patients with type 1 diabetes, the second group - 20 patients with type 2 diabetes, the third (control) group - 20 normal individuals, the fourth group - 20 newly discovered patients (10 patients with T1D and 10 patients with T2D). Electrodermal activity signals derived from 200 sensors have been collected from each individual involved in the study. Signals from the 200 sensors were captured and converted into image patterns at every 5 seconds over a period of 1 hour. During this period of 1 hour, a glucose tolerance test has been used at 1 minute (37g glucose) and at 30 minute (37g glucose) to test the EDA response from each patients. Each image pattern was then used to train a neural network responsible for diagnosis. In total, the neural network responsible for diagnosis has been trained using 57600 patterns. Thus, other 30 individuals whose diabetes diagnosis was known or expected in the near future, were then tested during a period of 1 hour against the neural network for prediction.

Results: The metabolic response differs from one individual to another. Nevertheless, the electrodermal activity shows clear specific signatures (patterns) for type 1 diabetes (T1D) patients, type 2 diabetes (T2D) patients and normal individuals. Moreover, EDA tests on the newly discovered diabetes patients (onset - 1 to 3 months) indicate specific signatures that predict type 2 diabetes several months before onset. By our estimates, the accuracy of prediction it is tied to the number of patterns used for training the neural network responsible for final diagnosis. Current estimates for our prototype show that correct diagnosis is made 98% of the time. The correct prediction before diabetes onset is made 92% of the time.

Conclusion: In our study we used the electrical patterns on the surface of the skin as a prediction method for diabetes onset. Future versions of the method aim to minimize diagnostic time from 1 hour up to 10-20 minutes.



Supported by: PN-II-ID-PCE-2011-3-0429
Disclosure: **C. Ionescu-Tirgoviste:** None.

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Influence of soluble leptin receptor on risk of gestational diabetes in a multi-ethnic population

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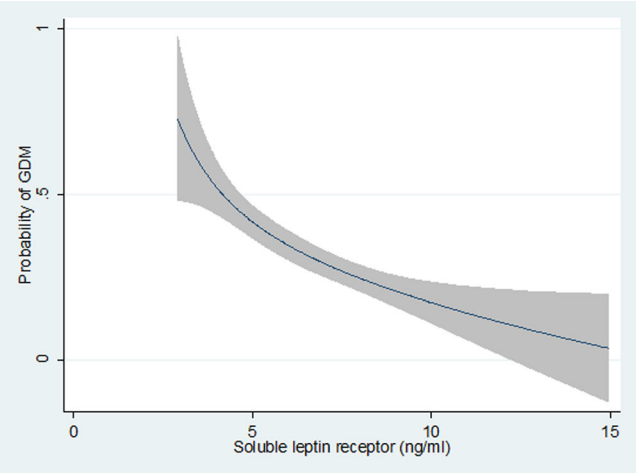
Background and aims: Low circulating levels of soluble leptin receptor (sOb-R) have been associated with increased risk of developing type 2

diabetes, independently of BMI and leptin levels. sOb-R is exclusively generated through proteolytic cleavage of the extracellular domain of membrane-anchored leptin receptor isoforms. sOb-R binds leptin in blood, influences leptin's bioavailability and metabolic effects and may be a marker of leptin sensitivity. We have previously shown that serum leptin is an independent predictor of gestational diabetes (GDM). In this study, we explored whether sOb-R was independently associated with GDM in a multiethnic cohort in Oslo, Norway.

Materials and methods: In a population-based prospective cohort study of 823 women, 680 (whereof 47.1% Europeans) had sOb-R, leptin and BMI measured in early pregnancy (week 15), an oral glucose tolerance test performed at 28 weeks' gestation and GDM diagnosed according to WHO 2013 criteria from fasting and 2-hour glucose. Skinfolds from triceps, subscapular and suprailiac sites were summarized and used as an indicator of subcutaneous fat.

Results: GDM was diagnosed in 211 (31%) women. Compared to non-GDM women, women with GDM had lower sOb-R (mean±SD; 6.3±1.9 vs. 7.4±2.1 ng/ml, p<0.001). sOb-R was inversely correlated with levels of leptin (r=-0.42, p<0.001), BMI (r=-0.51, p<0.001), and subcutaneous fat (r=-0.50, p<0.001). In a simple logistic regression analysis, sOb-R was inversely associated with risk of GDM (OR 0.76 [95% CI 0.69-0.83], p<0.001). In a multiple logistic regression analysis, sOb-R was associated with reduced risk of GDM (0.82 [0.74-0.91], p<0.001) after adjustment for age, parity, weeks' gestation, ethnic origin and leptin. After further adjustment for BMI or subcutaneous fat, sOb-R was still independently associated with reduced risk of GDM (0.84 [0.75-0.95], p=0.003 and 0.85 [0.76-0.95], p=0.006, respectively). Women within the highest decile of sOb-R levels had lower odds (0.25 [0.10-0.63], p=0.003) of developing GDM than women within the lowest decile, after adjustment for age, parity, weeks' gestation, ethnic origin and serum leptin.

Conclusion: Low serum levels of sOb-R measured early in pregnancy were associated with increased risk of developing GDM. This finding may indicate an important role for leptin and soluble leptin receptor in the metabolic aberrations characteristic of GDM.



Supported by: Aker and Ullevål Diabetes Research Fund and Norwegian Diabetes Association

Disclosure: **C. Sommer:** None.

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Lactation is associated with altered metabolomic signatures in women with gestational diabetes

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Background and aims: Lactation for > 3 months in women with gestational diabetes is associated with a reduced risk for type 2 diabetes that persists for up to 15 years postpartum. However, the underlying protective mechanisms are unknown. We examined whether lactation for > 3 months in women with a history of gestational diabetes is associated with altered metabolomic signatures postpartum.

Materials and methods: We enrolled 197 women with previous gestational diabetes participating in a postpartum assessment of glucose tolerance at a median time of 3.6 (IQR: 0.7;6.5) years after delivery. Targeted metabolomics profiles (including 156 metabolites) were obtained during glucose challenge. Information on lactation duration was collected by interview during the study visits. We assessed associations between lactation > 3 months and the homeostatic model assessment of insulin resistance (HOMA-IR), Matsuda-Defronza estimated insulin sensitivity index (ISI), and the metabolic profiles including selected sums and ratios of metabolites. Associations were adjusted for maternal body mass index and age at the postpartum visit, educational level, and time since delivery, and corrected for multiple testing. Additionally, we stratified our analyses for short- and long-term follow-up, using samples collected shortly after delivery (median 0.7 year) or several years postpartum (median 6.0 years), respectively.

Results: Lactation for > 3 months was not associated with HOMA-IR and ISI. However, lactation for > 3 months was associated with higher total lysophosphatidylcholine to total phosphatidylcholine ratios at 30 and 120 min during glucose challenge and, in women with short-term follow-up, additionally with lower leucine concentrations and lower sum of branched chain amino acids (BCAAs). Gaussian graphical modelling identified subgroups and networks of closely linked metabolites within the phosphatidylcholine and BCAA groups that were affected by lactation for > 3 months and were linked to the pathophysiology of type 2 diabetes in prior reports.

Conclusion: Lactation for > 3 months in women with gestational diabetes may correct several metabolic abnormalities associated with the early pathogenesis of type 2 diabetes, and may offer a safe and feasible low-cost intervention to prevent type 2 diabetes development in women with gestational diabetes.

Clinical Trial Registration Number: NCT01018602

Supported by: DZD e.V., BMBF, iMed, HORIZON 2020 DynaHEALTH action, DDG, Helmholtz HIRG

Disclosure: **D. Much:** Grants; DZD e.V., BMBF, iMed, HORIZON 2020 for DynaHEALTH action, DDG, Helmholtz HIRG.

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Are women with a history of gestational diabetes, without any current weight or glucose metabolism disorders, at a higher risk of cardiovascular disease?

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Background and aims: A history of gestational diabetes (pGDM) is associated with a 7-fold higher risk of developing type 2 diabetes and a 2-fold higher risk of cardiovascular diseases. It is not clear whether the increased risk is caused by being overweight, obese or having an impaired glucose tolerance (present significantly more often in this group of women at a later period), or perhaps other, so far unidentified, factors.
Materials and methods: The study included 79 women (av. age of 37.0 ±6.2 years) with normal weight (BMI 21.6±1.8 kg/m2) and normoglycemia in a recently performed glucose tolerance test (OGTT), with pGDM 7-12 years ago. The control group consisted of 24 women (av. age 35.1±5.2 years) with normal weight (BMI 21.6±1.8 kg/m2) and normoglycemia (results within the reference range of OGTT) who were pregnant around the same time as women from the pGDM group but did not have pGDM (C). The assessment included anthropometric parameters, body composition (Tanita SC-330S), measurement of blood pressure, OGTT with glucose and insulin; index of insulin resistance (HOMA-IR) and β-cell function (HOMA-%B), HbA1c, lipids, uric acid (UA), proinflammatory adipokines - tumor necrosis factor α (TNF-α) and interleukin 6.
Results: The study groups did not differ in age, number and duration of labour, anthropometric parameters, blood pressure, lipid metabolism and kidney function. In the pGDM group, despite fasting normoglycemia, glucose levels were significantly higher in 60 min of OGTT (6.7±1.5 vs 5.5±1.4 mmol/L, p=0.0005). Also a higher concentration of UA (232±48 vs 208±48 mmol/L, p=0.035) and TNF-α (4.6±1.6 vs 3.8±1.1 ng/mL, p=0.011) was noted.
Conclusion: Women with pGDM, even with normal body weight and normoglycemia, whose state is assessed many years after labour, have higher glucose levels in response to oral glucose tolerance test and higher concentrations of UA and TNF-α. Abnormalities of these parameters may have an influence on increasing the risk of cardiovascular diseases. The causes of these disorders remain unclear and require further studies.
Supported by: N 402 069 32/2047
Disclosure: **P. Molęda:** None.

PS 016 The complexity of beta cell signal transduction

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Dll1- and Dll4-mediated Notch signalling in adult pancreatic beta cells is essential for the structural integrity of islets and maintenance of glucose homeostasis

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Background and aims: The Notch signaling pathway is a short-range communication transducer involved in the regulation of many cellular processes. Genes of the pathway are expressed in different cell types and organs at different time points during embryonic development and adulthood. For example, the Notch ligand Delta-like 1 (DLL1) controls the decision between endocrine and exocrine fates of progenitor stem cells in the developing pancreas; and loss of Dll1 function leads to premature differentiation of the pancreatic endocrine cell lineage. The ligands DLL1 and DLL4 as well as other members of the pathway are also expressed in the adult pancreas. However, the role of Notch signaling in adult tissue homeostasis is not well understood.
Materials and methods: We developed inducible, Pdx1-CreERT based beta cell-specific mouse models containing loss- or gain-of function mutations within the Dll1 and/or the Dll4 gene. Gene recombinations were activated in weaned offspring for 4 weeks by Tamoxifen® containing chowder. As controls, Pdx1-CreERT positive wild-type mice were used. 8 weeks old animals were monitored for fasting blood glucose and plasma insulin levels as well as glucose tolerance performance. Pancreatic sections were used for immunohistochemical analysis of islet specific factors and markers to check for proliferation, apoptosis as well as Notch pathway patterns. Isolated islets were harvested for glucose stimulated insulin secretion and qRT-PCR analysis.
Results: Here, we describe cell-type specific, distinct expression of Notch pathway members in the adult murine pancreas. Using ligand-specific conditional loss- and gain-of-function mouse models we demonstrate alterations in islet morphology and effects on blood-glucose regulation as well as other metabolic parameters. β-cells lacking DLL1 as well as DLL4 mediated signaling reveal abnormal islet morphology and dysregulation in proliferation and apoptosis. Furthermore, we analyzed for a possible gain of function of DLL1. Here, we focused specifically on the intracellular domain of DLL1 (DICD), as it has been shown to have bi-directional properties parallel to the canonical extracellular domain-mediated receptor-ligand binding pathway. The overexpression of DICD in adult murine pancreatic β-cells resulted in decreased body weight and higher blood-glucose levels. Moreover, stimulation with glucose in vivo and in vitro showed impaired glucose tolerance and insulin secretion in βDICD mice.
Conclusion: We provide first insights into functional aspects of Notch signaling in the adult islet and its importance for maintenance of tissue homeostasis.
Disclosure: **M. Fuetterer:** None.

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Selective development of pancreatic beta cell insulin resistance upon different diets

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Background and aims: Obesity and type 2 diabetes mellitus (T2DM) have reached epidemic proportions and a connection between eating

behavior and disease development is discussed. Besides insulin resistance pancreatic β-cell dysfunction is central to the development of T2DM. Because the pancreatic β-cell itself is a target for insulin action, β-cell insulin resistance can contribute to β-cell dysfunction. Here we used a recently by us developed technique that reports on insulin resistance in pancreatic β-cells in vivo to investigate the dynamics of pancreatic β-cell insulin sensitivity/resistance in the context of bodyweight gain, whole body insulin resistance and glucose tolerance upon different diet interventions.

Materials and methods: C57BL/6J mice were fed a High Sucrose Diet (HSD) (32% sucrose in drinking water), High Fat Diet (HFD) (60% of the kcal from fat), High Fat High Sucrose Diet (HFHSD) (HSD+HFD) or control diet (normal chow diet) for 2 months. At different time points of the diet intervention (1 and 2 weeks and 1 and 2 months after treatment start), β-cell insulin resistance was measured by in vivo imaging of pancreatic islets transduced with a fluorescent insulin resistance biosensor and engrafted in the anterior chamber of the eye of recipient animals. The biosensor is based on the different subcellular localization of FoxO1-GFP, i.e. cytosolic in insulin responsive cells and nuclear at insulin resistance, and used to calculate a β-cell insulin resistance index (βIRI) representing the ratio of nuclear and cytoplasmic FoxO1-GFP localization. Glucose and insulin tolerance were measured by intraperitoneal glucose and insulin tolerance tests, respectively.

Results: A diet intervention time of 2 months did not change bodyweight, glucose tolerance, whole body and β-cell insulin sensitivity in mice fed a HSD compared to the control diet. In contrast HFD fed mice developed glucose intolerance during the first week of treatment, showed a significant increase in bodyweight after 2 weeks and became whole body insulin resistant at 1 month after treatment start, but they neither developed β-cell insulin resistance within 2 months of treatment. HFHSD caused glucose intolerance during the first week after treatment start, a significant increase in bodyweight, whole body and β-cell insulin resistance at 1 month of treatment (after 1 month of treatment: Control: $\beta IRI = 0.689 \pm 0.02$; HFHSD: $\beta IRI = 0.786 \pm 0.02$; $p < 0.01$).

Conclusion: This study provides in vivo evidence that pancreatic β-cell insulin resistance occurs upon HFHSD, but not HSD and HFD treatment. In general, this for the first time illustrates the development of β-cell insulin resistance in the context of increased bodyweight, glucose intolerance and whole body insulin resistance. These data provide the basis for further investigation of the molecular mechanisms underlying the different development patterns of organ specific insulin resistance and may lay the foundation for novel treatment strategies in diabetes.

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Oxidative stress-induced EGF and GLP-1 receptors cooperate towards pancreatic beta cell survival in response to inflammatory cytokines and glucolipotoxicity

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Background and aims: We have previously investigated the agonist-independent EGF receptor (EGFR) trafficking/signaling in response to oxidative stress in cancer cells, by which EGFR is internalised in a stress MAPK p38- and clathrin adaptor AP2-dependent manner, and segregated to a subset of signaling endosomes via endosomal actin regulator WASH, where it delays apoptotic onset. Here we examined 1) whether such a mechanism exists in pancreatic β-cells after exposure to pro-inflammatory cytokines or glucolipotoxicity, 2) whether similar agonist-independent effects apply to GLP-1 receptor (GLP-1R), and 3) possible receptor cross-talk, as shown for EGFR trans-activation by agonist-induced GLP-1R.

Materials and methods: Experiments were performed in mouse insulinoma MIN6B1 cells after O/N serum starvation. Stress conditions were 1) 2h exposure to cytokines (100 ng/ml TNFα, 200 ng/ml IL1β, 500 ng/ml IFNγ) or 2) O/N incubation with 500 μM BSA-palmitate in high (25 mM) glucose (glucolipotoxicity). EGFR trafficking was analysed by confocal and electron microscopy after transfection with SNAPf-tagged human EGFR, and labeling with fluorescent or biotinylated SNAP-tag probes. EGFR AP2 binding site was mutated by site directed mutagenesis to generate EGFRΔAP2. GLP-1R trafficking was analysed by FACS in SNAP-GLP-1R-expressing stable MIN6B1 cells. Signaling was assessed by WB. WASH and mouse EGFR were knocked-down (KD) by RNAi. Apoptosis was assessed by TUNEL. Histological analysis of donor human islet paraffin-embedded sections was performed after islet adenoviral infection.

Results: Exposure of MIN6B1 cells or human islets to cytokines or palmitate resulted in phosphorylation of pro-survival factors CREB and ERK, as well as p38. Selective inhibition of EGFR with gefitinib or GLP-1R with antagonist exendin 9-39, as well as p38 inhibition suppressed cytokine- and palmitate-induced CREB/ERK activation. Both stresses triggered agonist-independent EGFR and GLP-1R internalisation, which, for GLP-1R, resulted in a 25% decrease in surface receptor by 2h cytokine and a 41% decrease by O/N palmitate exposure. Stress-induced EGFR internalisation was abolished by mutating EGFR AP2-binding domain in MIN6B1 and human islets. Internalised EGFR was segregated by WASH to a subset of endosomes distinct to those harbouring EGF-bound receptor. EGFR overexpression decreased cytokine-induced apoptosis (3% vs. 8%, $p<0.01$). Conversely, apoptosis was increased by EGFR or WASH KD (1.98±0.12-fold (EGFR KD) and 1.71±0.05-fold (WASH KD)). Expression of wt human EGFR (but not EGFRΔAP2) following endogenous mouse EGFR KD restored cytokine-induced CREB/ERK activation, and reduced cytokine-induced apoptosis compared to EGFRΔAP2 (11% vs. 22%, $p<0.05$).

Conclusion: Both inflammatory and glucolipotoxic stress induce activation of ERK and CREB, two main targets of EGFR and GLP-1R signaling, respectively, in absence of agonists, with both receptor activities required, as well as p38. Stress exposure also triggers agonist-independent EGFR and GLP-1R trafficking, which results in AP2- and WASH-dependent EGFR sequestration in signaling endosomes. Manipulation of EGFR levels or trafficking under stress conditions modulates apoptotic responses, highlighting the importance of this mechanism in β-cell survival during T1 and T2 diabetes.

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Plasma membrane $PI(4,5)P_2$ controls Ca^{2+}-influx in clonal beta cells

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Background and aims: The lipid phosphatidylinositol 4,5-bisphosphate ($PI[4,5]P_2$) is a minor component of the plasma membrane that regulates the activity of numerous ion channels. $PI(4,5)P_2$ levels are metabolically regulated in beta cells, and in addition the lipid is hydrolyzed to IP_3 and DAG by receptor-triggered or Ca^{2+}-induced phospholipase C (PLC) activation. Consensus about the role of $PI(4,5)P_2$ in the regulation of the steps leading to insulin secretion, in particular Ca^{2+}-influx, is lacking, and there are support for both stimulatory and inhibitory roles. The aim of this study was to clarify the role of $PI(4,5)P_2$ in the regulation of Ca^{2+}-influx in beta cells.

Materials and methods: Clonal MIN6 beta cells were imaged by total internal reflection fluorescence (TIRF) microscopy and the Ca^{2+} and $PI(4,5)P_2$ concentration changes were measured using R-GECO and a fluorescence-tagged PH-domain from PLCδ1 (PH-PLC), respectively. Plasma membrane $PI(4,5)P_2$ levels were manipulated by muscarinic receptor-triggered activation of PLC or using optogenetic tools that enabled blue light-induced plasma membrane recruitment of either a 5'-phosphatase to deplete $PI(4,5)P_2$ or a PI4P5-kinase to synthesize $PI(4,5)P_2$.

Results: ATP synthesis is required to maintain plasma membrane $PI(4,5)P_2$ levels, and addition of the mitochondrial uncoupler FCCP caused an immediate 30±2% (n=26 cells) loss of PH-PLC fluorescence at the plasma membrane. Depolarization with 30 mM K^+ resulted in pronounced Ca^{2+}-influx that was accompanied by a smaller (10±2%; n=45 cells) drop in PH-PLC fluorescence. Application of 100 μM carbachol to activate PLC resulted in further 8±1% drop in PH-PLC fluorescence and caused a 27% suppression of the K^+-induced Ca^{2+}-influx. This suppression reached 62% in cells overexpressing the M1-receptor for carbachol. To more directly test the role of $PI(4,5)P_2$ in the regulation of Ca^{2+}-influx we used the light-regulated 5-phosphatase to acutely deplete $PI(4,5)P_2$ in the plasma membrane and found a linear correlation between loss of $PI(4,5)P_2$ and suppression of Ca^{2+}-influx (R^2=0.38; p<0.001; n=45 cells). When plasma membrane $PI(4,5)P_2$ was instead increased by light-induced recruitment of a 5-kinase, this amplified K^+-induced Ca^{2+}-influx by 21±8% (n=154 cells; p<0.001). Interestingly, whereas $PI(4,5)P_2$ loss suppressed tolbutamide-induced Ca^{2+}-influx to a similar extent as K^+-induced (83±5% suppression; n=24 cells; p<0.001 for tolbutamide and 68±3%; n=49 cells; p<0.001 for K^+), $PI(4,5)P_2$ synthesis had the opposite effect and instead suppressed tolbutamide-induced Ca^{2+}-influx by 34 ±12% (n=61 cells; p<0.001).

Conclusion: $PI(4,5)P_2$ is a positive regulator of Ca^{2+} influx in MIN6 cells, and depletion of $PI(4,5)P_2$ nearly completely blocks, whereas synthesis augments, K^+-induced Ca^{2+} influx. Tolbutamide-induced Ca^{2+}-influx was negatively affected by both $PI(4,5)P_2$ deficiency and excess, indicating that $PI(4,5)P_2$ is a regulator of both K_{ATP}-channels and voltage-dependent Ca^{2+} channels. These results suggest that conditions that alter $PI(4,5)P_2$ levels may have profound effects on beta cell Ca^{2+} homeostasis.

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Nutrient stimulation of rat pancreatic beta cells acutely improves their cytosolic and mitochondrial defenses against H_2O_2

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Background and aims: NADPH and H_2O_2 have been proposed as metabolic coupling factors in the glucose stimulation insulin secretion (GSIS) in pancreatic β cells. However, as NADPH favors enzymatic H_2O_2 degradation, their common operation is possible only if their production are spatially or temporally disconnected. Here, we used (mt-)roGFP2-Orp1 to monitor dynamic changes in cytosolic and mitochondrial H_2O_2 in β cells acutely stimulated by glucose and other nutrients. This ratiometric fluorescent probe is specifically oxidized by H_2O_2 owing to its Orp1 moiety, and is reduced by thioredoxin- and glutathione-dependent antioxidant systems.

Materials and methods: Male Wistar rat pancreatic islets were isolated and dispersed in clusters of ≥5 cells. (mt-)roGFP2-Orp1 was expressed (adenovirus infection, CMV promotor) in the cytosol or mitochondrial matrix of islet cells. The fluorescence ratio of the probe was measured every 30 s (λexc 405/485 nm; λem 535 nm) during perifusion with Krebs solution containing various glucose concentrations (Gn = n mM glucose), other nutrients and low concentrations of freshly added exogenous H_2O_2. The ratio was expressed relative to the ratios measured after sequential addition of 10 mM dithiothreitol (minimum ratio set to 0%) and 100 μM aldrithiol (maximum ratio set to 100%) at the end of each experiment.

Results: Both probes were mainly expressed in β cells: after coinfection with an adenovirus coding DsRed under the control of the rat insulin promoter, (mt-)roGFP2-Orp1 were expressed in 74-85% of DsRed-positive cells vs. 14-21% of DsRed-negative cells. RoGFP2-Orp1 was diffusely expressed in the cytosol and nucleus, and mt-roGFP2-Orp1 colocalized with MitoTracker Red (confocal microscopy, obj 100X). During perifusion with Krebs containing G10, roGFP2-Orp1 fluorescence ratio was low (~5% of min/max ratio) while mt-roGFP2-Orp1 was more oxidized (~15%), suggesting H_2O_2 is more concentrated in the mitochondrial matrix than cytosol of β cells. roGFP2-Orp1 was significantly oxidized by ≥2 μM exogenous H_2O_2 and mt-roGFP2-Orp1 by ≥15 μM H_2O_2, the effect of 15 μM on both probes being slowly reversible upon removal of H_2O_2. Under control conditions, roGFP2-Orp1 fluorescence ratio was unaffected by sequential changes from G10 to G0.5 then to G30. In contrast, roGFP2-Orp1 oxidation by 15 μM H_2O_2 was significantly higher at G0.5 than at G5, with no difference between G5 and G30. In the mitochondrial matrix, glucose also reduced mt-roGFP2-Orp1 oxidation, but this effect was maximal only at G10 (vs. G5 in the cytosol) and was observed both in the absence and presence of exogenous H_2O_2. Interestingly, the effects of glucose on (mt-)roGFP2-Orp1 oxidation were reproduced by addition of 20 mM monomethylsuccinate, 10 mM Leu + 10 mM Gln, or 10 mM alpha-ketoisocaproate (KIC) to G0.5 (cytosolic and mitochondrial probes) or G5 (mitochondrial probe only), either in the presence of 15 μM exogenous H_2O_2 (both probes) or in its absence (mitochondrial probe only). However, only KIC, a keto-acid that degrades H_2O_2, more effectively protected β cells against H_2O_2 than G10.

Conclusion: Glucose and other nutrients acutely improve the ability of rat pancreatic β cells to defend their cytosol and mitochondrial matrix against low concentrations of endogenous and exogenous H_2O_2. These results do not support a role for H_2O_2 as a metabolic coupling factor in GSIS.

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JNK3 is required for islet beta cell adaptation to obesity triggered by the GLP-1 mimetics

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Background and aims: cJun amino terminal kinase 3 (JNK3) is highly expressed in pancreatic beta cells and is involved in glucagon-like peptide 1 (GLP-1) protection against cytokines-induced beta cell death. Here, we investigated in mice the JNK3 putative role in the compensatory islet beta cell mass in response to obesity and to GLP-1 mimetics.

Materials and methods: Metabolic measurement including glucose and insulin tolerance tests were undertaken in wild type (WT) and JNK3-/- Knockout mice (KO) fed with a regular or high fat diet (HFD), with or without administration of liraglutide. Insulin secretion and content from mouse isolated islets and insulin producing cells were monitored. Cell viability and proliferation was quantified by TUNEL and Ki67 assays. Gene expression was monitored by luciferase reporter assay and quantitative real-time PCR.

Results: JNK3 expression was increased in islets of both ob/ob mice and mice fed with a HFD, as well as in human islets from non-diabetic obese individuals. Global suppression of JNK3 in mice resulted in glucose intolerance, with a marked decrease in plasma insulin levels. High glucose values under fasting and fed conditions were further exacerbated in JNK3 null mice fed with HFD for 16 weeks. While insulin sensitivity in JNK3 null and WT mice were similar, a significant impairment in functional beta cell mass was apparent in null mice with inefficient insulin secretion in response to glucose. Although alpha cell number was unaffected, beta cell death and proliferation were impaired in JNK3 null mice. In mice, liraglutide stimulates beta cell mass by CREB activation, which promotes beta cell survival and proliferation. Indeed CREB activity increased in islets from our WT mice treated with liraglutide. In contrast, JNK3 suppression hampered CREB activity and its known targets expression including Irs2, Icer and Atf3. Furthermore, defective CREB activity was

also found in INS-1E cells in which JNK3 expression was suppressed by interference RNA.
Conclusion: JNK3 is involved in mechanisms driving efficient beta cell mass compensation in rodent and human obesity by regulating the GLP-1 signaling.
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Disclosure: **M. Tenenbaum:** None.

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Lipid-induced hormesis in beta cells: triglyceride biosynthesis and storage in newly formed lipid droplets protect against free fatty acid toxicity

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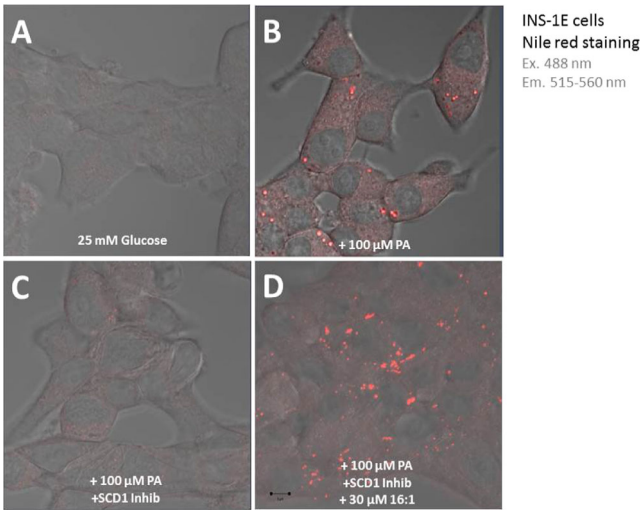
Background and aims: Beta cells operate hormetic mechanisms to alleviate cytotoxic effects of free fatty acids. We have found before that both high glucose and increasing palmitic acid levels induced substantial remodeling of membrane phospholipids and altered the relative abundance of saturated-, monounsaturated- and polyunsaturated fatty acids. This present study aimed at investigating the impact of the transformation of palmitic acid (16:0) to palmitoleic acid (9-cis 16:1) by the enzyme delta-9 desaturase (SCD1) on the ability of cells to sequester excess palmitic acid into triglycerides and store them in newly formed lipid droplets.
Materials and methods: Beta cells of the rat-derived INS-1E cell line were treated with increasing concentrations of glucose and/or palmitic acid in the absence or presence of CAY10566 (a selective SCD1 inhibitor) and palmitoleic acid. The cells were studied using (i) lipidomic analysis of fatty acids in membrane phospholipids, (ii) confocal microscopic analysis of TG stored in lipid droplets (visualized with the fluorescent probe Nile red), (iii) determination of total TG levels and (iv) the MTT viability assay.
Results: Increasing levels of palmitic acid in the incubation medium augmented the generation of palmitoleic acid and its accumulation in membrane phospholipids. This was accompanied with neogenesis of lipid droplets in the cells along with elevated TG accumulation (compare Fig. 1B with 1A). Inhibition of the transformation of palmitic acid to palmitoleic acid with CAY10566 blocked this effect (Fig. 1C) and caused massive cell death at high glucose levels. Exogenously added palmitoleic acid reversed the effect of SCD1 inhibition and restored TG biosynthesis and storage in newly-formed lipid droplets (Fig. 1D).
Conclusion: Studies in other cells showed that monounsaturated fatty acids (e.g., palmitoleic acid and oleic acid) are required to upregulate TG biosynthesis. Our results suggest that beta cells employ the same mechanism and use monounsaturated fatty acids, formed by SCD1 catalysis, to channel the damaging free palmitic acid into TG and sequester them in newly-formed lipid droplets. The mechanism by which monounsaturated fatty acids augment TG biosynthesis is currently being investigated. In conclusion, by altering fatty acid metabolism and desaturation and inducing TG biosynthesis beta cells ameliorate the potential detrimental effects of excessive internal free fatty acid levels.



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Disclosure: **S. Sasson:** None.

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The inhibitor of connexin 36 hemichannels, mefloquine, affects K_{ATP} channels and L-type Ca^{2+} channels in pancreatic beta cells

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Background and aims: Beta cells within an islet are connected via connexin Cx36 gap junctions. However, Cx36 may not only form cell-cell contacts, but may also form so-called hemichannels which open in response to a high extracellular K^+ concentration. Thus, hemichannels may be involved in the atypical response to K^+ depolarization, namely a strong further increase of insulin secretion, which occurs even when beta cells are already depolarized by complete K_{ATP} channel closure. Mefloquine has become the standard pharmacological tool to inhibit Cx36 gap junctions and hemichannels. Here, we have characterized the effects of mefloquine on membrane potential and Ca^{2+} currents of mouse beta cells.
Materials and methods: Primary mouse beta cells were isolated by a collagenase digestion technique and cultured for 24 - 48 h in RPMI 1640 (10 mM glucose until attachment, thereafter 5 mM glucose). The membrane potential was measured in the current clamp mode using the perforated patch configuration of the patch-clamp technique. Ca^{2+} currents were measured in the voltage clamp mode using the conventional whole-cell configuration.
Results: In the presence of 5 mM glucose 50 µM mefloquine slowly depolarized the beta cell plasma membrane by 33 ± 5 mV, but no action potential spiking resulted. At 10 µM mefloquine there was still a depolarizing effect (12 ± 3 mV). Under the same experimental conditions the K_{ATP} channel blocker tolbutamide (500 µM) established a plateau potential (depolarization by 24 ± 1 mV) with superimposed action potentials. These action potentials were abolished by 1 µM nisoldipine, showing that they resulted from the influx of Ca^{2+} via L-type Ca^{2+} channels. When mefloquine (50 µM) was added to the perifusion with tolbutamide the action potential amplitude slowly decreased, suggesting an inhibitory effect on Ca^{2+} influx. This hypothesis was tested by first exposing the beta cells to mefloquine and then adding 500 µM tolbutamide. In the presence of either 10 or 50 µM mefloquine, tolbutamide was unable to evoke action potential spiking. In voltage clamp experiments 50 µM mefloquine suppressed Ca^{2+} currents to a similar extent as 1 µM nisoldipine. Nisoldipine reduced Ca^{2+} currents down to 12%, mefloquine to 7%. The effect was partially reversible, however with more sluggish kinetics in the case of mefloquine.

Conclusion: In the same concentration range as it affects Cx36 gap junctions and hemichannels, mefloquine inhibits K_{ATP} channels (leading to depolarization) and L-type Ca^{2+} channels (suppressing action potentials) of the beta-cell. Thus, conclusions based on the use of this compound may require reconsideration. Conversely, future work on the role of hemichannels in insulin secretion would profit from a more specific tool.
Supported by: DDG
Disclosure: **N. Seemann:** None.

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The importance of newly discovered ER-resident glutathione peroxide isoforms for glucose-induced insulin secretion and insulin biosynthesis in INS-1E cells

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Background and aims: Reactive oxygen species (ROS) generated during metabolism of increased levels of glucose and free fatty acids are thought to contribute to the progressive loss of β-cell function and mass during the development of T2DM. In addition, ROS, especially hydrogen peroxide (H2O2), can also be generated during the oxidative folding of proinsulin. This process occurs in the endoplasmic reticulum (ER) and is accomplished by protein disulfide isomerase and ER oxidoreductin-1β, generating one molecule H2O2 per disulfide bond formed. Under conditions of high insulin requirement, as it prevails in the pathogenesis of T2DM, the ER-derived H2O2 might overwhelm the already low antioxidative capacity of β-cells finally leading to their failure. While it is well established that peroxiredoxin 4 (Prdx4) utilizes low H2O2 concentrations to generate disulfide bonds in a folding protein, the function of glutathione peroxide isoforms GPx7 and 8 in pancreatic β-cells remains uncharacterized. Therefore the aim of this study was to establish an expression profile of GPx7 and 8 in rat tissues and different islet cell types. Moreover, the impact of these GPxs on glucose-induced insulin secretion and insulin biosynthesis was investigated.
Materials and methods: The tissue-specific expression pattern of GPx7 and 8 was quantified using an external calibration curve based on plasmid DNA, while their expression in individual rat islet cells was assessed by in situ PCR. For stable overexpression of GPx7 or GPx8 in INS-1E cells, the specific hGPx7 or 8 cDNA was subcloned into lentiviral constructs. Thereafter cells were transduced with a lentiviral system. The overexpression of both enzymes was verified by Western blot analyses and their functionality by MTT assay in the presence of different H2O2 concentrations. Insulin content and secretion were determined by RIA and proinsulin content by a specific ELISA after stimulation with glucose (3, 10, 30 mM).
Results: Relative and absolute quantification of GPx7 and 8 showed that both GPx7 and 8 are expressed at a high level in liver followed by pancreas, islets, kidney, testis, and gut. Interestingly, insulin-secreting tissue culture cells, such as INS-1E neither expressed GPx7 nor GPx8. In situ PCR analyses in rat islets revealed that both GPxs are highly expressed in non-β-cells, whereas in β-cells only a faint expression of GPx8 and virtually no expression of GPx7 was detectable. Thus, the ER-resident GPxs are only expressed in non-β-cells. Specific overexpression of GPx7 or GPx8 resulted in a significant protection of INS-1E cells against exogenously added H2O2, whereas GPx8 showed a better antioxidative capacity than GPx7. Overexpression of GPxs partially improved insulin secretion after stimulation with 10 and 30 mM glucose compared to control cells but at variance from Prdx4 overexpression decreased proinsulin and insulin content when compared to control cells.
Conclusion: These data provide evidence that none of the ER-resident glutathione peroxide members is expressed in rat β-cells. An overexpression of ER-specific GPxs improved the antioxidative capacity but impaired the insulin biosynthesis. Thus, GPx7 and GPx8 are, at variance from Prdx4, not involved in oxidative insulin folding and preservation of beta cell function under conditions of high insulin requirement.
Disclosure: **I. Mehmeti:** None.

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Properties of voltage-gated sodium channels in pancreatic beta cells

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Background and aims: The role of voltage-gated sodium (Nav) channels in mouse pancreatic β-cells remains unclear due to the unique property of the channel in this cell. A characteristic feature of Nav channels is their voltage-dependent activation and inactivation. Nav channels in mouse β-cells undergo inactivation at very negative membrane potentials, rendering most Nav channels inactivate and thus unable to contribute to the electrical activity of the cell and other important cellular processes such as insulin secretion. Pancreatic β-cells express Nav1.3, Nav1.6 and Nav1.7. We hypothesised that these channels have different properties in β-cells compared to other cell types. The aim of the project was to characterise these channels in a β- and non-β-cell model and to identify potential modulators of Nav channel properties in β-cells.
Materials and methods: Nav channels were rendered resistant to tetrodotoxin (TTX), a Nav channel blocker. The channels were then transfected into a rat insulinoma cell line (INS1-832) and a human embryonic kidney (HEK) cells to model a β-cell and non-β-cell environment, respectively. TTX was applied to block any endogenous currents in the cells and to isolate the current that was made resistant to TTX. Using whole cell patch clamp the activation and inactivation properties of the Nav channel were measured.
Results: Half-maximal inactivation (V0.5) of Nav1.7 and Nav1.3 expressed in HEK cells was observed at -68 ± 2 mV (n=8) and -48 ± 2 mV (n=5), respectively. However when expressed in INS1-832 cells the V0.5 of Nav1.7 and Nav1.3 displayed a significant hyperpolarising shift to -93 ± 2 mV ($p<0.001$, n=8) and -86 ± 2 mV ($p<0.001$, n=13), respectively. This effect could also be observed in Nav channels that are not usually expressed in β-cells. For example the cardiac α-subunit Nav1.5 also produced a significant hyperpolarising shift in the V0.5 to -106 ± 2 mV (n=9) when expressed in INS1-832 cells, compared to the V0.5 of -77 ± 2 mV ($p<0.001$, n =5) observed in HEK cells. The shift in inactivation of all the channels occurred without a significant change in the activation properties of the channel. Receptor tyrosine kinases have been previously reported to modulate Nav channel current densities. The insulin receptor is a receptor tyrosine kinase and it was hypothesised that insulin secreted from β-cells may act via autocrine signalling on its own receptor to modulate Nav channel inactivation. However application of an insulin receptor antagonist had no effect on the gating properties of Nav1.7 or Nav1.3. Furthermore, modulation of downstream insulin receptor signalling molecules such as PIP2, which is known to alter the gating properties of the ATP-sensitive potassium channel, also did not affect Nav channel modulation.
Conclusion: These results suggest a β-cell-specific modulation of Nav inactivation properties. Preliminary data suggests that the KATP channel and the insulin signalling pathway do not affect channel modulation. Identification of this cell specific modulation warrants further investigation as it modulates the inactivation property of a broad spectrum of Nav channels and from a β-cell perspective recruitment of the channels could provide a novel mechanism for increasing insulin secretion in diabetes.
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Disclosure: **M. Godazgar:** None.

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Coixol from scoparia dulcis Linn stimulates insulin secretion through PKA and MEK kinase ERK½ signalling pathway

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Background and aims: There are claims and counter claims regarding the pathophysiology of type 2 diabetes; however, recent reports suggest that Asian non-obese type 2 diabetic subjects have predominant insulin secretory impairment. Taking advantages of in-house pure compounds, coixol (CX), isolated from Scoparia dulcis Linn, was identified as a new insulin secretagogue, enhances glucose-stimulated insulin secretion (GSIS) seems a better candidate than sulfonylureas. In the present study, we explored the possible insulinotropic mechanism(s) of CX focusing on K-ATP channels-dependent and -independent pathways.
Materials and methods: Islets or MIN6 cells were incubated for 1 h at 37°C in KRB buffer, containing 3 mM or 16.7 mM glucose in the absence or presence of CX and test substances and secreted insulin was measured by insulin ELISA kit. In vivo effects of CX on GSIS and insulinogenic index were evaluated in diabetic rats.
Results: CX improved GSIS distinctly different from sulfonylurea in isolated islets and MIN6 cells. CX stimulated insulin secretion only at high glucose concentrations (11.2-20 mM), but not at low glucose concentrations (3-5 mM). When islets were incubated with CX (100 μM) and diazoxide (50 μM) to keep K-ATP channels open, insulin secretion by CX was not modulated at 3 mM but inhibited significantly at 16.7 mM glucose (ng/islet/h; M ±SEM; 6.19 ± 0.55 vs. 70.15 ± 3.92, $P<0.001$). In depolarized islets by KCl (25 mM), in the presence of diazoxide, insulin secretory ability of CX was enhanced at both 3 mM (ng/islet/h; M±SEM; 24.64 ± 1.57 vs. 11.03 ± 0.67, $P<0.001$) and 16.7 mM (ng/islet/h; M±SEM; 97.38 ± 3.63 vs. 49.53 ± 1.95, $P<0.001$) glucose. The electrophysiological data in MIN6 cells revealed that, CX showed no significant effect on inward rectifying potassium currents (-95 ± 4.5 pA, n = 6) in comparison to tolbutamide (-32 ± 5.2 pA, n = 6, $P<0.001$). Verapamil (200 μM), an L-type Ca2+ channels blocker, showed no effect on insulin secretion by CX at 3 mM but showed inhibitory effect at 16.7 mM glucose (ng/islet/h; M ± SEM; 15.98 ± 0.94 vs. 62.5 ± 2.5, $P<0.001$). SQ22536 (25 μM), an adenylate cyclase inhibitor, moderately inhibited (ng/islet/h; M±SEM; 49.75 ± 2.80 vs. 59.93 ± 3.37, $P<0.05$) the CX-induced insulin secretion at 16.7 mM glucose. H-89 (30 μM), a protein kinase A (PKA) inhibitor, inhibited the CX-induced insulin secretion at 16.7 mM glucose (ng/islet/h; M±SEM; 37.75 ± 1.78 vs. 56.29 ± 3.56, $P<0.001$). U0126 (20 μM), a MEK kinase inhibitor, inhibited the CX-induced insulin secretion at 16.7 mM glucose (ng/islet/h; M±SEM; 38.73 ± 2.57 vs. 62.05 ± 3.37, $P<0.001$). In vivo studies revealed that CX enhanced glucose-stimulated plasma insulin and improved insulinogenic index (β-cell function) in non-obese type 2 diabetic rats. CX was subjected to in vitro cytotoxicity assay against MIN6 cells and islet cells, and in vivo acute toxicity test in mice that was found to be non-toxic.
Conclusion: CX, a new insulin secretagogue, stimulates GSIS only at high glucose concentrations both in vitro and in vivo. The CX-mediated insulin secretion seems through PKA and MEK/ERK½ signaling pathway, distal to the K-ATP channels coupled with stimulatory glucose.
Supported by: HEC, Pakistan
Disclosure: **A. Hameed:** Grants; HEC, Pakistan.

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FLIM-based pH measurements reveal incretin-induced rejuvenation of aged insulin secretory granules

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Background and aims: Insulin is stored in dense-core secretory granules (SGs) and is released from beta cells in two distinct phases upon glucose stimulation. Newly synthesized insulin SGs are secreted preferentially, but contribution of SG age to the different phases remains elusive. We previously developed a technique for imaging age-distinct SGs based on the pulse-chase fluorescent labeling of an insulin-SNAP reporter. With this approach we showed that young SGs display greater motility compared to their older counterparts, which are instead preferentially disposed in multigranular bodies/lysosomes. The relationship of SG age with their pH is also of particular interest: proinsulin conversion by prohormone

convertases follows the acidification of immature SGs by the vacuolar proton-translocating ATPase (v-ATPase). v-ATPase may also participate in the formation of the fusion pore for SG exocytosis, with intraluminal alkalinization inhibiting membrane fusion. The aim of our studies was to investigate for the first time the pH of individual insulin SGs also in relationship to their age.
Materials and methods: ECFP-based fusion constructs were used for high-accuracy measurements of the granular pH in INS-1 cells by fluorescence-lifetime imaging microscopy (FLIM). Luminal localization was confirmed by structured illumination super-resolution microscopy.
Results: Calibration of RESP18-HD-eCFP to pHs ranging from 5.0 to 7.5 confirmed its reliability as a pH reporter in lifetime microscopy (1600-2300 ns, respectively). Subsequent measurements and statistical analysis revealed two pH-distinct subpopulations among insulin SGs. Correlation of luminal pH and SG age indicated a pH of ~5.5 in 2-4 hour-old young and ~6.2 in 26-28 hour old SGs. Glucose stimulation prompted the collapse of the pH gradient in old but not young SGs. Remarkably, Exendin-4 re-acidified old SGs in a vesicular glutamate transporter (vGLUT)-dependent manner, while no change was observed in young SGs.
Conclusion: Previous studies examined the SG pH on a population level only. By examining individual SGs, we show that INS-1 cells contain two pH-distinct SG pools that correlate with their age. Further, glucose stimulation did not lead to the re-acidification of old SGs, consistent with young SGs being preferentially poised for exocytosis. However, Exendin-4 treatment induced the re-acidification of old SGs, potentially explaining incretin potentiation of insulin secretion.
Supported by: BMBF-DZD, DIGS-BB
Disclosure: **M. Neukam:** None.

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The diabetes-link factor HMG20A maintains islet beta cell metabolic maturity

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Background and aims: Genome-wide association studies have linked common small nucleotide polymorphisms (SNPs) in the HMG20A gene to higher risk of developing type 2 diabetes mellitus (T2DM). Intriguingly, HMG20A, a chromatin-remodeling factor, is important mainly for neuronal differentiation during development while in adults it exerts global genomic changes. Due to HMG20A described link with T2DM, the aim of the current study is to establish the possible role of HMG20A in beta cell function as well as to determine whether one of the SNP associated with diabetes impacts HMG20A expression.
Materials and methods: HMG20A transcript levels were assessed by QT-RT-PCR in various tissues as well as in blood samples from 118 human subjects from a semi-urban town of South Europe (Pizarra, Spain). Protein levels were also assessed by immunohistochemistry in islets. To evaluate the effect of HMG20A on beta cell function, expression levels of potential targets involved in insulin biosynthesis and secretion were measured 72 hours after siRNA-mediated repression of HMG20A in MIN-6 and INS-1E cells. The effect of the 3'UTR SNP rs7119, previously linked to T2DM, on HMG20A expression was tested by luciferase reporter assays.
Results: HMG20A transcript levels were increased by approximately 50% in T2DM patients as compared to non-diabetic subjects. Accordingly, we observed a transient increase of HMG20A in human islets (~2-fold at 72h), as well as in MIN-6 and INS-1E cells (~3-fold at 48h), when cultured in high glucose, showing a glucose-dependent regulation of HMG20A. A 60% knock down of HMG20A in INS-1E cells resulted in inhibition of NeuroD (70%), glucokinase (30%) and insulin (30%), as well as in a decline in glucose-stimulated insulin secretion correlating with diminished mitochondrial function. Luciferase reporter assays in human cell lines indicate that the diabetes-linked SNP rs7119 alters HMG20A expression, likely due to modification of a putative miRNA binding site.
Conclusion: Taken together, our results suggest that HMG20A is required for maintaining beta cell mature metabolic phenotype through regulation of metabolism-secretion coupling genes. T2DM-associated SNP rs7119 leads to altered HMG20A expression substantiating the importance of this gene in islet physiology and pathophysiology.
Supported by: ISCIII (PI13/00593 to BG) and MINECO(JCI-2012-12491 to EFM)
Disclosure: **J.M. Mellado-Gil:** None.

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Ultrastructural analysis of insulin secretory granule ageing by super resolution and transmission electron microscopy

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Background and aims: Pancreatic beta cells store insulin within secretory granules (SGs). Hyperglycemia elicits beta cells to release insulin in two phases: a short and rapid first phase followed by a sustained second phase. Lack of the first phase of insulin secretion is a hallmark of type 2 diabetes. Notably, newly synthesized insulin SGs are preferentially released, but the molecular reasons for this phenomenon remain obscure. Hence, our aim is to gain insight into the mechanisms the regulate insulin SG turnover in physiological and pathophysiological conditions. We have previously shown that a fusion construct between insulin and the SNAP tag is a reliable reporter for fluorescent labeling of age-distinct insulin SGs. SNAP is a 22kDa tag-polypeptide derived from human O6-alkylguanine-DNA alkyltransferase (AGT). In the SNAP tag, AGT is modified such that its catalytic cysteine covalently binds benzylguanine-containing substrates. SNAP-substrates, in turn, can be conjugated to a variety of probes, including cell permeable fluorophores. With this technique age-distinct insulin SG pools in beta cells can be independently labeled by applying fluorescent and non-fluorescent SNAP substrates to separate samples differing in the length of the time interval between the "pulse" and "chase" labeling.
Materials and methods: We labeled age-distinct insulin SGs in beta cells of pancreatic islets isolated from SOFIA (Study of insulin aging) mice, in which an insulin2-SNAP allele had been knocked-in in the Ins2 locus. Isolated islets were kept in standard culture medium containing 5.5 mM glucose and labeled for the detection of SG pools with ages ranging from several hours to 5 days. Islets were fixed and ultrathin frozen Tokuyasu sections were cut. Sections were first imaged by structured illumination microscopy (SIM), followed by transmission electron microscopy (TEM). Then, the corresponding fluorescence and TEM images were precisely overlaid and analyzed.
Results: By combining SIM and TEM for correlative light-electron microscopy (CLEM) we precisely tracked age-distinct insulin SGs in Tokuyasu sections of beta cells. Since in TEM sections between 100-

200 SGs/beta cell can be seen, the precise correlation achieved here was pivotal for distinguishing age-defined (i.e. labeled) from unlabeled SGs. We found a significant decrease in the number of labeled SGs starting from 3 days of age with a reduction of ~60% at an SG age of 5 days. This reduction was accompanied by the detection of SNAP-fluorescence in multi-granular bodies devoted to the intracellular degradation of aged SGs.

Conclusion: This novel approach allows for quantitative and topographic ultrastructural analyses of age-distinct insulin SGs in relationship to their life cycle in different metabolic conditions as well as the quantification by immunogold labeling of molecular markers connected to SG aging and degradation. Our CLEM approach is also applicable to a wide variety of cell biological questions, especially when the focus of interest is on subcellular compartments in small cell populations.

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Disclosure: **A. Mueller:** None.

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A novel long non-coding RNA involved in mouse beta cell proliferation

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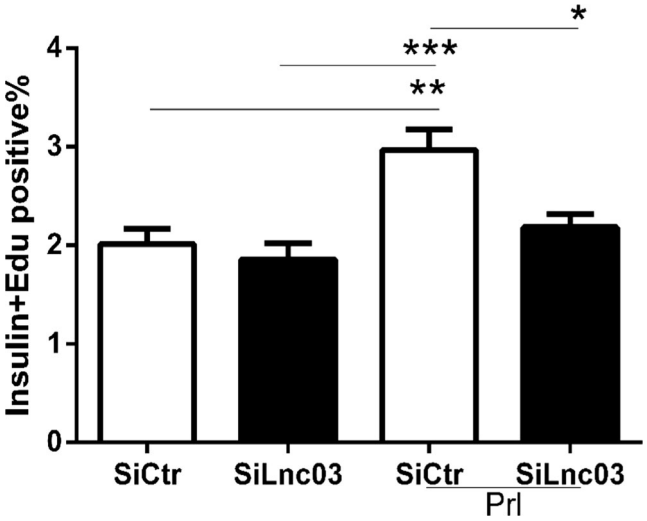
Background and aims: Long non-coding RNAs (LncRNA) constitutes a new class of non-coding transcripts with intriguing roles in β-cell biological processes. We previously demonstrated with in silico and in vivo/vitro approaches that differential expression of seven lncRNAs dominate at the β-cell proliferation peak during mouse pregnancy. An lncRNA on chromosome 15 referred to as Lnc03 has the most islet specific expression pattern and expression was regulated by Prolactin (Prl) in vitro. Here, we unveil the function of Lnc03 in regulating β-cell proliferation.

Materials and methods: The link between Prl and expression of Lnc03 was studied through inhibition of downstream components of the Prl receptor (PrlR) activation pathway. We investigated the cellular localization of Lnc03 RNA by extracting RNA from the nucleus and cytoplasm and evaluating its enrichment by real-time PCR. We used an siRNA silencing strategy to evaluate the role of Lnc03 in Min6c4 and dispersed mouse islet cell proliferation through cell count and 5-ethynyl-2'-deoxyuridine (EdU) incorporation.

Results: To evaluate Lnc03 expression induced by 500ng/ml Prl we inhibited the PrlR activation pathway in Min6c4 cells with Stat5 and PI3K inhibitors. We observed that expression of Lnc03 decreased significantly in the presence of the Stat5 or PI3K inhibitors compared to cells treated with Prl alone (2.8-fold, $p<0.0001$ and 1.2-fold, $p<0.05$, respectively). When investigating the intracellular localization of Lnc03, we observed an enrichment of Lnc03 in the cytoplasmic fraction when compared to the whole cell (1.7-fold, $p<0.05$) and nucleic fractions (3.3-fold $P<0.01$). Lnc03 knock-down using siRNA in Min6c4 cells led to a reduction in cell number compared to samples transfected with control siRNA in the presence and absence of 500ng/ml Prl ($p<0.05$). The percentage of EdU incorporation decreased ($p<0.01$) with the same treatment conditions. We also transfected dispersed mouse islets with Lnc03 siRNA, with and without 500ng/ml Prl. Lnc03 siRNA completely prevented the proliferative stimulation of Prl in β-cells ($p<0.05$, see figure).

Conclusion: We demonstrate that expression of Lnc03 in β-cells is driven by the PrlR through Stat5 and PI3K; Lnc03 is enriched in the cytoplasm; and it is regulating β-cell proliferation. Although further evidence is needed, the cytoplasmic localization of Lnc03 may represent a post-transcriptional regulatory role in β-cell proliferation, possibly involving Lnc03-protein interactions.



Disclosure: **B. Tyrberg:** None.

PS 018 Tinkering with islet transplantation: Can we do better?

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Intra-omental graft with a plasma hydrogel carrier: a novel alternative for islet transplantation

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Background and aims: Classically, islets infusion is performed through the portal vein. However, the hepatic site is the theater of inflammatory reactions which destroys almost half of the graft. Therefore, alternative sites have been investigated such as the omentum. It presents a lot of advantages among them high vascularization, respect of the first hepatic passage, and high malleable space. Nevertheless, injection of a liquid preparation is not feasible due to its anatomical conformation and a support has to be used. Previous results showed that culture islets in a hard fibrin scaffold can improve islet viability, but its transplantation as roll-up in the omentum results in no graft function, probably due to the lack of oxygen supply in the scaffold. To improve oxygenation of the graft, injection within the tissue with a technique derived from esthetic surgery could be used. The aim of this study was to test this technique using plasma hydrogels as carrier for islet transplantation in omentum in order to combined scaffold and recipient site benefices.
Materials and methods: Diabetic Lewis rats (glycaemia > 5g/L, and c-peptidemia <150pM), were under insulin treatment (3UI/day delivered using an insulin pellet) during 4 weeks after diabetes confirmation in order to reach an optimal weight (around 250g) for the graft. Pumps were retrieved at time of transplantation. Just before transplantation, 2300 IEQ were mixed with cellulose-based hydrogel alone (HPMC) or supplemented with Lewis plasma (HPMC Plasma), then loaded in a syringe with a specific atraumatic needle and injected in the omental tissue (n=8). Classical hepatic transplantation (Liver) was realized as control of islet graft efficiency (n=6). Metabolic study is ongoing and combined analyses of weight, glycemia, c-peptidemia and IpGTTs. Results are expressed ±SEM, ANOVA was realized and p-value <0.05 were considered significant.
Results: Glycaemia decreased significantly in all groups after graft (t0d Liver: 5.57±0.07, HPMC: 5.31±0.20g/L, HPMC Plasma: 5.215±0.14g/L and t7d, Liver: 2.26±0.42g/L, HPMC: 2.34±0.40g/L, HPMC Plasma: 2.00±0.33g/L ($p<0.05$ t0 vs t7)). A significant c-peptide increase is observed during the time; (t0d Liver: 53.67±21.48pM, HPMC: 60.00 ±18.76pM, HPMC Plasma: 60.75±20.25pM and t7d Liver: 881.11 ±149.87pM, HPMC: 796.65±121.84pM, HPMC Plasma: 1063.331 ±127.53pM ($p<0.05$ t0 vs t7)). HPMC plasma preserved the graft with higher c-peptide values and lower glycaemia than other conditions. IpGTT profiles shown a good graft function in all groups, AUC of HPMC Plasma group was lower that the two other groups (Liver: 341.5 ±43.08, HPMC: 351.8±16.91, HPMC Plasma: 335.15±51.98).
Conclusion: This study is a proof of concept of the feasibility of an intratissular graft in the omental site, and the power of plasma on islet function. Results showed that grafts are functional, with an advantage for HPMC plasma graft. Intra-omental islet graft is therefore a seductive alternative to hepatic graft, as it can integrate support beneficial for islet survival and function and can avoid danger of hemorrhages created by intra-portal infusion.
Supported by: Region Alsace
Disclosure: **A. Schaschkow:** None.

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Effects of human pancreatic ductal cells co-transplanted with islets in graft outcome

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Background and aims: Islet transplantation restores normoglycemia in type 1 diabetic patients, but with time hyperglycemia usually recurs. The cellular composition of the transplanted islet cell preparation is important in the outcome of the graft. Acinar cell contamination has been shown to have a negative impact, but the role of pancreatic ductal cells is not well established. Ductal cells produce tissue factor and cytokines that may be detrimental to islet survival. However, they also secrete angiogenic and growth factors that could improve islet survival and engraftment. *In vitro* studies have suggested both beneficial and negative effects of ductal cells on islets, but the effects have not been directly investigated *in vivo*. The aim of this study was to investigate the effect of human pancreatic ductal cells on experimental human islet transplantation.
Materials and methods: Pancreases of cadaveric organ donors were processed for islet isolation and the exocrine fraction was collected and dispersed into single cells. Ductal cells were purified by magnetic cell sorting with CA19.9 antibody and cultured in suspension for 3 days. Pancreatic ductal cells clustered into pancreatospheres. Isolated human pancreatic islets were used for transplantation and *in vitro* experiments. 1) Transplantation experiments: 800 human islets, a suboptimal beta cell mass, (Tx Group, n=9) and 800 human islet + 600 pancreatospheres (Co-Tx Group, n=9) were transplanted under the kidney capsule of immunodeficient nude mice. Diabetes was induced by repeated low-dose streptozotocin injections. Blood glucose was measured to determine the metabolic evolution, and beta and endocrine non beta cell mass was evaluated. 2) *In vitro* experiments islets were cultured alone or with pancreatospheres (Group 1: 100 islets, Group 2: 100 islets + 75 pancreatospheres, Group 3: 100 islets + 225 pancreatospheres) (n=5) for 48 hours to determine the effects of ductal cells on beta cell survival (TUNEL) and function (glucose-stimulated insulin secretion) (GSIS) (basal: 2.8 mM; stimulated: 20 mM).
Results: Normoglycemia was maintained in 67% of mice transplanted with 800 human pancreatic islets, whereas only 33% of co-transplanted mice remained normoglycemic after 30 days of follow up. Beta cell mass was 26% lower in grafts of co-transplanted mice ($p=ns$), and the ratio beta/endocrine non-beta cell mass was significantly decreased in co-transplanted mice (Tx Group: 2.05 ± 0.18, Co-Tx Group: 1.35 ± 0.15; $p<0.01$). After 48 hours in culture, beta cell apoptosis was similar (Group 1: 0.73± 0.31%; Group 2: 0.68±0.31%, Group 3: 0.68±0.38%), and GSIS and insulin content were lower in islets co-cultured with pancreatospheres, but differences were not significant: GSIS (stimulated index): Group 1: 7.42±1.39; Group 2: 5.74 ±1.43; Group 3: 5.76±2.31; Insulin Content: Group 1: 220±59.3 ng INS/μg DNA, Group 2: 201±49.6 ng INS/μg DNA, Group 3: 159 ±40.6 ng INS/μg DNA.
Conclusion: Human pancreatic ductal cells had a negative impact on the metabolic evolution and grafted beta cell mass in islet transplantation. *In vitro* co-culture experiments with islets and pancreatic ductal cells did not reveal changes in beta cell apoptosis and GSIS was not significantly reduced. The study of grafts in the initial days after transplantation may contribute to better understand the effects of pancreatic ductal cells in islet transplantation.
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Disclosure: **S. Marín:** None.

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Human adipose tissue-derived stem cells enhance survival and function of grafted islets in co-transplanted diabetic mice

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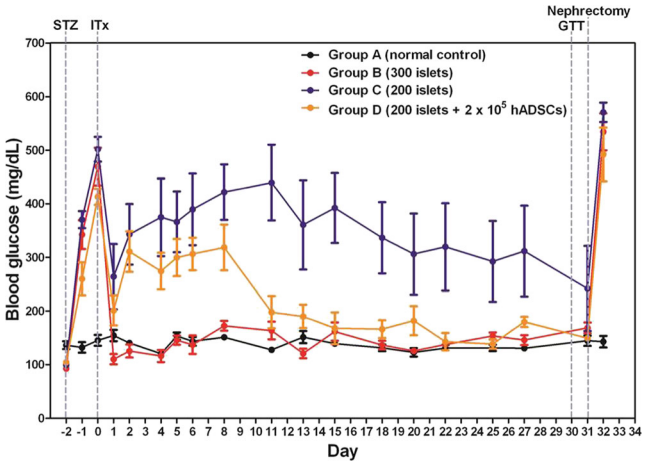
Background and aims: Pancreatic islet transplantation is a feasible treatment option to cure Type 1 diabetes. But there were some barriers like deficiency of islet supply due to shortage of pancreas and immune-mediated islet destruction after transplantation. Stem cells are considered as a substitutive tool to overcome the barriers. Multipotent adipose tissue-derived stem cells (ADSCs) are abundant in the human body and can differentiate into insulin-producing cells and improve engraftment and protection of donor islets when transplanted together. For these reasons, we evaluate the potentials of co-transplantation of human ADSCs (hADSCs) with mice pancreatic islets leading in the survival and function of grafted islets in diabetic animal model.
Materials and methods: Mice pancreatic islets obtained from 10 week old male C57BL/6 mice were infused into the renal capsule of streptozotocin induced diabetic recipients. The recipients were randomized into four groups: normal control, diabetes with 300 islets transplantation, diabetes with 200 islets and 2x105 hADSC cells co-transplantation and diabetes with 200 islets transplantation. We checked body weight, glucose and serum insulin. Also, we performed immunofluorescence staining for insulin, BrdU and human chromosomal marker in islet transplanted kidney.
Results: Mice that were transplanted with 200 islets alone exhibited failed to ameliorate hyperglycemia. In contrast, mice transplanted with 200 islets and 2x105 hADSC cells exhibited significantly improved blood glucose levels, and achieved normoglycemia like normal control and 300 islet transplanted group. Also, insulin and BrdU was well stained in transplantation section in kidney.
Conclusion: In this study, we have demonstrated that co-transplantation of hADSCs with pancreatic islets improved glucose profile and islet function and survival in STZ induced diabetic mice. The protocol of stem cell islet co-transplantation may open the window of opportunity to allow more successful islet transplantation and could have a significant clinical impact on the treatment of T1D.



Disclosure: **B. Kim:** None.

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Induction of immune tolerance site under the skin by agarose rods with cyclic peptide and islets transplantation into the sites

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Background and aims: Clinical islet transplantations still have some serious problems to be solved. Because immunosuppressive drugs, which are used to prevent graft rejection, might compromise ability to resist infection and increases probability of onset of cancer, a method to transplant islets without immunosuppressive drugs has been desired. In current islet transplantation, islets are transplanted in to the liver via portal vain, but liver is not suitable site for islet transplantation. Especially, high efficient methods to differentiate islets from induced pluripotent stem cells or embryo-stem cells (quasi-islets) were reported, and those methods will be applied to treat diabetic patients in near future. It is difficult to deny the possibility of tumor formation by contaminated undifferentiated cells, when quasi-islets are transplanted into the liver. A subcutaneous site is suitable for islet transplantation, because the graft can be observed by various methods and easily removed under local anesthesia. There are few reports for success of islet transplantation under the skin. Islet cannot survive under the skin due to insufficient oxygen, because vascular density is scarce in the subcutaneous site.
Materials and methods: In this study, we prepared pre-vascularized and immune tolerance sites under the skin for islet transplantation. A solution of 4.5% (w/v) agarose was collected in a tube and induced gelation on room temperature. The agarose gel rods was cut into rods of 4 mm in diameter and 25 mm in length, frozen overnight and dried under reduced pressure. The SEK-1005, cyclic peptide to be known as TGFβ1 inducer, solution in ethanol was applied onto the freeze- dried agarose rod. After volatilization of ethanol, saline was dropped onto the agarose rod. Recipient ACI rats were rendered diabetic with an intraperitoneal injection of a streptozotocin (STZ) solution. An agarose rod with SEK (SEK- rod) was implanted into each of the two dorsal subcutaneous sites of the STZ treated ACI rat. Upon removal of the rods at day 10, 1500 islets isolated from F344 were transplanted into the two dorsal SEK-treated pockets, respectively. Blood glucose levels were monitored to see the graft function.
Results: SEK- rods were implanted into the dorsal subcutaneous sites of the STZ treated ACI rat for 10 days, and vascularization was induced at the subcutaneous site. By transplanting F344 islets into the site formed with SEK- rods, the blood glucose levels reversed to normal levels about 10 days after transplantation, and 8 of 10 diabetic ACI rats with 3000 F344 islet demonstrated stable normoglycemia for a long period (Figure. 1).
Conclusion: These results indicated that the implantation of SEK- rods under the skin can successfully construct vascularized immune tolerance sites. This method would have a potential to form a suitable site for allogeneic islet transplantation without immunosuppressive drug.

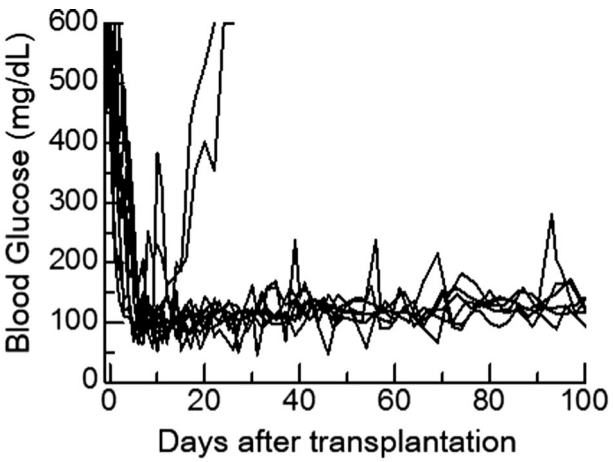

Figure 1. Blood glucose levels of STZ-ACI rats transplanted with 3000 F344 islets into subcutaneous spaces pre-vascularized by SEK- rods.

Disclosure: **R. Kuwabara:** Grants; Grant-in-Aid for Scientific Research on Innovative Areas "Nanomedicine Molecular Science" (No. 2306) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

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Dipeptidyl peptidase-4 inhibitor LAF-237 improves glucose tolerance and expends graft beta cell mass in diabetic mice transplanted with a sufficient number of islets

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) inhibitors increase circulating levels of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide which promote β-cell proliferation and survival. This study tested if DPP-4 inhibition with LAF237 is beneficial for diabetic recipients with a marginal or sufficient number of islets.
Materials and methods: We syngeneically transplanted 150 or 300 C57BL/6 mouse islets under the kidney capsule of each inbred streptozotocin-diabetic mouse, and then treated recipients with or without LAF237 (10 mg/kg, po) for 6 weeks. Before and after transplantation, recipients' blood glucose, body weight and intraperitoneal glucose tolerance test (IPGTT) were measured. At 6 weeks, the grafts were removed to determine insulin content and β-cell mass.
Results: In mice transplanted with 150 islets, blood glucose levels decreased in both groups after transplantation, but there were no significant differences between two groups. The area under the curve (AUC) of IPGTT at 2, 4 and 6 weeks were comparable between the LAF237-treated group and controls. Both groups exhibited increased body weights over time, but the differences between two groups were not significant. At 6 weeks after transplantation, the graft insulin content and β-cell mass of grafts were not significantly different between LAF237-treated group and controls. In mice transplanted with 300 islets, blood glucose levels decreased in both groups after islet transplantation, but there were no significant differences between two groups. The AUC of IPGTT at 4 and 6 weeks was lower in the LAF237-treated group than controls (28720 ± 1780 vs. 34840 ± 2136 mg-min, p=0.033 and 26330 ± 1748 vs. 33340 ± 2125 mg-min, p=0.015, respectively). Both groups exhibited increased body weights over time, but the differences between two groups were not significant. At 6 weeks after transplantation, the insulin content of grafts was not significantly different between LAF237-treated group and controls. However, LAF237-treated group had more graft β-cell mass than controls (0.53±0.09 vs. 0.31±0.06 mg, p=0.043).
Conclusion: Our results indicate posttransplant LAF237 treatment improves glucose tolerance and expands graft β-cell mass in diabetic recipients transplanted with a sufficient number of islets.
Supported by: CMRPG1A0541-2
Disclosure: **J. Juang:** None.

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Continuous blood glucose monitoring in a rat model of islet transplantation

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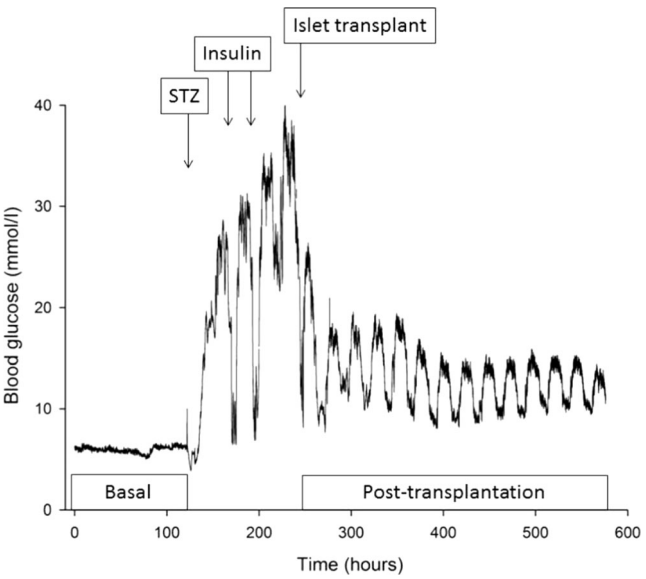
Background and aims: Rodent models of diabetes are often used in preclinical islet transplantation studies. In most studies, blood glucose is measured daily and often the time-point is not reported. The aim of this study was to measure blood glucose continuously in a rat model of islet transplantation to assess blood glucose excursions in the night when the rats are active and are feeding.
Materials and methods: Three male Lewis rats were implanted intraperitoneally with state of the art continuous glucose telemetry devices with blood glucose measured in the descending aorta in accordance with manufacturer's instructions. Following complete recovery from surgery blood glucose monitoring started, with recordings taken every 10 seconds. After five days of baseline measurements the rats were injected with 65mg/kg streptozotocin (STZ) to induce diabetes. Five days later, 2000-3000 Lewis rat islets were implanted under the kidney capsule and animals were maintained for a further 14 days. Each rat generated 207355 individual blood glucose measurements, and as such for purposes of statistical analysis mean blood glucose concentrations for each day (7am-7pm) and night cycle (7pm-7am) were calculated.
Results: In the non-diabetic state, the mean difference in blood glucose concentrations between day and night was minimal (day: 6.9 ±0.6 mmol/l vs night: 7.2±0.7 mmol/l, p=0.764, t-test. Mean day-night difference: 0.42±0.1mM). After STZ injection, rats became hyperglycaemic (>20 mmol/l) within 13-22h and day to night blood glucose excursions increased (day: 24.7±2.8 mmol/l vs night: 33.0 ±4.8 mmol/l; mean excursion: 8.3±1.2 mmol/l, p<0.0001 vs pre-STZ, t-test. Data within 5 hours of an insulin injection were excluded). After transplantation, two rats showed improved blood glucose concentrations but day vs night glucose excursions were still significant (day time mean of 10.5 mmol/l and 11.0 mmol/l vs night mean of 14.6 mmol/l and 14.8 mmol/l respectively, p<0.0001 vs respective mean day glucose concentrations, t-test. Mean excursion: 3.8 mmol/l and 4.2 mmol/l respectively). The graph below shows the trace of one of these rats. The third rat remained hyperglycaemic and showed substantial day vs night glucose excursions, similar to that seen after STZ-treatment (mean day blood glucose: 25.2 mmol/l vs night blood glucose 38.1 mmol/l, p<0.0001, t-test. Mean excursion 12.0 mmol/l).
Conclusion: During basal conditions, differences in mean blood glucose concentrations between the night and day are minimal in male Lewis rats. In an islet transplantation model, blood glucose excursions during the night are significantly pronounced after STZ administration. Despite the improvement in glycaemic control following transplantation, the significant night time glucose excursions persist. This may lead to an over-estimation of the efficacy of an intervention, as most researchers measure blood glucose during the day. The data also highlights the importance of consistent glucose monitoring time-points both within a study and when comparing studies.



Supported by: DSI
Disclosure: **A.J.F. King:** Non-financial support; Blood glucose monitoring probes were provided by Data Sciences International.

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Intrapancreatic injection of human mesenchymal stem/stromal cells ameliorated hyperglycaemia in diabetes mice via regulating macrophages polarisation

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Background and aims: Transplantation of human mesenchymal stem/stromal cells (hMSCs) has been used to alleviate hyperglycemia of Type 1 diabetes mellitus (T1DM). However, effective treatment regimens and underlying mechanisms are still unclear. We compared the therapeutic effect of intrapancreatic (ipan) or intravenous (iv) injection of hMSCs on mice with streptozotocin (STZ)-induced T1DM. Furthermore, we evaluated macrophage activation to determine the underlying mechanisms.
Materials and methods: Adult C57/BL6 mice were injected with STZ (115 mg/kg, i.p.) to induce T1DM. Seven days later, hMSCs (1 million cells per mouse), provided by healthy donors, or vehicle, were administered to hyperglycemic mice (220-500 mg/dL) by either ipan or iv (into the jugular vein) injections. Some mice were given hMSCs again by ipan at 28 days. The other T1DM mice were treated with fluorescence-labeled hMSCs by ipan or iv injections to observe the distribution of cells in the body and tissues. During experimental periods, non-fasting blood glucose (BG) levels were measured in a time-dependent fashion. Blood and pancreases were subjected to measure blood insulin levels and to the histological examination, when the animals were sacrificed.
Results: Before STZ treatment, mean BG levels were 169 ± 4 mg/dL. Seven days after STZ treatment, the levels increased to 317±12 mg/dL. The BG levels in vehicle-injected animals were further increased and kept at approximately 400 mg/dL during the experimental periods. Iv-hMSCs injection temporarily, but insignificantly decreased BG levels. By contrast, ipan-hMSCs injection decreased significantly the BG levels to 280 mg/dL or less. By in vivo tracing of fluorescence signals of hMSCs, the mice treated with ipan-hMSCs injection displayed greater fluorescence signals in the subphrenic left lateral abdomen which fitted closely with pancreas, and the signals were hard to observe 14 days after the injections. In the pancreas, the fluorescence signals were mainly recognized in interlobular spaces of the pancreas, but not in peri-region of endocrine glands. Multiple ipan-hMSCs injections further decreased the BG levels to 233 ± 26 mg/dL at 56 days [$p<0.01$, vs vehicle treatment (416 ± 34 mg/dL)]. In these mice, blood insulin levels, pancreas weight, and the density of pancreatic insulin positive cells were improved. Immunostaining with pan-macrophage marker Iba1 and M2 type macrophage marker CD206 in the pancreases with ipan-hMSCs injection showed that hMSCs treatment decreased Iba1 positive cells in endocrine pancreas. Moreover, the hMSCs treatment increased number of CD206-positive cells.
Conclusion: These results suggested that ipan-hMSCs injection in T1DM mice displayed greater hypoglycemic effect than iv one. Moreover, ipan-hMSCs injection might contribute to modulate macrophages polarization as the one of the underlying mechanisms.
Disclosure: **N. Murai:** None.

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GRP44 inhibition improves islet function and survival in human islet xenografted mice

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Background and aims: GPR44 is a receptor for prostaglandin D_2 (PGD_2) that is highly expressed in human beta cells but with unknown function. The aim of this study was to investigate the role of the PGD_2-GPR44 axis in human islet function and survival in vitro and in vivo.
Materials and methods: Human islets (from Prodo Labs) were cultured for 24 h with a stable PGD_2-analogue (15(R)-15-methyl-PGD_2) or cytokines (IL-1β, IFNγ and TNFα) with and without addition of a specific GPR44 antagonist (AZ compound, 1 or 10 μM). Apoptosis was measured as caspase 3/7 activity by luminescence. In parallel experiments, PGD_2 secretion and glucose-stimulated insulin secretion (GSIS) were measured after culture of human islets for 24 h in 5.8 or 22.2 mM glucose with and without IL-1β (20 ng/ml) or a GPR44 antagonist (10μM). Islets were analyzed for mRNA expression of GPR44 and enzymes in the pathway producing PGD_2 (COX1,COX2, L-PGDS). The in vivo effect of GPR44 inhibition was investigated by transplanting a suboptimal human islet graft under the kidney capsule to alloxan diabetic NMRI nu/nu mice. Mice (n=8/group) were dosed orally twice daily with the GPR44 antagonist (50mg/kg) or with vehicle, starting at the day of transplantation and continued for 16 days. Blood glucose was monitored regularly and human C-peptide at day 2 and 16. At termination, the islet graft was harvested and analyzed for insulin and TUNEL immunofluorescence staining.
Results: The specific GPR44 antagonist significantly reduced apoptosis (caspase 3/7 activity by luminescence) in human islets in response to PGD_2 (19.7±3.2 vs.9.9±0.8 RLU, $p<0.01$) and to cytokines (17.2±1.6 vs. 10.5±1.2 RLU, $p<0.05$). Secretion of PGD_2 was increased in response to high glucose (518±29 vs. 902±101 pg/ml, $p<0.01$) and to cytokines (6150±650 pg/ml, $p<0.0001$). This correlated with increased mRNA expression of COX2 at high glucose (~10-fold, $p<0.001$) and in combination with IL-1β (~200-fold, $p<0.001$), while GPR44 and L-PGDS were reduced after incubation with IL-1β ($p<0.01$ for both). Incubation of human islets with a GPR44 antagonist for 24 h partly restored high glucose-induced impairment in GSIS. Administration of the GPR44 antagonist for 16 days to diabetic nu/nu mice transplanted with a suboptimal human islet graft resulted in increased human C-peptide levels (379±80 pM vs. 804±145 pM at day2 and 16, respectively, $p<0.001$) compared to vehicle (297±55 pM vs. 97±23 pM at day 2 and 16, respectively). Apoptotic cells within the islet graft, detected using TUNEL staining, were low and showed no difference between the vehicle and the treatment group. However, the insulin area was significantly increased by the treatment (98.3±10.8 vs. 37.5 ±5.9 μm^2/graft, $p<0.001$).
Conclusion: Human islets produce and secrete PGD_2 in response to a diabetic milieu in vitro resulting in apoptosis and impaired GSIS. Inhibition of GPR44 improved human islet survival and function both in vitro and in vivo. We conclude that GPR44 antagonism may be beneficial to islet survival following transplantation of human islets.
Disclosure: **M. Sörhede Winzell:** Employment/Consultancy; Several authors are employees at AstraZeneca.

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Effect of glycaemic control on exendin-based beta cell mass quantification

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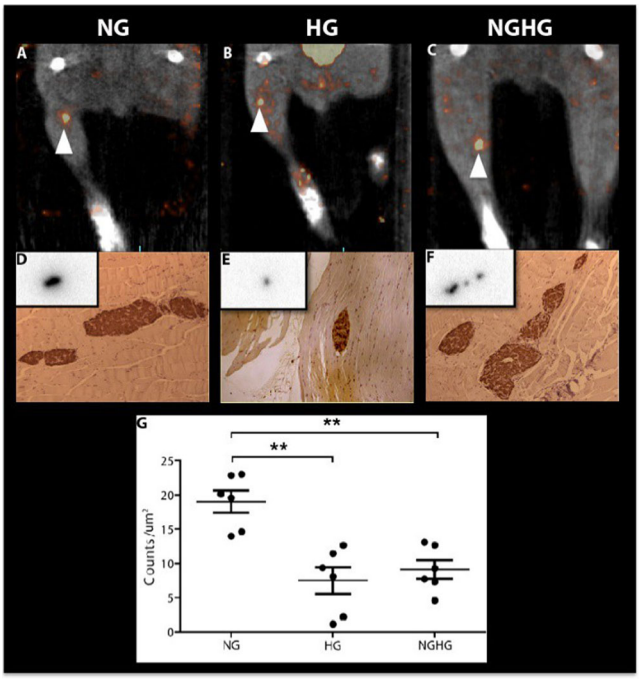
Background and aims: Targeting of the glucagon-like peptide-1 receptor (GLP-1R) with radiolabeled exendin, a GLP-1R agonist, is an attractive approach to determine the beta-cell mass (BCM). Intravenous injection of 111In-exendin, followed by SPECT imaging was demonstrated as a promising strategy to quantify BCM in the pancreas of healthy and diabetic subjects, as well as in islet transplants. It was reported that chronic hyperglycemia reduces GLP-1R expression. Hence, the effect of glycemic control on exendin-based BCM quantification remains to be elucidated. Here, we investigated

the effect of chronic hyperglycemia on 111In-exendin uptake in diabetic mice with intramuscular islet transplants.
Materials and methods: Syngeneic transplantations of 200 islets were performed in the calf muscle of alloxan-induced diabetic C3H mice. Glycemic state was regulated using insulin pellets. Three groups of glycemic state were analyzed; 4 weeks of normoglycemia (NG, blood glucose levels 25mM, n=6), and 2 weeks of normoglycemia followed by four weeks of hyperglycemia (NGHG, n=6). Mice were injected intravenously with 15 MBq of 111In-exendin and scanned 1h post-injection by SPECT. Exendin uptake was determined by autoradiography and was normalized for insulin area. Data were presented as mean±SEM.
Results: Islet grafts were clearly visualized by SPECT in all recipient groups. Tracer uptake was significantly influenced by glycemic state with an accumulation of 19.0 ± 1.6 counts/insulin area for the NG mice and 7.5 ± 2.0 and 9.1 ± 1.3 for the HG and NGHG mice, respectively ($p<0.01$, ANOVA with a bonferroni correction for multiple group comparisons, figure). Despite this two-fold reduction in tracer accumulation, tracer uptake linearly correlated with insulin area (Pearson's Correlation Coefficient r for NG= 0.75, HG= 0.95 and NGHG=0.82).
Conclusion: Our results indicate that exendin uptake is influenced by glycemic control. The observation that tracer uptake linearly correlates with insulin area, regardless of glycemic control, indicates that BCM quantification using exendin is a valid technique provided that tracer uptake will be corrected for the glycemic state of the subject. At the moment we are investigating whether tracer uptake can be restored when glycemic control is re-established after a period of chronic hyperglycemia. These results have significant clinical implications for the interpretation of exendin scans in subjects with poorly controlled diabetes.



A-C) SPECT images of islet grafts of NG, HG and NGHG mice (white arrows). D-F) Histological evaluation of islet grafts of NG, HG, and NGHG mice. Inserts represent corresponding autoradiography images. G)Radioactive uptake per insulin positive area of islet grafts of NG, HG, and NGHG mice

Disclosure: **M. Buitinga:** None.

PS 019 Islet development

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Manipulating Kras signalling alters endocrine differentiation by changing cell cycle length during mouse embryonic pancreas development

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Background and aims: The composition and final function of the adult pancreas is dependent on the precise control of the proliferation and differentiation of progenitor cells. During early mammalian pancreas development, the Pdx1+Cpa1+ tip multipotent progenitor cells give rise to exocrine, endocrine and ductal cells, while the trunk Pdx1+Cpa1- cells are fated to only the endocrine and ductal lineages. Recent work in our laboratory demonstrated that the cell cycle length increases 1.5-fold between embryonic day (E)11.5 and E13.5 and that this is due to a specific increase in the length of the G1 phase of the cell cycle. Moreover, the G1-length of the Pdx1+Cpa1- bipotent trunk progenitor cells, from which Ngn3+ endocrine progenitors arise, is significantly longer than the tip cells, suggesting G1 lengthening is required for Ngn3 activation.
Materials and methods: To investigate the importance of cell cycle length in the formation of Ngn3+ endocrine progenitors, the $Kras^{LSL\text{-}G12D}$ mouse model was used. Embryos heterozygous for this allele have only one functional copy of Kras ($Kras^{LSL}$) while Pdx1-Cre+; $Kras^{LSL\text{-}G12D/wt}$ mice have a single amino acid mutation resulting in constitutively active Kras signaling in all pancreatic cells ($Kras^{G12D}$).
Results: As Kras is upstream of many signaling pathways involved in regulating cell division, the effect of altering Kras signaling on the cell cycle was first investigated by injecting pregnant dams with thymidine analog 5'-Ethynyl-2'-deoxyuridine (EdU) for 3.5 hours to label dividing cells and the proportion of EdU+ progenitor cells was quantified using immunofluorescence. In Pdx1+ epithelium at E12.5, the proportion of EdU+ cells was reduced to 0.32±0.04 (n=6) in the $Kras^{LSL}$ model compared to 0.45±0.03 (n=6) in littermate controls, suggesting that loss of one allele of Kras is sufficient to reduce the number of cells in S phase and lengthen the G1 phase. Consistently, the proportion of EdU+ multipotent progenitors was significantly reduced to 0.45±0.03 in $Kras^{LSL}$ compared to wild type littermates (0.75±0.07 EdU+ cells) and the proportion of EdU+ bipotent progenitors was reduced to 0.28±0.04 compared to wild type controls (0.39±0.02 EdU+ cells). Interestingly, increasing Kras signaling using $Kras^{G12D}$ did not significantly increase the proportion of EdU cells in any of the progenitor populations. Neither model of altered Kras signaling resulted in significant differences in number of nuclei, Pdx1+ or Sox9+ cells, or changed the proportion of multipotent to bipotent progenitor cells. To investigate the consequences of preventing S-phase transition and lengthening the G1 phase by manipulating Kras signaling, the number of Ngn3+ endocrine progenitors was quantified at E13.5. Compared to control, the number of Ngn3+/Sox9+ trunk epithelial cells was significantly increased 1.18±0.01 fold in $Kras^{LSL}$ and was reduced 0.58±0.03 fold in $Kras^{G12D}$, suggesting the manipulating cell cycle length during mouse pancreas development can affect endocrine cell formation.
Conclusion: Manipulating Kras signaling during pancreas development alters the length of the G1 phase of the cell cycle leading to significant changes in endocrine cell formation, supporting the hypothesis that the cell cycle directly regulates progenitor cell differentiation. This has important implications for producing functional insulin-producing cells from human embryonic stem cells and implies that cell cycle progression and differentiation are incompatible.
Supported by: JDRF, CFRI
Disclosure: **N.A.J. Krentz:** None.

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Dpy30 is essential for pancreas and endocrine progenitor cell specification

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Background and aims: Pancreas development begins at embryonic day 9 (E9) with expression of Pdx1, Sox9 and Cpa1 in multipotent progenitor cells capable of generating acinar, duct and endocrine cell lineages. Around E12.5, the branching pancreas epithelium is differentiated into "tip" precursors that lose Sox9 and maintain Cpa1 expression, and bipotent "trunk" precursors that lose Cpa1 and maintain Sox9 expression. At E14.5, the "tip" precursors specify to Cpa1-positive exocrine progenitors, and the "trunk" precursors specify to Sox9-positive duct progenitors, or Ngn3-positive endocrine progenitors. This process, as well as the further differentiation of progenitors to mature duct, acinar, and endocrine islet cell types, requires the appropriate activation of a cascade of different regulatory factors. However, exactly how these factors are activated during pancreas development is largely unknown. The Trithorax group (TrxG) complexes are chromatin remodelers that promote gene activation by catalyzing histone H3 lysine 4 methylation, and we hypothesized that these complexes are necessary for the activation of factors critical to progenitor cell specification during pancreas development.
Materials and methods: To test our hypothesis, we targeted the TrxG core protein Dpy30 that is essential for full activity of all TrxG complexes. For this, we generated pancreas (Pdx1-Cre) and endocrine (Ngn3-Cre) Dpy30 knock-out mice and examined the role of Dpy30 in pancreas and endocrine progenitor specification using these mice.
Results: In Pdx1-Cre:Dpy30flox/flox mice (Dpy30pko), Cpa1-positive Sox9-negative "tip" precursors are prematurely specified, and are diminished in number. Instead, many cells at the tips fail to downregulate Sox9, or to maintain Cpa1 expression. Consistent with this, the number of acinar cells generated at E18.5 is significantly reduced in Dpy30pko mice. Further, many of the generated acinar cells appear to have an immature phenotype, or a phenotype reminiscent of cells undergoing acinar-to-ductal metaplasia. Dpy30pko pancreata have a 50% reduction in Ngn3-positive cells ($n=3$, $p<0.05$) suggesting the ability of Sox9-positive bipotent precursors to be specified to the endocrine lineage is also impaired. At E18.5, the number of insulin-positive β-cells and glucagon-positive α-cells is decreased by ~80% ($n=3$, $p<0.001$), and not surprisingly, Dpy30pko mice are diabetic prior to weaning ($n=7$, $p<0.01$). In contrast, the number of duct cells appears to be expanded in Dpy30pko mice. Meanwhile, in Ngn3-Cre:Dpy30flox/flox mice (Dpy30nko), the relative number of α-cells is increased at the expense of the number of β-cells, and multi-hormonal cells are relatively frequent, indicating that islet cell type specification is impaired. The islets in these mice also show morphological defects, and the mice become hyperglycemic ($n=5$ $p<0.01$) by seven weeks of age, suggesting their islet cells have failed to reach functional maturity.
Conclusion: These results suggest that the core TrxG complex protein Dpy30 is essential for differentiation of pancreas progenitors into endocrine and exocrine, but not duct, cell lineages, and is further required for normal endocrine lineage-specification and maturation.
Supported by: CIHR, CFRI Canucks for Kids Fund, BC Transplant Training Program
Disclosure: **S.A. Campbell:** None.

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Neural crest cells are required for autonomic innervation of the endocrine pancreas

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Background and aims: Autonomic innervation has been implicated as an important modulator of pancreas development and glucose homeostasis. Despite efforts to determine the role of sympathetic/parasympathetic nerves, whether innervation directly controls endocrine development and function remains controversial. Our current study focused on the characterization of innervation dynamics during endocrine pancreas development and maintenance.
Materials and methods: The zebrafish is well suited for the in vivo study of islet development with time-lapse imaging and has several advantages over other vertebrate models, including rapid embryogenesis, transparency during embryonic and early larval stages, and high regenerative capacity. Innervation studies in rodents and humans have been limited to specific snapshots in time and depth of the tissue, not allowing one to capture the temporal and three-dimensional spatial interactions that define the dynamic nature of innervation as the endocrine pancreas develops and matures. With reporters labelling specific neuronal and endocrine cells, we determined how innervation is initiated during embryonic pancreas development using time-lapse confocal microscopy. The use of two-photon laser ablation allowed for further analysis of key neurons potentially involved in signalling to the endocrine cells.
Results: Pancreatic islet innervation has been observed in human and mouse with differences in the extent of innervation and specific cell types that are innervated. Our studies in zebrafish revealed that parasympathetic innervation of the endocrine pancreas begins early in development, prior to fusion of the dorsal and ventral pancreatic buds. Neural crest cells immediately adjacent to the primary endocrine cell cluster are involved in recruitment of the vagal nerve. A dense network of islet innervation was observed by four days post fertilization, as nerve extensions towards the secondary islets begin to develop. Sympathetic nerves from the celiac ganglion were seen at five days post fertilization. Interestingly, in the absence of the vasculature, the density of islet innervation remained unchanged, suggesting that the initial establishment of islet innervation can occur independently of islet vascularization.
Conclusion: Our study on innervation dynamics in zebrafish helps elucidate the cellular interactions involved in the establishment of islet innervation. Further studies on the signalling molecules and downstream mechanisms mediating cell recruitment and migration are underway. The results of this research can have a major impact on our understanding of pancreatic innervation dynamics, signalling, and function.
Supported by: Postdoctoral fellowships from HFSP, CIHR, and EMBO
Disclosure: **Y.H.C. Yang:** None.

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Defining the role played by GPR56 in regulating islet mass and beta cell proliferation

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Background and aims: We have identified that GPR56, an adhesion G-protein-coupled receptor (GPCR) that is activated by the extracellular matrix protein collagen III, is the most abundant GPCR in human islets. Its function in islets remains elusive, but previous studies have shown that GPR56 is important in the proliferation of neural stem cells and it is highly enriched in endocrine progenitor cells. The aim of this study was to identify its role in β-cell proliferation and islet size using GPR56 knock-out (KO) mice, and MIN6 β-cells in which GPR56 was overexpressed.
Materials and methods: GPR56 expression in mouse and human pancreas was determined by fluorescence immunohistochemistry. Age-matched GPR56 WT and KO littermates underwent intraperitoneal glucose tolerance tests and mice at postnatal days 9 and 56 (P9, P56) were given BrdU intraperitoneally. Islet size and proliferating β-cells were quantified in fixed, immunostained (BrdU/insulin and Ki67/insulin) pancreas samples, and insulin content of isolated islets was determined by radioimmunoassay. MIN6 β-cells were transfected with plasmid encoding full length GPR56, after which β-cell proliferation and

apoptosis were determined by BrdU-ELISA and caspase-Glo 3/7 assay respectively.
Results: GPR56 was expressed by mouse and human islets, where it co-localised to insulin-expressing β-cells. Studies with GPR56 KO mice indicated that deletion of GPR56 did not affect mouse body weight (WT: 23.5 ±0.8g; KO: 23.9±1.2g n=7, p>0.2). There was a trend of mild glucose intolerance in adult male and female KO mice (AUC: WT vs KO males: 1,749.4 ±126.8 vs 1,976.6±173.3; females: 1,166.9±60.7 vs 1,254.4±61.9), but there was no difference in islet insulin content (ng/islet, WT: 103.8±18.6; KO: 101.6±5.5, n=7, p>0.2). Islets in pancreas sections from adult (P56) KO mice were smaller than those from age-matched WT mice (μm2, WT: 10,870 ±1,579; KO: 7,053±2,697) and this was more pronounced at P9 (μm2, WT: 4,154±358; KO: 2,485±316, p=0.001). The number of β-cells actively replicating in the cell-cycle was significantly greater in the P9 WT islets compared to those from P9 KO mice (Ki67+ β-cells/islet, WT: 3.17±0.41; KO: 1.79±0.36, p=0.01). Transient transfection of 1.2μg GPR56 plasmid into MIN6 β-cells resulted in a 20 fold increase in GPR56 expression compared to non-transfected cells and overexpression of GPR56 in MIN6 cells stimulated β-cell proliferation (absorbance, basal: 0.47±0.03, +12ng GPR56: 0.44 ±0.02, +1.2μg GPR56: 0.55±0.02, n=8, p<0.05) and reduced apoptosis (caspase 3/7 luminescence, basal: 112,666±7,439, +12ng GPR56: 104,283 ±5,164, +1.2μg GPR56: 81,281±4,415, n=4, p<0.05).
Conclusion: This study provides insight into mechanisms by which the extracellular matrix protein collagen III may interact with islets to regulate β-cell function. Our data indicate that GPR56 is expressed by mouse and human islet β-cells, and they support a role for this adhesion GPCR in islet mass regulation as a consequence of increased β-cell proliferation and decreased apoptosis.
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Disclosure: **O.E. Olaniru:** None.

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The E3 SUMO ligase PIASy regulates the transcriptional factor activity and stability of hepatocyte nuclear factor 1- alpha

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Background and aims: Hepatocyte nuclear factor 1 alpha (HNF-1A) is a transcription factor regulating the expression of several liver- and pancreas-specific genes. Mutations in HNF1A are associated with Maturity- Onset Diabetes of the Young (MODY3). The post-translational regulation of HNF-1A may be important in the development of diabetes disease. Since SUMOylation is a post-translational regulatory mechanism previously shown to modify key proteins in glucose metabolism, the aim of this study was to investigate the relevance and function of HNF-1A SUMOylation in target cells.
Materials and methods: In vitro SUMOylation of HNF-1A was performed after expression and [35S] Met- labeling by the TnT® T7 Quick Coupled Transcription/Translation System. HNF-1A SUMOylation in HeLa and HEK293 cells was assessed by transfection with constructs encoding proteins of the SUMOylation cascade, followed by immunoprecipitation and SDS-PAGE/immunoblotting. Effect of SUMOylation on the transcriptional activity of HNF-1A was investigated in MIN6 beta-cells by a Luciferase reporter assay system in the presence/absence of constructs of the SUMOylation cascade. Moreover, effect of SUMOylation on HNF-1A protein stability was investigated by cycloheximide treatment in HEK293 cells. Furthermore, the interaction between fluorescently labeled PIAS1 and HNF-1A recombinant proteins was assessed by MicroScale Thermophoresis analyses.
Results: In this study we find that HNF-1A is SUMOylated both in vitro and in cellulo. Moreover, the level of SUMOylation is increased in the presence of PIASy. Further, in MIN6 beta-cells, HNF-1A transactivation on the rat albumin promoter was reduced in the presence of SUMO-1, Ubc9 (E2) and PIASy. Furthermore, PIASy increased HNF-1A expression and protein stability. A direct interaction between HNF-1A and PIAS1 was confirmed by MicroScale Thermophoresis analyses.
Conclusion: SUMOylation of HNF-1A represents a novel post translational regulatory mechanism affecting HNF-1A transcriptional activity. The relevance of HNF-1A SUMOylation and PIAS interaction on the regulation of beta-cell genes and insulin secretion, however, is presently unknown.
Supported by: KG Jebsen Foundation and the Norwegian Diabetes Association
Disclosure: **A. Kaci:** None.

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Transgenic pigs with green fluorescent protein-labeled pancreatic beta cells

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Background and aims: Rodent models with fluorescently labeled beta-cells in islets enabled in vivo monitoring of beta-cell development and provided important insights into islet biology. However, extrapolation of findings in rodents to humans is sometimes hampered by major species differences in beta-cell physiology. As an alternative to rodents, islets from pigs might be more useful to study beta-cell biology. Furthermore, xenotransplantation of porcine islets is even seen as a realistic option for the future treatment of type 1 diabetic patients with labile metabolic condition. We have generated transgenic pigs by somatic cell nuclear transfer (SCNT) that express green fluorescent protein (eGFP) under the control of the porcine insulin gene promoter. Offspring of two founder animals with high and intermediate levels of beta-cell specific eGFP expression were used to characterize the functionality of such transgenic pig islets.
Materials and methods: INS-eGFP transgenic pigs were generated by SCNT and founder animals were analyzed for reporter gene expression. Two founders, one with high and one with intermediate level of beta-cell specific eGFP expression were successfully reproduced by SCNT to set up breeding lines. Neonatal islet cell clusters (NICCs) from F1 offspring were characterized i) in vitro in glucose-stimulated insulin secretion (GSIS) assays and ii) in vivo by monitoring of NICC maturation, engraftment as well as restoration of normoglycemia after transplantation into diabetic mice.
Results: INS-eGFP transgenic pigs exhibited eGFP expression specifically in islet beta-cells: eGFP was neither detected in glucagon positive alpha-cells nor in somatostatin positive delta-cells as demonstrated by double-immunofluorescence analyses. However, the F0 transgenic founder pigs differed in their eGFP expression intensity. Breeding lines were set up from two founders, one with high and one with intermediate transgene expression level. The GSIS stimulation index (SI; insulin at 15 mM Glc/insulin at 3 mM Glc) of NICCs from transgenic (tg) F1 animals were similar as in NICCs from wild-type (wt) littermates (3.4 vs. 3.1; n=4 per genotype, 8 replicates each). In addition to, augmentation of insulin secretion by the sulfonylurea glimepiride (100 nM) was comparable between tg and wt islets (SI at 3 mM Glc: wt 3.1, tg 3.7; SI at 15 mM Glc: wt 6.8, tg 6.9, each compared to insulin secretion at 3 mM Glc without glimepiride). In vivo monitoring of transplanted tg NICCs in the anterior eye chamber of NOD^{scid} mice demonstrated engraftment of NICCs and maturation of beta cells. Further, transplanted transgenic NICCs in a streptozotocin-induced diabetic mouse were able to restore normoglycemia.
Conclusion: eGFP transgene expression in pig beta-cells under the pig INS promoter did not influence the functionality of porcine islets. Therefore, INS-eGFP transgenic pig islets might be a novel valuable tool

for studying porcine beta-cell biology and enable efficient and specific FACS sorting of beta-cells. Further, they are highly suitable for monitoring and optimizing conditions of NICC maturation in culture as well as for in vivo imaging of transplanted islets.
Supported by: Sanofi-Aventis
Disclosure: **E. Kemter:** Other; support by Sanofi-Aventis.

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Beta-arrestin2 expression levels control insulin induced-nuclear exclusion of FOXO1 in mouse pancreatic beta cells

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Background and aims: We have recently reported that the scaffold protein β-arrestin2 (ARRB2) is required for insulin signaling and pancreatic β-cell mass plasticity. The autocrine action of insulin, through the activation of the PI3K/Akt cascade, has been shown to be critical for the maintenance of normal pancreatic β-cell mass, notably by the phosphorylation of the transcription factor FOXO1 leading to its nuclear exclusion. The aim of our study is to investigate the molecular mechanisms by which ARRB2 contributes to insulin signaling in mouse pancreatic β-cells.
Materials and methods: Experiments were performed using islets isolated from wild-type, Arrb2+/+, Arrb2-/- that were either 4- or 12-month-old, but also from prediabetic (7-week-old) and diabetic (12-week-old) db/db mice. Islets were exposed to 100nmol/l insulin for 10 min. Expression levels, phosphorylations or subcellular distributions of proteins were measured by western blot or immunocytochemistry.
Results: In Arrb2-/- mouse β-cells, insulin-induced phosphorylation of Akt was reduced by 70% ($p<0.001$) and the nuclear exclusion of FOXO1 was suppressed ($p<0.001$). By contrast the re-expression of ARRB2 in Arrb2-/- β-cells, using an adenovirus encoding ARRB2-GFP, completely restored the nuclear exclusion of FOXO1 induced by insulin ($p<0.001$). ARRB2 is known to recruit Src to allow a full insulin-induced Akt activation in liver. In mouse islets, Src inhibition using PP2 reduced by 70% ($p<0.05$) the phosphorylation levels of Akt and completely suppressed the nuclear exclusion of FOXO1 ($p<0.001$) induced by insulin, indicating that not only ARRB2 but also Src is required for an efficient insulin signaling in mouse β-cells. The islet ARRB2 expression levels were found to be significantly downregulated in diabetic db/db mice. By contrast, ARRB2 expression levels were found to be upregulated in islets from prediabetic db/db mice or in aged animals (12-month-old). The upregulation was associated with an enhancement of the insulin signaling and an increase in large islet number in 12-month-old animals compared to 4-month-old animals. By contrast in 12-month-old Arrb2-/- mice, insulin was still unable to exclude FOXO1 from the nucleus, unless ARRB2 was adenovirally reintroduced.
Conclusion: Our study highlight a new molecular mechanism involved in the insulin signaling, where ARRB2 and Src are both required to induce a full Akt activation and FOXO1 nuclear exclusion. A change in ARRB2 expression levels in aging, prediabetic or diabetic states might be an important contributor to the modulation of insulin signaling and pancreatic β-cell mass plasticity.
Disclosure: **M.A. Ravier:** None.

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The hypervascularisation of islets in type 2 diabetes is not mediated by angiopoetin-2

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Background and aims: Changes in the islet vasculature have been implicated in the regulation of β-cell survival and function during the progression to type 2 diabetes (T2D). Failure of the β-cell to compensate for the increased insulin demand in obesity eventually leads to diabetes; as result of a complex interplay of genetic and environmental factors (e.g. ongoing inflammation within the islets) and impaired vascular function. The Angiopoietin/Tie (Ang/Tie) angiogenic system maintains vasculature and is closely related to organ inflammation and angiogenesis. In this study we aimed to identify whether the vessel area within the islets change in diabetes and whether such changes would be triggered by the Tie-antagonist Ang-2.
Materials and methods: Immunohistochemical and qPCR analyses to follow islet vascularization and Ang/Tie levels were performed in human pancreatic autopsies and isolated human and mouse islets. The effect of Ang-2 was assessed in β-cell-specific Ang-2 overexpressing mice during high fat diet (HFD) feeding.
Results: Islet vessel area was increased in autopsy pancreases from patients with T2D. The vessel markers Tie-1, Tie-2 and CD31 were upregulated in mouse islets upon HFD feeding from 8 to 24 weeks. Ang-2 was transiently upregulated in mouse islets at 8 weeks of HFD and under glucolipotoxic conditions (22.2 mM glucose/ 0.5 mM palmitate) in vitro in human and mouse islets, in contrast to its downregulation by cytokines (IL-1β, IFN- and TNF-α). Ang-1 on the other hand was oppositely regulated, with a significant loss under glucolipotoxic condition, a trend to reduce in islets from patients with T2D and an upregulation by cytokines. Modulation of such changes in Ang-2 by its overexpression or the inhibition of its receptor Tie-2 impaired β-cell function at basal conditions but protected islets from cytokine induced apoptosis. In vivo, β-cell-specific Ang-2 overexpression in mice induced hypervascularization under normal diet but contrastingly led to hypovascularized islets in response to HFD together with increased apoptosis and reduced β-cell mass.
Conclusion: Islet hypervascularization occurs in T2D. A balanced expression of the Ang1/Ang2 system is important for islet physiology. Ang-2 prevents β-cell mass and islet vascular adaptation in response to HFD feeding with no major influence on glucose homeostasis.
Supported by: ERC
Disclosure: **K. Maedler:** None.

PS 020 What can we learn from human islets?

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The lincRNA GAS5 is highly-enriched in human pancreatic islets and is involved in glucocorticoid-mediated inhibition of insulin secretion

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Background and aims: Analysis of RNA-seq data from 89 human pancreatic islet samples reveals the expression of 493 long intergenic non-coding RNA (lincRNA) genes, in which the Growth Arrest-Specific 5 (GAS5) is the most abundantly expressed. Previous studies in HeLa cells have shown GAS5 to interact with the glucocorticoid receptor (GR) in a dexamethasone dependent-manner. The diabetogenic effect of glucocorticoids (GCs) is widely recognized, for instance in steroid-induced diabetes mellitus. This led us to investigate the potential role of GAS5 in GC-induced dysfunction in human pancreatic islets and insulin-secreting human beta cell line.
Materials and methods: Previous human islets RNA-seq data (N=89) were used to analyze lincRNA expression. GAS5 knock-down (GAS5 KD) was done in human EndoC-BH1 cells and islets using LNA-gapmeRs (Exiqon). The cells and islets were treated with 2 uM dexamethasone (DEX) with or without the GR inhibitor RU486, for 24/48 hours prior to stimulation with 20/16.7 mM glucose or 500 μM IBMX. Gene or protein expression levels were determined by qPCR or western blot, respectively. Insulin secretion and content were determined using ELISA (Mercodia). Apoptosis was assayed using a cell death detection kit (Roche) and validated by measuring the cleaved caspase-3.
Results: Co-expression analyses between GAS5 and all expressed transcripts in human pancreatic islets revealed significant negative correlations with beta cell transcription factors PDX1 and NKX6-1, and the exocytotic gene SYT13. We attained 70% knock-down of GAS5 in the EndoC-BH1 cells, leading to 30% decrease in the expression of PDX1 ($n=4$, $p< 0.01$) and 40% decrease in SYT13 ($n=4$, $p< 0.05$) at the protein level. We also observed reduction in both mRNA ($n=3$, $p< 0.05$) and protein expression of GR ($n=4$, $p< 0.01$), and ~80% increase in mRNA expression of the serum- and glucocorticoid-regulated kinase 1 (SGK1) ($n=3$, $p<0.01$) upon GAS5 knock-down. GAS5 KD resulted in increased apoptosis, and lead to ~30% reduction in insulin secretion upon glucose stimulation ($n=3$; $p<0.001$) and IBMX treatment ($n=3$; $p<0.05$) without significant difference in insulin content. In EndoC-BH1 cells, DEX reduced the expression of GAS5 (~20%, $n=4$, $p< 0.01$), PDX1 (~90%, $n=4$, $p< 0.001$), NKX6-1 (~90%, $n=4$, $p< 0.001$) and SYT13 (~40%, $n=4$, $p< 0.001$) at mRNA level with corresponding 25% reduction in insulin secretion ($n=4$, $p< 0.01$). Furthermore, similar to GAS5 KD, we observed decreased expression in GR and increased expression of SGK1 upon DEX treatment. DEX combined with RU486 resulted in partial or complete recovery of aforementioned gene expression levels and insulin secretion. In human islets, 48hrs in DEX resulted in 60% reduction of insulin secretion ($n=2$), while GAS5 KD reduced insulin secretion by ~30% ($n=1$).
Conclusion: The lincRNA GAS5 is highly-enriched in the human pancreatic islets and negatively-correlated with genes important for beta cell function (PDX1, NKX6-1 and SYT13). Here we showed that the glucocorticoid-induced dysfunction in both EndoC-BH1 cells and human islets may be acting through GAS5-mediated transcriptional regulation of the GR itself and other important beta cell genes potentially regulated by glucocorticoids.
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Disclosure: **J.K. Ofori:** None.

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Thrombospondin 1 protects beta cells from proinflammatory cytokines through MANF induction

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Background and aims: Progressive autoimmune β-cell destruction leads to type 1 diabetes. Novel approaches that preserve β-cell mass might represent new disease-modifying therapies. Thrombospondin 1 (THBS1) is a secreted glycoprotein. In other tissues THBS1 modulates the ATF6 pathway of the endoplasmic reticulum (ER) stress response and binds membrane receptors. THBS1-/- mice have glucose intolerance and impaired glucose-stimulated insulin release. We have recently observed that THBS1 promotes β-cell survival under lipotoxic conditions by upregulating the antioxidant stress response. In this study we examined whether THBS1 regulates proinflammatory cytokine-induced β-cell death.
Materials and methods: Apoptosis was evaluated using nuclear dyes, mRNA expression by qPCR, and protein expression/phosphorylation by Western blot. Gene silencing and overexpression were achieved by RNA interference and adenoviral vectors, respectively. All data shown are based on 3-5 independent experiments.
Results: Treatment with the proinflammatory cytokines interleukin 1β (IL) plus interferon γ (IFN) reduced THBS1 protein expression in human islets by 74±10% ($p<0.05$) and in clonal rat β-cells by 75±6% ($p<0.05$). THBS1 knockdown (by 80±3%) sensitized rat β-cells to IL+IFN-induced caspase 3 activation (by 2.1±0.4 fold, $p<0.05$) and apoptosis (from 25±2% to 48±2%, $p<0.05$), while its overexpression (by 18±1-fold) was protective (decreased caspase 3 cleavage by 64±4%, $p<0.05$ and apoptosis from 24±2% to 18±2%, $p<0.05$). This was confirmed in human islet cells, where THBS1 silencing potentiated (from 32±4% to 42±4%, $p<0.05$) and overexpression reduced cytokine toxicity (from 30±5% to 20±5%, $p<0.05$). Conditioned medium from THBS1-overexpressing cells was not cytoprotective suggesting that intracellular THBS1 (but not its secreted form) is protective. We next characterized the molecular pathways involved in THBS1-mediated β-cell survival. The antioxidant and ER stress responses and the anti-apoptotic proteins BCL-2 and BCL-XL were not changed by THBS1 gain- or loss-of-function. Interestingly, IL+IFN reduced expression of mesencephalic astrocyte-derived neurotrophic factor (MANF, by 60±7%, $p<0.05$), an anti-apoptotic ER protein recently shown to be deficient in NOD mouse islets. THBS1 overexpression partially prevented MANF degradation (from 0.56±0.07 in cells infected with control adenovirus to 0.94±0.06 in THBS1-overexpressing cells, $p<0.05$). MANF silencing per se (by 53±3%, $p<0.05$) sensitized β-cells to IL+IFN (apoptosis from 20±1% to 30 ±2%, $p<0.05$). The protection conferred by THBS1 overexpression was lost in MANF depleted cells. Silencing of the pro-apoptotic BCL-2 protein BIM fully protected β-cells from THBS1 deficiency (IL+IFN-induced apoptosis from 31 ±1% in THBS1 silenced cells to 11±1% in THBS1/BIM siRNA transfected cells, $p<0.05$), identifying BIM as the final downstream apoptosis effector.
Conclusion: THBS1 prevents MANF degradation, thus orchestrating an anti-apoptotic response during exposure to proinflammatory cytokines. These data indicate that THBS1 protects β-cells against apoptosis induced by lipotoxicity and cytokines by different mechanisms, and suggest that induction of the THBS1-MANF pathway may be an interesting therapeutic target for type 1 diabetes.
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Disclosure: **D.A. Cunha:** None.

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Dynamic profiling of insulin secretion and ATP generation in isolated human and mouse islets reveals differential glucose sensitivity

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Background and aims: Murine islets represent a valid and extensively used model to study endocrine pancreas physiology in mammals, but

some morphological and functional differences exist between mouse (MI) and human islets (HI). The aim of this study was to compare the glucose concentration-dependence of elevations in ATP and dynamic insulin secretion in MI and HI and evaluate the expression and role of glucose transporters (GLUTs) in these functional responses.

Materials and methods: Islets were isolated from CD1 mice (male, 8 weeks, 25-28g) and from heart beating organ donors (6 male, 2 female, 44 ±4 years, 28±1 BMI) by collagenase digestion. Dynamic insulin secretion was assessed by perifusion (40 islets per chamber) and radioimmunassay, and islet ATP levels were measured by luminescence assay (5 islets per well). Expression of GLUTs by islets was evaluated by semi-quantitative western blotting (WB) and the effects of GLUT1 inhibition on insulin secretion and ATP were determined using the specific inhibitor STF-III.

Results: Profiling of dynamic insulin secretion indicated that HI had a left-shifted glucose concentration dependency compared to MI. Perifused HI challenged with incremental glucose concentrations from 2mM (basal) to 20mM, showed elevated insulin release at 3.5mM glucose (231±32% basal; $p<0.0001$) and reached maximum secretion at 7.5mM glucose (322 ±87% basal) without further increase at 10 or 20mM glucose (330±42% and 352±10% respectively, $p\geq0.2$ vs 7.5mM glucose).Only 2 of the 8 batches of HI tested showed further potentiation at 10 and 20mM glucose (420±11% and 525±12% basal, respectively). The HI insulin secretory profile was accompanied by glucose-dependent elevations in ATP production (% basal at 3.5, 5, 7.5, 10 and 20mM glucose: 149±11%; 131 ±12%; 158±13%; 160±9%; 198±12%, n=7, $p<0.01$). In parallel experiments perifused MI showed maximum glucose-induced secretion at 20mM glucose in all batches tested (540±72% basal, $p<0.01$), despite elevations in ATP being detected at glucose concentrations as low as 5mM (187±15% basal, $p<0.05$), with a stable plateau from 7.5 to 20mM glucose (220±14%, $p<0.05$). WB of purified HI lysates revealed high expression of GLUT1 and GLUT3, with very little GLUT2, while GLUT2 was the predominant isoform in isolated MI. The use of 5μM STF-III to inhibit GLUT1 activity in HI resulted in inhibition of glucose-induced ATP synthesis (ATP luminescence units, 2mM glucose: control, 8.0±0.5*105, +STF-III, 7.8±0.1*105; 7.5mM glucose: control, 1.3 ±0.2*106, +STF-III, 1.0±0.1*106, $p<0.05$) and it also impaired, but did not abolish, glucose-induced insulin secretion (3.5mM glucose: control, 229±55% basal, +STF-III: 236±3.1%; 5mM glucose: control, 393±67% basal, +STF-III: 275±18%; 7.5mM glucose: control, 479±100% basal, +STF-III 280±21% basal, $p<0.001$).

Conclusion: The in vitro dynamic glucose responsiveness of isolated HI in the range of 3.5-7.5mM glucose is consistent with the circulating glucose concentrations in humans, while the right-shifted profile in MI reflects the elevated basal and stimulated blood glucose concentrations in mice. The expression of low Km GLUT1/3 in HI and high Km GLUT2 in MI also fits with human and rodent physiology. Inhibition of GLUT1 activity in HI did not completely inhibit glucose-induced ATP generation or insulin secretion, suggesting that both GLUT1 and GLUT3 are required for appropriate glucose transport in HI.

Disclosure: **A. Pingitore:** None.

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Reversibility of human beta cell functional damage induced by glucolipotoxic conditions

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Background and aims: Prolonged exposure of beta cells (Bc) to increased concentrations of glucose and/or certain free fatty acids (palmitate in particular) is associated with reduced glucose-stimulated insulin secretion (GSIS) and increased Bc death, possibly contributing to the onset and progression of type 2 diabetes (T2D). In the present study we performed a comprehensive assessment of the effects of several different gluco-lipotoxic conditions (GLTc) on GSIS from human islet Bc and evaluated whether Bc functional damage induced by GLTc may be reversible.

Materials and methods: Isolated islets (Ii) were prepared from the pancreas of 33 non-diabetic organ donors (age: 73±2.5 yrs, mean±SE; M/F: 12/21; BMI: 24.7±0.6 Kg/m^2) by collagenase digestion and density gradient purification, and then cultured for 2 days in the presence or absence (3 to 6 different Ii preparations per condition) of 0.5 mM palmitate (P), 11.1 mM glucose (g), 22.2 mM glucose (G), 0.5 mM palmitate+11.1 mM glucose (P+g), 0.5 mM palmitate+22.2 mM glucose (P+G), 1.0 mM palmitate+oleate, (1:2, P+O), 1.0 mM palmitate+oleate+11.1 mM glucose (P+O+g) and 1.0 mM palmitate+oleate+22.2 mM glucose (P+O+G). Acute GSIS (at 3.3 and 16.7 mM glucose) was assessed basally (Bas), after 2 days (2d) of incubation with GLTc and following additional 4 day culture in normal medium (wash-out, WO). Control Ii (Ctrl) were cultured in normal medium (with 5.5 mM glucose). Statistical analysis was performed by the Student's t-test or the ANOVA test followed by the Bonferroni correction.

Results: Overall, acute insulin release from Ii at Bas in response to 3.3 and 16.7 mM glucose was 53.4±3.2 and 154.9±11.7 μU/ml (n: 33, $p<0.01$), corresponding to an insulin stimulation index value (ISIv) of 3.1±0.19. No significant change in GSIS occurred during the study period with Ctrl, g, P+O and P+O+g. However, ISIv declined significantly at 2d with P, G, P+g, P+G and P+O+G, due to either increased (at 3.3 mM glucose) and/or reduced (at 16.7 mM glucose) insulin release. Interestingly, statistically significant improvements of insulin release were observed after WO vs 2d with P, G and P+g (with GSIS results similar to those at Bas), whereas no significant difference of WO vs 2d was found with P+G and P+O+G.

Conclusion: These results show that, at our experimental conditions: 1) several of the tested GLTc had deleterious effects on GSIS from human Bc, depending on the type and concentration of the stressors and their combinations; and 2) reversal of Bc functional derangement occurred after removal of some the damaging GLTc. The assessment of the molecular features associated with these changes might allow better prevention and treatment of T2D through protective interventions on the Bc.

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Disclosure: **M. Suleiman:** None.

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Biphasic electric coupling of islets determines secretion profile

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Background and aims: Islets secrete insulin upon stimulation in a biphasic and oscillatory pattern, and both are disturbed early in type 2 diabetes. β-cell coupling and signal propagation are important features but we still do not understand the biphasic nature of secretion. Limited time resolution of methods used (<1 Hz), use of complex algorithms to explore coupling with inherent risk of bias, as well as supra-physiological glucose/hormone levels have hampered understanding. Moreover detailed high-resolution analyses of these kinetics have not been performed. We have therefore investigated the relation between the 2 phases on extracellular multi-electrode arrays by high-resolution long-term recordings. This approach takes advantage of β-cell specific signals, the slow potentials (SPs), that directly reflect multicellular coupling and avoids use of external algorithms.

Materials and methods: SPs from mouse and human islets were recorded at 10 kHz with high-density arrays of electrodes (5-15 electrodes per islet). Changes in their frequencies, amplitudes and synchrony were analysed with MC_Rack and Matlab softwares.

Results: SPs occur at a sub-second frequency, are glucose-dependent (EC50 7.2 mM) and disapear in absence of coupling. Concomitant recording via extracellular electrodes and intracellular sharp electrodes at different positions within an islet confirm that SPs are integrative signals generated by short depolarization bursts (≤1 s) and their amplitude reflects the degree of coupling. Raising glucose from 3 to 8.2 mM, the frequency of SPs increases during the first 10 min from 0 to 0.77±0.07 Hz, amplitudes are small (52±1 μV) and signals are poorly synchronized (correlation-based measure of SP timing reliability). This indicates activities of multiple independent units. After a subsequent lag phase (5-8 min) characterized by a very reduced activity, SPs synchronize with a concomitant increase in amplitude (147±23 μV) and decrease in frequency (0.47±0.02 Hz). Finally, after prolonged exposure to 8.2 mM glucose (≥60 min), large oscillations appear (periods 4.2±0.5 and 5.4±0.6 min in murine and human) similar to insulin secretion. If directly after the 1st phase glucose was reduced to 3 mM for 5 min and raised subsequently to 8.2 mM, again sequential 1st and 2nd phases were observed. This indicates an obligatory passage through the 1st phase. GLP-1 (10 pM) differentially affects the 2 phases. Preliminary data demonstrate that whereas maximum frequency and amplitude of the 1st phase were unaltered, duration of its peak was reduced by 2/3. In contrast, during the 2nd phase frequency was reduced (from 0.45 to 0.25 Hz) and amplitudes increased considerably (from 130 to 230 μV) leading to very prominent pulses.
Conclusion: High resolution and multisite recordings at physiological glucose/hormone levels provide direct analysis of electric signaling/coupling during 1st and 2nd phase. The presence of a 1st phase seems to be obligatory. This phase is reduced by GLP-1, which may reflect indirect action of the incretin via other cell-types, whereas the 2nd phase is considerably potentiated. As SPs are upstream of exocytosis, it is the biphasic shape of this integrative signal that dictates the secretion profile.
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Disclosure: **M. Raoux:** None.

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Relationship between intra-pancreatic fat content and beta and alpha cell mass in humans with and without diabetes

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Background and aims: It has been reported that ectopic fat deposits in pancreas induce beta cell apoptosis in vitro and in vivo studies of rodents, called lipotoxicity hypothesis. Whereas some human studies have shown that pancreatic fat content is associated with decreased beta cell function by imaging assessment such as ultrasonography, CT and MRI. However, effect of ectopic fat in pancreas on beta cell mass (BCM) remains unclear. The aim of this study was to clarify the effects of intra-pancreatic fat on beta and alpha cell mass in humans with and without diabetes.
Materials and methods: We analyzed human pancreas at autopsy from 72 Japanese non-diabetic adults (group of NDM-1; age 47 ± 11 years, body mass index (BMI) 24.1 ± 5.0 kg/m2, HbA1c 5.5 ± 0.6% (mean ± S.D)) and pancreas samples from 99 diabetic and non-diabetic adults who underwent pancreatic surgery (NDM-2; n = 50, age 64 ± 14 years, BMI 22.5 ± 2.7 kg/m2, HbA1c 5.6 ± 0.5%, DM; n = 49, age 67 ± 9 years, BMI 21.9 ± 3.5 kg/m2, HbA1c 7.8 ± 1.6%). Pancreatic sections were stained for insulin or glucagon and hematoxylin, and fractional beta (BCA) or alpha cell area (ACA) was measured, respectively. Fractional intra-pancreatic fat area (PFA) was also quantified by using imaging software. In addition, we evaluated the density of islets, islet size, scattered beta cells (a cluster of three or fewer beta cells in acinar tissue), insulin-positive duct cells and beta cell replication.
Results: Although the PFA varied among the individuals (range 0.01-7.29%), there was no difference in PFA between NDM-1 and NDM-2 groups (1.01 ± 1.44% vs. 0.79 ± 0.77%, P = 0.28). In NDM-1, PFA was significantly correlated with age (r = 0.37, P = 0.001) but not correlated with HbA1c or BMI. In NDM-2, PFA was correlated with neither age, HbA1c nor BMI, but tended to be positively correlated with BMI (r = 0.24, P = 0.10). In DM, BCA was significantly decreased compared to NDM-2, but there was no significant difference in PFA between the groups (1.14 ± 1.34% vs. 0.79 ± 0.77%, P = 0.12). In DM, PFA was significantly correlated with BMI (r = 0.32, P = 0.03). In all groups, PFA was not significantly correlated with BCA, ACA or the ratio of ACA to BCA. In NDM-1 and NDM-2 groups, there was no significant correlation between PFA and islet density, mean islet size or beta cell turnover, while in DM group, PFA was positively correlated with mean islet size (r = 0.37 , P = 0.01).
Conclusion: PFA was positively correlated with age and BMI in humans. There was no significant difference in PFA between humans with and without diabetes. In only subjects with diabetes, PFA was associated with mean islet size. There was no significant association between PFA and BCA, ACA or beta cell turnover in subjects with or without diabetes. These findings suggest that there is little effect of fat deposits in pancreas on beta cell mass and development of diabetes in humans.
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Disclosure: **R. Murakami:** None.

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Glucotoxicity activates autophagosome formation but hampers autophagy flux in human islets and INS-1(832/13) cells

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Background and aims: The role of autophagy in β-cell dysfunction and Type 2 Diabetes (T2D) is incompletely understood. Autophagy serves a critical role in cellular adaptation to stress through clearance of toxic, misfolded or accumulated proteins via lysosomal pathways. These processes release macromolecules that provide an intracellular pool of energy upon an increased metabolic demand. In fact, autophagy may be crucial for maintaining β-cell function and survival, when cells are exposed to metabolic stress. In view of this, the present study investigated the occurrence of autophagy in clonal β-cells and human islets from non-diabetic and T2D individuals, to determine whether autophagy is deregulated in a disease state.
Materials and methods: Dispersed human islets (4 non-diabetic and 5 T2D islet donors) and INS-1(832/13) cells (n=4) were cultured in 0.5mM Palmitate, 30mM Glucose, or a combination of the two, for 24 hours, to mimic gluco-, lipo- and glucolipotoxicity. To examine autophagy, we utilized an EGFP-tagged baculoviral vector containing LC3, a marker of autophagy and a lysosomal marker. Subsequently, treated cells were infected and quantitatively analyzed by microscopy. Human islet cells were counter-stained with insulin and glucagon to determine cell identity. To assess effects of treatment and induction of autophagy, we measured insulin secretion, expression of autophagy-related (ATG) genes and genes involved in membrane fusion of autophagosomes/lysosomes.
Results: Untreated INS-1(832/13) cells showed very little signs of autophagy (visualized as LC3 positive areas) while cells treated with glucose, palmitate, or both, displayed an increase in LC3-positive staining. Palmitate and the combination of palmitate and glucose induced autophagy, but not to the same extent as glucose (p=0,02). In contrast, glucose stimulation evoked a large induction of autophagosome formation but very little fusion with lysosomes (p=0,07). Interestingly, dispersed human islet cells from T2D islet donors displayed a similar pattern with increased LC3-positivity (p=<0,0001), as compared to non-diabetic islet cells; autophagosome/lysosome fusion (p=<0,0001) was rarely observed. Expression of the autophagic/lysosomal fusion genes LAMP2 and Syntaxin17 was upregulated after lipotoxicity but downregulated in a glucotoxic state in INS-1(832/13) cells. Similarly, expression of LAMP2 (p=0.014), Syntaxin17 (p=0.033) and UVRAG (p=0.00083)

was downregulated in human T2D islets (n=144) as compared to non-diabetic controls (n=27). Moreover, high glucose treatment markedly reduced expression of ATG3, ATG5, ATG7, ATG9A, ATG10 and ATG12 while palmitate increased the expression of the same genes. Glucose-stimulated insulin secretion decreased with both glucose and palmitate treatment.

Conclusion: Our in vitro findings, using human islets and INS-1 (832/13) cells, indicate that glucotoxicity is a powerful activating condition for the autophagy process. This was coupled to a hampering of autophagy flux. In addition, glucotoxic conditions downregulated genes important for the autophagosome/lysosome fusion, thus disabling cells to complete the autophagy cycle. This suggests that deregulation of autophagy is involved in β-cell dysfunction in T2D and that metabolic stress in pancreatic islets may promote this negative situation. If such negative events could be prevented or reversed, β-cell function would be sufficient to control whole body metabolism.

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Inhibition of microRNA-33 promotes beta cell proliferation and reduces beta cell apoptosis

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Background and aims: Islet β-cells are capable of compensatory expansion in response to physiological challenges such as during gestation and obesity, and failure in this leads to the onset of diabetes. MicroRNA-33 (miR-33) belongs to a class of non-coding RNA molecules that act as sequence-specific suppressors of their target messenger RNAs (mRNAs). In silico prediction using TargetScan software has revealed that miR-33 targets a network of genes that are known to play key roles in regulating β-cell expansion such as calcium-calmodulin kinase 4, insulin receptor substrate 2, and cyclin M1. The aim of this study was therefore to investigate the role of miR-33 in maintaining functional β-cell mass.

Materials and methods: miRNAs were isolated from isolated mouse and human islets using a miRNA extraction kit. Expression of miR-33 was quantified using primers specific for human and/or mouse miR-33 with the miScript SYBR Green PCR System and normalised to the small nucleolar RNA RNU6B. In some experiments expression of miR-33 was suppressed by transfection of isolated mouse islets with miRIDIAN miR-33 inhibitors. Islet cell caspase 3/7 activities were quantified using a luminescent assay following exposure of isolated mouse islets to a cytokine cocktail (1U/μl IFNγ, 1U/μl TNFα, 0.05U/μl IL-1β). MIN6 β-cell proliferation was determined by quantifying BrdU incorporation into replicating DNA using a BrdU ELISA.

Results: Human and mouse islets express miR-33 and its expression is significantly down-regulated in islets isolated from mice with diet-induced obesity (relative to $RNU6Bx10^4$; standard chow: 5.7±0.9; high fat diet: 3.0±0.6; n=4; P<0.05) in which β-cell proliferation is increased prior to the onset of a significant diabetic phenotype (BrdU positive β-cells/total β-cells; standard chow: 3.5%±0.6%; high fat diet: 7.3%±1.0%; n=12; P<0.05). These data suggest a potential role for miR-33 in the negative regulation of β-cell proliferation. Transfection of mouse islets with miRIDIAN miR-33 inhibitors caused a significant reduction in miR-33 levels (relative to $RNU6Bx10^4$; control: 4.8±0.3; miR-33 inhibitor: 3.9 ±0.1; n=2; P<0.05) and this was accompanied by decreased caspase 3/7 activities (luminescence units; control: 10,203±699; miR-33 inhibitor: 7,604±541; n=3; P<0.05), suggesting a pro-apoptotic role of this miRNA in β-cells. miRNAs are targets for anti-depressant drugs such as fluoxetine and, consistent with this, exposure of human islets to fluoxetine for 72h significantly reduced miR-33 expression (relative to $RNU6Bx10^4$; control: 0.24±0.01; 0.1μM fluoxetine: 0.12±0.01; n=3, P<0.001). Furthermore, exposure of MIN6 β-cells and mouse islets to fluoxetine for 72h dose-dependently increased BrdU incorporation (OD450nm; control: 0.155±0.002; 0.1μM fluoxetine: 0.169±0.004; 1μM fluoxetine: 0.171±0.005; n=10; P<0.05) and reduced caspase 3/7 activities (luminescence units; control: 45,918±3,685; 0.1μM fluoxetine: 36,771±2,538; 1μM fluoxetine: 27,845±2,570; n=8; P<0.001).

Conclusion: These data are consistent with miR-33 being expressed by islets and playing a pivotal role in regulating functional β-cell mass through repression of β-cell proliferation and stimulation of apoptosis. Inhibition of miR-33 expression by fluoxetine represent a potential novel strategy in maintaining β-cell mass for the treatment of both type 1 and type 2 diabetes.

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Disclosure: **B. Liu:** None.

PS 021 Beta cells under stress

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The haem-regulated eIF2α kinase HRI promotes pancreatic beta cell survival through modulation of the Akt/BAD and JNK pathways

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Background and aims: The eukaryotic translation initiation factor 2 alpha (eIF2α) is part of the initiation complex that drives the initiator amino acid methionine to the ribosome, a crucial step in protein translation. Under stress conditions and as part of the unfolded protein response (UPR), eIF2α is phosphorylated, thereby inhibiting global protein synthesis. In parallel, there is induction of ATF4 and CHOP that, if prolonged, may lead to cell death. Heme-regulated eIF2α kinase (HRI) is a member of the eIF2α kinase family that regulates protein translation under heme deficiency and oxidative stress conditions. HRI can also activate the PI3K/Akt pathway. Our RNA sequencing data of human islets and primary rat beta cells indicated that HRI is the most abundantly expressed eIF2α kinase family member. The aim of this study was to identify the role of HRI in pancreatic beta cells.
Materials and methods: Gene silencing in INS-1E and primary rat beta cells was done by siRNAs while overexpression was achieved using adenoviral vectors. Apoptosis was detected using nuclear dyes. mRNA levels were assessed by qPCR and protein expression and phosphorylation by Western blot.
Results: HRI knockdown with two different siRNAs (H1 and H2) in INS-1E cells induced 10-15% apoptosis in basal condition and sensitized cells to palmitate (from 22±1% apoptosis to 50±2% with H1 and 38±2% with H2, $p<0.05$, n=4) or IL1β+IFNγ (from 17±1% to 31±3% with H1 and 29±3% with H2, $p<0.05$, n=4). HRI silencing in primary rat beta cells also potentiated cytokine-induced apoptosis (from 12±1% to 19±3%, $p<0.05$, n=3). HRI deficiency did not alter expression of CHOP and ATF4, two proteins usually regulated by eIF2α phosphorylation. HRI knockdown decreased phosphorylation of Akt (by 47±9% with H1 and 57±9% with H2, $p<0.05$, n=4). The phosphorylation of the Akt target BAD was also decreased (by 43±9% with H1 and 54±8% with H2, $p<0.05$, n=3), which can lead to activation of this pro-apoptotic BCL-2 protein. Overexpression of constitutively active Akt in HRI-deficient beta cells partially rescued the cells from apoptosis (from 21 ±3% to 17±1%, $p<0.05$, n=3), and BAD silencing fully protected HRI-deficient cells (from 17±1% to 8±1% apoptosis, $p<0.05$, n=4). HRI silencing induced JNK phosphorylation (by 60±15% with H1 and by 153±43% with H2, $p<0.05$, n=3) and JNK knockdown protected cells against HRI deficiency (from 15±2% to 8±1% apoptosis, $p<0.05$, n=8).
Conclusion: Using transcriptomic studies and gain- and loss-of-function approaches we identified the abundantly expressed HRI as a crucial pro-survival factor in beta cells under both basal or diabetogenic conditions. HRI promotes beta cell survival through modulation of the Akt/BAD and JNK pathways. HRI may thus be an interesting, potentially druggable, target to modulate beta cell fate in diabetes.
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Phlda3 is a novel stress-responsive gene in beta cells that regulates cytokine and oxidative stress-induced apoptosis

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Background and aims: Beta cell death is implicated in the loss of functional beta cell mass in type 1 and type 2 diabetes, but the mechanisms are not clear. Pleckstrin homology-like, domain family A, member 3 (Phlda3) has been identified as a tumor suppressor in pancreatic neuroendocrine tumors. However, its role in beta cells has not been investigated. Here we investigated: 1) whether Phlda3 expression is regulated by stress in beta cells, and 2) the role of Phlda3 expression in beta cell pathophysiology under stress conditions.
Materials and methods: Phlda3 expression was assessed in islets from diabetic db/db mice and age-matched lean control db/+ mice. MIN6 cells were exposed for 24h to various stress stimuli including cytokines [TNFα (100 U/ml) + IFNγ (250 U/ml) + IL1β (100 U/ml)], thapsigargin (300 nM, 1 μM), tunicamycin (1-5 μg/ml), H2O2 (200 μM), ribose (50 mM) and modulators of Ca2+ influx diazoxide (250 μM) and nifedipine (10 μM) . Phlda3 was inhibited using siRNA. Gene expression was assessed by real-time RT-PCR. Apoptosis was measured by DNA fragmentation ELISA.
Results: The mRNA levels of Phlda3 were markedly upregulated in vivo in the islets of diabetic db/db mice by ~4,6-fold ($p<0,001$). In vitro, exposure of MIN6 cells to cytokines upregulated Phlda3 mRNA levels by ~4-fold ($p<0,01$) in parallel with the induction of ER stress (Ddit3 and Trb3) and antioxidant (Hmox1) genes. Interestingly, thapsigargin treatment, but not tunicamycin treatment, significantly upregulated Phlda3 mRNA levels despite marked upregulation of ER stress genes by both agents. This implicates perturbation of Ca2+ homeostasis in the regulation of Phlda3 expression. Diazoxide and nifedipine treatment reduced Phlda3 mRNA levels by ~50% ($p<0,05$). On the other hand, exposure of MIN6 cells to ribose, which induces oxidative stress, strongly upregulated Phlda3 mRNA levels by ~13-fold ($p<0,001$). Interestingly, siRNA-mediated knockdown of Phlda3 potentiated cytokine-induced apoptosis by ~2,6-fold ($p<0,05$). This effect was associated with ~1,5-fold further upregulation of iNos mRNA levels ($p<0,01$) and downregulation of antioxidant genes Gpx1 ($p<0,01$) and Srxn1 ($p<0,05$) and adaptive UPR genes Xbp1 ($p<0,001$), Hspa5 ($p<0,05$) and Fkbp11 ($p<0,01$), whereas beta-cell enriched genes Glut2, Pc and Mafa as well as Ddit3 were unchanged. These findings suggest that the inhibition of Phlda3 potentiates the effects of cytokines to induce nitrosative stress and depletes defenses against oxidative and ER stress. Finally, Phlda3 knockdown also potentiated H2O2 and ribose-induced apoptosis ($p<0,05$).
Conclusion: We have identified Phlda3 as a novel stress-response gene in beta cells that plays an important protective role under stress conditions. Phlda3 may regulate the nitrosative, oxidative and ER stress response in beta cells via the maintenance of antioxidant and adaptive UPR gene expression and repression of iNos. The induction of Phlda3 may promote beta cell survival under stress conditions in diabetes.
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Disclosure: **M. Bensellam:** None.

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Heterogeneity of autophagy status in pancreatic beta cells under metabolic stress

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Background and aims: It has been reported that autophagy plays a pivotal role in intracellular quality control through degradation of subcellular damaged organelles and components. We have previously reported that autophagic dysfunction in β-cell specific Atg7-deficient mice cause impaired glucose tolerance accompanied by progressive reduction of β cell mass. In Atg7-deficient mice and Lprdb/db mice, only part of damaged β cells exhibited accumulation of p62/SQSTM1, a specific substrate of the autophagy, suggesting that β cells are heterogeneous in autophagy status. In addition, Western blotting with isolated islets from Lprdb/db mice revealed the elevated expression of microtubule-associated protein light chain 3 (LC3) type 2, showing that some islets cells exhibited elevated autophagic flux. Thus, although autophagic status in whole islets can be altered under metabolic stress, it remains elucidated how different stages of autophagic flux individual β cells have.

Materials and methods: To address this question, we introduced GFP-LC3 reporter mice, which can visualize autophagic status in each β cell as green-fluorescent puncta, and cross the mice with Lprdb/db diabetic mice.
Results: After 16-hour fasting, part of β cells in GFP-LC3; Lprdb/db mice exhibited green-fluorescent puncta, most of which overlapped with p62, whereas fewer GFP-puncta were observed in the islets of GFP-LC3; Lprdb/+ non-diabetic mice. When GFP-LC3 mice were treated with a low dose of S961 (5 nmol/week), which antagonized insulin signaling without inducing hyperglycemia, part of β cells were positive for GFP, but negative for p62, in contrast with the findings in GFP-LC3; Lprdb/db mice. In the mice treated with a high dose of S961 (10 nmol/week), which induced hyperglycemia, there were extremely-dense GFP puncta observed in part of β cells. Western blot analysis with the isolated islets from S961-treated mice resulted in the elevated expression of LC3 type 2, compared to those from the mice treated with phosphate-buffered saline (PBS), which is consistent with the patterns of GFP puncta in S961-treated GFP-LC3 mice.
Conclusion: These findings suggest that pancreatic β cells under metabolic stress were heterogeneous in autophagy status, which could be affected by insulin resistance and hyperglycemia.
Disclosure: **M. Kamitani:** None.

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HSPB1 mediates PRL action on cell death inhibition, restoration of mitochondrial function and modulation of ER stress on beta cells

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Background and aims: Maintaining islet cell in vitro appears as an attractive strategy to increase the outcome of pancreatic islet transplantation. However, islet fate in culture is determined by the balance between pro- and anti-apoptotic mediators. We have previously shown HSPB1 levels are increased by prolactin (PRL) on pancreatic beta cells. Since HSPB1 role in beta cells has not been directly studied, we set out to explore the molecular mechanisms by which HSPB1 mediates PRL-induced beta cell cytoprotection.
Materials and methods: Lysates from cytokine and/or PRL treated Min6 cells were subjected to HSPB1 immunoprecipitation. Co-precipitated proteins were identified by electrophoresis coupled to mass spectrometry. Wild type or HSPB1 silenced (siHSPB1) Min6 cells were used for confirmation of cell viability, mitochondrial bioenergetic efficiency and for monitoring activation levels, by western blot, of members of the three main unfolded protein response pathways.
Results: Our results showed that PRL-treated cells presented an enrichment of both protein degradation and carbohydrate metabolism related proteins (i.e. GRP78, ubiquitine-like modifier enzyme and malate dehydrogenase) co- precipitating with HSPB1. Using the XF24 respirometer platform, we were able to show that cytokine treatment of Min6 cells induced an increase of the uncoupled mitochondrial levels, which were significantly restored to almost normal levels ($p<0.05$) upon PRL treatment. HSPB1 is a key mediator of this increase since its lack significantly abrogated PRL-induced mitochondrial function recovery ($p<0.05$). Since we have found several proteins involved in protein degradation interacting with HSPB1, we investigated whether PRL could promote beta cell cytoprotection of Min6 cells subjected to tunicamycin (tun) or thapsigargin (thag)-induced ER stress. Our experiments indicated that ER stressors-induced beta cell death was significantly inhibited by PRL ($p<0.05$; control: 5±0.3%; tun: 66±1%; thag: 92±1%; tun+PRL: 25 ±2%; thag+PRL: 9,7±0.9%). Moreover, this pro-survival effect is dependent on HSPB1 presence ($p<0.05$; control: 3.6±0.5%; tun: 87±5%; thag: 55±9%; tun+PRL: 84±4%; thag+PRL: 53±9%). Kinetic experiments monitoring activation levels of members of the three main unfolded protein response pathways (pPERK, peIF2alpha, ATF4, ATF6 and IRE1alpha) showed that PRL is able to anticipate the response elicited by cytokines or ER stressors treatment, ultimately leading to a decrease in CHOP levels. This behaviour was also lost in the absence of HSPB1.
Conclusion: We have shown the importance of HSPB1 on both PRL pro-survival effects against ER stressors-induced beta cell death as well as on mitochondrial efficiency recovery mediated by pro-inflammatory cytokines. These results are in accordance with the PRL-induced enrichment of HSPB1 interacting proteins displaying functions related to either protein degradation or carbohydrate metabolism. Finally, our results outline the importance of further studies aiming at a deeper understanding of HSPB1 functions on beta cells, since they could lead to the mitigation of beta cell death through the up-regulation of an endogenous protective pathway, which is not dependent on the modulation of the immune system.
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Disclosure: **L.F. Terra:** None.

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Palmitoylation of L-type calcium channel β2 subunit induces apoptosis in beta cells

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Background and aims: The importance of voltage-gated calcium channels (Cav) in pancreatic beta cells lies in their ability to trigger insulin secretion. Abnormal pore-forming Cavα1 subunits increase the risk of developing type 2 diabetes. In addition, auxiliary Cavβ subunits regulate Cavα1 subunit through transport and stability. Examples of this include the negative effect of Cavβ3 on Ca2+ oscillations, and contribution of Cavβ1 to mouse beta cell survival. Despite this, the functions of many of these auxiliary subunits remain unknown. Interestingly, Cavβ2 is the only Cavβ subunit which undergoes palmitoylation, a post-translational modification regulating proteins. To examine the mechanism by which Cavβ subunits contribute to the function of Cavα1, we have investigated the role of Cavβ2 in relation with palmitoylation in beta cells.
Materials and methods: Insulin secretion. Calcium imaging. Immunoblotting. Viability assay. Mitochondrial Permeability Transition Pore (MPTP) assay.
Results: CACNB2, -1, & -4 are shown, using microarray data from human islets, to correlate with EIF3E which recently was shown to regulate Cavα1 translocation to the cell surface. We have investigated this further by overexpressing mutant non-palmitoylated *Cacnb2* with both palmitoylation sites altered by exchanging 2 cysteines with 2 serines. This resulted in a 62% ($P<0.001$) reduction of Cavα1 translocation to the plasma membrane as illustrated by confocal imaging. This reduction in Cavα1 on the plasma membrane, however, did not affect glucose-stimulated insulin secretion in INS-1 (832-13) cells. Next, we studied the effect of overexpression of wild-type Cavβ2 on cytoplasmic Ca2+ concentration ([Ca2+]i). A 2-fold increase ($P<0.01$) in basal [Ca2+]i was observed in INS-1 (832-13) cells overexpressing wild-type Cavβ2 compared to the mutant non-palmitoylated protein; however, this did not affect insulin secretion. In addition, an increase in apoptosis was observed by immunoblotting (4-fold increase in cleaved caspase-3) as well as confocal imaging (5.15±0.97 vs 12.55±1.16 percentage cells; $P<0.01$) in cells transfected with wild-type, but not mutant non-palmitoylated, Cavβ2 suggestive of Ca2+ toxicity. Further investigation using mitochondrial permeability transition pore (MPTP) assay, and mitochondrial Ca2+ dye, Rhod-2, revealed an increase in mitochondrial Ca2+ concentration ([Ca2+]mt) in wild-type, but not mutant non-palmitoylated, Cavβ2 in pancreatic beta cells.
Conclusion: A balance in the number of Cavβ2 is essential for a normal Ca2+-homeostasis in beta cells, and affects cell functions differently. An increase in Cavβ2 expression enhances mitochondrial and cytoplasmic Ca2+ signalling, but fails to affect insulin secretion. However, elevated apoptosis is also observed, probably as a consequence to Ca2+ toxicity.
Supported by: Swedish Research Council;Swedish Diabetes Society;EXODIAB Human Tissue Lab
Disclosure: **A.S. Kazim:** None.

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MicroRNA-708 links endoplasmic reticulum stress and beta cell dysfunction

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Background and aims: MicroRNAs (miRNAs) have emerged as key factors in gene expression regulation. Recent studies have uncovered a link between miRNAs modulation and environmental stress responses. However, miRNAs dynamics in stressed pancreatic islets have been poorly investigated. The β-cell transcriptome is sensitive to extracellular stimuli. Low and very high glucose concentrations can foster endoplasmic reticulum (ER) stress, thus inducing changes in β-cell gene expression and function. The aim of the current study was to identify miRNAs that are modulated by stress settings in pancreatic islets and, in turn, to study how these miRNAs can affect β-cell gene expression and functionality.
Materials and methods: miRNA expression profiling of mouse isolated islets cultured at low (3 mM) and standard (11 mM) glucose concentrations was performed using Exiqon 384-well panels. Potential miRNA targets were identified by using TargetScan and TargetRank. Gene expression was analysed by RT-qPCR. Small interfering RNA was carried out to assess the role of Chop in modulating miRNA expression in β-cells. Selected miRNAs were transduced using adenovirus to study their role in pancreatic islets and dissociated islet cells. β-cell functionality was assessed by glucose-stimulated insulin-secretion (GSIS), and cell viability and apoptosis were evaluated by MTT and TUNEL assays.
Results: We identified intronic miR-708 as the most upregulated (8.1±0.4 fold change) miRNA in pancreatic islets cultured at low glucose concentrations, a setting that triggers a potent stress response. The expression pattern of miR-708 paralleled those of its host gene Odz4 and their transcriptional activator Chop. miR-708 was also identified as the most upregulated miRNA when islets were exposed to 1μM thapsigargin, a chemical ER inducer. Accordingly, mir-708 induction by stress settings was blocked by treatment with the chemical ER stress reliever 4-phenyl pyruvate (PBA, 2.5 mM) and by siRNA of Chop. An integrative analysis of global gene expression profiles and miRNA-target-prediction algorithms identified neuronatin (*Nnat*) as a potential target of mir-708 that is also repressed under low glucose levels. *Nnat* is an ER membrane protein that regulates intracellular Ca2+ levels. Interestingly, Nnat expression was inversely correlated with miR-708 in different settings and was reduced by 45.2±10.3% following miR-708 overexpression. Moreover, forced miR-708 expression impaired the secretory capacity of pancreatic β-cells, consistent with the known role of *Nnat* in insulin secretion. Finally, miR-708 overexpression also induced apoptosis and decreased cell viability by 28.0±0.94% in pancreatic β-cells.
Conclusion: We uncover miR-708 as the most upregulated microRNA in pancreatic islets exposed to low glucose concentrations that result in ER stress. We propose miR-708 as a key mediator of apoptosis and loss of insulin secretory capacity in β-cells exposed to stress settings.
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Mig6 suppresses endogenous repair mechanisms in pancreatic beta cells

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Background and aims: Type 1 diabetes (T1D) is caused by autoimmune-mediated beta cell destruction. Following beta cell injury, the pancreas initiates a cellular repair or regeneration program, which includes epidermal growth factor receptor (EGFR) signaling. However, upon irreparable beta cell damage, EGFR signaling is dampened, disrupting attempts to restore functional beta cell mass and maintain normoglycemia. We have previously demonstrated that Mitogen-inducible gene 6 (Mig6), a negative feedback inhibitor of EGFR, is induced by the pro-inflammatory cytokines central to beta cell destruction in T1D. We also established that pro-inflammatory cytokines suppress EGFR activation, and siRNA-mediated suppression of Mig6 restores EGFR signaling. Thus, we hypothesized that a loss of pancreatic Mig6 would protect mice from a chemically-induced form of diabetes by enhancing EGFR signaling and promoting cellular repair.
Materials and methods: Mice lacking pancreatic Mig6 (PKO) and their wild-type littermates (WT) were treated with multiple low doses of streptozotocin (STZ; 35 mg/kg body weight) to induce beta cell death and diabetes or with saline as a control. Glucose and insulin tolerance testing as well as analysis of pancreatic beta cell mass and proliferation were assessed over time. Relevant cellular signaling was examined via immunoblotting in isolated islets and beta cell lines.
Results: Whereas STZ-treated WT mice became hyperglycemic and had reduced beta cell mass, STZ-treated PKO mice remained euglycemic and glucose tolerant, due to preserved beta cell mass. In addition, nitric oxide (NO) synthase inhibition blocks both the induction of Mig6 and cytokine-impaired EGFR signaling, and treatment with an NO donor alone induces Mig6. Interestingly, beta cell mass was maintained in STZ-treated PKO mice without increases in beta cell proliferation, suggesting increased resistance to cell death or enhanced cellular repair. Exposure to high glucose or pro-inflammatory cytokines increases Src phosphorylation at tyrosine 416, which is required for Src-mediated activation of Mig6, and resultant inhibition of EGFR signaling.
Conclusion: Thus, Mig6 ablation promotes beta cell damage repair, hence abating the progression to diabetes. Our work suggests that Mig6 may be a novel therapeutic target for preserving beta cell mass following damage and destruction in T1D.
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PS 022 Beta cells need protection

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Combined transcriptome and proteome profiling of the beta cell response to palmitate to unveil novel mediators of lipotoxicity

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Background and aims: Chronic exposure to saturated free fatty acids impairs insulin secretion and induces β-cell death, contributing to type 2 diabetes. To identify the molecular mediators of lipotoxic β-cell demise, we performed RNA-sequencing of human islets and time course proteomic profiling of β-cells following exposure to palmitate, the most common circulating saturated fatty acid in man.
Materials and methods: Six human islet preparations were RNA-sequenced after exposure to palmitate for 48h (average depth 38 million reads). Rat insulin-producing INS-1E cells exposed to palmitate for 4, 16 or 24h were proteome profiled using iTRAQ (n=2 at each time point). Genes modified by palmitate at both mRNA and protein level were analyzed by IPA and DAVID bioinformatic tools, to assess enriched pathways and transcription factor binding sites. Regulatory networks were inferred from palmitate-modified genes and known regulators described in the literature. Selected genes were silenced by RNA interference in clonal and primary rat β-cells. Data are based on 3-5 independent experiments.
Results: Comparison of the transcriptome and proteome of palmitate-treated β-cells revealed 93 up- and 133 downregulated genes/proteins common to both datasets. IPA indicated that upregulated genes were involved in fatty acid oxidation, ERK/MAPK signaling and oxidative stress, whereas downregulated genes were involved in cell cycle regulation, cAMP signaling and cytoskeleton remodeling. Manual curation into functional categories showed that palmitate-modified genes regulate fatty acid metabolism, endoplasmic reticulum (ER) stress, apoptosis and amino acid transporters. We confirmed by qPCR that palmitate induces the amino acid transporters CAT1 and LAT1 (by 2.1- and 2.4-fold, $p<0.05$), but silencing of these genes did not protect β-cells from palmitate. This stands in contrast with a previous suggestion that their induction mediates ER stress-induced apoptosis. Given the important role of the ER stress response in lipotoxic β-cell death, we examined the function of CREB3L2 and MANF, two ER-resident cytoprotective proteins. Upregulation of CREB3L2 by palmitate was confirmed in INS-1E cells (by 2.7-fold, $p<0.01$). CREB3L2 silencing induced apoptosis in INS-1E cells (from 6±0.7% to 31±3.7%, $p<0.05$). Induction of MANF by palmitate was confirmed in INS-1E cells (by 1.4-fold, $p<0.01$). Knockdown of MANF in INS-1E and primary rat β-cells did not alter palmitate-induced apoptosis, indicating that MANF is not β-cell cytoprotective under lipotoxic conditions. Network inference analysis suggested that JunD, APP, ARNT and FoxO1 are key mediators of lipotoxicity. ARNT and FoxO1 are known to modulate insulin secretion and β-cell survival. JunD silencing potentiated palmitate-induced apoptosis in INS-1E cells (from 27±3.5% to 40±2.9%, $p<0.01$).
Conclusion: The present study is the first to combine transcriptomic and proteomic profiling of palmitate-treated β-cells. Our data point to novel mediators of lipotoxicity, and uncover regulatory networks predicted to play a role in β-cell failure in type 2 diabetes.
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Disclosure: **M. Lytrivi:** None.

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Perixiredoxin6 deletion impairs mitochondria function and decreases insulin secretion in pancreatic beta cells

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Background and aims: Mitochondrial metabolism is essential for cellular function by regulating ATP production, redox signaling, and calcium (Ca2+) handling. Moreover, in pancreatic beta cells mitochondria are responsible for glucose sensing insulin secretion (GSIS). Recent data suggest that beta cells normally contain a dynamic mitochondrial network due to regular Fission/Fusion ratio; furthermore, when mitochondria become largely fused or fragmented, GSIS resulted impaired. In our previous study, we demonstrated as a deletion of an antioxidant enzyme, Peroxiredoxin 6 (Prdx6), significantly reduced insulin secretion by altering the morphology of the Langerhans islets. Therefore, the main goal of the present study was to evaluate the potential role of Prdx6 in altering beta cells function by impairing mitochondrial homeostasis.
Materials and methods: We generated a murine insulinoma beta cell line (BTC6) stably silenced for Prdx6 (Prdx6KD) and we compared all results obtained with control scramble (scr) cells. Insulin secretion and ATP production were evaluated after glucose (30 mM) stimulation, at different time points, by using commercial kit. Mitochondrial fission and fusion proteins were analyzed by Western Blot analysis. Ultrastructural mitochondrial analysis was performed by using Transmission Electron Microscopy (TEM).
Results: We observed decreased insulin secretion in Prdx6KD cells at 15 min after glucose stimulation compared to scr ($p<0.0005$). At the same time point, we showed significant reduction in ATP production in association with lower levels of mitochondrial membrane potential ($\Delta\Psi m$) in Prdx6KD cells. We, also, demonstrated that ATP alteration induces a reduction in intracellular Ca2+ influx, which contributed to an impairment in granuli exocytosis machinery. Ultrastructural analysis revealed in scr cells, mostly rounded or slightly elongated mitochondria, with normally oriented cristae. In Prdx6KD, mitochondria appeared irregular in the shape, sometimes very elongated with abnormal bifurcations, and disorganized cristae. The elongated, tubular, interconnected mitochondrial showed in knockdown cells are typical of alteration in Fission/Fusion ratio. Then, we evaluated the protein expression of several factors regulating the mitochondrial network process. In particular, we analyzed the Fission protein 1 (Fis1) and the Dynamin-Related Protein 1 (Drp1) for fission, and the Mitofusins 1 and 2 (Mfn1 and Mfn2) and Optic atrophy 1 (Opa1) for fusion. All these proteins resulted significantly reduced in knockdown cells compared to control cells, suggesting that Prdx6 regulates the Fission/Fusion dynamic network and mitochondrial metabolism.
Conclusion: For the first time we demonstrated as Prdx6 is directly associated to mitochondrial structure and function in beta cells. The impairment of mitochondrial Fission/Fusion machinery is, also, responsible for altered insulin secretion. All these data highlight a novel important role of Prdx6 that may open new horizon for the therapy against diabetes and that need to be further investigated.
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Disclosure: **F. Pacifici:** None.

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A comparative study on lipotoxicity in insulin-producing rodent RINm5F cells and in the new human beta cell line EndoC-βH1

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Background and aims: In the field of experimental diabetology murine β-cell lines are established as model cell lines to study the pathological

mechanisms of diabetes development. With the EndoC-βH1 β-cell line there is now a new human tissue culture cell line available that has physiological and biochemical characteristics comparable to those of primary human β-cells. Chronically elevated levels of non-esterified fatty acids (NEFA) are discussed as one of the major reasons for type 2 diabetes development due to their ability to induce β-cell dysfunction and β-cell apoptosis. The aim of this study was to compare lipotoxicity of saturated and unsaturated NEFA in the novel human β-cell line EndoC-βH1 with the established insulin-producing rodent cell line RINm5F.

Materials and methods: Insulin-producing RINm5F and EndoC-βH1 tissue culture cells were incubated with saturated and unsaturated NEFA. Toxicity was determined by using caspase-3 assay. The formation of β-cell-toxic hydrogen peroxide (H2O2) by NEFA was analyzed by expression of the fluorescent sensor protein HyPer. The respective ER-Stress marker genes were quantified by qRT-PCR using specific primers.

Results: Palmitic acid and other long- and very long-chain saturated NEFA were toxic to rat RINm5F cells and human insulin-producing EndoC-βH1 cells. In RINm5F cells only the incubation with long-chain saturated NEFA caused an induction of caspase-3 and an increase in H2O2 production, unsaturated NEFA were not toxic and showed no H2O2 production. Unexpectedly, we observed in EndoC-βH1 cells both strong toxicity not only of saturated but also of oleic acid and other unsaturated long-chain NEFA as documented by caspase-3 activation. The toxicity correlated with the intracellular H2O2 production. In contrast to RINm5F cells, in which long-chain unsaturated NEFA protected against the long-chain saturated NEFA-mediated lipotoxicity, in EndoC-βH1 cells unsaturated long-chain NEFA revealed no protection against the toxicity of long-chain saturated NEFA. In the rat β-cell line RINm5F only long-chain saturated NEFA induced gene expression of ER-stress marker genes, whereas unsaturated NEFA had no effect on the gene expression of any analyzed ER-stress marker genes. This is in analogy to human EndoC-βH1 cells, in which only saturated NEFA revealed ER-stress.

Conclusion: Human EndoC-βH1 and rat RINm5F cells exhibit significant differences in their sensitivity to lipotoxicity. Long-chain unsaturated NEFA reveal toxic effects in EndoC-βH1 cells at variance from the rodent β-cell line RINm5F. These analogous differences can be observed also between the primary rodent β-cells and human islets. These results have important consequences for the understanding of the mechanisms of lipotoxicity in human β-cells.In view of these differences, it can be concluded that effects of fatty acids obtained in rodent β-cells represent both in in vitro and in vivo studies inadequate models for the understanding of mechanisms of lipotoxicity in human β-cells. Thus the concept of a protection by unsaturated NEFA against toxicity of saturated NEFA is not valid for the human β-cell.

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Crucial role of the histone acetyl transferase p300 in pancreatic beta cell survival

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Background and aims: Type 2 diabetes is characterized by chronic hyperglycemia due in part to a deficit in pancreatic β-cells. It is therefore crucial to preserve a functional β-cell mass to maintain glucose homeostasis. The preservation of an adequate β-cell function and survival is made possible by a fine regulation of gene expression in response to physiological stimuli. Among the mechanisms involved in gene regulation, remodelling of chromatin structure by epigenetic mechanisms, such as histone acetylation, is a fundamental process. The histone acetyl transferase p300 is a key activator of the transcriptional machinery. The aim of this study is to understand the role of p300 as well as the cellular/molecular mechanisms that control its function in healthy β-cells and in β-cells exposed to pathological conditions (such as glucotoxicity, proteotoxiciy and pro-inflammatory cytokines).

Materials and methods: Experiments were performed with the pancreatic β-cell line (INS-1E), isolated mouse pancreatic islets and mouse pancreatic sections (transgenic mice expressing the toxic human islet amyloid polypeptide, h-TG vs. control mice). p300-interacting partners were identified by immunoprecipitation. p300 and histone H4 acetylation levels were evaluated by western blot and immunofluorescence. Apoptosis was assessed with the cleaved form of caspase-3. Glucose-induced insulin secretion was assessed by Homogeneous Time Resolved Fluorescence (HTRF) technology.

Results: Knock-down of p300 by siRNA (50 nM for 48h) led to β-cell apoptosis (3.5 fold increase in cleaved caspase-3 vs. control RNA, $p<0.01$) followed by an altered glucose-induced insulin secretion. Among the mechanisms involved in the anti-apoptotic role of p300, we found that acute stimulation by glucose and/or GLP-1 led to p300 interaction with the active form of CREB (cAMP-Responsive Element Binding protein), a transcription factor crucial for β-cell survival. Interestingly, p300 and histone H4 acetylation levels were both decreased under chronic exposure to high glucose concentrations (33.5±3.4% decrease vs. control, $p<0.001$) and pro-inflammatory cytokines (48.6±6.5% decrease vs. control, $p<0.001$). Moreover, nuclear fractionation and immunofluorescence experiments reveal that nuclear levels of p300 were decreased in β-cells of h-TG mice (73±11% decrease vs. control mice, $p<0.01$), a murine model of proteotoxicity recapitulating β-cell deficits in human type 2 diabetes.

Conclusion: This study reveals for the first time the involvement of the histone acetyl transferase p300 in β-cell survival, as well as its alteration in β-cells exposed to physiopathological conditions mimicking the diabetic environment.

Disclosure: **L. Ruiz:** None.

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Oleate protects beta cells from the toxic effect of palmitate by restoring pro-survival pathways of the ER stress response

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Background and aims: Long-term exposure of beta cells to saturated fatty acids impairs insulin secretion and increases apoptosis. In contrast, unsaturated fatty acids protect beta-cells from the long-term negative effects of saturated fatty acids. The aim of the study was to identify the mechanisms underlying this protective action of unsaturated fatty acids.

Materials and methods: To address the aim, insulin-secreting MIN6 cells were exposed for 48 hours to 0.5 mM palmitate in the absence or presence of 0.5 mM oleate. After treatment, proteins were extracted from the cells and digested by trypsin. The peptides were labeled using reductive dimethylation and then separated by nano-LC MS/MS based proteomic approach. Proteins were identified using MASCOT search engine against the SwissProt database. Proteins quantified in at least 4 out of 8 biological replicates were selected for further analysis. The differentially expressed proteins were annotated using UniProt database. The effect of fatty acids on the ER stress was determined by measuring the markers of the ER stress response BiP, PDI, p-eIF2alph and CHOP by western blotting (WB). The effect of fatty acids on beta-cell function was determined by measuring glucose-stimulated insulin secretion (GSIS) and apoptosis (cytoplasmic oligonucleosomes).

Results: Treatment of MIN6 cells with palmitate abolished GSIS and increased apoptosis. When oleate was also present during culture, GSIS was partially restored whereas apoptosis was decreased to control level. Proteomic analysis identified 34 proteins differentially expressed in the

presence of palmitate compared to control samples. These proteins play a role in insulin processing, mitochondrial function, metabolism of biomolecules, calcium homeostasis, exocytosis, receptor signalling, ER protein folding, antioxidant activity and anti-apoptotic function. When oleate was also present during culture, expression of 15 proteins was different from the expression in the presence of palmitate alone. All of the proteins affected by oleate play a pro-survival role in beta cells by improving protein folding, antioxidant activity and anti-apoptotic function. The role of ER stress in the protective effects of oleate was confirmed by measuring markers of the ER stress response by WB.
Conclusion: Our study suggests that restoration of the pro-survival pathways of the ER stress response is a major mechanism that underlies the protective action of oleate in palmitate-treated beta cells.
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Disclosure: **E. Sargsyan:** None.

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Role of the (macro)autophagy in the postnatal regeneration of endocrine pancreas and its relationship with IGF-2

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Background and aims: The developing endocrine pancreas undergoes substantial remodeling during the postnatal period associated to changes in the type of nutrition, which triggers a transformation from a fetal phenotype of beta-cells to adult phenotype with altered glucose thresholds and the ability to rapidly release insulin. This beta-cell turnover is achieved by a transient wave of apoptosis that is temporally associated with a lack of expression of IGF-2 within islets. (Macro)Autophagy is a dynamic and highly inducible catabolic process that responds to environmental and hormonal cues, and can drive important functions in neonatal tissues, including programmed cell remodeling. Because IGF-1R pathway is upstream of mTORC1, a negative regulator of autophagy, we expected autophagy to be activated in beta-cells during the neonatal period. Thus, our study aimed to characterize the basal autophagic activity during endocrine pancreas remodeling and its contribution to neonatal beta-cell apoptosis.
Materials and methods: Autophagy markers were measured by Western blot in the pancreas of rats on postnatal (PN) day 4, 14 and 23. Autophagosome formation was also analyzed by electron microscopy (TEM). Neonates were treated with rapamycin (3 mg/kg, 5 days) and beta-cell apoptosis was quantified by TUNEL assay on PN14. In order to determine the effect of IGF-2 on autophagy, in vitro studies were performed in INS-1E cells.
Results: Under basal conditions, a significant decrease of LC3II levels was observed in the pancreas of neonates on PN14 and PN23 compared with PN4. This decrease in LC3II levels was accompanied by an accumulation of the specific autophagic substrate p62 (>2-fold vs. PN4). Pharmacological inhibition of lysosomal degradation with chloroquine (CQ; 30 mg/kg, 24 h), confirmed the blockage of autophagic flux on PN14 and 23. Similarly, TEM revealed a reduction in the number of autophagic vacuoles per beta cell in PN14 (49.7%) and PN23 rats (64.24%) compared with PN4 rats. Sub-chronic treatment with rapamycin, induced a significant reduction ($p<0.01$) in the rate of beta-cell apoptosis observed on PN14 suggesting a crosstalk between beta-cell autophagy and apoptosis during the neonatal period. Finally, kinetic experiments showed that long- but not short-term supplementation of INS-1E cells with IGF-2 resulted in a significant increase in LC3-II formation.
Conclusion: The blockage of autophagic activity in the endocrine pancreas seems to be required during postnatal remodeling in order to ensure the correct beta-cell turnover by apoptosis. This blockage might be associated to disappearance of islet IGF-2 expression.
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Disclosure: **E. Fernández-Millán:** None.

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Inhibition of the MAP3 kinase Tpl2 protects beta cells from apoptosis and prevents chronic hyperglycaemia in db/db mice

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Background and aims: We recently reported that the MAP3 kinase tumor progression locus 2 (Tpl2) is expressed in pancreatic beta-cells, activated and overexpressed in response to proinflammatory cytokines. We further demonstrated that specific inhibition of Tpl2 may be a promising strategy to promote beta-cell survival by suppressing apoptosis induced by proinflammatory conditions. Here, we wanted to validate Tpl2 as a therapeutic target and assessed the potent efficacy of Tpl2 inhibition against other diabetogenic stresses, as well as in vivo in type 2 diabetic mice.
Materials and methods: INS-1E beta-cells were exposed for 48 h to different diabetogenic stresses (IL-1beta (20 ng/ml), TNFalpha (50 ng/ml), and high glucose concentration (30 mM glucose), and treated with Tpl2-inhibitor (3 micromol/l). Protein expression/phosphorylation were measured by Western-Blotting. For in vivo experiments, db/db mice (and db/+ control mice) were injected intra-peritoneally with 2.5 mg/kg of Tpl2 inhibitor or vehicle (DMSO) for 2 weeks, starting at 6 weeks of age.
Results: In INS-1E cells, inhibition of Tpl2 decreased JNK activation and apoptosis induced by chronic exposure to IL-1beta, TNFalpha, and high glucose. Tpl2 expression was found to be increased in pancreatic islets isolated from db/db mice (7 weeks of age). db/db mice treated with the Tpl2 inhibitor for 2 weeks presented an improvement in ip glucose tolerance, a decrease in fasting blood glucose (from 258.6 ± 40.7 to 144.0 ± 15.5 mg/dL, $p<0.01$) and fasting serum insulin levels (from 11.3 ± 1.2 to 4.3 ± 0.4 ng/ml, $p<0.001$), as well as reduction in random fed glycemia (from 490 ± 8.4 to 385.6 ± 14.7 mg/dL, $p<0.01$). Body weight was unchanged, and insulin tolerance was not markedly improved with the inhibitor, suggesting that an improvement in beta-cell survival and/or function is responsible for the metabolic benefits of Tpl2 inhibition.
Conclusion: These results suggest that Tpl2 inhibition protects pancreatic beta-cells from the deleterious effects induced by cytokines and chronic hyperglycemia, and may be a relevant therapeutic strategy to preserve pancreatic beta-cell mass and/or function from various diabetogenic stresses, and could be harnessed to treat type 2 diabetes.
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Disclosure: **D. Muller:** None.

PS 023 Beta cells need help to survive

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Alkalinization by phosphate uptake via PIT-1/2 participates in high phosphate-induced oxidative stress and defective insulin secretion

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Background and aims: Elevated plasma level of inorganic phosphate (Pi) is harmful, causing among other complications vascular calcification and defective insulin secretion. The underlying molecular mechanisms of these complications remain poorly understood. Here, we demonstrate the role of Pi transport across the plasmalemma on Pi toxicity in insulin-secreting cells.
Materials and methods: To investigate the role of sodium-phosphate cotransporter (NaPi), we performed knockdown of NaPi in INS-1E cells as well as dispersed rat pancreatic islet cells. Electrophysiology and fluorescence imaging were used to measure cytosolic and mitochondrial changes by high Pi application.
Results: PiT-1 and -2, isotypes of type III NaPi, are the predominant Pi transporters expressed in insulin-secreting cells. Transcript and protein levels of PiT-1 and -2 were upregulated by high Pi incubation. In patch clamp experiments, extracellular Pi elicited a sodium-dependent inwardly rectifying current, which was markedly reduced under acidic extracellular conditions. Cellular uptake of Pi elicited cytosolic alkalinization and, intriguingly, this pH change facilitated Pi transport into the mitochondrial matrix. Increased mitochondrial Pi uptake accelerated superoxide generation, mitochondrial permeability transition and ER stress-mediated translational attenuation, leading to reduced insulin content and impaired glucose-stimulated insulin secretion. Silencing of PiT-1 and -2 prevented all the Pi-induced pathogenic alterations including restorations of insulin secretion and content.
Conclusion: Pi transport across plasma membrane and consequent cytosolic alkalinization could be a therapeutic target for protection from Pi toxicity in insulin secreting cells but also other cell types.
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Disclosure: **K. Park:** None.

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Lipocalin 2 induces pancreatic beta cell dysfunction

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Background and aims: Lipocalin 2 (LCN2) is a small secretable protein, which has been reported to regulate iron-transport and exert immunomodulatory, pro-inflammatory and pro-apoptotic effects. LCN2 is secreted from white adipose tissue (AT) and has been implicated in the pathophysiology of insulin resistance and type 2 diabetes. LCN2 levels are elevated in serum and white AT of obese, insulin resistant and type 2 diabetes patients, and positively correlate with elevated fasting glucose, insulin resistance and presence of inflammatory markers. However, the role of LCN2 in regulation of pancreatic β-cell function, and thus evidence of a direct role in onset of overt type 2 diabetes has yet to be examined.
Materials and methods: We investigated LCN2 expression changes in (a) islets, white AT and liver isolated from diabetic high-fat fed mice (HFD; 60% fat, 10 weeks), (b) islets isolated from control mice and exposed to TNFα/IL1β (48 h; 5 ng/ml), (c) islets and white AT isolated from the offspring of mice maintained on 8% (MPR) or 20% protein (control) diet during pregnancy (offspring were fed standard diet) and (d) islets isolated from non-diabetic and type 2 diabetic human donors. In addition, islets were isolated from control C57Bl/6 mice and exposed to recombinant LCN2 (24h; 250 - 500 ng/ml) with islet function assessed by glucose-stimulated insulin secretion (GSIS). Finally, control C57Bl/6 mice were administered recombinant LCN2 (250 ng/ml/day for 10 days) or saline equivalent, and islets were collected for GSIS analysis.
Results: LCN2 levels were markedly increased in (a) islets (3 fold; $P<0.001$), WAT (≈20 fold; $P<0.05$), liver (7 fold; $P<0.05$) of HFD-mice, (b) mouse islets incubated with TNFα/IL1β (5 fold; $P<0.001$), (c) islets (7 fold; $P<0.05$) and WAT (270 fold; $P<0.05$) isolated from the offspring of MPR mice, and (d) in islets isolated from type 2 diabetic donors compared to non-diabetic donors (2 fold). In all four models, elevated LCN2 expression was associated with impaired ex vivo and/or in vivo GSIS. Furthermore, exposure of isolated mouse islets to recombinant-LCN2 markedly suppressed GSIS (80%; $P<0.001$), whilst administration of recombinant-LCN2 to C57Bl/6 mice suppressed GSIS in isolated islets by 85% ($P<0.05$).
Conclusion: Using three distinct models of pancreatic islet dysfunction, and human T2D islets, our data identify LCN2 as a novel mediator of β-cell failure that may represent a novel therapeutic target for treatment of type 2 diabetes
Disclosure: **P.W. Caton:** None.

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ZBED6 negatively regulates insulin content, glucose-stimulated insulin secretion, neuronal differentiation and cell aggregation in MIN6 cells

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Background and aims: We have recently observed that knockdown of Zbed6 in mouse βTC-6 cells resulted in increased insulin production and decreased proliferation rates. The aim of the present study was to further investigate the role of ZBED6 in insulin-producing cells, using mouse MIN6 cells, and to evaluate the effects on Zbed6 knockdown on basal beta-cell functions such as morphology, transcriptional regulation, insulin content and release.
Materials and methods: Lentiviral shRNA-mediated stable Zbed6-silenced cells and controls were characterized with a range of methods including RNA sequencing, ChIP sequencing, insulin content and release, sub-plasma membrane Ca2+ measurement, cAMP determination and morphological studies.
Results: We identified more than 4,000 putative ZBED6-binding sites in the MIN6 genome, including several beta-cell specific transcription factors, such as Pdx1, MafA and Nkx6-1. The highest scoring motif identified was a 12 bp motif with a core GCTCG sequence consistent with the previously established motif for ZBED6. More than 700 genes showed differential expression in response to Zbed6 knock-down. MafA, Nkx6-1 and Pdx1 were among the significantly upregulated genes. The up-regulation of these genes was further verified by real-time PCR. Immunoblotting and staining supported the up-regulation of PDX1 at the protein level and nuclear localization of PDX1. Zbed6 knockdown increased MIN6 cells insulin content and glucose stimulated insulin release, which was paralleled by increases in sub-plasma membrane calcium concentrations. Interestingly, our RNA-seq results showed that Zbed6 knockdown resulted in increased levels of mRNA coding for adenylyl cyclases Adcy1 and Adcy9 and decreased levels for phosphodiesterases Pde3a/5a/6a/8a/10a. When stimulated with forskolin, an enhanced cAMP content was observed in Zbed6-silenced cells. Several Gene Ontology categories were significantly enriched by analyzing the differentially

expressed genes (FDR-corrected P<0.05), including neuronal differentiation and projection, cell projection, regulation of neuronal differentiation, generation of precursor metabolites, axon guidance molecules, MAPK signaling pathway and cell adhesion. We also observed altered morphology/growth patterns of Zbed6-silenced cells as indicated by increased cell clustering and in the appearance of axon-like neurofilament-M positive protrusions.

Conclusion: We conclude that ZBED6 acts as a transcriptional regulator in MIN6 cells and that its activity supresses insulin production and release, cell aggregation and neuronal-like differentiation.

gene	shMock	shzbed6	log2(fold change of shzbed6 to shMock)	p_value	significant
Adcy9	15,1754	20,3737	0,424974	5,92E-05	yes
Adcy1	2,24748	6,11523	1,4441	0,00114	yes
Pde6a	2,65377	1,69154	-0,649701	0,00116	yes
Pde5a	1,28682	0,787112	-0,709171	0,00135	yes
Pde10a	7,77087	6,0643	-0,357735	0,00171	yes
Pde8a	12,5159	9,43184	-0,408149	0,00243	yes
Pde3a	1,29783	0,648056	-1,00191	0,00044	yes

Table.1 RNA-sequencing results of adenylyl cyclases and phosphodiesterases in control (shMock) and Zbed6-silenced (shzbed6) cells. The average mRNA expression levels (RPKM) of triplicates were listed in the table.

Disclosure: **X. Wang:** None.

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DNA damage in pancreatic beta cells is connected with loss of cell mass and function in an ERCC1-deficient mouse model

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Background and aims: Beta cell mass loss and impaired function are key features of Type 2 Diabetes (T2D). Patients with hyperglycemia also show increased levels of DNA damage, suggesting a potential connection between these events. Our research aimed to study the impact of DNA damage on beta cell mass and function using a mouse model with deficient DNA repair.

Materials and methods: Glucose metabolism, beta cell mass, proliferation and apoptosis were studied in Ercc1$^{-/\Delta}$ mice, in which ERCC1-XPF repair endonuclease is knocked-down systemically to increase the burden of endogenous DNA damage, and control littermates. Glucose metabolism and beta cell function were also measured in Ercc1$^{-/fl}$;Rip-Cre$^{+/-}$ mice, in which ERCC1-XPF is deleted in pancreatic beta cells only, and control littermates.

Results: As expected, beta-cells of Ercc1$^{-/\Delta}$ mice showed signs of persistent DNA damage, indicated by increased γH2AX staining. Ercc1$^{-/\Delta}$ mice had a 62% reduction in beta-cell area (control mice 0.60±0.14 vs. Ercc1$^{-/\Delta}$ mice 0.23±0.07; p<0.01) with increased apoptosis, whereas beta cell proliferation was unaffected. Isolated islets from Ercc1$^{-/\Delta}$ mice showed a 2-fold increased sensitivity to apoptosis induced by high glucose. Glucose tolerance was reduced in Ercc1$^{-/fl}$;Rip-Cre$^{+/-}$ mice model in comparison to controls. Additionally, isolated islets from Ercc1$^{-/fl}$;Rip-Cre$^{+/-}$ mice showed reduced insulin secretion compared to controls after culture in high glucose concentrations (16.7mM vs 2.8mM).

Conclusion: Our data suggest that endogenous DNA damage when not repaired in beta cells induce apoptosis, which contributes to loss of beta cell mass. In addition, DNA damage impairs glucose metabolism affecting insulin secretion. Therefore, therapies aimed to decrease DNA damage, such as antioxidants, may contribute to the preservation of beta cells during T2D.

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Disclosure: **A.P. Huerta Guevara:** None.

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Carbamazepine protects pancreatic beta cells and reduces type 1 diabetes incidence in non-obese diabetic mice

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Background and aims: Type 1 diabetes is an autoimmune disorder in which the pancreatic β cells are selectively destroyed by the host immune system. Although many approaches are focused on suppressing aberrant immune activity, studies aimed at the direct cellular protection of β cells are limited. To address this knowledge gap, previous work from our laboratory employed unbiased high-throughput screening to identify the use-dependent sodium channel blocker carbamazepine as a compound that directly protected β cells from a toxic cytokine cocktail in vitro. In this study, we tested whether carbamazepine could protect β cells in a mouse model of type 1 diabetes.

Materials and methods: Female, non-obese diabetic (NOD) mice were weaned onto standard LabDiet 5053 alone (n = 20) or supplemented with 0.5% w/w carbamazepine (n = 22) and were fed ad libitum. Incidence of diabetes was determined by two consecutive measurements of 4 hr fasting blood glucose above 16 mmol/l up to 25 weeks of age. Serum carbamazepine was quantified by ELISA from cardiac puncture blood at experimental endpoint. Formalin fixed, paraffin embedded pancreatic sections were obtained for immunohistochemical measurements of insulin-positive β cell area and insulitis scoring. A chase cohort of carbamazepine treated (n = 4) NOD mice was euthanized at 7 weeks of age for pre-diabetic IPGTT using 2 g/kg glucose and histology.

Results: Incidence of persistent fasting hyperglycemia diverged at 16 weeks of age between carbamazepine treated and control female NOD mice. By 25 weeks of age, approximately 40% of carbamazepine treated mice developed diabetes in comparison to the ~80% of control 5053 fed animals. Serum carbamazepine levels measured at experimental endpoint were highly variable (14.98 ± 3.19 μM), but consistent with the concentrations used previously by our group in vitro (~8.5 μM). Insulitis was also highly variable in between mice within the same treatment group. No significant differences in insulitis were found between control and drug treated animals, suggesting that carbamazepine did not alter lymphocyte homing or aggregation to the pancreatic islets. Carbamazepine treated mice exhibited increased β cell area as compared to controls, consistent with a direct protective effect of this drug on the β cells. Fasting blood glucose in the pre-diabetic NOD mice at 6 weeks of age was 3.6 ± 0.19 mmol/l in carbamazepine treated animals versus 4.4 ± 0.12 mmol/l in control mice ($p < 0.01$). IPGTT challenge of these mice revealed a statistically significant improvement in glucose homeostasis in carbamazepine treated mice (AUC 474.6 ± 21.8) versus control mice (AUC 676.2 ± 40.7; $p < 0.01$).

Conclusion: Taken together, our data suggest that carbamazepine reduces, but does not eliminate, the development of type 1 diabetes in NOD mice. Our evidence suggests that this effect is due, in part to, a higher functional capacity for responding to glucose challenge and maintenance of insulin-producing cells.

Supported by: SCN Drug Discovery Grant

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Imatinib inhibit streptozotocin induced Rac1-nadph oxidase mediated pancreatic beta cell death via activation of phosphatidylinositol 3-kinase signalling

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Background and aims: Beta cell failure plays a fundamental role in the onset and progression of type 1 (T1D) and type 2 diabetes (T2D).

Evidences in cell lines and animal models suggest that abnormal receptor tyrosine kinase signaling may involve in the beta-cell death in Type 1 and Type 2 diabetes. Recent development of tyrosine-kinase inhibitor (TKI) based drugs demonstrates promising outcomes for treatment of diabetes. But, the mechanisms whereby tyrosine-kinase inhibitors (TKI) exert their effects of pancreatic beta cell failure are not fully understood. Rac1 (Ras-related C3 botulinum toxin substrate 1), a small G-protein belonging to the Rho family regulate a plethora of critical cellular functions, such as oxidative stress, cellular contacts, migration, and proliferation. Because of its diverse functions, Rac1 plays both positive and negative roles in pancreatic beta cells. Our present study evaluates the effects of c-Abl inhibitor Imatinib on stress induced by streptozotocin (STZ), and its potential implication on Rac1 signaling.

Materials and methods: To address this question, INS-cells were pretreated with Imatinib (10uM) for 2h and then exposed to STZ (1mM) for 8 hours. Apoptosis was measured by TUNEL-Nick End Labeling assay. Rac1 activation was measured by non-radioactive Rac1 activation kit (Millipore). NADPH oxidase activity was measured by Lucigenin based chemiluminescence assay. Protein expression levels of insulin and ERK signaling measured by western blot analysis. C57BL/6 mice were received Imatinib (150mg/kg body weight) day before and 2h after STZ injection i.p (150mg/kg body weight) and day after the injection.

Results: Exposure of INS-1 cells to STZ (1mM) increased the expression of active Rac1-GTP resulted in elevated NADPH oxidase activity (4 fold $p<0.001$) compared with control cells. The effects on Rac1-GTP were associated with a 60% increase in cell apoptosis, evaluated by TUNEL-assay. Further, NADPH oxidase activity potentiated STZ-induced mitochondrial dysfunction. Imatinib (10uM) pretreatment blocked STZ-induced Rac1-GTP activation which was significantly blocked NADPH oxidase activity ($p<0.001$) and mitochondrial dysfunction mediated cell death. Moreover Inhibition of Rac1-GTP using NSC23766 (50uM) inhibited STZ-induced NADPH oxidase activity ($p<0.001$) and apoptosis. Imatinib pretreatment stimulated ERK (thr202/tyr204) and phosphatidylinositol 3-kinase (PI3K) activation. Inhibition of ERK by U0126 (10uM) enhanced Imatinib effect on phosphatidylinositol 3-kinase activation and completely blocked STZ action. Interestingly, inhibition of phosphatidylinositol 3-kinase activation (PI3K) using PX-866(1uM) inhibited Imatinib protective effect against STZ. Finally, Imatinib treated mice showed reduced blood glucose levels as well as apoptotic beta cells compared to STZ-treated mice. Immunohistochemistry analysis showed enhanced insulin positive areas in the pancreatic section of Imatinib treated mice compared to STZ.

Conclusion: STZ- triggers the Rac1- mediated upregulation of NADPH oxidase activity in INS-1 cells. This is associated with increased mitochondrial dysfunction with cell apoptosis. Imatinib decreased STZ-induced Rac1-NADPH oxidase activity and apoptosis via enhancing phosphatidylinositol 3-kinase signaling.

Disclosure: **U. Karunakaran:** None.

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The role of MCPIP1 in insulin-producing cells

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Background and aims: Type 1 diabetes mellitus is an autoimmune disease, characterized by a progressive destruction and death of pancreatic beta cells. The process is induced by proinflammatory cytokines released by activated immune cells. Cytokines modulate several intracellular pathways in beta cells, including the NFkB axis. Recently a novel antiinflammatory protein MCPIP1 was described to inhibit the NFkB pathway and cytokine production in different cell types. The aim of this study was to characterize the role of MCPIP1 in insulin-producing cells upon exposure to cytokines.

Materials and methods: MCPIP1 expression was measured in rat tissues by real-time RT-PCR. Human MCPIP1 was overexpressed in rat insulin-producing INS1E cells using pcDNA3 vector or pLVX-EF1α-Tet3G-Zs-Green1 lentiviral vector (doxycycline inducible system). MCPIP1 suppression was achieved by shRNA. Positive clones were selected by resistance to G418 and puromycin. Cells were treated with a cytokine mixture (IL-1β 60 U/ml, TNFα 185 U/ml and IFNγ 14 U/ml). Glucose-induced insulin secretion was measured by RIA, viability by MTT assay, apoptosis by caspase-3-Glo assay, NFkB activation by SEAP reporter gene assay. iNOS protein expression was analyzed by Western blotting and nitrite by the Griess method.

Results: Measurements of MCPIP1 gene expression revealed that in rat islets and insulin-producing cells the level of MCPIP1 is low as compared to other tissues. The MCPIP1 expression in untreated INS1E was increased several-fold after exposure to cytokines. Overexpression of MCPIP1 in INS1E cells resulted in the protection against cytokine-mediated cell viability loss (INS1E-control cells 48 ± 2 vs. INS1E-hMCPIP1 75 ± 3%) and inhibition of cytokine-stimulated caspase-3 activation (INS1E-control cells 157 ± 9, INS1E-hMCPIP1 98 ± 3%). The transcription factor NFkB was strongly activated by cytokines in control INS1E cells (620 ± 20%), while in INS1E-MCPIP1 cells a significant reduction was observed (3-fold). Consequently, cytokine-mediated iNOS protein expression as well as nitrite accumulation was decreased in cells overexpressing MCPIP1. Interestingly, the gene expression of insulin in INS1E-MCPIP1 cells was significantly lower than that of control INS1E cells. Basal insulin secretion at 3 mM Glc was similar in control INS1E and INS1E-MCPIP1 cells. MCPIP1 overexpression resulted, however, in a significant enhancement of GSIS after incubation with 10 mM Glc or 30 mM Glc. The observed effects of MCPIP1 overexpression depended on the expression level as shown using an inducible MCPIP1 system. Suppression of the basal rat MCPIP1 expression had opposite effects to the overexpression.

Conclusion: MCPIP1 is strongly stimulated by cytokines in insulin-producing cells. MCPIP1 protects insulin-producing cells against cytokine-mediated toxicity by inhibition of the NFkB-iNOS pathway and prevention of caspase-3 activation. The observed lower transcription level of insulin together with a potentiating effect on GSIS upon cytokine exposure suggests that cytokine-mediated MCPIP1 overexpression may serve as a protective feedback mechanism by i) inhibition of cell death pathways, and also by ii) reduction of the physiological overload of insulin production, though maintaining proper glucose responsiveness.

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In type 2 diabetes, microRNA miR-184 is reduced in human pancreatic islets and targets CRTC1, a molecule that counteracts palmitate-induced apoptosis and functional damage

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Background and aims: MicroRNAs are small endogenous non-coding RNAs that negatively regulate gene expression. They are involved in the pathogenesis of several diseases, including type 2 diabetes (T2D). Recent studies have highlighted the role of miR-184 in β cell growth as a potential protective/compensatory mechanism in T2D. However, the molecular mechanism(s) that regulates this compensatory response is not entirely known. Aim of this study was to gain insight into the potential role of miR-184 in protective/compensatory response in T2D

Materials and methods: miR-184 expression was evaluated by Taqman RT Real-time PCR in purified human islets from 7 T2D and from 12 non-diabetic multiorgan donors. TargetScan7.0 algorithm was used to identify miR-184 predicted target genes. Taqman RT Real-time PCR was performed to evaluate the expression predicted target genes of potential interest in human islets from non-diabetic and T2D donors; luciferase assay was used to validate the identified target gene of interest [i.e. CRTC1 (CREB Regulated Transcription Coactivator 1)] as actual miR-184 target. CRTC1 functional effects were evaluated by overexpressing it in MIN6 β cell line by transfection with a CRTC1 encoding plasmid, in the presence or not of palmitate (0.5mM for 48h). Apoptosis was investigated by Caspase-3 staining quantified by FACS, while basal and glucose stimulated insulin secretion (GSIS - 2mM and 20mM Glu) was analyzed by ELISA. Statistical analyses were performed using Mann-Whitney U test

Results: miR-184 was significantly reduced in T2D vs non-diabetic control human islets (p=0.038); its expression did not correlate with age or BMI and was not affected by in vitro exposure of non-diabetic human islets to high glucose (22mM for 48h). Predicted target gene evaluation revealed CRTC1 as a potential target gene of miR-184. CRTC1 expression was higher in T2D vs non-diabetic human islets, in accordance with the pattern of expression of its predicted negative regulator, i.e. miR-184. The direct binding of miR-184 to CRTC1 was demonstrated by luciferase assay, thus revealing that miR-184 directly regulates CRTC1 expression. Accordingly, inhibition of miR-184 in 1.1B4 human pancreatic islet cell line resulted in increased expression of CRTC1, further demonstrating the regulation of CRTC1 expression by miR-184. As for the potential functional role of CRTC1 in β cells under stressful condition, MIN6 cells transfected with CRTC1 showed a protection from Caspase-3-mediated apoptosis (Ctr: nt 2,21±1,28% vs palm 5±5,9% p=0,0426; CRTC1-transfected: nt 1,41±2.3% vs palm 1.8±1.5%, p: NS). Moreover, CRTC1 overexpression in MIN6 cells prevented the functional damage of palmitate on GSIS [Stimulation Index (ratio between 20mM and 2mM Glucose)- Ctr: nt 9.1±3.0 vs palm 6.1±1.3, p=0,02; CRTC1-transfected: nt 7.09±4.9 vs palm 8.2±5.1, p: NS)

Conclusion: In conclusion, we hypothesize that the downregulation of miR-184 observed in T2D islets, leading to hyperexpression of CRTC1, a molecule that counteracts palmitate-induced apoptosis and functional damage, may represent a potential protective/compensatory mechanism during metabolic stress, thus identifying novel candidates for β cell rescue in T2D

Disclosure: **G. Sebastiani:** None.

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Crosstalk of fatty liver with fatty pancreas impairs islet function in humans

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Background and aims: The fatty liver produces fetuin-A, which amplifies insulin resistance and inflammation in a TLR4-dependent manner. Circulating fetuin-A concentrations are increased in obese patients with non-alcoholic fatty liver disease. In humans, a positive correlation between plasma fetuin-A concentrations and insulin resistance depends on high fatty acid levels, indicating an interaction between fetuin-A and fatty acids. Pancreatic steatosis was recently recognised as a potential factor in the pathogenesis of T2D. Elevated pancreatic fat content (up to 9% of the gland) associates with impaired insulin secretion in patients with impaired glucose tolerance. It is proposed that fatty pancreas curtails insulin secretion, but how pancreatic fat modulates islet function remains to be clarified. This work examines the crosstalk between fatty pancreas and fatty liver and deciphers its impact on islet function.

Materials and methods: 60 human pancreatic resections were immunohistochemically stained for insulin, glucagon and somatostatin. The pancreatic adipocytes were identified by oil red and adiponectin staining. Plasma fetuin-A, glucose and insulin levels were determined. Insulin secretion was estimated on the basis of disposition index (DI) obtained from oral glucose tolerance tests. Isolated human islets were co-cultured with pancreatic preadipocytes or differentiated adipocytes and the fatty liver-fatty pancreas crosstalk was mimicked by addition of fetuin-A. Insulin secretion was evaluated by ELISA and RIA.

Results: In 70% of the patients a variable degree of pancreatic steatosis, with sporadical fat infiltration of the islets, was detected. Interestingly, even islets surrounded by adipocytes displayed unaltered architecture and hormone distribution. In accordance, no correlation between pancreatic steatosis and BMI, HbA1c or DI was observed. Most interestingly, the DI negatively correlates with plasma fetuin-A levels. The co-culture of isolated human islets with pancreatic preadipocytes or differentiated adipocytes did not alter insulin secretion, while fetuin-A inhibited secretion. This effect of fetuin-A was independent of TLR4, since the inhibition of GIIS (glucose-induced insulin secretion) was still visible in TLR4 KO mouse islets. Moreover, fetuin-A inhibition of GIIS was counteracted by the JNK inhibitor SP600125, suggesting that the stress kinase JNK mediates the effect of fetuin-A on insulin secretion. Indeed, fetuin-A stimulated JNK phosphorylation in a TLR4 independent manner.

Conclusion: The hepatokine fetuin-A impairs glucose-induced insulin secretion, which, in addition to the fetuin-A-induced local inflammation, may accelerate the development of diabetes.

Disclosure: **F. Gerst:** None.

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Osteocalcin: a novel therapy to reverse the decline in functional beta cell mass in type 2 diabetes

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Background and aims: Insulin producing β-cells exposed to increased glucose levels develop abnormalities in insulin secretion, and undergo de-differentiation, with changes in gene expression and in structural and functional characteristics. Such changes are accompanied by

abnormalities in β-cell morphology that results from the loss of β-cell transcription factor such as MAFA, MAFB, NEUROD1, SIRT1 and PDX1. β-cell de-differentiation is increasingly recognized as a pathway for hyperglycemia-induced β-cell dysfunction, adaptation, and decompensation. The bone hormone osteocalcin (OCN) was found in animal models to induce β-cell proliferation and to have positive effects on peripheral insulin sensitivity, hepatic metabolism and on visceral fat and energy expenditure. Since the publication of these data, several studies examined, albeit indirectly, the relationship between serum OCN levels and glucose, lipids, and atherosclerosis in humans. In this study we examined whether OCN can reverse pre-diabetes and diabetes induced changes in human islets.

Materials and methods: For in-vitro function, human islets from healthy, obese and T2DM pancreata were cultured for 7 days with or without OCN (4.5ng/ml). Glucose-stimulated insulin release and insulin content was quantified by ELISA. For in-vivo function, an aliquot of 500IEQ of each isolated islets was transplanted under the renal capsule of NOD/SCID mice (n=5/donor islets/treatment) and assessed for human insulin and c-peptide release at 30 minutes post glucose challenge. Western blot and PCR were used to characterize the transcriptional changes caused by obesity and diabetes. Histological examination of the islets for total β-cell mass and proliferation was done by double staining of insulin and Ki67.

Results: We found that treating obese, pre-diabetic and type 2 diabetic human islets with osteocalcin restored insulin secretion in response to glucose stimulation, significantly increased insulin content and insulin protein expression. Most importantly, OCN induced an increase in human β-cell mass through proliferation. We also found, using western blot and real time PCR that osteocalcin restored expression of MAFA, MAFB, NEUROD1, SIRT1 and PDX1. Finally we showed that continuous exposure to OCN strongly enhances the metabolic response of human islets to glucose challenge, in terms of insulin and C-peptide secretion, in-vivo, following transplantation into NOD-scid mice.

Conclusion: Our results show that OCN not only restored the loss of β-cell transcription factor such as MAFA, MAFB, NEUROD1, SIRT1 and PDX1, but also increase β-cell mass through proliferation. Understanding the role of OCN in the regulation of glucose homeostasis, insulin secretion and β-cell proliferation in human islets could have immediate and wide ranging relevance to the treatment of diabetes.

Disclosure: **O.M. Sabek:** Grants; Vivian Smith Foundation.

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Eukaryotic translation initiation factor 2A (eIF2A) saves pancreatic beta cells "lost in translation" during ER stress-induced apoptosis

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Background and aims: Endoplasmic reticulum (ER) stress-induced unfolded protein response (UPR) is a well-known mechanism mediating beta cell apoptosis in diabetes. Inhibition of general protein synthesis via phosphorylation of eIF2α is key event during UPR. eIF2A has been implicated in the translation of specific mRNAs under stress conditions, such as viral infection, when general translation is similarly suppressed. Therefore, we hypothesized that eIF2A plays a key role in regulation of mRNA translation during UPR in beta cells.

Materials and methods: Thapsigargin (Tg, 1 μmol/l), palmitate (PA, 0.5 mmol/l in BSA) were used to induce UPR in MIN6 cells, primary mouse and human islets. Real-time RT-PCR and Western Blot were used to assess gene expression. Cells were infected with lentivirus encoding GFP-tagged eIF2A or just GFP; experiments were performed 72 hours post infection. Apoptosis was assessed by live cell imaging of propidium iodide stained cells. To assess protein synthesis cells were treated with 35S labeled Met/Cys media for 30 min, protein lysates collected, gel separated and radiation quantified.

Results: We showed that pancreatic islets had highest eIF2A abundance compared to other tissues. Both Tg and PA dose-dependently induced mRNA (3.5±0.4-fold and 2.0±0.3, n=6) and protein (2.2±0.2-fold and 2.1±0.2 n=6) expression of eIF2A in primary mouse and human islets, suggesting an association with the UPR. We observed the highest levels of eIF2A protein levels 24 hours after induction of UPR, which coincided with highest induction levels of ER stress markers Bip and IRE1α. We showed 84.7±6.9% (n=3) and 63.7±7.8% (n=5) reduction in thapsigargin- and palmitate-induced apoptosis, respectively, in primary mouse islet cells overexpressing eIF2A (3-fold). These finding were confirmed using primary human islets (n=3). The reduced cell death by stable eIF2A overexpression in MIN6 cells (3-fold) was associated with a 88.3±8.4% (n=5) decrease in protein expression of UPR pro-apoptotic marker CHOP. However there was no effect on the steady-state levels of CHOP mRNA during UPR, suggesting a specific role of eIF2A in the inhibition of ER stress-induced translation of CHOP mRNA. Additionally, we observed a 50.4±10.1% decrease in XBP1 splicing suggesting selective inhibition of the IRE1α branch of UPR. MIN6 cells showed decreased protein synthesis rate by 80.2±10% (n=3) after 2 hours of Tg treatment but it was unchanged in cells overexpressing eIF2A, despite normal ER stress-induced eIF2α phosphorylation. Immunoprecipitation of eIF2A showed an increase in the interaction of eIF2A with eIF2α during UPR with the maximum of 3.9±1.1 fold increase (n=4) after 24 hr of Tg treatment, which may explain uncoupling of translational initiation from eIF2α phosphorylation in cells overexpressing eIF2A.

Conclusion: We conclude that eIF2A prevents UPR-induced apoptosis in beta cells via inhibition of translational repression. We identified a novel protective role for eIF2A in the context of ER-stressed beta cells via the selective inhibition of CHOP mRNA translation and XBP1 splicing.

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Disclosure: **E. Panzhinskiy:** None.

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Comparative analysis of cytoprotective enzyme equipment in human pancreatic beta cells and non beta cells

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Background and aims: The pancreatic beta-cell is a cell type particularly vulnerable to oxidative insults due to its low antioxidative defense enzyme equipment. In particular pancreatic beta-cells are very sensitive to the beta-cell toxicity of pro-inflammatory cytokines in T1DM and lipotoxicity in T2DM. Human pancreatic beta-cells however are often considered to be better protected than rodent beta-cells. This assumption is based on the circumstantial evidence obtained in experiments with isolated intact human islets. But islets also contain non-beta-cells, which are thought to be better protected against toxicity induced by diabetogenic agents. Since human islets contain more non-beta-cells than rodent islets this may have resulted in such studies in an overestimation of the cytoprotective potential of human beta-cells.

Materials and methods: Therefore we developed a new method for quantitative in-situ PCR allowing an exact analysis of the cytoprotective enzyme equipment of each individual non-beta- and beta-cell in the islets in human pancreas. The gene expression of the cytoprotective enzymes in human non-diabetic pancreatic sections was performed by in situ PCR analysis with a specific thermal cycler. The incorporated digoxigenin-labeled nucleotides were visualised by fluorescence staining and thereafter immunostained for insulin to identify beta- and non-beta-cells in the same section. Gene expression of the enzymes was quantified

densitometrically in the beta and non-beta-cells with a computer-assisted method.
Results: Quantitative analysis of cytoprotective enzyme expression in individual islet cells revealed a clearly better protective equipment of non-beta-cells than of beta-cells. This is true for the situation in all subcellular locations with the exception of the endoplasmatic reticulum (ER). The superoxide radical inactivating isoenzymes Cu/ZnSOD in the cytosol and MnSOD in the mitochondria are highly expressed in beta-cells, not much less than in non-beta-cells (2/3). The hydrogen peroxide inactivating enzymes GPx1 in cytosol and mitochondria and catalase in peroxisomes, the subcellular sites of pro-inflammatory cytokine and lipotoxicity, respectively, are very weakly expressed (33% and less than 10% of non-beta-cells, respectively). This documents for the first time convincingly the imbalance between hydrogen peroxide generating and inactivating antioxidative enzyme capacity in human beta-cells. The only exception is the ER where the cytoprotective enzymes PRX4 and GPx7 are as strongly expressed in beta-cells as in non-beta-cells. This ER protection is also significantly higher than that in rodent beta-cells with much lower expression values in the beta-cells. No significant differences were observed between the different non-beta islet cell types.
Conclusion: These results thus explain why human pancreatic beta-cells are comparatively sensitive to pro-inflammatory cytokine and lipotoxicity but are significantly better protected against ER-stress.
Disclosure: **A. Jörns:** None.

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Metabolic stress induces aberrant activation and mislocalisation of Ras-related C3 botulinum toxin substrate 1 (Rac1) in rodent and human beta cells

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Background and aims: Metabolic activation of small molecular mass G-proteins [Rac1] has been implicated in islet beta cell function including physiological insulin secretion. However, significant knowledge gaps exist with regard to the fate of these signaling proteins, specifically Rac1, in pancreatic beta cells exposed to metabolic stress [hyperglycemia; HG]. Herein, we investigated the functional status of Rac1 [activation and subcellular targeting] in clonal beta [INS-1 832/13] cells, normal rodent and human islets under the duress of metabolic stress.
Materials and methods: INS-1 832/13 cells, rodent and human islets were incubated either under basal [2.5-5 mM] or HG [20-30 mM; 24 hr] conditions. Rac1 activation was assessed by the pull-down assay [Cytoskeleton, USA]. Purified nuclear fractionation was isolated using NE-PER kit [Thermoscientific, USA]. Human islets are from Prodo Labs [USA]. Rac1 constructs were from cDNA Resource Center [Bloomberg University, USA].
Results: Metabolic stress significantly promoted activation of Rac1 [Rac1-GTP] in INS-1 832/13 cells, normal rodent and human islets. Under these conditions, we observed a marked increase in the accumulation [translocation] of Rac1 with the nuclear fraction [2.77±0.51 fold over basal; n=3; $p<0.05$] in these cells. Interestingly, the translocation of Rac1 to the nucleus under HG conditions is significantly reduced [48±8.5% inhibition; n=3; $p<0.005$] in INS-1 832/13 cells overexpressing a dominant negative mutant of Rac1 [N17Rac1] suggesting that prior activation of Rac1 is requisite for its nuclear translocation. Furthermore, pharmacological inactivation of Rac1 [GDP-bound conformation] using EHT 1864 [a known inhibitor of Rac1] significantly prevented [44±5.5% inhibition; n=4; $p<0.005$] HG-induced nuclear translocation of Rac1 in these cells. Our hypothesis that metabolic stress induced sustained activation of Rac1 is requisite for its nuclear accumulation is further validated by our data demonstrating a sizable increase in the nuclear translocation of constitutively active Rac1 mutant [V12Rac1].
Conclusion: We provide the first evidence in rodent and human beta-cells that metabolic stress induces preferential translocation of active, GTP-bound Rac1 to the nuclear compartment. We propose that HG conditions promote sustained activation and alterations in the subcellular distribution [nuclear translocation] of Rac1, culminating in the activation of nuclear events, which could contribute to cellular dysfunction of the islet beta cell.
Supported by: VA and NIH
Disclosure: **A. Kowluru:** None.

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Plasma membrane PI(4,5)P_2 is required for normal glucose-stimulated insulin secretion

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Background and aims: Insulin secretion from pancreatic beta cells is stimulated by elevated blood glucose levels and occurs through Ca^{2+}-triggered exocytosis. The steps leading to exocytosis involves multiple ion channels in the beta cell plasma membrane, including K_{ATP} channels and voltage-dependent Ca^{2+} channels. Many ion channels are regulated by the phospholipid phosphatidylinositol 4,5-bisphosphate (PI[4,5]P_2), which can stabilize the open conformation of the channels and facilitate conductance. PI(4,5)P_2 levels in beta cells are regulated by cytosolic ATP and Ca^{2+} concentrations and can therefore vary over time, but to what extent this lipid is required for beta cell function is not well established. The aim of the present study was to investigate if plasma membrane PI(4,5)P_2 is a regulator of insulin secretion in beta cells.
Materials and methods: Recombinant adenoviral vectors carrying a light-regulated PI(4,5)P_2 phosphatase (Opto-5ptase), a PI(4,5)P_2 biosensor (PH-PLCδ1-mRFP) and a red-light emitting Ca^{2+} indicator (R-GECO) were used to transduce primary mouse and human beta cells and islets. Mouse (MIN6) and rat (INS-1E) beta cells lines were instead transfected with plasmids encoding the same fusion proteins. All cells were imaged with total internal reflection fluorescence (TIRF) microscopy. Activation of Opto-5ptase was achieved at the stage of the microscope by blue-light-induced translocation of the PI(4,5)P_2 phosphatase to the PI(4,5)P_2-rich plasma membrane. A growth hormone assay was used to measure secretion from MIN6 cells expressing Opto-5ptase.
Results: Blue-light illumination of dispersed mouse beta cells expressing Opto-5ptase resulted in the immediate loss of co-expressed PH-PLCδ1-mRFP from the plasma membrane, indicating loss of PI(4,5)P_2. In MIN6 and INS-1E cells, light-induced PI(4,5)P_2 depletion suppressed depolarization-induced Ca^{2+}-influx by 68±3% (n=49, $p<0.001$) and 72 ±3% (n=53, $p<0.001$), respectively. Somewhat less pronounced suppression was also observed in beta cells within intact human islets (51±5%, n=22, $p<0.01$). The degree of suppression correlated linearly with the loss of plasma membrane PI(4,5)P_2 ($R^2=0.84$, $p<0.001$, n=44 cells), and the Ca^{2+} influx was almost completely inhibited in cells with the most pronounced loss of PI(4,5)P_2. Stimulation of dispersed mouse beta cells with 20mM glucose resulted in pronounced oscillations of the cytoplasmic Ca^{2+} concentration that were reversibly suppressed by 41±5% (n=22, $p<0.001$) following PI(4,5)P_2 depletion. Similar suppression was observed in glucose-stimulated MIN6 cells (69±4%, n=31, $p<0.001$). Measurements of growth hormone secretion from MIN6 cells expressing Opto-5ptase revealed 49±4% (n=4, $p<0.05$) suppression of glucose-stimulated secretion following illumination.
Conclusion: Acute light-induced depletion of plasma membrane PI(4,5)P_2 suppressed glucose-induced Ca^{2+}-influx in, and secretion from, beta cells. The degree of suppression correlated with the degree of lipid depletion, such that even small changes in PI(4,5)P_2 had an impact on Ca^{2+}-influx. Conditions resulting in altered beta cell metabolism, such as diabetes, will have an impact on the metabolically regulated PI(4,5)P_2 and may help to explain the impaired Ca^{2+} signalling and insulin secretion in diabetes.
Supported by: Swedish Research Council, GG foundation, MoLPS
Disclosure: **B. Xie:** None.

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LDL receptor expression and function in human pancreatic beta cells

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Background and aims: High dose statins slightly enhance the risk of type-2-diabetes (T2D) in predisposed individuals. It is not known whether PCSK9 inhibitors may also increase this risk. We aimed to evaluate if modulations of the LDL receptor (LDLR) pathway by statins and PCSK9 alter glucose-stimulated insulin secretion (GSIS) in beta cells.
Materials and methods: EndoC-BH1 human pancreatic beta cell line were maintained in serum-depleted culture conditions and subsequently deprived in glucose (2.8mM) with or w/o mevastatin (10μg/ml), with or w/o recombinant PCSK9 (600ng/ml). Cell surface LDLR expression and fluorescent LDL uptake were measured by flow cytometry. PCSK9 secretion was measured in culture medium. GSIS assays were performed for 1h in Krebs Ringer buffer with increasing concentrations of Glucose (2.8-15mM) with or w/o Exendin-4 (0.1μM) and IBMX (500μM). Insulin was measured in culture supernatants and cell lysates.
Results: Cell surface LDLR expression was significantly up-regulated by mevastatin (+134%, $p<0.05$), whereas PCSK9 significantly reduced LDLR expression (-83%, $p<0.05$). LDL cellular uptake followed LDLR expression patterns. Similar GSIS levels were observed with or w/o mevastatin treatment and/or PCSK9 supplementation (Fig 1). Surprisingly, native EndoC-BH1 cells secrete significant amounts of PCSK9 (7.4±1ng/106 cells), which was further increased by mevastatin treatment (+112%).
Conclusion: Our in vitro data indicate that human pancreatic beta cells secrete PCSK9 and that statins and PCSK9 modulate LDLR expression and function in beta cells without apparently altering GSIS.

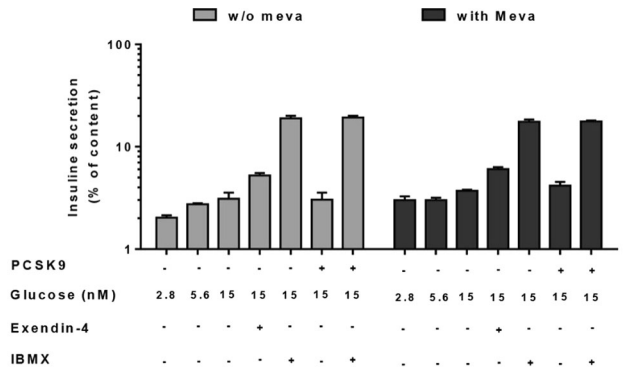

Figure 1) Impact of statin and PCSK9 on GSIS by EndoC-BH1 cells.

Supported by: Sanofi-Regeneron
Disclosure: **G. Lambert:** Grants; Sanofi-Regeneron. Honorarium; Sanofi-Regeneron.

PS 025 How do beta cells work?

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Increased SGLT2 expression with PPARγ agonist in pancreatic alpha cells reversed glucagon increase at hyperglycaemia

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Background and aims: One recent study showed that inhibition of the sodium glucose transporter 2 (SGLT2) increased glucagon secretion in pancreatic alpha cells. Troglitazone, Peroxisome proliferator-activated receptor γ (PPAR-γ) agonist, was reported to increase SGLT2 in renal proximal tubule cells, but its role on pancreatic alpha cells have not been reported. We investigated that the effect of troglitazone on SGLT2 expression in alpha cell and subsequent glucagon regulation in hyperglycemia.
Materials and methods: An Alpha TC1-6 cell line was cultured in control or hyperglycemia (15mM) for 72 hours. We applied troglitazone with or without PPARγ antagonist (GW9662 10μM). To investigate the involvement of PI3K/Akt pathway, we applied troglitazone with or without Wortmanin. We measured sodium glucose transporter 2 (SGLT2) and glucagon (GCG) mRNA and protein expression. PPAR gamma, PI3K and Akt protein were also measured.
Results: Exposure of alpha TC cells to hyperglycemia (HG) for 72h increased glucagon mRNA and protein expression. HG decreased SGLT2 mRNA and protein expression. Troglitazone significantly reversed HG-induced reduction of SGLT2 expression and increase of glucagon secretion. PPARγ antagonist (GW9662 10μM) decreased the expression of SGLT2 and increased glucagon as HG did. Hyperglycemia increased PI3K and p-Akt expression in alpha cell. Wortmanin (PI3K inhibitor, 1μM) reversed HG-induced SGLT2 decrease and glucagon increase. Troglitazone treatment decreased PI3K and p-Akt expression in HG.
Conclusion: In conclusion, PPARγ agonist, troglitazone improved glucose transport SGLT2 dysfunction and subsequent glucagon dysregulation in alpha cell under hyperglycemia. Those effects were through the involvement of PI3K/pAkt signaling pathway. This study may add one more reasons for ideal combination of PPARγ agonist and SGLT2 inhibitor in clinical practice.
Disclosure: **M. Kim:** None.

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Fluoxetine treatment impairs E-cadherin-mediated cell adhesion and altered calcium homeostasis in pancreatic beta cells

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Background and aims: Selective serotonin reuptake inhibitors (SSRIs) are the most common prescribed drugs for anxiety and mood disorders. Long term use of SSRIs is associated with an increased risk of diabetes, but the mechanism(s) underlying this association is not fully elucidated. Evidences showed that E-cadherin-mediated cell adhesion plays an important role on glucose-stimulated insulin secretion (GSIS) in pancreatic β-cells. Calcium signaling is essential for the release of insulin granules. The aims of the current study were to investigate the underlying mechanism(s) by which fluoxetine induces pancreatic beta cell dysfunction.
Materials and methods: MIN6 beta cells were used to investigate the structure of adheres junction and the distribution of E-cadherin by using immunohistochemistry. The effects of fluoxetine on membrane localization of E-cadherin were studied in MIN6 cells using biotin surface protein isolation assay and immunostaining. E-cadherin-mediated adhesion was

investigated by using chimeric proteins made of functional E-cadherin ectodomains fused to the Fc fragment of immunoglobulin (E-cad/Fc). Insulin secretion, single cell calcium measurement were determined.
Results: Fluoxetine significantly reduced GSIS of mouse insulin secreting MIN6 cells. MIN6 cells formed smaller colonies of loosely packed cells with reduced cell-cell contact after fluoxetine treatment. Immunohistochemistry revealed that E-cadherin largely accumulates in cytosol, mainly in Golgi apparatus. Fluoxetine reduces the membrane-localized E-cadherin due to increase of its endocytosis. Fluoxetine inhibits spreading of β cells attached to E-cad/Fc as well as disrupts E-cadherin-mediated actin filament. Furthermore, single cell calcium measurement indicated that fluoxetine significantly suppresses ER calcium release and the activation of store-operated calcium entry (SOCE) via reduction of ER calcium storage and inhibition of STIM1 trafficking after ER calcium depletion respectively.
Conclusion: Our results suggested that exposure to fluoxetine results in impaired β-cell function, occurring in concert with reduction of E-cadherin-dependent cell adhesion and alterations of calcium homeostasis.
Supported by: Ministry of Science and Technology
Disclosure: **Y. Chen:** None.

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Nanoscale topography promotes the survival and proliferation of human beta cells in vitro

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Background and aims: Intense research in recent years has aimed at generating sources of human b-like insulin-producing cells for drug discovery and cell transplantation therapy in diabetes. b-cell expansion in tissue culture represents the most obvious approach for the generation of insulin producing cells; however, it has been shown to be difficult. Indeed, cultured human b-cells undergo dedifferentiation and do not replicate. Although the architecture and physical interactions within and surrounding islets are complex and not completely understood, there is significant evidence that islets are heavily influenced by cell-ECM interactions. Aim of the proposed research was to evaluate the suitability of nanostructured scaffolds to support long term culture of human b-cells and islets of Langerhans in vitro.
Materials and methods: We used metal oxide layers with tailored nanoscale roughness to fabricate scaffolds for human islet cultures in vitro. We then investigated the suitability of these scaffolds to support long term culture of human islets, through assessment of β-cell differentiation, survival and function in vitro. Data were compared to islets grown on gelatin supports (control). Finally, using a proteomic approach we evaluate the molecular mechanisms involved.
Results: We found that nanostructured substrates significantly increased the number of β-cells and insulin content relative to control (25±2% insulin content; $p<0.05$; 4 different islets preparations), in long term cultures (up to 25 days). The increased survival of β-cells on nanostructured substrates was due to decreased apoptosis and necrosis, compared to control substrates (15±2% and 20±2% decrease, respectively. $P<0.05$; 4 different islets preparations). Interestingly, some proliferation was also detected (ki67 positive cells), thus suggesting that these substrates also allow β-cell replication in vitro. Furthermore, only islet β-cells grown over nanostructured scaffolds conserved intact cell morphology with several dispersed insulin granules and the glucose-sensitive insulin secretion in long term cultures. Proteomic analysis confirmed activation of a proliferative program. The process was promoted by a mechanotransductive signalling driven by nanostructured topology via remodelling of the actin cytoskeleton and nuclear architecture.
Conclusion: Understanding how to manipulate human β-cell survival and proliferation ex vivo may lead to the identification of new biotechnological or pharmacological strategies to treat diabetes
Disclosure: **C. Perego:** None.

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Brain derived neurotrophic factor induced acute and transient RhoA activation probably through truncated TrkB receptor (TrkB-t1) on MIN6 cells

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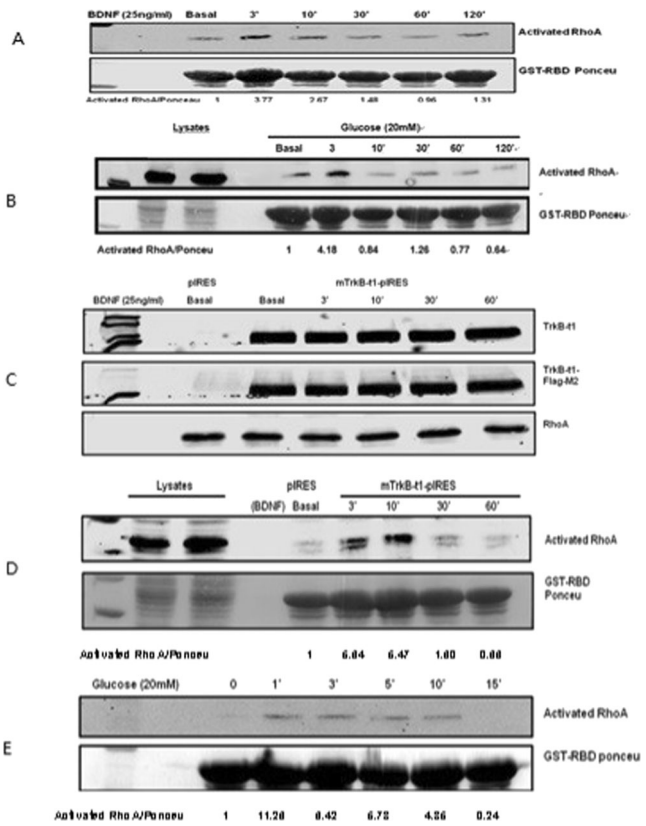
Background and aims: We previously validated that truncated TrkB receptor (TrkB-t1) was abundantly expressed on human islets and multiple rodent islet cell lines (MIN6, INS-1, and aTC-1), the function of which were largely unknown.
Materials and methods: Traditional pull down assay was used to examine the activity of RhoA in MIN6 cells treated with BDNF (25ng/ml) or glucose (20mM) for 3, 10, 30, 60, 120min respectively. CRISPR-CAS9 system and fluorescent cell sorting technology were employed to select single colonies knocking out of TrkB receptor isoforms on MIN6 cells. Cripsr TrkB knockout cells rescued w/o TrkB-t1 were stimulated with either BDNF or glucose.
Results: RhoA activities increased by more than 3 folds in MIN6 cells in response to BDNF or glucose stimulation, and the activation initiated as early as 3 minutes, and decreased back to basal level after 30 minutes. RhoA activity in MIN6 cells without TrkB expression still maintained acute and marked response to glucose stimulation. Crispr TrkB knockout cells rescued with TrkB-t1 showed a more than 5 folds of RhoA activation at 3 and 5 minutes after BDNF stimulation, and turned down to basal level at 30 minutes, the pattern of which was similar to that seen in parental MIN6 cells.
Conclusion: In contrast to what has been reported in the literature, we showed that BDNF induced acute and transient RhoA activation in MIN6 cells, which probably through TrkB-t1 receptor. The activation pattern was similar to that seen with glucose stimulation, however the latter was independent on TrkB receptor. The significance of the acute and transient RhoA activation in MIN6 cells is yet to be investigated.



Disclosure: **Z. Huang:** None.

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Comparison of alpha cell and beta cell in the response to hyperglycaemia

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Background and aims: Glucagon and pancreatic alpha cell are more and more focused as pathophysiology and treatment of type 2 diabetes. The effects of hyperglycemia on alpha cell have not been studied much in contrast to beta cells. Several studies separately reported that FoxO1 translocation in alpha cell and beta cell under normoglycemia and hyperglycemia, which were opposite. We compared pancreatic alpha cell and beta cell under hyperglycemia because FoxO1 is important to cellular stress response.
Materials and methods: Alpha TC cell and Beta TC cell were incubated with control (11mM Glucose) or high glucose (33mM Glucose) with or without PI3K inhibitor. Immunostaining of BrdU and FoxO1 was detected by green fluorescence microscope and confocal microscope. Beta cell specific transcription factors, PAX4 and MAFA, and alpha cell specific transcription factors, PAX6 and MAFB, were measured. We assessed phosphorylated FoxO1 (pFoxO1), PI3K and pAkt in both cells.
Results: Hyperglycemia decreased proliferation of beta TC cell, but increased proliferation of alpha TC cell. PAX4 and MAFA in beta cell were increased and PAX6 and MAFB in alpha cell were decreased under hyperglycemia compared to normoglycemia. Hyperglycemia decreased PI3K and p-AKT in beta cell, but increased in alpha cell. FoxO1 localizations between alpha cell and beta cell were opposite.
Conclusion: In conclusion, hyperglycemia increased proliferation and decreased differentiation in alpha cell which are opposite to beta cell. Those differences may be related to contrasting PI3K/pAkt changes in both cells and subsequent FoxO1 activation.
Disclosure: **E. Lee:** None.

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Mechanisms of the induction of incretin/cAMP responsiveness in insulin secretion: study by pseudoislets

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Background and aims: Normal regulation of insulin secretion is retained by cell-to-cell interactions between pancreatic beta-cells and/or other cell types within islets. We previously reported that formation of pseudoislets (PIs) induced incretin responsiveness in incretin-unresponsive clonal beta-cells (MIN6-K20). In this study, we attempted to elucidate the mechanisms of the induction of incretin responsiveness in PIs.
Materials and methods: PIs were formed from MIN6-K20 by seven-day culture on ultra-low attachment dishes. Morphological analysis by electron microscopy was performed on monolayer-cultured MIN6-K20 (MCCs), PIs, and mouse pancreatic islets. Insulin secretion and insulin content were measured by the batch incubation method with stimulation of incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) and the cell-permeable cAMP analogue 8-Br-cAMP. ATP and cAMP production were measured by commercial kits. Gene expressions of GLP-1 receptors and GIP receptors were measured by qPCR with TaqMan probe. Glutamate content was measured by Capillary Electrophoresis/Mass Spectrometry (CE/MS). The effect of oxamic acid (OA), an inhibitor of the malate-aspartate shuttle, on incretin- and cAMP analogue-induced insulin secretion was evaluated by batch incubation.
Results: By formation of PIs, the number of insulin granules was increased and the mitochondria were well developed: insulin content and ATP production were increased markedly. In the presence of a high concentration of glucose (16.7 mM), both GLP-1 and GIP potentiated insulin secretion from PIs in a concentration-dependent manner. Gene expression of the GLP-1 receptor did not change, but that of the GIP receptor had a tendency to increase in PIs. GLP-1-stimulated cAMP production was markedly increased in PIs. The cAMP analogue potentiated insulin secretion from PIs but not from MCCs, suggesting that the downstream signal of the cAMP pathway was also activated in PIs. Glutamate content was significantly increased in PIs. OA treatment did not affect glucose-induced insulin secretion in PIs, whereas the treatment abolished both incretin- and cAMP analogue-induced insulin secretions.
Conclusion: Our results indicate that both glutamate production through the malate-aspartate shuttle in glucose metabolism and incretin/cAMP signaling are increased by formation of PIs. Thus beta-cell to beta-cell interaction is critical for the induction of incretin/cAMP responsiveness in insulin secretion.
Supported by: MEXT
Disclosure: **M. Hashim:** None.

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The RabGAP TBC1D1 regulates glucose-stimulated insulin secretion in isolated mouse islets by modulating K^+-ATP channel function

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Background and aims: The RabGTPase-activating protein (RabGAP) TBC1D1 has been described for its role in both glucose and lipid metabolism in skeletal muscle. TBC1D1 is also expressed in pancreatic beta-cells but its role in glucose-stimulated insulin secretion (GSIS) remains to be determined. We investigated the function of TBC1D1 in GSIS in isolated islets from Tbc1d1-deficient mice and transgenic mice overexpressing Tbc1d1 in beta-cells, respectively.
Materials and methods: We isolated mouse pancreatic islets and assessed static secretagogue-induced insulin secretion, islet morphometry and expression of candidate genes by qPCR. Statistics were calculated with either t-test, one-way ANOVA or two-way ANOVA.
Results: We found that pancreatic islets exclusively express the short variant of TBC1D1 whereas skeletal muscle contains only the long isoform of TBC1D1. We generated transgenic mice on a C57BL/6J background (Tg-RIP2-D1) overexpressing the short variant of Tbc1d1 specifically in pancreatic beta-cells under control of the RIP2 promoter. TBC1D1 protein expression in Tg-RIP2-D1 islets was 2.6-fold higher compared to controls. GSIS at 25 mM glucose stimulation was substantially increased in islets from Tbc1d1-deficient mice (120.5 ± 12.7 vs. 178.1 ± 24.0 pg insulin/islet/60 min. n=7. $p<0.05$), whereas the overexpression of Tbc1d1 had no effect on GSIS (136.4 ± 26.0 vs. 135.1 ± 42.2 pg insulin/islet/60 min, n=7). In both cases insulin secretion at basal glucose (2 mM) was unchanged. Insulin secretion was still increased in Tbc1d1-deficient islets after three repetitive glucose stimulations (415.1 ± 44.1 vs. 589.4 ± 69.2 pg insulin/islet/60 min. n=5. $p<0.05$). No differences were found in general islet morphometry and integrity due to the lack of Tbc1d1, assessed by immunohistochemistry. Stimulation of islets with 1 μM of the K^+-ATP channel inhibitor glibenclamide at 2 mM glucose resulted in an increased insulin secretion in islets with Tbc1d1-deficiency (154.4 ± 10.4 vs. 229.1 ± 13.8 pg insulin/islet/60 min. n=7. $p<0.01$). Moreover, glibenclamide potentiated GSIS at 25 mM glucose in wildtype islets, but not in Tbc1d1-deficient islets. In contrast, exposure of isolated islets to 30 mM KCl or to 5 μM calcium ionophore A23187, respectively, had no genotype-dependent insulin secretion effect at basal

glucose levels. In addition, mRNA expression showed no genotype-dependent alterations in a variety of genes responsible for insulin exocytosis. Finally, we could also demonstrate that additional knockout of the close TBC1D1 homologue TBC1D4 had no additive effect on GSIS compared to the single knockout of Tbc1d1 (96.8 ± 17.3 vs. 172.8 ± 25.4 pg insulin/islet/60 min. n=7. $p<0.05$).
Conclusion: We demonstrated that the signaling protein TBC1D1 represents a novel component in the regulation of GSIS in pancreatic beta-cells and presumably modulates the function of K^+-ATP channels.
Supported by: vivid Research Training Group
Disclosure: **T. Stermann:** Grants; vivid Research Training Group.

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Conditional deletion of the (pro)renin receptor causes disturbed pancreatic beta cell homeostasis and the onset of diabetes

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Background and aims: The (pro)renin receptor (PRR) was identified a decade ago as a receptor for (pro)renin, the initiating molecular of the renin-angiotensin system (RAS). This discovery generated an enormous amount of research in the diabetes field, because of the involvement of the RAS in promoting diabetic complications such as retinopathy and nephropathy. However subsequent transgenic and conditional knockout animal models have failed to show a causal link between PRR and these conditions, and instead point towards an essential role for PRR in regulating cellular homeostasis and development. These effects appear to be central to the molecular association of PRR with the vacuolar (V) H+-ATPase, a ubiquitously expressed proton-pump. The precise function of PRR remains unknown. Here, we have employed an inducible and conditional knockout model to analyse the function of PRR in pancreatic β-cells; a cell type reliant on V-ATPase activity for proper insulin processing and secretion.
Materials and methods: We bred ATP6AP2flox/+ females with male mice expressing Cre recombinase under the tamoxifen-inducible rat insulin promoter (RIP-CreER). Male ATP6AP2flox/y;RIP-CreER offspring and controls were aged to 8 weeks to allow for normal pancreatic development, before being implanted sub-cutaneously with tamoxifen-releasing pellets to induce the conditional deletion of PRR from pancreatic β-cells.
Results: PRR β-cell knockout mice (PRR βKO) exhibited severe diabetes due to a reduction in beta-cell insulin content, coupled with a disturbance in glucose-stimulated insulin secretion. This phenotype was not due to a loss of V-ATPase acidification activity, as both insulin processing and potassium-stimulated glucose secretion in PRR βKO islets were preserved. Islet histological analysis revealed the appearance of incredibly large LC3B+ and p62+ vacuolated structures in PRR KO β-cells, which upon further inspection were found to be single-membraned, and therefore not consistent with the formation of autophagosomes. Furthermore, autophagic flux was preserved, as isolated islets cultured ex vivo with a V-ATPase inhibitor (Bafilomycin) demonstrated accumulation of LC3B-II.
Conclusion: These data identify PRR as a molecule essential for the maintenance of β-cell homeostasis; the phenotype described here is incredibly severe and observed after only 3 weeks of deletion of PRR from mature, normally developed β-cells. Molecularly, PRR appears to be a major regulator of the synthesis of multi-vesicular bodies, including autophagosomes, a phenotype which is highly reminiscent of depletion or loss of phosphatidylinositol 3-phosphate (PI3P) formation.
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Disclosure: **K.J. Binger:** None.

PS 026 Type 1 diabetes in animal models

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Altered gut morphology and microbiome composition characterise Non-Obese Diabetic (NOD) mice with accelerated diabetes progression

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Background and aims: Metabolic alterations may accelerate the onset of type 1 diabetes (T1D). However, the mechanisms underlying diabetes-promoting metabolic disorders remain largely unknown. In NOD mice, a model of T1D, accelerated diabetes development occurs after deletion of the Toll-like receptor 4 (TLR4). TLR4 may play a central role in the control of both autoimmunity and energy homeostasis and affect metabolism and interactions of the host with its microbial environment by binding free fatty acids (FA) and bacterial lipopolysaccharides. Thus, we hypothesized that accelerated progression of T1D in the absence of TLR4 entails metabolic alterations favoring diabetes development.
Materials and methods: Gut morphology and microbiome composition, triglycerides (TG), FA, parameters of glucose homeostasis and mitochondrial respiratory activity were analyzed in 70-90 days old female TLR4-expressing ($TLR4^+$) and TLR4-deficient ($TLR4^-$) normoglycemic (prediabetic) NOD mice.
Results: $TLR4^-$ NOD mice developed diabetes 56 d earlier than $TLR4^+$ mice ($p<0.001$) and featured reduced thickness of the small intestinal muscle layer ($TLR4^-$ 43±1 μm; $TLR4^+$ 49±2 μm ($p<0.01$)), suggesting reduced gut motility. Next generation sequencing showed striking differences of gut microbiome composition between $TLR4^+$ and $TLR4^-$ NOD mice as documented by a 2.2fold lower firmicutes/bacteroidetes ratio in the colon and cecum of $TLR4^-$ NOD mice ($p<0.05$). $TLR4^-$ NOD mice further had higher plasma levels of TG and FA than $TLR4^+$ mice (all $p<0.05$). Intraperitoneal glucose and insulin tolerance tests showed impaired glucose tolerance and insulin responsiveness in $TLR4^-$ NOD mice as revealed by 2.7fold greater AUC and a reduced glucose lowering effect of insulin in $TLR4^-$ NOD (all $p<0.001$). High-resolution respirometry revealed a 60-86% higher rate of mitochondrial oxygen flux in livers ($p<0.05$) but not in soleus muscles of $TLR4^-$ than $TLR4^+$ NOD mice.
Conclusion: In the absence of intact TLR4 signaling, altered gut microbiota and morphology result in a metabolic profile associated with tissue-specific oxidative capacity, which accelerates diabetes progression in this model of insulin-deficient diabetes.
Supported by: DDG, DGMIM, DZD
Disclosure: **M. Simon:** None.

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MiR-409-3p, an IFN-γ targeting miRNA, is reduced in plasma of new-onset diabetic NOD mice and mirrors its in situ pancreatic islet expression

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Background and aims: MicroRNAs (miRNAs) are small non-coding RNAs, which regulate post-transcriptionally the expression of their target genes by mRNA degradation or translational repression. miRNAs are not only widely expressed in cells and tissues, but are also secreted, thus being detectable in body fluids including plasma. Previously, it has been demonstrated that miR-409-3p targets and regulates the expression of interferon-gamma (IFN-γ), a pro-inflammatory cytokine of major importance in the development of autoimmune type 1 diabetes. Therefore the aims of this study were: (i) to characterize the expression of circulating miR-409-3p in plasma of diabetes-prone NOD mice; (ii) to analyze its expression in islet endocrine tissue and in lymphocytic infiltrates of the same mice in order to establish whether circulating miR-409-3p mirror its in situ islet expression.
Materials and methods: Total RNA from plasma of new-onset diabetic NOD mice (n=5; 12-22 weeks of age), age-matched normoglycemic NOD mice (n=5) and NOD-scid mice (n=5; 3 weeks of age) was extracted using miRNeasy kit. The expression of miR-409-3p was evaluated by real time RT-PCR and data normalized using exogenous miRNA ath-miR-159a and endogenous miRNAs miR-30e and miR-195. From the same mice analyzed for circulating miR-409-3p, laser capture microdissection (LCM) of islet endocrine tissue and lymphocytic infiltrates was performed, separately analyzing the captured islets according to the degree of infiltration. RNA was extracted from LCM-captured tissues and miR-409-3p and selected genes were evaluated using real time RT-PCR.
Results: The analysis of murine plasma samples revealed that the expression of circulating miR-409-3p was significantly lower ($p<0.001$) in new-onset diabetic NOD mice compared to age-matched disease non-progressor mice and to NOD-scid mice. Interestingly, analysis of both LCM-islet endocrine tissue and -islet lymphocytic infiltrates revealed a clear reduction of miR-409-3p expression solely in insulitic lesion of new-onset diabetic NOD mice compared to age-matched disease non-progressor mice, suggesting that the differential expression of circulating miR-409-3p mirrors that of islet-infiltrating cells. Moreover, the expression levels of miR-409-3p target gene, IFN-γ, were upregulated in LCM-islet lymphocytic infiltrates from new-onset diabetic NOD mice compared to age-matched disease non-progressor mice, showing, as expected, a miR-409-3p/IFN-γ inverse expression pattern, typical of miRNA-target gene relationship. Interestingly, in-situ IFN-γ expression in islet lymphocytic infiltrates was also inversely correlated to miR-409-3p plasma levels, both in disease progressor and non-progressor mice, thus suggesting a potential role for circulating miR-409-3p as a potential biomarker of in-situ islet inflammation.
Conclusion: In conclusion, miR-409-3p may represent a valid circulating candidate biomarker of autoimmune diabetes, mirroring the in-situ conditions and likely to be involved also in the pathogenesis of diabetes as direct regulator of IFN-γ expression.
Disclosure: **G. Ventriglia:** None.

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Exosomal microRNAs from human islet-derived progenitor cells regulate immune cell proliferation

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Background and aims: Transplantation of cadaveric islets is currently the only clinical cell replacement therapy for diabetes. One of the major factors limiting this therapy to many of the potential recipients is the need for immunosuppression. We have established and extensively characterized human islet-derived progenitor cells (hIPCs), which are generated following epithelial-to-mesenchymal transition (EMT) and proliferation of human islet cells. Characterization of hIPCs indicates that they exhibit most of the mesenchymal stem cell markers, including expression of CD73, CD90 and CD105. It is however not yet known if hIPCs exhibit immuno-regulatory properties similar to those of bona-fide mesenchymal stem cells. microRNAs are non-coding RNA molecules that are shown to fine-tune several biological processes. Very recently, they are demonstrated to shuttle between cells and are reported to be involved in inter-cellular gene regulation. We hypothesized that microRNAs are novel mediators of immune regulation and aim to understand if i) hIPCs can regulate immune cell proliferation in vitro and ii) if the miRNAs are one of the possible underlying mechanisms in this regulation.
Materials and methods: We established an in vitro co-culture system of hIPCs and human peripheral blood monocytes (PBMCs). PBMCs were stimulated using phytohemagglutinin (PHA) or anti-CD3 antibody and T cell proliferation was monitored using CFSE dilution method on a flow cytometer. miRNAs were quantified using real time quantitative PCR method. Unstimulated PBMCs, hIPCs alone and PBMCs alone were used as controls in all experiments.
Results: We observed that hIPCs can significantly inhibit in vitro proliferation of different immune cell subsets (CD4+ T, CD8+ T, CD19+ B) of (PHA)-stimulated PBMCs in a co-culture system. We observed that this inhibition is most effective when there is direct cell-cell contact, although significant inhibition is also retained in a transwell system. When microRNAs were analysed in hIPCs alone and in hIPCs co-cultured with PBMCs, we found a small subset of significantly altered microRNAs. These microRNAs showed increase in abundance in PBMCs from co-culture setting and were identified to be actively released in to the culture supernatant. Inhibition of exosomal packaging machinery (to prevent the transfer of microRNAs from hIPCs) in this co-culture setting, rescued T-cell proliferation confirming that hIPCs inhibit T-cell proliferation via transfer of exosomal microRNAs from hIPCs to T-cells.
Conclusion: We demonstrate here for the first time that hIPCs exhibit immune-regulatory properties through a novel mechanism involving inter-cellular transfer of microRNAs via exosomes. Present experiments are focused on understanding the potential of exosomal microRNA mimics in protection of transplanted islets into mice with Type 1 diabetes. We believe that this study will further help in understanding potential role of these novel immunoregulatory molecules for improving graft survival in islet transplantation for diabetes.
Supported by: Diabetes Australia Research Trust
Disclosure: **M.V. Joglekar:** None.

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CD4 immunomodulation by monoclonal RIB5/2 antibody in LEW.1AR1-iddm rats: long-term protection against islet infiltration and beta cell destruction

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Background and aims: The LEW.1AR1-iddm rat is an animal model of spontaneous autoimmune diabetes. Islet infiltration occurs within a narrow time range between 40 and 60 days after birth with proinflammatory cytokine peaks in blood PBMCs. The rat-specific anti-CD4 antibody RIB5/2 confers temporal internalization of the T-cell receptor complex without depletion of T-cells. The aims of this study were to investigate how short-term RIB5/2 treatment would affect (1) islet infiltration with manifest diabetes and (2) T-cell subpopulations in the peripheral blood.
Materials and methods: Normoglyceamic prediabetic LEW.1AR1-iddm rats (n= 17) were treated with the monoclonal anti-CD4 antibody RIB5/2 between day 40 - 50 (5 x 5 mg ab/kg b.w. i.p.). Rats were monitored up to

manifestation of diabetes or in case of normoglycaemia until day 120. Pancreatic sections were characterized for insulitis and beta cell mass (T-cells, cytotoxic T-Cells, activated T-cells, macrophages, insulin positive cells). Blood samples were taken at day 50, 60, 75, 120 or directly after diabetes manifestation for the quantification of CD4+, CD8+, CD4+/CD25+ and CD8+/CD25+ T-cells.

Results: The short-term application of the RIB5/2 antibody significantly reduced diabetes incidence in LEW.1AR1-iddm rats from 60% to 11%. Immune modulation by the RIB5/2 antibody resulted in a significant reduction of CD4+ T-cells from 43% to 15%, which regenerated after treatment within 10 days lasting until day 120. During treatment CD4 modulation was accompanied by a weak depletion of CD4+ T-cells and a compensatory expansion of CD8+ cytotoxic T-cells (26% - 30%) in the peripheral blood of LEW.1AR1-iddm rats. Sham-treated rats showed no changes in CD4+ and CD8+ T-cell populations. After day 60 T-cell populations remained stable with 43% CD4+ T-cells and decreased levels of 15% CD8+ T-cells. Normoglycaemic RIB5/2-treated rats were completely devoid of islet infiltration and preservation of beta cell mass. In contrast the insulitis score in diabetic rats was not different to untreated controls with massive infiltrations by macrophages and T-cells (TCR+ cells, CD8+ T-cells, CD25+ T-cells, CD68+ cells).

Conclusion: Temporal CD4+ T-cell silencing by anti-CD4 RIB5/2 antibody treatment conferred immune tolerance without significant immunosuppressive side effects in the LEW.1AR1-iddm rats. The treatment induced permanent tolerance against beta cell autoimmunity in 80% of the treated rats with prevention against islet infiltration and beta cell destruction. CD4+ T-cells pools completely recovered after treatment. T-cell silencing by modulating antibodies may be an attractive option for prevention of human T1DM in autoantibody-positive high-risk individuals.

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Disclosure: **T. Schoeppe:** Grants; EFSD/GSK.

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Experimental gene therapy of type 1 diabetes

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Background and aims: Insulin-dependent diabetes mellitus is associated with a nearly complete destruction of insulin-producing pancreatic beta-cells, which leads to insulin deficiency in the body. In spite of the fact that type 1 diabetes is a multifactor disorder, to date there is no other method of treatment of the disease except insulin therapy. A radical treatment may be provided only by gene therapy. A therapeutic effect of injected genetic constructions is reached at the expense of additional synthesis of proteins in cells as a result of the injected gene's expression.

Materials and methods: We have optimized the system of in vivo delivery of a constructed vector containing human proinsulin gene, to hepatocytes of animal models using polyplexes. The construction consists of two modules: a sequence of bacterial plasmid that allows its replication in Escherihia coli, and an expression cassette with a target gene of proinsulin. We have used a streptozotocin (STZ) model of diabetes in Wistar rats and C57BL/6j mice. Male animals were injected for a period of 5 days with STZ 50 mg/kg. The effect of injected recombinant molecules was assessed according to glucose in blood. Determination of human insulin, C-peptide in blood serum of rats was performed using reagent kits "Insulin ELISA", "C-Peptide ELISA" ("DRG Instruments GmbH", Germany).

Results: The experiment has shown - under conditions of administration to diabetic mice of a preproinsulin gene - a significant decrease in blood glucose level in 85% of experimental animals. A tendency to a decrease in glucose levels has also been revealed in experiments with plasmid control. Proinsulin gene transfection led to a decrease in blood glucose levels in C57BL/6j mice and Wistar rats with different duration of STZ diabetes: 16 days and 36 days. After administration of a plasmid with preproinsulin gene to mice on day 16 and 36 of STZ diabetes development, a decrease was noted, in glycemia level from 18.78±1.74 to 9.78±1.08 mmol/L, and from 23.63±2.10 to 10.97±2.22 mmol/L (respectively) two weeks after experiment started. Proinsulin target gene administration to rats on day 16 and 36 of STZ diabetes development led to a significant decrease in blood glucose levels from 24.77±1.49 to 8.0±1.79 mmol/L, and from 31.10±1.84 to 13.40±3.67 mmol/L (respectively) two weeks after experiment started. Administration to liver parenchyma of rats with STZ diabetes of different concentrations of DNA preparations (within the range 30 to 40 μg of DNA per 200g of body weight) allowed to determine optimum DNA doses that led to a significant decrease (normalization) of blood glucose levels. Single administration of different concentrations of DNA preparations into rat liver (25 to 60 μg) and mouse liver (10 to 15 μg) allowed to determine optimum DNA doses which led to a significant and steady (2 months) decrease in hyperglycemia in diabetic animals. A positive correlation has been revealed between the levels of human insulin and C-peptide in blood serum of rats with STZ Diabetes.

Conclusion: A more pronounced decrease in glucose levels and a higher survival rate in mice was noted when using DNA at a dose 15 μg/20 g of body weight, compared with the group of mice which were administered with a dose 10 μg/20 g of body weight. These results show that regression of diabetes has been achieved after a single course of gene therapy for STZ diabetes in rodents.

Disclosure: **O. Kovzun:** None.

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Engineering of a novel beta cell specific single chain variable fragment (ScFv) for imaging

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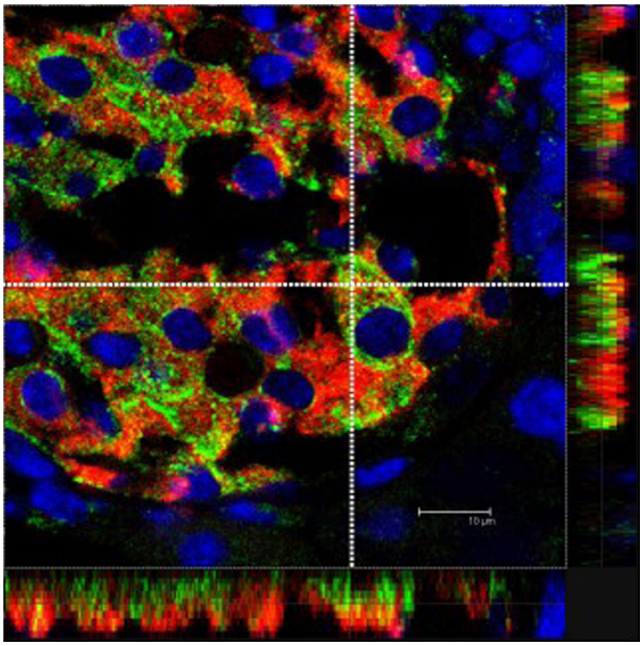
Background and aims: To assess the fate and outgrowth of endogenous as well as transplanted β-cells, minimally invasive/noninvasive probes are desirable to allow their repeated qualitative and quantitative assessment in the living animal. To this end, we generated a recombinant β-cell specific single chain variable fragment (ScFv) in a mammalian expression system and investigated its properties.

Materials and methods: The recombinant ScFv was expressed in 3T3 mouse fibroblasts and then purified from conditioned media (CM) by ammonium sulphate (AS) fractionation, metal ion affinity chromatography (IMAC) and gel filtration (SEC). Its purity was analyzed by SDS-PAGE/Western Blot (WB) and SEC, and the specific targeting of β-cells was assessed by immunofluorescence (IF) analysis on the murine β-cell line MIN6 as well as primary pancreatic sections from C57BL/6 mice.

Results: SDS-PAGE and WB analysis identified the integrity of recombinant ScFv and showed that the relative molecular weight was 35 and 70kD in CM fractions from 40 to 70% AS, as well as in the yields after IMAC and SEC purification, indicating that the recombinant protein may exist in several forms. Nevertheless, the recombinant protein preparations were able to recognize cytoplasmic structures in MIN6 cells and in insulin positive cells in murine pancreatic sections. Below, a confocal image from a murine pancreatic section is shown. It depicts the different spatial distribution of the structures stained with the ScFv (green) and anti-insulin (red).

Conclusion: A secretable form of a recombinant ScFv specific for pancreatic β cells has been generated, and its unique characteristics allow further potential applications as an immunodiagnostic and immunotherapeutic reagent.



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The impact of extracellular and cell-autonomous regulation of beta cell replication differs with ageing

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Background and aims: β-cell replication is the major mechanism for β-cell mass expansion in the young pancreas, but it significantly declines with aging. We analyzed the contribution of extracellular and cell-autonomous regulation of β-cell replication during aging and the molecular targets that could be potentially valuable for defining strategies to induce β-cell regeneration.
Materials and methods: β-cell replication was determined by BrdU incorporation in islets from 6-8 weeks (young, Y), 12 months (middle-aged, M-A) and 18 months old (old, O) rats exposed to several extracellular signals. Ex vivo, β-cell replication was assessed in islets exposed to high glucose (22mM) and IGF-2-overexpression (Ad-Igf-2). In vivo, β-cell replication was assayed in islets transplanted to hyperglycemic (Hy) or normoglycemic (No) recipients, and in islets transplanted heterochronically (Hc; aged to young) or isochronically (Ic; age-matched donors and recipients). Subpopulations of replicating β-cells were identified by sequential labelling with thymidine analogues (EdU and BrdU). The gene expression profile related to β-cell replication was determined in FACS-sorted replicating vs quiescent β-cells.
Results: β-cell replication in the pancreas of middle-aged and old rats was significantly lower than in young rats (Y: 1.28±0.16%; M-A: 0.13 ±0.03%; O: 0.11±0.02%; $p<0.05$ Y vs M-A and O). Ex vivo, β-cell replication was significantly increased in middle-aged and old islets exposed to high glucose and IGF-2 (M-A; 5mM: 1.41±0.07%; 22mM: 5.36 ±1.41%; Ad-igf2: 2.96±0.48%; $p<0.05$, 5mM vs 22mM and Ad-Igf2) (O; 5mM: 0.21±0.05%; 22mM: 0.89±0.26%; Ad-Igf2: 0.48±0.14; $p<0.05$, 5mM vs 22mM and Ad-Igf2). Middle-aged islets completely rejuvenated in terms of β-cell replication upon stimulatory conditions, and showed similar replication levels than young islets (Y; 5mM: 1.48±0.23%, 22mM: 4.7±0.7%, Ad-Igf-2: 1.66±0.34%). In contrast, β-cell replication of old islets was significantly lower compared with young and middle-aged islets ($p< 0.05$). In vivo, β-cell replication was significantly increased in middle-aged and, to a lesser extent, in old islets under hyperglycemic and heterochronic transplantation settings (M-A; Ic-No: 0.29±0.01%, Ic-Hy: 1.37±0.05%, Hc: 0.51±0.04%; $p< 0.05$) (O; Ic-No: 0.11±0.04%, Ic-Hy: 0.92±0.11%, Hc: 0.28±0.01%; $p< 0.05$). Ex vivo sequential labelling of replicative β-cells revealed that β-cell proliferation uniformly occurred across the β-cell lineage in young islets, as previously shown in vivo. In contrast, the β-cell replicative pool was significantly reduced in old islets. The gene expression profile of purified β-cells revealed differential expression of transcription factors and epigenetic modulators in replicating β-cells, along with the expected changes in cell cycle regulators and insulin.
Conclusion: β-cells from middle-aged and old rats retain the capacity to proliferate in response to extracellular signals. In middle-aged islets, the β-cell mitotic index primarily depends on the extracellular milieu. In contrast, in old islets, cell-autonomous mechanisms also regulate β-cell replication and may contribute to the aging-related reduction of the β-cell proliferative pool. These results suggest that to achieve a robust potentiation of β-cell replication in older subjects, intracellular targeting may be required.
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Disclosure: **N. Tellez:** None.

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Porcine neonatal pancreatic cell clusters maintain their pluripotency in culture and after transplantation

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Background and aims: Surrogate transplantable β cells pave the way to correct β-cell deficit in diabetic subjects. Previous data showed that transplantation (Tx) of isolated porcine neonatal pancreatic cell clusters (NPCCs) in diabetic mice required more than 8 weeks to ameliorate hyperglycemia and the frequent detection of ductal epithelial cells in grafts arguing that initial NPCC grafts could possibly exhibit progenitor-like phenotype.To further clarify the cell identity of NPCCs grafts, we examined the molecular profile of NPCCs from 1-day neonatal pigs before and after Tx.
Materials and methods: Pancreases obtained from 1-day-old neonatal pigs were cut into fragments, digested by collagenase and then maintained in culture. Six hundred NPCCs were transplanted under the kidney capsule of nondiabetic nude mice. The mRNA expression of insulin, glucagon and carboxypeptidase B (CPB) in isolated NPCCs were detected by semi-quantitative RT-PCR.Isolated NPCCs and NPCCs grafts were fixed, sectioned and stained with immunofluorescence for insulin, glucagon, somatostatin, pancreatic polypeptide, CPB, PDX-1, SOX-9 and Ki67.
Results: In agreement with previous finding, RT-PCR and immunofluorescence staining analysis showed up-regulated endocrine and decreased exocrine protein/mRNA expression over 4-day cultivation implying gradual enrichment of endocrine population in cultured NPCCs. Among all endocrine cells, the dual hormonal (insulin+/glucagon+, insulin+/somatostatin+ and insulin+/pancreatic polypeptide+) precursor cells were detected in cultured NPCCs. Strikingly, major population of cultured NPCCs were positive for progenitor markers of Pdx1 (83.7%, 72.7%, 77.3% and 77.1% in 1-, 2-, 3- and 4-day culture, respectively) and Sox9 (66.5%, 75.4%, 78.5% and 76.4% in 1-, 2-, 3- and 4-day culture, respectively) indicating that pluripotent pancreatic progenitors were quickly activated after isolation and could be propagated in vitro. On the contrary, the preliminary data showed a gradual increase of insulin+ cells (8.64% and 10.06% in 9-day and 16-day NPCC grafts, respectively) in accompany with a decreased glucagon immunoreactivity (8.15% and 3.70% in 9-day and 16-day NPCC grafts, respectively) post-Tx. Interestingly, Pdx1+ cells

in NPCC grafts were down-regulated in NPCC grafts (40.70% and 46.49% at 9 and 16 days post-Tx, respectively) compared to those in NPCC cultures implying that cultivated pancreatic progenitors quickly loss their pluripotency and undergo differentiation within the first 2 week post-Tx. The presence of insulin+ki67+ cells and insulin+ cells budding out from Pdx1+/Sox9+ ductal epithelium in NPCCs grafts further suggested that both replication and neogenesis could be possible underlying mechanisms for NPCCs maturation after Tx.

Conclusion: Our results demonstrated that islet precursors could be activated during NPCCs isolation. NPCCs derived progenitors maintain their pluripotency in culture and in early stage post-Tx providing a useful platform to examine potential insulintropic promoters involving in NPCCs differentiation.

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Disclosure: **W. Li:** None.

PS 027 The control of insulin sensitivity by the endocrine system

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Circulating levels of cartilage intermediate layer protein 2(CILP-2) are elevated and associated with insulin resistance in patients with type 2 diabetes

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Background and aims: A large-scale gene-centric meta-analysis of 39 multiethnic T2DM association studies identified Cartilage intermediate layer protein 2 (CILP-2) as a T2DM risk loci. However, to date, no study has demonstrated a relationship between CILP-2 and insulin resistance in humans. This study was designed to investigate whether the CILP-2 is a secreted protein and identify its association with insulin resistance via cross-sectional and interventional studies.

Materials and methods: A total of 300 subjects, including 103 newly diagnosed patients with T2DM (nT2DM group), 92 subjects with IGT (IGT group) and 105 age-matched healthy controls, were recruited in this study. CILP-2 was identified as a secreted protein via an in vitro secretion study. Creating the plasmid pIRES2- CILP-2-Flag and transfect HEK-293T cells. Circulating CILP-2 was determined by an ELISA and was compared with various parameters related to insulin resistance in subjects with healthy, IGT and nT2DM subjects. EHC was performed in 20 normal subjects. 30 patients were recruited from the nT2DM group received subcutaneous Exenatide (5μg) injections twice daily for 12 weeks. Skeletal muscle and visceral (omental) adipose tissues were obtained from 15 patients with T2DM and 15 controls undergoing intra-abdominal surgery. Real-time PCR and western blotting were used to assess the mRNA and protein expression of CILP-2.

Results: CILP-2 was detected in both conditioned medium and lysates of HEK-293T cells transfected with an overexpressed vector and in human blood. In human study, there were significantly higher circulating CILP-2 levels in IGT and nT2DM relative to normal controls. Circulating CILP-2 positively correlated with WHR, TG, HbA1c and HOMA-IR, but negatively with HDL-C in all population. The relative risks for T2DM were significantly elevated along with increasing CILP2 quartiles. CILP-2 was also affected by oral glucose challenge, hyperinsulin and exenatide treatment. The mRNA and Western blot results showed that CILP-2 was expressed in both human skeletal muscle and adipose tissues. In addition, CILP-2 expression was significantly increased in muscle tissues in patients with T2DM when compared with controls, but not in adipose tissues.

Conclusion: CILP-2 may be a cytokine associated with insulin resistance in humans.

Clinical Trial Registration Number: ChiCTR-OCS-13003185
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Disclosure: **S. Wu:** None.

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Metabolic impact of normal thyroid hormone levels: a prospective study

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Background and aims: While both hyperthyroidism and hypothyroidism have been shown to be associated with a degree of insulin resistance, the relationships between thyroid hormone levels within the normal range

and metabolism are controversial. We explored these relationships systematically both in cross-sectional data and longitudinally in a large cohort of non-diabetic subjects.
Materials and methods: In 1,016 male and female participants (771 with normal glucose tolerance (NGT), 149 with impaired fasting glycaemia (IFG) and 96 with impaired glucose tolerance (IGT)) followed up for 3 years with complete data, we measured insulin sensitivity (by a euglycaemic clamp) and beta cell function (by C-peptide modelling of the OGTT). Free triiodothyronine (fT3), free thyroxine (fT4) and thyroid-stimulating hormone (TSH) were measured in fasting serum samples.
Results: At baseline, fT3 levels were higher in IFG/IGT than NGT ($p<0.03$), while fT4 and TSH were similar. Across quartiles of increasing fT3 levels, there emerged clinical and metabolic features of insulin resistance (male sex, higher BMI, waist circumference, heart rate, systolic and diastolic pressure, fasting and insulin-suppressed non-esterified fatty acids, and triacylglycerols, and lower HDL-cholesterol levels). Underlying these abnormalities were significant reciprocal relationships between fT3 and insulin sensitivity ($p<0.0001$) and beta cell glucose sensitivity ($p=0.0042$). In multivariate analysis adjusting for centre, sex, familial diabetes, age, BMI and waist girth (i.e., the main correlates of insulin sensitivity), fT3 was reciprocally, and fT4 was directly, related to clamp-derived insulin sensitivity (partial r's of -0.09 and 0.10, $p=0.004$ and 0.002, respectively), but neither hormone nor TSH was related to beta cell glucose sensitivity. When analysing 3-year data, baseline fT3 was positively related to changes in (a) fasting glucose, (b) 2-hour glucose, (c) fasting insulin, and (d) systolic blood pressure, and inversely related to changes in insulin sensitivity ($p<0.003$) and beta cell glucose sensitivity ($p<0.01$) after controlling for baseline covariates. Subjects in the top quartile of fT3 levels (3.2±0.3 pg/mL) had an adjusted odds ratio of 1.74 [95%CI:1.16-2.62] vs those in the bottom quartile (2.3±0.2 pg/mL) of having a follow-up change in fasting glucose within the top quartile of its distribution (+0.7±0.4 mmol/L).
Conclusion: In the non-diabetic and prediabetic state, serum fT3 levels within the normal range are independently associated with insulin resistance, and high-normal fT3 predicts deterioration of glucose tolerance and blood pressure.
Disclosure: **E. Ferrannini:** None.

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Stimulatory effects of the long-acting kisspeptin analogue TAK-448 on serum testosterone in men with type 2 diabetes and hypogonadotropic hypogonadism

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Background and aims: Exogenous kisspeptin stimulates testosterone in hypogonadal men with type 2 diabetes (T2DM); long-acting kisspeptin analogues may have therapeutic potential. The study aimed to evaluate serum testosterone (ST) responses to different doses and frequencies of TAK-448, an oligopeptide analogue of the fully active 10-amino acid C terminus of kisspeptin-54.
Materials and methods: Open-label, adaptive design assessing ST responses to varying doses of TAK-448 in hypogonadotropic hypogonadism (HH) males recruited at a Clinical Research Unit Centre for Diabetes, Endocrinology and Metabolism. Fifteen overweight men with T2DM and HH, defined as two morning total ST values ≤12 nmol/L and normal luteinizing hormone, were given TAK-448 0.3, 1.0 or 3.0 mcg once- or twice-weekly. Primary outcome measures were trough ST concentrations and area under the effect curve (AUEC)(0-72h) percentage changes after 4 weeks of TAK-448 administration.
Results: TAK-448 was well tolerated. First dose mean ST AUEC(0-72h) responses ranged from 11% after 0.1 mcg to 53% after 3 mcg. In contrast, after the last dose, a marginal benefit (9%) was seen for 1 mcg once-weekly with a reduction (-4%) for 3 mcg once-weekly. Following 0.3 mcg weekly or 0.1 mcg twice weekly similar levels of responses were seen after the first and last dose. 0.3 mcg given twice weekly showed a reduction in response compared to baseline (-4.4%). No regimen increased mean post dose trough ST levels above 10.4 nmol/L. Once-weekly 3.0 and 1.0 mcg dosing produced trough ST levels below baseline, indicating desensitization. Twice-weekly 0.3 and 0.1 mcg dosing also showed desensitization. Once-weekly 0.3 mcg did not show desensitization overall but did for some individual profiles.
Conclusion: TAK-448 stimulated ST secretion acutely in a dose dependent manner. Repeated administration produced desensitization of ST responses for all regimens except 0.3 mcg once-weekly. No dosing regimen that maintained testosterone secretion within the normal range could be identified.
Clinical Trial Registration Number: NCT02369796
Supported by: Takeda Development Centre Europe Ltd
Disclosure: **J.T. George:** None.

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The power of thyroid stimulating hormone to predict cardiovascular mortality in non-obese patients is significantly modulated by type 2 diabetes

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Background and aims: Elevated thyroid stimulating hormone (TSH) is associated with cardiovascular risk factors, in particular with hypercholesterolemia, diabetes and obesity. Our aim was to investigate the association between TSH and cardiovascular mortality in non-obese patients with our without type 2 diabetes (T2DM).
Materials and methods: We measured TSH in a high-risk cohort of 1741 non-obese patients undergoing coronary angiography for the evaluation of suspected coronary artery disease. Prospectively, the incidence of vascular events was recorded over 10 years.
Results: At baseline, TSH was not significantly different between patients with T2DM (n=502) and those who did not have T2DM (2.31±4.59 vs. 2.06±2.66 μU/ml; $p=0.446$). During follow-up, 553 patients suffered vascular events; the event rate was significantly higher in patients with T2DM than in non-diabetic subjects (40% vs. 29%; $p<0.001$). TSH proved to be a strong and independent predictor of vascular events in non-obese patients without T2DM (standardized adjusted HR 1.34 [1.03-1.66]; $p=0.013$), but not in those with T2DM (HR 0.963 [0.894-1.037]; $p=0.315$). An interaction term TSH x T2DM was significant ($p=0.026$), indicating that TSH was a significantly stronger predictor of vascular events in non-obese subjects without T2DM than in those with T2DM.
Conclusion: We conclude that the power of thyroid stimulating hormone to predict cardiovascular mortality in non-obese patients is significantly modulated by the presence of T2DM.
Disclosure: **A. Vonbank:** None.

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The combined effect of PTH and vitamin D on glucose homeostasis in elderly prediabetics: it takes two to shine

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Background and aims: The aim of this study was to investigate the potentially combined effect of 25-hydroxyvitamin D [25(OH)D] and

parathyroid hormone (PTH) on b-cell function, insulin homeostasis and glycemia, in an elderly prediabetic cohort compared to healthy age-matched controls.
Materials and methods: A total of 144 prediabetic patients (age >65 years) (Pre DM group) and 81 healthy age - matched controls (control group) were included in this cross-sectional study. Anthropometric parameters [age, body mass index (BMI), waist circumference] and dietary intake of calcium and vitamin D (vit D) were recorded. A morning fasting plasma sample was obtained for fasting plasma glucose (FPG), insulin, PTH, 25(OH)D, calcium and phosphorus measurements. Homeostatic model assessment of insulin resistance (HOMA-IR) and b-cell function (HOMA-β %) were calculated accordingly.
Results: All participants were initially stratified into groups according to 1) vitamin D status and 2) tertiles of PTH, respectively. Vit D status was classified as per Institute of Medicine guidelines as vitamin D deficient [25(OH) D ≤20 ng/ml] or vit D sufficient [25(OH) D ≥ 20ng/ml]. The tertiles of PTH were defined as 1st tertile (PTH ≤30 pg/ml), 2nd tertile (PTH >30 pg/ml and ≤ 40 pg/ml) and 3rd tertile (PTH ≥40 pg/ml). The effect of 25(OH)D and PTH status alone (PTH or Vit D status) and in combination (PTH and vit D status) on glucose homeostasis in both groups (FPG, insulin HOMA-IR and HOMA-β %), was examined by ANCOVA and Wilcoxon rank sum test for continuous variables and the x2 test for categorical variables. No statistically significant difference in age, BMI, 25(OH) D, PTH or calcium concentrations was detected between groups. Both groups were vit D deficient [control vs Pre DM group: 17.88 vs 19.59 ng/ml respectively (p=0.365)]. Participants with VitD deficiency in both groups did not differ in glucose homeostasis markers compared to those with vit D sufficiency. In the Pre DM however, participants in the 3rd tertile of PTH had significantly higher values of HOMA-IR and FPG [(3.06 ±0.21, p=0.045) and (103.28 (101.28-105.27),p=0.011,respectively] compared to Pre DM in the 1st and 2nd tertile of PTH. Such an effect was not evident in the control group. Finally, when the impact of vitD and PTH status was assessed in Pre DM group in combination, participants with vit D deficiency /PTH in 3rd tertile (n=72) demonstrated [103.51 (101.52-105.51) mg/dl] significantly higher (p=0.027) FPG concentrations compared to the reference group (vitamin D sufficiency and PTH in the 1st/2nd tertile). Individuals, at the vit D sufficiency/PTH in 3rd tertile group, also demonstrated significantly (p=0.015) higher FPG concentrations [102.80 (100.8-104.79) mg/dl] compared to the reference group, but significantly lower (p=0.024) FPG, compared to the group of vit D deficiency /PTH in 3rd tertile (p=0.018).
Conclusion: Findings of this study indicate that PTH concentration could significantly affect the impact of hypovitaminosis D in elderly prediabetics. Potential deteriorating effects on glucose homeostasis in prediabetics with vitamin D deficiency/insufficiency in conjunction with lower PTH, were not observed. However, evaluation of vitamin D deficiency in conjunction with PTH status, in concentrations ≥40 pg/ml, might better reflect the potential dysglycemic effects of vitamin D/PTH axis in prediabetic cohorts.
Disclosure: **K. Kotsa:** None.

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Neuregulin-1 decreases hepatic glucose production in db/db mice

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Background and aims: Previous studies from our laboratory had shown that neuregulin-1 (NRG1) has a role in glucose homeostasis, improving glucose tolerance in db/db mice. A predominant role of the liver has been suggested, since NRG1 receptors were activated only in the hepatic tissue, but not in skeletal muscle or adipose tissue. Our aim was to study whole body insulin sensitivity and hepatic response to an acute treatment with NRG1.
Materials and methods: Three hour fasted 9-week old db/db mice were injected with NRG1 (50μg/kg) or saline (SAL) as control. After 2h, a hyperinsulinemic-euglycemic clamp was performed. A different group under the same conditions, was used to study hepatic signalling after the NRG1 or SAL injection.
Results: Two hours after injection, insulin levels were lower in db/db mice injected with NRG1 (38±9 SAL vs 18±4 NRG1, ng/ml). As consequence, this group of mice showed lower glucose infusion rate (GIR) during the clamp (21±2 SAL vs 13±3 NRG1, mg/kg•min). Despite lower insulin levels, glucose turnover (Rd) and hepatic glucose production (endo-Ra) were significantly decreased in the presence of NRG1 (Rd: 32±3 SAL vs 24±2 NRG1, mg/kg•min. EndoRa: 10±1 SAL vs 7±1 NRG1, p<0.05; mg/kg•min). Tissue-specific glucose uptake was not different. Liver Akt phosphorylation (Ser 473) ratio was significantly higher in db/db mice 2h after NRG1 treatment (1±0.4 SAL vs 4.5±0.9 NRG1, p<0.05, arbitrary units). GSK3-β phosphorylation (Ser9) ratio was not different. Hepatic gene expression of G6pc was lower in db/db mice treated with NRG1 (Rq 1±0.03 SAL vs 0.6±0.1 NRG1). Hepatic glycogen content was not different among groups.
Conclusion: Acute (2h) treatment with neuregulin-1 caused a decrease in insulin levels, glucose turnover, and hepatic glucose production during the hyperinsulinemic-euglycemic clamp. The stronger inhibition in endo-Ra with lower insulin levels, may reflect an increase in hepatic insulin sensitivity and/or a direct effect of NRG1 on hepatic glucose production. The increased Akt phosphorylation ratio and decreased G6pc gene expression could be involved in the mechanism used by NRG1
Supported by: UBP (YFO), DK059637 (MMPC)
Disclosure: **Y.F. Otero:** None.

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Chronic leptin treatment in insulin-deficient mice does not induce resistance but leptin is inefficacious in obese insulin-deficient mice

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Background and aims: Leptin regulates body weight and glucose homeostasis. Superphysiological levels of leptin reverse hyperglycemia in insulin-deficient mice without altering body weight. Obese rodents and humans are hyperleptinemic and this may cause leptin resistance. We sought to determine whether chronic hyperleptinemia in insulin-deficient mice leads to resistance in the glucose lowering actions of leptin.
Materials and methods: We administered a vehicle (controls) or plasmid encoding the mouse *Obese* gene by hydrodynamic gene delivery to streptozotocin (STZ)-diabetic mice at 3 different doses (0.5, 5, and 50 μg) and body weight and blood glucose levels were tracked for 4.5 months. We performed glucose tolerance tests (days 16 and 112). A separate cohort of mice was rendered obese and hyperleptinemic with 12 weeks of high-fat diet feeding then injected with STZ. We then delivered vehicle or plasmid encoding leptin and tracked body weight and blood glucose levels for 7 days.
Results: STZ induced hyperglycemia and body weight loss compared to non-diabetic controls. Hydrodynamic delivery of the mouse *Obese* gene increased plasma leptin to physiological levels by day 2 in STZ-mice for the low dose compared to vehicle controls (3.3±0.4 vs 0.3±0.04 ng/ml) similar to that of healthy mice (3.7±0.4 ng/ml) and to superphysiological levels for the medium and high doses compared to vehicle controls (51.5 ±11.6 vs 0.2±0.3 ng/ml and 511.8±104.9 vs 0.3±0.1 ng/ml). Despite restoration of leptin to non-diabetic levels, blood glucose levels were not lowered by day 8 with low dose leptin compared to vehicle (20.2±2.1 vs 24.1±2.8 mM). In contrast, superphysiological levels of leptin robustly lowered blood glucose levels by day 8 at medium and high doses (6.0±1.1 and 2.5±0.3 mM) compared to vehicle controls (21.8±3.7 and 24.9±2.5

mM), without differences in body weight. Plasma leptin levels remained within the superphysiological range at medium and high doses until the end of our study (day 123; 38.2±6.5 and 101.6±35.4 ng/ml), and leptin continued to normalize blood glucose levels (day 123; 12.9±1.5 and 10.5 ±1.3 mM) despite prolonged hyperleptinemia. During glucose tolerance tests on days 16 and 112, the vehicle controls were severely glucose intolerant. On day 6, glucose excursion was significantly improved in medium and high dose leptin groups mirroring the excursion of non-diabetic controls and similar improvements were observed post chronic leptin therapy on day 112. Diet-induced obese mice were hyperleptinemic (114.5±7.1 ng/ml) and mildly hyperglycemic (12.8±0.6 mM). STZ injection lowered plasma leptin levels (32.4±3.4 ng/ml) and led to severe hyperglycemia (23.3±0.8 mM). Hydrodynamic delivery of the *Obese* gene increased plasma leptin to superphysiological levels (100.5±12.3 vs 13.6±4.9 ng/ml) but did not change body weight or lower blood glucose levels compared to vehicle controls (25.1±0.9 vs 21.9±0.8 mM).

Conclusion: Superphysiological levels of leptin are required to lower blood glucose levels in insulin-deficient mice and leptin continues to lower blood glucose levels and improve glucose tolerance long-term. In contrast, therapeutic levels of leptin do not lower blood glucose levels in mice with pre-existing hyperleptinemia. These data suggest that chronic hyperleptinemia does not lead to resistance of the glucose lowering actions of leptin but obesity related leptin resistance may block such actions of leptin.

Supported by: CIHR

Disclosure: **M.M. Kwon:** None.

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Androgens potentiate the adverse metabolic effects of glucocorticoids

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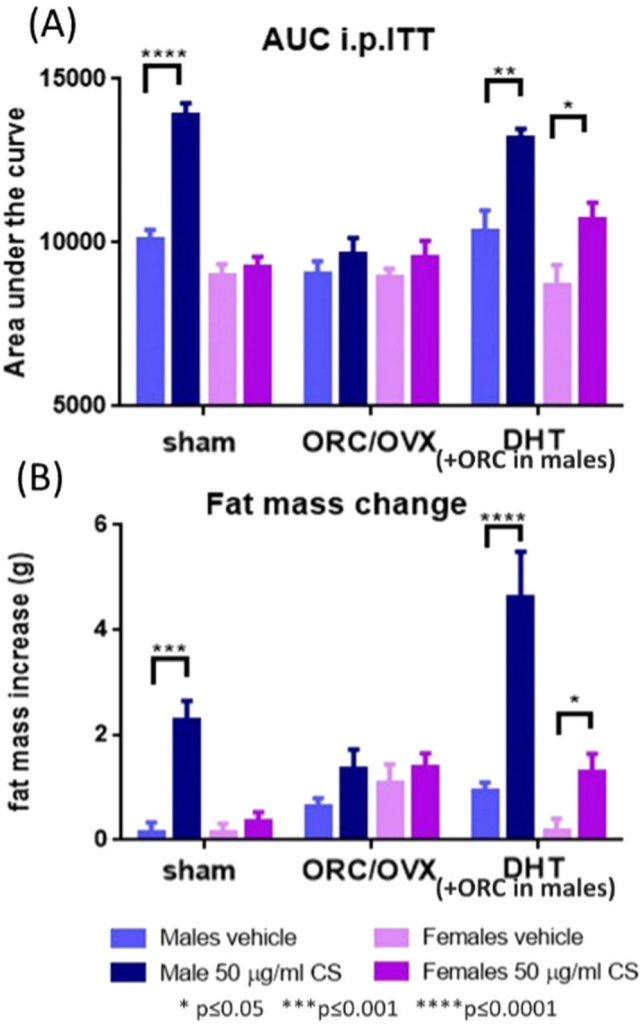
Background and aims: Glucocorticoid excess, whether pharmacological or endogenous, is clinically associated with adverse metabolic effects such as central obesity, insulin resistance, hypertension and hyperlipidaemia. This often limits their therapeutic use and thus a greater understanding of the mechanisms underlying the pathophysiology of these side-effects is paramount. As male rodents have been shown to respond more robustly to the anti-inflammatory effects of glucocorticoids, we aimed to determine whether there are also sex differences in the metabolic response to exogenously administered glucocorticoids.

Materials and methods: Eight week-old CD1 mice were treated with vehicle (1% ethanol) or 50 μg/ml corticosterone (CS) in the drinking water for 4 weeks. Insulin tolerance (by i.p. insulin tolerance test) and body composition (by DXA) were assessed in intact, castrated and DHT treated (0.5 cm silastic DHT implants) male and female mice with or without CS treatment. All statistics are one way ANOVA with Tukey's multiple comparisons test.

Results: Intact male mice rapidly developed severe insulin resistance (fig. 1A) and increased adiposity as a result of CS treatment (fig. 1B, Fat mass in Males; vehicle +0.2 g vs. CS +2.3 g p≤0.001). In contrast, both intact and overiectomised females maintained normal insulin sensitivity and body composition in the presence of CS treatment (fig. 1A&B), indicating that the gender difference in metabolic CS-sensitivity is not due to a protective effect of estrogens. However, when male mice were orchidectomized (ORC), treatment with CS no longer resulted in insulin resistance or abnormal fat accrual (fig. 1A&B). To assess if the resilience of both females and ORC males to the metabolic side-effects of CS was due to a lack of androgens, ORC males as well as intact female mice were implanted with the minimally aromatizable androgen, dihydrotestosterone (DHT). While DHT replacement alone had no effect on insulin resistance or fat accrual in either females or ORC males, combination of DHT with CS treatment rendered the female mice moderately insulin resistant (fig. 1A) and caused significant fat accrual (fig. 1B, Fat mass in Females+ DHT; vehicle +0.2 g vs. CS +1.3 g p≤0.05). Further, DHT and CS treatment in ORC males caused severe insulin resistance (fig. 1A) and induced an increase in fat mass even greater than seen in intact males (fig. 1B, Fat mass in Male CS +2.3 g vs. ORC+DHT CS +4.6 g fat p≤0.001)

Conclusion: Mice demonstrate a strong dichotomy in their metabolic response to excess glucocorticoids, with males being more sensitive than females. Our data indicate that androgens potentiate the adverse metabolic side effects of excess glucocorticoids.



Disclosure: **S.J. Gasparini:** None.

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Identifying whole body and tissue-specific insulin sensitivity using serum metabolomic profiling

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Background and aims: Access and cost-effectiveness on metabolomics profiling has rapidly improved recently. The aim of this study was to identify metabolites that are associated to whole body and tissue insulin sensitivity and improve widely used traditional indices for insulin sensitivity by using metabolomic measures, BMI, age and gender.
Materials and methods: The study group was 179 non-diabetic subjects (91 females and 88 males) with a median age of 51 [range 20-77 years], and BMI of 28 [20-51 kg/m^2], in whom insulin sensitivity of glucose uptake (GU) was measured using euglycemic-hyperinsulinemia, ^{18}F-fluorodeoxyglucose and positron emission tomography in whole body (M-value) and in skeletal muscle, abdominal subcutaneous and intraperitoneal adipose tissue. Metabolomic profiling of fasting plasma samples was done using high-throughput NMR, which included various amino acids and fatty acid measures (26 measurements). Statistical analyses was done using lasso/OLS regression with leave-one-out cross-validation to create models for identifying whole body and tissue insulin sensitivity based on Matsuda index (measurements at 0 and 120 min) or reciprocal of fasting insulin (1/f-ins) and metabolites, age, BMI and gender.
Results: Alanine, phenylalanine, tyrosine, histidine, glycoprotein, glucose, serum triglycerides, acetate and acetoacetate were negative regressors for insulin sensitivity, while glycine, glutamine, creatinine, HDL2-cholesterol, citrate and omega-6-fatty acids/total fatty acids were positive regressors. These results were based on models identifying insulin sensitivity in whole body, skeletal muscle and intraperitoneal and subcutaneous fat. The improved models had higher ability to identify insulin sensitivity than the commonly used surrogate measures of insulin resistance: 1/f-ins and Matsuda insulin sensitivity index (R^2 of the models presented in Table 1).
Conclusion: These results suggest that adding circulating metabolites, BMI, age and gender to traditional indices of insulin sensitivity may improve detection of insulin resistance and tissue insulin sensitivities. In all, the evidence presented here may serve as modern tools that can rapidly and cheaply identify insulin resistance status in at risk individuals.

Table1. Adjusted R^2 for models of insulin stimulated glucose uptakes.

	Whole body GU	Skeletal muscle GU	Intraperitoneal fat GU	Subcutaneous fat GU
1/f-ins only	0.298	0.259	0.045	0.098
Matsuda only	0.581	0.491	0.132	0.199
Improved models with 1/f-ins included	0.758	0.645	0.490*	0.463*
Improved models with Matsuda included	0.773	0.671	0.524	0.486

*1/f-ins was not included in the final model.

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Disclosure: **M. Honka:** None.

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The impact of adrenal hyperandrogenism on insulin resistance and lipid profile in women with polycystic ovary syndrome

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Background and aims: Polycystic Ovary Syndrome (PCOS) is characterized by chronic anovulation and hyperandrogenism. It is often accompanied by insulin resistance and abnormal lipid profile, which are positively correlated to the degree of hyperandrogenism. Androgens are mainly of ovarian origin but in 30% of the patients they originate from adrenals and this is mainly expressed by increased dehydroepiandrosterone sulfate (DHEA-S) levels. The effect of this androgen on the metabolic profile of these women has not yet been clarified. The aim of this study was to investigate the impact of adrenal hyperandrogenism on insulin resistance and lipid profile in women with PCOS and its correlation with clinical and laboratory metabolic parameters.
Materials and methods: We studied 372 patients with PCOS according to the NIH criteria. Two hundred thirty two age and BMI matched healthy women with normal cycles and without clinical or biochemical characteristics of hyperandrogenism served as the control group, in order to define adrenal hyperandrogenism (DHEAS levels > 95th percentile of control women). Then, patients with PCOS were divided into two groups, group A (n = 108) with adrenal hyperandrogenism (PCOS-AH) and group B (n = 264) without adrenal hyperandrogenism (PCOS-NAH). Anthropometric measurements including weight, height and waist circumference (WC) were recorded. Fasting glucose, insulin, lipids, sex hormone binding globulin (SHBG) and androgens [total testosterone (TT), Δ4-androstenedione (Δ4A), DHEA-S] levels were determined. Free androgen index (FAI) and HOMA-IR index were calculated in all patients. Statistical analysis of the data was performed using SPSS 16.00.
Results: Women with PCOS-AH were younger than PCOS-NAH ($p<0.001$), but did not differ in the degree and type of obesity (BMI and WC, $p>0.05$). No differences were found in HOMA-IR (3.4 ± 2.3 vs 3 ± 1.7), total cholesterol (183 ± 37 vs 185 ± 34 mg/dl), HDL cholesterol (51.3 ± 13 vs 53.8 ± 14 mg/dl), LDL cholesterol (112 ± 35 vs 111 ± 32 mg/dl) and triglycerides (83.1 ± 35 vs 87.3 ± 49 mg/dl) levels (in all comparisons, $p>0.05$). Women with PCOS-AH had lower SHBG (29.2 ± 13.8 vs 32.4 ± 11.8 nmol/l, $p=0.025$) and higher TT (0.96 ± 0.2 vs 0.8 ± 0.4 ng/ml, $p=0.05$) and Δ4A (3.9 ± 1.2 vs 3.4 ± 1 ng/ml, $p=0.007$) levels as well as FAI (14.1 ± 8 vs 10.2 ± 5, $p<0.001$). These results were confirmed by a multiple regression analysis model, which resulted in a negative correlation between adrenal hyperandrogenism with age ($p<0.001$) and SHBG ($p=0.02$), while no correlation was found with metabolic parameters (in all comparisons, $p>0.05$).
Conclusion: Women with PCOS and adrenal hyperandrogenism do not exhibit any deterioration of insulin resistance and lipid profile, despite the higher degree of hyperadrogenism. Therefore, it remains to be clarified whether higher levels of DHEA-S are an adaptive mechanism to insulin resistance or they exert a protective role on the metabolic profile of women with PCOS.
Disclosure: **S.A. Paschou:** None.

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HOMA-IR: the two faces of the same value

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Background and aims: Insulin resistance (IR) is a risk factor for type 2 diabetes and cardiovascular diseases. HOMA-IR, being the simple

product of fasting plasma glucose (FPG) and fasting plasma insulin (FPI) values, is the most widely used parameter for detection of IR. However, very different levels of FPG and FPI can generate the same HOMA-IR value. We have evaluated to which extent these differences may identify specific phenotypes in spite of similar HOMA-IR.

Materials and methods: Out of 989 subjects at high risk of diabetes participating in the Genetics, Physiopathology, and Evolution of Type 2 diabetes (GENFIEV) study, we have identified 30 subjects (53% women; age 51±12 years; BMI 29.9±4.4 kg/m^2) with the same HOMA-IR value (corresponding to the median value of the study population) and divided them into two groups: one with FPG>75° and FPI<25° percentile (H-FPG), and other with FPG<25° and FPI>75° percentile (H-FPI). In these subjects cardiovascular risk profile was evaluated, plasma glucose, insulin and C-peptide were determined in the fasting state and during a 2h 75 g OGTT for assessment of β-cell function (minimal model analysis).

Results: Individuals in H-FPG group (n=15) had higher FPG (119±11 mg/dl) and lower FPI (8.4±0.8 μU/ml) and those in H-FPI group (n=15) had lower FPG (77±6 mg/dl) and higher FPI (13.0±0.8 μU/ml) (p<0.0001 for both). HOMA-IR was identical in two groups (2.45±0.23 vs. 2.45 ±0.23). H-FPG were older than H-FPI (58±7 vs. 45±13 years; p<0.01). After adjustment for age, H-FPI individuals had lower HbA_1c (5.5±0.3% vs. 6.2±0.7%; p<0.05), higher waist circumference (105±9 vs. 97±12 cm; p<0.05), with no difference in BMI, blood pressure and lipid profile. β-cell function parameters were lower in H-FPG: HOMA-B (22.7±3.4 vs. 56.0±10.3; p<0.0001), Insulinogenic Index (0.006±0.104 vs. 0.109 ±0.084; p<0.01), basal (pre-hepatic) insulin secretion rate (77±22 vs. 122±33 pmol/m^2; p<0.01), β-cell glucose sensitivity (670±545 vs. 1961 ±732 pmol/m^2 BSA per mM/min; p<0.0001) and stimulus-response curve of insulin secretion rate at incremental glucose (4.0 mM: 77±22 vs. 142 ±51; 5.5 mM: 94±51 vs. 311±95; 8.0 mM: 243±186 vs. 682±234; and 11.0 mM: 503±348 vs. 1128±425 pmol/m^2 BSA per mM/min; all p<0.001), after adjustment for age and waist circumference.

Conclusion: Similar HOMA-IR value can result from significantly different FPG and FPI levels. These diversities underlie major differences in age, adipose tissue distribution and β-cell function. This calls for caution in interpreting HOMA-IR values. Further efforts in the quest for a more precise, but still simple IR index are needed.

Clinical Trial Registration Number: NCT00879801

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Disclosure: **D.S. Popovic:** Grants; FoRiSID - Rome and Eli Lilly, Italy.

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PGC-1α ameliorates insulin resistance through regulation of STARS expression in high-fat diet rat

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Background and aims: PGC-1α expression correlates with fatty acid metabolism and insulin sensitivity. We sought to investigate the molecular mechanism of PGC-1a and its pathway including STARS and CPT-1β, in the development of abnormal fatty acid metabolism and insulin resistance.

Materials and methods: We detected the lipid profiles and expression of PGC-1alpha, STARS, insulin signaling related genes of SD rats fed by high-fat diet. We also detected the protein and gene expressions of PGC-1alpha, STARS, CPT-1beta,ACC and genes or proteins involved in insulin signaling in L6 cells after upregulating or knockdowning the expression of PGC-1alpha by means of transfecting plasmid or siRNA. The serum BG, INS, TC and TG were significantly higher in high-fat diet fed rats . The expression of PGC-1a, IRS-1, AKT and GLUT4 mRNA in HF group was significantly lower, and STARS mRNA was obviously higher comprared with C group. After upregulating PGC-1alpha, STARS mRNA and protein were decreased significantly and the expression of CPT-1b ,IRS-1, AKT and GLUT4 mRNA were significantly increased.Phosphorylation ACC, phosphorylation AKT and GLUT4 increased, nevertheless phosphorylation IRS-1(Ser307) was significantly decreased. After knockdowning PGC-1alphathe expression of STARS mRNA and protein increased significantly, the expression of CPT-1b mRNA and phosphorylation ACC decreased in si-PGC-1a group (all P<0.05). IRS-1, AKT and GLUT4 mRNA expression were significantly decreased in si-PGC-1a group, the expression of phosphorylation IRS-1 significantly increased, while the expression of GLUT4 and phosphorylation AKT were decreased significantly in si-PGC-1a group (all P<0.05).

Results: In conclusion, Regulating the expression of PGC-1a by transfection plasmid or siRNA could change the expression of STARS, meanwhile the fatty acid oxidation related genes and the indexes involved in insulin signaling pathway changed, thus suggesting PGC-1a might ameliorate insulin resistance through STARS and fatty acid oxidation in skeletal muscle cells.

Conclusion: Expression of PGC-1α and insulin signaling pathway related genes were decreased, and STARS was increased in L6 muscle cells cultured by palmitic acid. Expression of STARS was lower and insulin signaling related genes were higher by up-regulating the expression of PGC-1α. It suggested PGC-1αmay improve insulin sensitivity in skeletal muscle cells through regulating the expression of STARS.

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Disclosure: **H.J. Ma:** None.

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Characteristics of disturbances in glucose metabolism during treatment of acromegaly

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Background and aims: In acromegaly, carbohydrate metabolism disorders (CMD) are frequently observed. The effect of therapy of acromegaly on pathogenesis of CMD is not clear. We aimed to assess the effect of somatostatin analogues (SSA) on pathogenesis of carbohydrate metabolism disturbances in acromegaly.

Materials and methods: 112 acromegaly patients were examined (33 men, 79 women; age 51 [IQR 42-60] years; 89 had newly diagnosed acromegaly (NA), 13 receiving SSA, 10 after transsphenoidal surgery (TSS). We analyzed fasting plasma insulin and glucose levels (FPI, FPG), HbA1c, the Matsuda and HOMA-IR indices, area under insulin curve in the first 30 minutes (AUCins.30) and from 30 to 120 minutes of oral glucose tolerance test (AUCins.30-120). In 27 NA patients we assessed these parameters after 3 and 6 months of SSA therapy (19 patients) and after TSS (8 patients).

Results: Mean age (55.0-58.0 yrs) and mean BMI (29.4-29.5 kg/m2) were comparable between the 3 groups. Median of % upper limit normal of IGF1 was similar between the SSA and TSS groups (-13.7[-26.2 -3.1] vs. -22.3 [-77.1—4.2]), but severely elevated in NA patients (p=0.0001). In the SSA group, prevalence of CMD was 93%, whereas it was 50% in the NA group and 10% in TSS group. NA patients were mainly insulin-resistant, with high fasting plasma insulin of 90 [60-172] pmol/l, high HOMA-IR 3.4 [2.0-7.3] and low Matsuda index (3.0[1.8-5.2]. In the SSA and TSS groups the HOMA-IR (1,9, [0,7-4.5] and 0.8 [0.5-1.9]) and Matsuda indices (5.1 [2.9-9.5] and 6.5 [4.7-20.6]) were comparable. We observed the lowest level of the AUC ins 30 min in SSA patients in compare with NA and TSA groups (1.0 [0.3-1.9], 5.1 [2.2-9.7] and 4.6 [1.8-10.7] respectively). The levels of HbA1c, FPG and AUC glu. were higher in SSA patients in comparison with NA and TSA groups. Among 19 prospectively followed SSA-treated patients, the reduction of IGF1 after 6 months coincided with a decrease of FPI and HOMA-IR (both p<0.01), and an increase of the Matsuda index (both p<0.01). SSA treatment resulted in a considerable reduction of early phase insulin secretion (AUCins.30) during an OGTT compared to the TSS group (p=0.06),

while the reduction of AUCins.30-120 was similar between these groups (p=0.4).
Conclusion: Despite reduction of IGF1 levels and insulin resistance during SSA therapy and after TSS, the decrease of the first phase of insulin secretion on SSA therapy leads to the development of CMD but not after TSS.
Disclosure: **I.V. Trigolosova:** None.

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Fetuin-B levels in relation to metabolic/hormonal profile and carotid intima media thickness (cIMT) in women with polycystic ovary syndrome (PCOS)

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Background and aims: PCOS is a prevalent and multifaceted endocrinopathy, associated with an increased risk of insulin resistance, type 2 diabetes, dyslipidemia, subfertility and cardiovascular events. Fetuin-B, a highly expressed, liver-derived protein originally implicated in fertilization as a potent ovastacin inhibitor, is newly recognized for its roles in the modulation of glucose metabolism and of atherosclerotic plaque formation/stability. Prompted by these recent findings, we developed a working hypothesis that fetuin-B metabolism may be altered in patients with PCOS. We also tested whether in these patients, circulating levels of this protein are correlated with metabolic disturbances and cIMT.
Materials and methods: We conducted a cross-sectional study in a tertiary referral center in Turkey during a period of twelve months. 280 women (140 with PCOS and 140 age- and BMI-matched controls) were consecutively recruited. Using all three of Rotterdam consensus criteria, we defined PCOS as the presence of oligo- or anovulatory menstrual dysfunction, clinical and/or biochemical hyperandrogenism and ≥12 follicles measuring 2-9 mm in diameter or an ovarian volume >10 ml. Fetuin-B was measured using a commercial ELISA (intra-assay CV<6%, inter-assay CV<8%) and cIMT was measured by ultrasonography. Insulin resistance was estimated using the homeostatic model assessment, HOMA-IR. Demographic and laboratory characteristics of the studied women with and without PCOS were compared using 2-tailed independent samples t-test and association between fetuin-B and other variables were assessed with Pearson correlation.
Results: Circulating fetuin-B levels were significantly elevated in women with PCOS compared with controls (1124.12 ± 407.13 vs. 640.27 ± 285.60 ng/ml, p<0.001). Fetuin-B levels positively correlated with BMI, HOMA-IR, free androgen index (FAI), high-sensitivity C-reactive protein (hs-CRP), triglycerides and cIMT. Multivariate logistic regression analyses revealed that the OR for PCOS was 3.49 for patients in the highest quartile of fetuin-B compared with those in the lowest quartile (95% CI=2.48-4.91, p=0.003). Furthermore, multiple regression analyses revealed that BMI, HOMA-IR, hs-CRP, FAI and triglycerides were independent factors influencing serum fetuin-B levels.
Conclusion: To the best of our knowledge, our study is the first to illustrate a link between fetuin-B and PCOS. Our findings suggest that increased levels of this hepatic protein are significantly associated with metabolic disturbances, as well as with cIMT in PCOS. Further investigations addressed to explore the underlying mechanisms are warranted.
Disclosure: **P. Yesil Senses:** None.

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History of anabolic androgenic misuse is associated with impaired insulin sensitivity and increased abdominal fat among younger men

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Background and aims: Android fat distribution is characterised by central obesity and strongly linked with reduced insulin sensitivity (IS). The association between hyperandrogenism and increased android fat distribution is well-known in women. However, the impact of supraphysiological concentrations of androgens remains unresolved in men, since central obesity is mainly associated with male hypogonadism. Misuse of anabolic androgenic steroids (AAS) cause a dramatic increase in plasma androgens and side-effects have predominantly been investigated in relation to disturbances in the hypothalamus-pituitary-gonadal axis but metabolic side-effects remain to be illuminated. The objective of this study was to investigate the impact of AAS misuse on abdominal fat distribution and IS in young men.
Materials and methods: Cross sectional case-control study among young men (≤50 years) including three study groups: current AAS misuse, former AAS misuse and age-matched healthy controls who had never used AAS. All participants were engaged in recreational strength training. A 120 min OGTT was performed after a minimum of 8-hour overnight fasting. Plasma glucose and insulin were obtained at five time points during the OGTT: 0, 30, 60, 90 and 120 min. The Matsuda index was calculated as a measure of IS. Body composition was assessed by a DEXA-scan including measurements of abdominal fat distribution, divided into visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) by a pre-defined and validated method using a 5 cm high transverse slice at L4/L5 level.
Results: A total of 100 participants were included: current AAS misuse, n = 37 (age 31.4 ± 1.4 years, mean (SE)) and former AAS misuse, n = 33 (age 34.8 ± 1.2 years), duration since AAS cessation (geometric mean (95%CI)) of 2.6 years (1.7 - 3.7) and healthy control participants, n = 30 (age 31.5 ± 1.2 years). Participants with current AAS misuse and former AAS misuse had clearly lower Matsuda index reflecting impaired IS as compared with the control group (geometric mean (95%CI) 6.49 (4.27 - 9.85) vs. 5.09 (3.34 - 7.75) vs. 8.51 (7.14 - 10.15), P < 0.01). Despite that men with current AAS misuse had the greatest lean body mass and lowest total body fat % as compared with the healthy controls and subjects with former AAS misuse (25.4 ± 0.4 kg/m2 vs. 21.3 ± 0.3 kg/m2 and 21.9 ± 0.5 kg/m2, P < 0.01) and (14.1 ± 0.4% vs. 17.3 ± 0.7% and 19.4 ± 0.6%, P < 0.01). Interestingly, VAT volume was higher in the groups with current AAS and former AAS misuse than in the controls (388 ± 17 cm3 vs. 347 ± 17 cm3 and 290 ± 12 cm3, P < 0.01). Moreover, the three groups differed significantly in SAT volume, as participants with former misuse of AAS had the highest SAT volume (962 ± 71 cm3) and men with current AAS misuse had lowest volume (546 ± 28 cm3) vs control participants (748 ± 75 cm3) (P < 0.01). In linear regression analysis VAT and SAT had strong inversely associations with Matsuda index (P < 0.05) in the pooled study cohort, as expected.
Conclusion: The current data suggest that a history of AAS misuse leads to impaired IS, even several years after AAS cessation, compared with healthy controls who had never used AAS which could be mediated by increased VAT as the primary metabolically active fat tissue.
Supported by: AntiDoping Denmark, Danish Heart Foundation, University of CPH, Herlev Hosp
Disclosure: **J.B. Rasmussen:** None.

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Transforming growth factor beta-like stimulated clone 22 D4 promotes diabetic hyperglycaemia and insulin resistance

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Background and aims: Insulin resistance represents the core component of the so-called Metabolic Syndrome, ultimately promoting glucose intolerance, pancreatic beta cell failure, and overt type 2 diabetes. Based on substantial side effects of existing pharmacological approaches, efficient and safe insulin sensitization and glucose control remain critical therapeutic aims to prevent diabetic late complications. The goal of this study is to characterize the role of Transforming Growth Factor beta-like Stimulated Clone (TSC) 22 D4 protein in insulin resistance and diabetes.
Materials and methods: In order to investigate the metabolic function of TSC22D4 in vivo, we employed liver-specific TSC22D4 knockdown and overexpression approaches both in wild type and diabetic mouse models (db/db and NZO mice).
Results: We identified TSC22D4 as a critical molecular determinant of insulin signaling and glucose handling. Hepatic TSC22D4 knockdown enhanced insulin signaling in liver, while hepatic TSC22D4 overexpression blunted insulin signaling. Consequently, hepatic TSC22D4 inhibition both prevented and reversed hyperglycemia, glucose intolerance, and insulin resistance in diabetic mouse models (db/db and NZO mice). In addition, we found out that TSC22D4 exerts its effects on systemic glucose homeostasis -in large parts- through the transcriptional regulation of the small secretory protein lipocalin (LCN) 13 as demonstrated by in vivo chromatin recruitment and genetic rescue experiments.
Conclusion: In support of our hypothesis and findings, diabetic human patients have elevated hepatic TSC22D4 levels correlating with decreased insulin sensitivity and hyperglycemia. Thus, our results establish TSC22D4 as a promising and attractive target for the treatment of diabetes mellitus.
Supported by: SFB1118
Disclosure: **B. Ekim Ustunel:** None.

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In vivo high throughput screening to identify insulin-independent modulators of metabolism

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Background and aims: Given the socioeconomic significance of type 2 diabetes, a thorough understanding of β cell function and dysfunction holds great significance. Under diabetic conditions, peripheral tissues start losing sensitivity to circulating insulin, resulting in pancreatic β cells attempting to secrete increasing amounts of insulin, and ultimately causing β cell dysfunction. However, increasing evidence suggests that these effects can be reversed to an extent by maintaining glycemic control. Molecules capable of modulating metabolism or promoting glucose uptake independent of insulin would open new avenues to managing and treating diabetes. We aim to perform high throughput screens for identifying insulin-independent modulators of metabolism by recapitulating some of the features of late stage diabetes, while capitalizing on using a highly amenable model organism like the zebrafish.
Materials and methods: A guide RNA was designed targeting the genomic region encoding the signal peptide of preproinsulin and was co-injected with Cas9 mRNA in to one cell embryos. Injected animals were raised to adulthood, out-crossed to wild-type animals, and a 16 bp deletion allele was isolated in the F1 generation. All experiments were done using F2 or F3 animals. Glucagon, Somatostatin and Insulin were detected by immunofluorescence using standard antibodies.
Results: The genomic lesion results in a complete absence of detectable Insulin protein in the pancreatic islet. Although mutant animals develop normally at initial stages, they begin to die at around 9 days post fertilisation (dpf), most likely due to metabolic defects. Insulin reporter expressing cells were detected in the islet of the mutant pancreas, with disrupted islet architecture lacking the typical β cell localization in the core and α cells at the periphery. In the mutants, α cells appeared in the core of the islet, while β cells were scattered throughout. The mutants also show metabolic defects, beginning as early as 3 dpf, which can be partially rescued by PPARγ agonists.
Conclusion: In addition to understanding the role of insulin during pancreas morphogenesis, we are developing tools to perform high throughput screens aimed at identifying molecules which promote glucose uptake in peripheral tissues, and to find insulin-independent modulators of metabolism. Such a screen will unravel novel signaling pathways which can potentially be manipulated to treat diabetes, including cases where insulin resistance has already set in.
Disclosure: **S. Mullapudi:** None.

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Gut hormones, rather than glucose or insulin, are the main drivers of diminished bone resorption in the postabsorptive state

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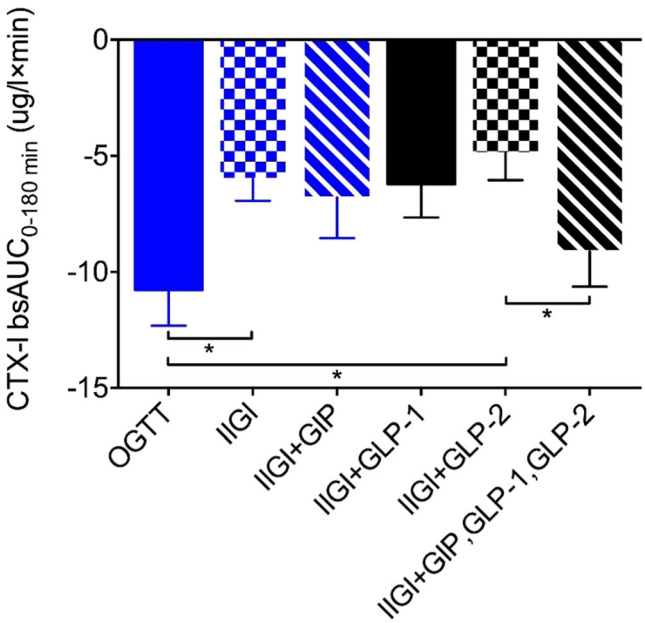
Background and aims: Following ingestion of nutrients an acute reduction in bone resorption is observed. The mechanisms behind this

phenomenon are uncertain, but may involve increments in plasma glucose, insulin and/or the gut hormones glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2). We aimed to dissect the individual effect of these potential mediators of bone resorption.

Materials and methods: We measured plasma levels of the bone resorption marker C-terminal telopeptide of type I collagen (CTX-I) during three experimental settings: 1) 75g-OGTT and isoglycaemic iv glucose infusion (IIGI) in 10 patients with type 2 diabetes (T2D) and 10 non-diabetic control subjects (CTRLs); 2) 75g-OGTT and IIGI in 10 totally pancreatectomised (PX) patients (devoid of endogenous insulin production) and 10 CTRLs and; 3) 50g-OGTT and five IIGIs with iv infusion of saline and physiological doses of GIP, GLP-1, GLP-2 and a combination of the three in 10 patients with T2D.

Results: OGTT induced a robust reduction in CTX-I in both patients with T2D and CTRLs as opposed to a marginal reduction during IIGI (baseline-subtracted AUCs (bsAUC)$_{0\text{-}240min}$ (mean±SEM): -24.4±5.3 vs -6.4 ±2.7 μg/l×min, p=0.002 (T2D); -27.8±4.0 vs -8.9±1.8 μg/l×min, p<0.001 (CTRLs)). Postabsorptive reduction in CTX-I was also present in PX patients during OGTT (bsAUC$_{0\text{-}180\ min}$: -19.3±4.7 μg/l×min). Combined infusion of GIP, GLP-1 and GLP-2 during IIGI in patients with T2D mimicked the suppression of CTX-I observed during OGTT in these patients (bsAUC$_{0\text{-}180min}$: -10.7±1.4 (OGTT) vs -9.0±1.4 μg/l×min (IIGI+GIP,GLP-1,GLP-2), p=0.60) (Fig. 1).

Conclusion: These results indicate that gut hormones, rather than glucose or insulin, represent the main drivers of diminished bone resorption in the postabsorptive state.*Figure 1. CTX-1 expressed as baseline-subtracted AUC (bsAUC) (mean±SEM) during 50g-OGTT and IIGIs + GIP, GLP-1, GLP-2 and a combination of the three hormones in patients with T2D. Asterisks (*) indicate significant (p<0.05) differences.*



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Disclosure: **A. Lund:** None.

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Association of metabolic flexibility under glucose clamp and postprandial conditions with glucose control

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Background and aims: Metabolic flexibility, defined as the capacity for the organism to adapt fuel oxidation to fuel availability, has been proposed as an index of metabolic health. However, evidence supporting that notion remains elusive. Unfortunately, most of the studies evaluating metabolic flexibility have not taken into consideration potential confounding factors, such as the extent of insulin-stimulated glucose disposal rate (GDR) or body size. Furthermore, metabolic flexibility has been mainly assessed under supra-physiological conditions (i.e. euglycemic-hyperinsulinemic clamp [EHC]), which may not reflect a true physiological state. We therefore aimed to compare metabolic flexibility using an EHC vs. a standard 75-g oral glucose dose after adjusting for GDR and body size, respectively. We then compared the adjusted metabolic flexibility to markers of glucose control including fasting and 2-h glycemia, insulin sensitivity and insulin secretion.

Materials and methods: In 30 non-diabetic individuals (15 males; 35±12 y old; 27±4 kg/m2), a 75-g oral glucose load was supplied. Circulating glucose, insulin and C-peptide concentrations and gas exchange were measured before and for 4 hours after glucose. Two days later, a 2-h EHC (120 mU/m2/min) was performed and gas exchange was assessed under fasting and steady-state conditions. Metabolic flexibility was estimated from the steady-state non-protein respiratory quotient (npRQ) at the end of the EHC, and as the increase in npRQ after 60 min oral glucose loading. Insulin sensitivity was determined by the OGIS index from the 3-h oral glucose response, and insulin secretion through C-peptide deconvolution. In another study, a 75-g oral glucose load was administered to 51 non-diabetic individuals (24 males; 34±9 y old; 27±4 kg/m2). Circulating glucose, insulin and C-peptide concentrations and gas exchange were measured before and for 3 hours after glucose loading. Regression analysis was used to calculate residual values of metabolic flexibility during the EHC or oral glucose adjusted for GDR or body size, respectively. Association between metabolic flexibility and glucose control was analyzed through Spearman correlation testing.

Results: At the end of the EHC, GDR correlated with npRQ (r=0.56; p=0.001) and metabolic flexibility was therefore expressed as npRQ adjusted for GDR. In turn, the change in npRQ after 60 min glucose loading related with the glucose dose per kg of body weight (r=0.55; p=0.002). Thus, metabolic flexibility (change in npRQ) after glucose loading was adjusted for the glucose dose per kg body weight. Both metabolic flexibility indexes were correlated (r=0.54; p=0.003). However, none of these markers of metabolic flexibility related with fasting or 2-h glycemia, OGIS or insulin secretion. From the second study, we confirmed the association between the change in npRQ after 60 min glucose loading and glucose dose (r=0.46; p<0.001). Similarly, metabolic flexibility adjusted for glucose dose did not correlate with fasting and 2-h glycemia, OGIS or insulin secretion.

Conclusion: Metabolic flexibility is impacted by the amount of exogenous glucose administered, thus requiring an adjustment for the glucose dose. Once proper adjustment is conducted, metabolic flexibility assessed during the EHC or in response to oral glucose are correlated. However, metabolically flexibility is not associated to glucose homeostasis, which challenges its (patho)physiological relevance for metabolic health.

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Disclosure: **J.E. Galgani:** Grants; FONDECYT 1130217.

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Discrimination between advanced glycation endproducts of endogenous and dietary origin in overweight and obese non-diabetic subjects

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Background and aims: Accumulation of advanced glycation endproducts (AGEs) has been implicated in development of insulin

resistance, impaired glucose tolerance and increased risk cardiovascular disease in overweight and obesity populations. It is also implicated in the development of vascular complications of diabetes. A non-invasive measure of AGE exposure is the flux of AGE free adducts (glycated amino acids) excreted in urine. A confounding factor is the proportion of AGEs absorbed from digestion of AGE-modified proteins in food. AGE-modified proteins formed endogenously are hydrolysed to AGE free adducts and excreted in urine together with AGEs absorbed as free adducts from food. The AGE pyrraline is formed exclusively at high temperatures of cooking and hence is sourced only from food. The aim of this study was to assess the correlation of urinary AGE free adducts with flux of pyrraline and explore regression with extrapolation of AGE flux to zero pyrraline content to thereby deduce the mean endogenous formation of AGEs in overweight and obese subjects. This was then validated for the methylglyoxal (MG)-derived AGE, MG-H1, by therapeutic intervention with an inducer of glyoxalase 1 (Glo1) that decreased endogenous MG and MG-H1 formation.

Materials and methods: AGE free adducts were assayed in urine by stable isotope dilution analysis liquid chromatography-tandem mass spectrometry (LC-MS/MS) and normalised to creatinine. Urine samples were collected from 29 overweight and obese subjects as urine second void after overnight fasting. The Glo1 inducer was evaluated in a randomised, placebo-controlled, double-blinded crossover clinical trial in the same overweight and obese subjects. Treatment was with Glo1 inducer or placebo once daily for 8 weeks with 6 week washout period before crossover.

Results: The median urinary excretion of pyrraline was 8.2 - 12.1 nmol/mg creatinine throughout the intervention study. Total urinary excretion of MG-H1 free adduct correlated positively with urinary pyrraline for all 4 study visits, $r = 0.43 - 0.63$, $P<0.02$. Regression of urinary excretion of MG-H1 free adduct on urinary excretion of pyrraline free adduct gave a non-zero intercept on the urinary MG-H1 excretion axis which is the endogenous formation of MG-H1. For example, at baseline of the placebo arm the regression equation was: Urinary MG-H1 (nmol/mg creatinine) = (0.592 ± 0.180) x urinary pyrraline (nmol/mg creatinine) + (13.4 ± 2.1); $P = 0.003$. The flux of endogenously-generated MG-H1 adducts was 13.2 nmol per mg creatinine at baseline and decreased by 14% with Glo1 inducer treatment ($P<0.01$) but not with placebo.

Conclusion: For urinary excretion of AGE free adducts that correlate positively with urinary excretion of food-derived pyrraline, linear regression of urinary excretion of AGE on urinary excretion of pyrraline extrapolated to zero pyrraline gives an estimate of mean endogenous formation of AGEs.

Clinical Trial Registration Number: NCT02095873

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Disclosure: **N. Rabbani:** Grants; Yes.

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IgG N-glycan patterns are associated with type 2 diabetes in independent European populations

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Background and aims: Type 2 Diabetes (T2D) results from intricate interplays between environmental and genetic factors. Glycans on proteins are known to reflect genetic, metabolic and environmental factors. IgG N-glycosylation changes have been associated with BMI, blood pressure and lipids. However, associations with T2D have not been described. In this study, we compared IgG N-glycans patterns of patients with T2D to healthy subjects.

Materials and methods: In the DiaGene study, (1886 patients with T2D and 854 controls) 76 IgG glycosylation traits were measured and associations with T2D were replicated in independent European samples (163 cases of T2D and 3164 controls from the Korcula, VIS and ORCADES studies). Results from both populations were meta-analyzed. Bonferroni correction was applied. AUCs of Receiver Opererator Characteristic (ROC) curves were calculated using 10-fold cross validation for prediction of T2D based on clinical characteristics, IgG glycans and the combination of both.

Results: In the discovery population, 14 glycan peaks (GP) showed significant associations with T2D corrected for age and sex. After extensive correction for covariates (age, sex, BMI, smoking, creatinine, HDL-cholesterol and non-HDL cholesterol)l, 3 associations remained (GP6, GP8 and GP9). These associations were confirmed by replication and remained significant after meta-analysis. Adding all glycan peaks to a model with age and sex increased the AUC on test data from 0.542 to 0.734 for predicting T2D. Adding the glycan peaks to the most extensive covariate model (age, sex, BMI, smoking, creatinine, HDL-cholesterol and non-HDL cholesterol) increased the AUC from 0.892 to 0.895. The AUC for IgG glycans alone was 0.729.

Conclusion: Several glycan traits were firmly associated with the presence of T2D and replicated in an independent population. In addition, IgG glycans showed improvement of the AUCs for models predicting T2D in this case-control setting. For the most extensive model this improvement was limited, however IgG glycan peaks showed quite good prediction alone. This may indicate that IgG glycans capture much of the information of the combined clinical covariates. The IgG glycan associations found can yield important insights in T2D pathophysiology and should be further investigated for their biomarker potential.

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Disclosure: **R.F.H. Lemmers:** None.

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The relationship between branched-chain amino acids, metabolic syndrome and cardiovascular risk profile in a Chinese population: a cross-sectional study

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Background and aims: Branched-chain amino acids (BCAAs) are strongly correlated with visceral obesity and insulin resistance (IR) and are predictors of diabetes. However, it is not clear whether BCAAs could be regarded as independent risk factors for cardiovascular disease (CVD). This study aimed to evaluate the relationship between BCAAs, metabolic syndrome (MS), and other cardiovascular (CV) risk factors in middle-aged and elderly Chinese population at high risk for the development of CVD.

Materials and methods: A total of 1302 subjects aged from 40 to 79 years old were enrolled from a Diabetes Prevention Program. Anthropometric and biochemical examinations were performed and stratification was done on the basis of BCAAs quartiles. MS was defined according to the the Adult Treatment Panel III criteria. Serum BCAAs (leucine, isoleucine and valine) concentrations were measured by hydrophilic interaction chromatography-tandem mass spectrometric method.

Results: BCAAs levels were positively correlated with MS, its components and CV risk profile. After adjusting for several traditional risk factors, the odds ratio (OR) for MS among subjects in the fourth quartile of BCAAs levels showed a 2.17-fold increase compared with those in the first quartile. Multivariate logistic regression analysis revealed that BCAAs were independently associated with high Framingham risk score even after adjusting for MS and its components (OR 1.03, 95% CI 1.00-1.04; $P<0.0001$), Additionally, the OR for high CV risk were 3.20 fold (95% CI 1.83-5.61; $P<0.0001$) in participants in the fourth BCAAs quartile with MS compared with participants in the first BCAAs quartile without MS.

Conclusion: Increased BCAAs levels are independent risk factors of MS and CVD in addition to the traditional factors in middle-aged and elderly Chinese population. The development of CVD in MS patients with high level BCAAs is accelerated. Intervention studies are needed to investigate whether the strategy of BCAAs reduction has impacts on endpoints in patients with higher CV risk.
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Disclosure: **Z. Hongwen:** None.

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Dysregulated iron homeostasis in type 2 diabetes
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Background and aims: Iron and diabetes are strictly related. This is clinically evident in patients affected by hereditary hemochromatosis, in which the prevalence of diabetes is about 20%. On the other side, recent studies demonstrate that in diabetic patients plasma iron and/or ferritin levels could be prognostic factors for the development of the disease. The aim of this study is to understand the molecular mechanisms that cause diabetic iron overload and to elucidate the role of increased iron levels in the pathogenesis of diabetes and in the generation of the diabetic late complications.
Materials and methods: Patients with diabetes mellitus type 2 (T2DM) from the HEIDIS cohort were analysed for systemic iron parameters and hepcidin expression. Lepr(db/db) mice were used as a model of T2DM to identify the mechanisms by which diabetes affects iron homeostasis. Tissue iron content and distribution have been assessed in histology while alterations in the expression of iron-related genes have been analysed in qRT-PCR and western blot. Systemic iron content and hepcidin levels have been measured using the BDA method and with a specific ELISA. To assess how increased iron levels affect the pathogenesis of diabetes, Lepr(db/db) mice have been mated with the FpnC326S mouse model of hereditary hemochromatosis type 4 and diabetic and iron parameters have been measured.
Results: 115 T2DM patients and 26 healthy controls were analyzed for systemic iron parameters. CRP>2 was used as exclusion criteria to avoid inflammatory interferences. Diabetic patients show a significant increase in serum iron content (p=0,035), transferrin saturation (p=0,025) and ferritin levels (p=0.01). Serum hepcidin levels were inappropriately low (p<0,0001) explaining the elevated systemic iron levels. To study how diabetes affects iron homeostasis, we analyzed the Lepr(db/db) mouse model of T2DM. 10-week old mice show the hallmarks of the early T2DM: obesity, hyperglycemia, hyperinsulinemia and increased HbA1c. Consistent with the patients, these mice have elevated serum iron, transferrin saturation and ferritin levels while circulating hepcidin is significantly reduced. Analysis of the liver revealed an overall iron deficiency despite the elevated systemic iron content and the increased TfR1 mRNA expression. Interestingly, the hepatic levels of the iron storage protein ferritin are unchanged. Molecular analysis revealed the typical BMP/SMAD activation pattern that mediates hepcidin upregulation upon systemic iron overload. FpnC326S/Lepr(db/db) hemochromatotic diabetic mice show a similar phenotype when compared to hemochromatotic non-diabetic controls, having a 1,5-fold increase in systemic iron levels and a decrease in non-heme liver iron content.
Conclusion: This study revealed that hepcidin levels are inappropriately decreased both in patients and in Lepr(db/db) mouse model of T2DM, causing elevated systemic iron levels. T2DM causes a lack of systemic iron uptake by the liver, generating hepatic iron deficiency. This phenotype persists in hemochromatotic/diabetic mice, which show decreased hepatic iron content and increased systemic iron levels compared to hemochromatotic non-diabetic mice. Experiments are ongoing to identify the consequences of increased systemic iron content in organs affected by the diabetic late complications.
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Disclosure: **S. Altamura:** None.

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Carotid sinus nerve transection restores glucose and lipid homeostasis in prediabetes animal models
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Background and aims: We have recently described that the carotid sinus nerve (CSN) resection prevents the development of insulin resistance and hypertension. Moreover, we have also observed that CSN denervation restored insulin sensitivity and fasting glycemia and insulinemia in hypercaloric animal models of insulin resistance. The aim of this work was to investigate the effect of CSN resection on glucose and lipid metabolism in prediabetes animal models.
Materials and methods: Six groups of Wistar rats (9-12 weeks) were used. The control group fed a sham diet, the HSu group fed 35% of sucrose in drinking water and the HF group fed a 45% lipid-rich diet. The groups have been randomly divided and half was submitted to CSN bilateral transection and the other half to a sham surgery. CSN resection in HF and HSu rats was performed after 21 and 28 days of diet, respectively. At a terminal experiment, 3 weeks after the surgery, blood was collected to quantify total cholesterol, LDL, HDL and triglycerides. Skeletal muscle, adipose tissue and liver were collected to evaluate, by western blot, alterations in the expression of Glut4 and Glut2. In vivo glucose uptake was evaluated in several tissues (liver, soleus and gastrocnemius muscle, pancreas and adipose tissue) by an intravenous glucose tolerance test (500mg/kg body weight of glucose mixed with 100μCi/kg body weight of 2-deoxy-D-[1,2-3H]-glucose) in control and HF animals with or without CSN resection.
Results: HSu diet decreased Glut4 expression by 45.67% in skeletal muscle, an effect that was restored with chronic CSN resection. HSu and HF diets significantly decreased Glut4 expression in adipose tissue by 20.99 and 15.71%, respectively, an effect that is normalized with CSN resection. In liver, the hypercaloric diets did not modify Glut2 expression, however CSN resection increased it expression in HF animals by 39.88%, relative to control values. HF diet decreased glucose tolerance, an effect that is restored by the CSN resection. In HF animals glucose uptake decreased in liver and pancreas by 41.64 and 43.26%, respectively, and CSN resection almost restored glucose uptake in liver. Glucose uptake also decreased in HF perienteric adipose tissue by 44.95%, an effect that is reversed with CSN cut. HSu and HF diets did not modify total cholesterol or LDL. In contrast, HDL was diminished in HF animals by 27.72%, and CSN denervation restored it to control values. Also, HSu and HF diets increased triglycerides by 74.77 and 46.15%, respectively, an effect that is restored by the CSN resection.
Conclusion: CSN chronic resection restores glucose and lipid homeostasis in prediabetes animal models and might represent a putative therapeutic target for the treatment of metabolic diseases.
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Disclosure: **J.F. Sacramento:** None.

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Chronic exercise alleviates diet-induced metabolic dysfunction via enhancing FGF21 sensitivity in adipose tissues

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Background and aims: Fibroblast growth factor 21 (FGF21) is a hormone mainly derived from liver and acts on adipocytes by activating FGF21 receptors (FGFR1 and β-Klotho) mediated intracellular signalling. FGF21 can modulate lipolysis and stimulate adiponectin production in adipose tissues. Several lines of evidence shows that exercise can regulate FGF21 action on target tissues by affecting FGF21 expression and sensitivity. This study aims to investigate whether the beneficial effects of exercise on metabolic function are mediated by enhanced FGF21 action on adipose tissues and clarify involving molecular mechanisms.
Materials and methods: FGF21-KO mice, adipoese β-Klotho specific KO mice and their WT littermates were fed with high fat diet (HFD) for eight weeks and then divided into sedentary and exercised groups. Exercised mice were subjected to treadmill running for another 4 weeks. Body composition and glucose homeostasis were monitored. Blood and tissue samples were collected for biochemical, histological and molecular analysis.
Results: Chronic exercise could reverse HFD-induced downregulation of FGF21 receptors in adipose tissues and enhance adipose FGF21 sensitivity. Chronic exercise could significantly improve glucose homeostasis of WT mice, but only slightly in FGF21-KO mice. Besides, exercise could also significantly reduce serum and tissue lipid levels in WT mice, but such effects were refractory in FGF21-KO mice.Exercise-trained FGF21-KO mice displayed more active lipolysis but less adiponectin production and lipid disposal compared with exercised WT littermates.The mice lacking β-Klotho in adipose showed similar phenotypes as FGF21-KO mice after exercise training.
Conclusion: Chronic exercise increases FGF21 receptors expression and enhances FGF21 action on adipose tissues. FGF21 actions on adipose tissues play an important role in mediating the beneficial effects of chronic exercise on alleviating systemic lipotoxicity, glucose tolerance and insulin resistance.
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Disclosure: **L. Geng:** None.

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Exercise training diminishes the effect of acute exercise on insulin sensitivity in man

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Background and aims: Several studies have shown that both a single bout of exercise as well as repeated bouts of exercise (exercise training) increase insulin stimulated glucose uptake in skeletal muscle of man. Yet, it is not well described how the improvement in insulin sensitivity with exercise training affects the ability of a single bout of exercise to increase insulin sensitivity further. Thus, the aim of this study was to investigate the effects of acute exercise on insulin stimulated glucose uptake before and after a training period.
Materials and methods: We investigated nine healthy, lean, young men who performed an acute bout of one-legged exercise at the same relative intensity (1h at 80% of peak workload (PWL) including 3×5 min intervals at 100% PWL) before and after 12 weeks of exercise training (indoor cycling 4×1h per week at 75-90% of maximal heart rate). On each experimental day (48 hrs after the last training session), after having performed the acute bout of one-legged exercise, the subjects underwent a euglycemic hyperinsulinemic clamp (EHC) ([insulin] ~100 μIU/mL). Insulin stimulated leg glucose uptake was measured in both legs ([glucose]A-V differences × blood flow (measured by Doppler ultrasound technique)) before and during the EHC. Muscle biopsies were taken immediately before (i.e. 4 hrs after acute exercise) and after the EHC.
Results: After 12 weeks of exercise training subjects had increased their VO2peak significantly from 43.6±1.6 to 50.9±1.2 ml/kg/min ($p<0.001$) and glucose infusion rate during EHC was also improved by exercise training (5.1 ±0.4 to 6.1±0.4 mg/min/kg, mean last 60 min of the EHC, $p<0.05$). In the control leg insulin stimulated glucose uptake was increased from 34±6 to 45 ±6 μmol/min/kg lean leg mass (mean last 60 min of the EHC, $p<0.01$) by exercise training. Interestingly, the increment in insulin stimulated leg glucose uptake with acute exercise was not further increased by exercise training (68 ±6 vs. 62±5 μmol/min/kg lean leg mass, no statistical difference). Thus, the difference in insulin stimulated glucose uptake between the exercise leg and control leg was 50% lower after training compared to before training ($p<0.01$). This was seen despite that glycogen degradation by acute exercise was similar before and after the training period.
Conclusion: In conclusion, these results suggest that the ability of acute exercise to increase insulin stimulated glucose uptake in the previous exercised muscle is diminished in trained subjects. By exploring the molecular mechanisms behind this phenomenon we anticipate further insight to our understanding of the beneficial effects of exercise with regards to insulin sensitivity not only in healthy subjects but also in patients with insulin resistance and type 2 diabetes.
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Disclosure: **D.E. Steenberg:** None.

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Circulating skeletal muscle-derived microvesicles expressing fatty acid transporters are increased by acute exercise in type 2 diabetes

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Background and aims: Skeletal muscle is a secretory organ important for intercellular metabolic communication. We propose that microvesicles (MVs) shed from skeletal muscle (SMMVs) may be involved. Further that acute exercise (AE) induces release of SMMVs, in particular SMMVs expressing the metabolically important fatty acid transporter protein 4 (FATP4) and CD36. The aim of this study was to investigate the presence of circulating SMMVs, and to study the impact of AE on SMMVs and SMMVs expressing FATP4 and CD36 in particular. In addition, the influence of T2DM was explored since fatty acid uptake is increased in T2DM.
Materials and methods: Obese males with (N=13) and without (N=14) T2DM underwent a 60 min bicycle exercise test (70% VO2max). Blood samples were collected before and immediately after exercise. MVs (size-range 100-1000 nm) were analyzed by flow cytometry using fluorescence threshold triggering. SMMVs were identified as particles positive for Lactadherin-binding and muscle-specific sarcolemmal beta-Sarcoglycan and either FATP4 or CD36. Study groups were compared by Mann-Whitney U test and exercise-induced MV release by Wilcoxon signed-rank test. The study was approved by the local ethical committee and informed consent was obtained before inclusion.
Results: At baseline total MV numbers were increased by 10% in T2DM compared to controls ($p<0.05$) and SMMVs tended to be increased ($p=0.06$), whereas SMMVs expressing FATP4 or CD36 were unaltered. In both study groups total SMMVs were unaltered after AE. However, in T2DM subjects SMMVs carrying FATP4 or CD36 were increased relatively 1.58 fold ($p<0.02$) and 1.36 fold ($p<0.02$), respectively. In controls, SMMVs carrying FATP4 were unaltered after AE, whereas CD36-positive SMMVs were increased 1.36 fold ($p<0.003$).
Conclusion: This study present novel data demonstrating the presence of circulating SMMVs. AE triggers the release of SMMVs expressing fatty acid transporter proteins important in fatty acid uptake, in particular in T2DM. We hypothesize that SMMVs shed upon muscle contraction may be involved in intercellular communication and provide novel insight to the metabolic condition of their parental skeletal muscle cells.
Disclosure: **M.H. Nielsen:** None.

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HOMA-AD and inflammatory profile: the role of different types of physical exercise in obese adolescents

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Background and aims: It has shown that adolescents with obesity present high prevalence of insulin resistance (83%) and hypoadiponectinemia associated to an increased atherosclerotic process. Adiponectin, an adipose tissue-specific factor, clearly, is a major modulator of glucose and lipid metabolism, which improves insulin sensitivity and inhibit vascular inflammation. The cycle, between the pro/anti-inflammatory states may regulate the metabolic health and the amelioration, is partially dependent of the clinical approach choose to obtain the effectiveness in therapy, including different types of exercise training. Recently it has showed that the Homeostasis Model Assessment-Adiponectin (HOMA-AD) is an importante sensitive predictor of insulin resistance12 however; it was not clear the role of different kinds of exercise training in their improvement in obese adolescent. Since, we hypothesized that in a long-term weight loss therapy, aerobic plus resistance training (AT+RT) was more effective than aerobic training (AT) alone, in ameliorate this risk factor in obese adolescents.
Materials and methods: 148 obese adolescents (15-19y) were enrolled in the long-term interdisciplinary weigh loss therapy (one year) and was randomized in two groups, aerobic training (n=51) and aerobic plus resistance training (n=97). The study was approved has by the Local Ethics Committee and performed in accordance with the Helsinki Declaration. Blood samples was collected to analyze adiponectin, glucose and insulin concentrations. Homeostasis Model Assessment-Adiponectin (HOMA-AD) and Homeostasis Model Assessment Insulin Resistance Index (HOMA-IR) measured the Insulin Resistance. The adopted significant value was $\alpha \leq 5\%$. To analyze the effects of therapy was applied ANOVA two way test (repeated measures) post hoc Bonferroni. Delta values were calculated by the difference between baseline and end values of variables.
Results: Both kinds of exercise training promoted a decrease in body mass, body mass index, and fat mass, visceral and subcutaneous fat. However, only aerobic plus resistance training was effective to reduce compared with aerobic training: fat mass (kg and %) (Δ AT= -7.41 vs Δ AT+RT= -10.86, $p \leq 0.05$) and (Δ AT= -5.04 vs Δ AT+RT= -7.64, $p \leq 0.05$), respectively. Subcutaneous fat (cm) (Δ AT= -0.70 vs Δ AT+RT= -0.90, $p \leq 0.05$); insulin (μg/l) (Δ AT= -1.81 vs Δ AT+RT= -5.04, $p \leq 0.05$); HOMA-IR (Δ AT= -0.45 vs Δ AT+RT= -1.04, $p \leq 0.05$) and HOMA-AD (Δ AT= -0.17 vs Δ AT+RT= -12.51, $p \leq 0.05$). In addition to increased lean body mass (kg and %) (Δ AT= -0.96 vs Δ AT+RT= 2.65, $p \leq 0.05$) and (Δ AT= 5.02 vs Δ AT+RT= 7.64, $p \leq 0.05$) vs aerobic training alone.
Conclusion: The aerobic plus resistance training was more effective than aerobic training alone to reduce HOMA-AD and inflammatory profile, suggesting clinical application on diabetes, obesity, atherosclerosis and metabolic syndrome control in the pediatric population.
Clinical Trial Registration Number: NCT 0135/7883
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Disclosure: **A.R. Dâmaso:** None.

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Effect of 12-week combined exercise programme on metabolic health and skeletal muscle phenotype in elderly individuals

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Background and aims: Age-related decline in skeletal muscle mass and function may predispose to lower mobility and impaired metabolic health often associated with ageing. Exercise has been shown to improve insulin sensitivity, glucose tolerance, physical fitness and quality of life independent of age. We aimed to investigate the effect of 12-week exercise program in seniors on skeletal muscle and whole-body metabolic phenotype.
Materials and methods: Fourteen volunteers (8M/6F; age=64.9±7.6yrs; BMI=28.0±4.1kg/m2) underwent 12 weeks of supervised combined aerobic-resistance training (3x/week). OGTT, indirect calorimetry and quadrupedal bioimpedance were performed to assess metabolic and anthropometric phenotypes. Myofibrillar ATPase activity was measured in native cryosections of m. vastus lateralis by histochemistry to evaluate skeletal muscle fiber type & fiber size. Rockport walking test was used to assess aerobic fitness. Muscle strength was evaluated by dynamometry, motor functions by 10m walk test and chair stand test. Glucose, lactate, myoglobin and creatin kinase (CK) levels were measured at rest and acutely after 40min bout of aerobic exercise both before and after the intervention.
Results: Twelve week exercise training reduced % body fat ($p<0.05$), improved glucose tolerance (AUC oGTT; $p<0.005$, 2h glucose; $p<0.05$) while increasing % lean body mass (LBM; $p<0.05$), maximal aerobic capacity (VO2max; $p<0.001$), resting energy expenditure (REE; $p<0.001$), preferred walking speed ($p<0.005$) and maximal voluntary contraction (MVC) force of knee extension ($p<0.05$). In contrast to slow-twitch (type I) muscle fibers, abundance of fast-twitch (type II) fibers increased on average by 10.2% ($p<0.005$). Neither type I nor type II fiber size was modified by training ($p>0.05$). However, type II fiber size correlated with 2h glucose (oGTT, $p<0.05$; $R=-0.41$), LBM ($p<0.005$, $R=0.49$), handgrip strength ($p<0.001$, $R=0.56$), MVC of knee flexion ($p<0.001$, $R=0.61$) and extension ($p<0.01$, $R=0.45$), MVC on horizontal leg press ($p<0.005$, $R=0.49$), VO2max ($p<0.01$, $R=0.47$) and chair stand test speed ($p<0.05$, $R=-0.37$). Type II fiber size also positively associated with both resting and acute exercise-induced levels of myoglobin ($p<0.05$, $R=0.43$ vs. $p<0.05$, $R=0.42$) and CK ($p<0.001$, $R=0.62$ vs. $p<0.001$, $R=0.64$) and negatively with acute post-exercise lactatemia ($p<0.005$, $R=-0.80$, $n=12$) and glycemia ($p<0.05$, $R=-0.35$).
Conclusion: Body composition, glucose tolerance and physical fitness improved after 12-week exercise program in seniors. Training induced an increase in fast-twitch fibers abundance, which is typically reduced with ageing. Relationships between muscle fiber size and markers of muscle damage, muscle strength, physical fitness and glucose metabolism indicate that characteristics of muscle fibers is important not only for metabolic and functional state of skeletal muscle but also for the whole body metabolic health.
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Disclosure: **Z. Janakova:** None.

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The higher insulin resistance the lower cardiac output in adults with type 1 diabetes during maximal exercise test

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Background and aims: Insulin resistance may have a direct effect on the function of the heart and blood vessels. Only few reports analyze this phenomenon during exercise in patients with type 1 diabetes. The aim of this study was to evaluate the insulin resistance effect on cardiac output analyzed during maximal exercise test.
Materials and methods: The study included 30 men with type 1 diabetes, the median age was 33.5 years (IQR 28.3-38.3), with a median duration of diabetes of 6.7 years (IQR 6.1-7.3), median HbA1c 6.9% (IQR 6.3-7.3), median daily insulin dose: 0.4 U/kg/day (IQR 0.3-0.5), median BMI 25.7 kg/m2 (IQR 24.4-28.3), with declared moderate physical activity (according to International Physical Activity Questionnaire (IPAQ). The study excluded patients with hypertension, chronic complications of diabetes, acute inflammation, with episodes of severe hypoglycemia or

DKA in one month before the test. Insulin sensitivity was assessed using normoglycemic-hyperinsulinemic clamp with measurement of glucose disposal rate (GDR) 48-56 hours before exercise test. Patients were divided into insulin sensitive group (G1) GDR≥4.5mg/kg/min, and insulin resistant (G2) GDR<4.5 mg/kg/min. Assessment of cardiac output was performed using analysis of bioimpedance of the chest with the device PhysioFlow (PF-07 Enduro, Manatec Laboratories, Paris, France) during graded exercise test on cycle ergometer, conducted to voluntary exhaustion.

Results: Groups G1 and G2 did not differ significantly in age, BMI, duration of diabetes, HbA1c and daily doses of insulin. Tested groups reached the maximum fatigue after 11.7 minutes of exercise, the group G1 after 12.3 min (IQR 9.7-14.0) and the group G2 after 11.1 min (IQR 9.7-12.0), p=0.12. G1 has reached up to 240 watt (IQR 200-260) and the group G2: 200 watt (IQR 180-220), p=0.007. The maximal heart rate at the maximal effort, was in G1: 177/min (IQR 169-187), and G2: 172/min (IQR 158-180), p=0.12. Group G2 was characterized by a lower cardiac output (CO): during exercise (8.6 vs 12.8 l / min; p <0.0001) and at the maximal effort (13.1 vs 18.6 l/min; p=0.001). In addition a significant positive correlation of GDR and cardiac output was observed: during exercise test (Rs 0.63, p=0.0002) and on the top of maximal effort (Rs 0.56, p=0.001).

Conclusion: The higher insulin resistance, the lower cardiac output analyzed during maximal exercise test in adults with type 1 diabetes.

Disclosure: **P. Niedzwiecki:** None.

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The effect of mechanical squat exercise on muscle function and body composition in sarcopenic and non-sacropenic women

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Background and aims: Sarcopenia, a newly recognized geriatric syndrome, is characterized by age-related decline of skeletal muscle plus low muscle strength and/or physical performance. Squat exercise is known be a strengthening exercise. Body composition in known to be changed by exercise. However, exercise type, characteristics of subjects can be impact on the body composition. The aim of this study is to exam the impact of squat exercise using motorized medical device on muscle function and body composition in sarcopenic and non-sarcopenic women.

Materials and methods: Community dwelling women over 60 years were enrolled (n=30). They were assigned to exercise(n=20) and control group(n=10). We checked whole body composition and grip strength, knee extensor, and 3 min walking distance before and after the squat exercise. The exercise was done by mechanical assisted squat, 30 minutes, three times a week, for 6 weeks. Approved by the Institutional Review Board.

Results: After the 6-week exercise, grip strength and knee extensor, and 3 min walking distance was improved in both sarcopenic and non-sarcopenic group (P<0.005, P<0.001, respectively). After the exercise, leg regional fat percent was decreased in both sarcopenic and non-sarcopenic group (p=0.047, p=0.029, respectively). The lean mass of leg and total was increased in non-sarcopenic group (p=0.030, p=0037). The appedicular skeletal muscle mass was also increased only in non-sarcopenic group (p=0.013). Oxidized LDL was significantly decreased in subjects with sarcopenia (p=0.037).

Conclusion: Squat exercise using motorized medical device could improve muscle function in both sarcopenic and non-sarcopenic group. Oxidative stress can be improved in sarcopenic patients by squat exercise. Even though, muscle mass was not increased after 6 week squat exercise, we could observe increased muscle function and anti-oxidative effect in sarcopenic group.

Characteristics	Sarcopenia (n=10)			Nonsarcopenia(n=20)		
	Pre	Post	P value	Pre	Post	P value
Age (years)	75,4±5,3			73,0±6,2		
Height (cm)	149,0±4,4			153,6±3,7		
Weight (kg)	55,9±6,4	56,7±6,7	0,059	58,1±6,9	58,7±6,9	0,001
BMI (kg/cm2)	25,3±3,2	25,7±3,5	0,032	24,7±3,1	24,9±3,1	0,084
Lt HG(kg)	18,1±3,4	19,1±3,3	0,005	21,8±4,0	23,3±4,0	0,000
Rt HG(kg)	16,9±1,8	19,3±2,6	0,005	22,7±3,3	24,7±3,8	0,000
3MWD(m)	226,3±47,2	241,1±39,2	0,005	261,7±33,3	275,2±33,7	0,000
TUG (s)	7,41±1,02	5,92±0,78	0,005	7,04±1,03	5,84±0,84	0,000
Lt knee extensor (g)	121,5±34,9	170,7±41,4	0,005	135,2±26,2	195,4±52,9	0,000
Rt knee extensor (g)	114,2±41,5	158,3±48,9	0,005	144,0±35,7	203,3±47,6	0,000
LDL (mg/dl)	106,4±48,1	106,9±50,1	0,959	116,1±37,8	111,9±35,4	0,285
TG (mg/dl)	188,4±171,9	161,9±83,8	1,000	110,0±54,4	106,4±34,5	0,940
FFA (mg/dl)	569,7±292,9	532,9±168,6	0,646	660,4±246,6	691,9±325,9	0,911
GGT (mg/dl)	21,9±8,3	21,0±7,0	0,676	20,2±8,9	22,0±13,2	0,622
TC (mg/dl)	192,3±47,3	183,7±48,7	0,721	189,0±45,0	180,0±42,0	0,014
25(OH)D (mg/dl)	42,3±10,2	37,8±11,7	0,241	48,9±19,4	47,8±21,7	0,968
oxidzed LDL (mg/dl)	60,9±15,4	51,5±10,4	0,037	48,5±17,4	44,3±14,8	0,296
Arm regional fat percent	35,2±5,8	37,0±5,6	0,017	34,1±6,5	33,5±6,1	0,156
Leg regional fat percent	36,8±8,0	35,7±7,5	0,047	33,4±6,4	32,3±6,7	0,029
android lean(g)	2406±228	2416±201	0,683	2488±409	2554±429	0,019
gynoid lean (g)	4225±311	4437±324	0,017	4785±611	4884±598	0,009
Leg lean (kg)	9,4±0,8	9,5±0,8	0,445	10,7±1,4	10,9±1,4	0,037
Total lean (kg)	32,4±2,0	33,0±1,6	0,093	36,1±4,5	36,7±423	0,030
ASM (kg)	11,8±1,1	12,1±1,0	0,114	13,4±1,8	13,7±1,7	0,013

HG: hand grip strength, 3MWD: 3min walking distance, TUG: time to Up and Go test, ASM: appendicular skeletal muscle mass

Clinical Trial Registration Number: H-1504-015-029

Disclosure: **M. Shin:** None.

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Reported high physical activity predicts long partial remission in adults with new onset type 1 diabetes

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Background and aims: Physical activity was associated with improved glycaemic control in some studies in patients with established type 1 diabetes. Little is known about the impact of physical activity on the initial course of disease in patients with new onset type 1 diabetes (NOT1DM). Therefore, the aim of this study was to investigate the association between physical activity and duration of partial remission in this group.

Materials and methods: We included 106 patients (36 women and 70 men, aged 18-35 years, mean age 25 ± 5 years), admitted to the teaching hospital with autoimmune NOT1DM (confirmed with positive result of at least one of the autoantibodies: ICA, GADA or IA-2). 20 patients presented with diabetic ketoacidosis (DKA), mean (±SD) glycaemia at diagnosis was 23.8 ± 8.9 mmol/l, HbA1c 10.8 ± 2.3% and BMI 22.1 ± 3.1 kg/m2. Remission was defined as the time when the patients met all of the the following criteria: HbA1c <6.5%, daily dose of exogenous insulin <0.3 U/kg body weight and serum C-peptide concentration >0.5 ng/ml. Patients were divided into those with remission shorter than one year (short remission, n=56) and those with remission at or exceeding 1 year (long remission, n=50). Physical activity was assessed by self-report. High physical activity was defined as regular practice of sport (at least 1 hour 3 times per week) or daily work associated with strenuous physical activity. Statistical analysis was performed using Statistica 10 software.

Results: In univariate logistic regression reported high physical activity increased the odds of long remission (OR: 8.54, 95%CI: 3.52-20.69, p<0,0001), while the following factors were negative predictors: DKA at diagnosis of diabetes (OR:0.22, 95%CI: 0.07-0.71, p=0.01), cigarette smoking (OR: 0.29, 95%CI: 0.13-0.68, p=0.004), and HbA1c value (OR: 0.78, 95%CI: 0.65-0.95, p=0.01). In multivariate logistic regression model, the association between reported high physical activity and long remission was independent from age, sex, cigarette smoking, BMI at diagnosis, number of specific autoantibodies, presence of DKA, HbA1c value and glycaemia at diagnosis (OR: 3.56, 95%CI: 1.31-18.50, p<0.0001).

Conclusion: High physical activity may be considered as important predictor of long partial remission of type 1 diabetes, independent from potential confounders.

Clinical Trial rEgistration Number: NCT02220257

Supported by: Grant from Polish Ministry of Science and Higher Education

Disclosure: **S. Pilacinski:** None.

PS 031 Action of insulin and other agents on target tissues: new insights

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Characterisation of the cardiac function of human insulin, the long-acting insulin analogues M1-glargine and degludec in adult rat ventricular cardiomyocytes

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Background and aims: Important aspects for the long-term use of insulin analogues are cardiovascular efficacy and safety data. Previously we have shown that under steady-state conditions, insulin glargine (IGla) and its active metabolite M1 (IGlaM1) as well as insulin degludec (IDeg) all elicit a comparable signalling and contractility response in different cardiomyocyte cell models. Indeed, under steady-state conditions all insulins displayed a similar AKT phosphorylation and contractility of electrically paced adult rat ventricular cardiomyocytes (ARVM) after 15 min. However, for IDeg, a delayed onset of action could be observed. In the current study, we investigated the early contractility response of human insulin (HI), IGlaM1 and IDeg in electrically paced ARVM.
Materials and methods: ARVM were isolated by enzymatic digestion, starved overnight, and treated with 100 nM insulin or insulin analogues. ARVM were paced at 1 Hz, 15 V, 0.5 ms with the IonOptix MyoPacer Cell Stimulator System to assess sarcomeric shortening.
Results: In the early phase (1-5 min), only HI and IGlaM1 were able to stimulate fractional shortening of total cardiomyocytes length in a comparable dose-dependent manner (1, 10, and 100 nM) with a maximal shortening at 100 nM of 8.8% ± 1.1% and 7.3% ± 0.3%, respectively. In contrast, IDeg displayed no clear dose response up to 1000 nM and a maximum increase of only 3.6% ± 0.3% at 1000 nM. The difference might be explained by a different binding behaviour of IDeg toward the cell membrane-embedded insulin receptor (IR). The IC50 values observed in radioactive insulin displacement assays differ between detergent-solubilised (S-IR) and membrane-embedded IR (M-IR) preparations (IC50 values [nM] S-IR vs M-IR: 0.4/0.6 [HI], 0.7/0.9 [IGlaM1], 2.3/18.2 [IDeg]), which could result from a different association kinetic.
Conclusion: In summary, the data demonstrated that IDeg and IGlaM1 show a different onset of action in AVRM, most probably due to a different IR kinetic. However, under steady-state conditions a similar efficacy can be observed. Whether those differences might translate to in vivo conditions needs further investigation.
Supported by: Sanofi
Disclosure: **P. Wohlfart:** Employment/Consultancy; Sanofi.

501

Novel insulin analogues with improved therapeutic benefits: dicarba insulin analogues exhibit reduced mitogenic potential

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Background and aims: Treatment of Type 1 and late stage Type 2 diabetes requires the use of insulin. Current use of rapid- and long-acting insulin analogues has vastly improved the ability to emulate a normal biphasic insulin response to food intake. However, in addition to the undesirable metabolic-mitogenic profile of some of the commonly used insulin analogues, existing insulin mimetics also have poor in vivo stability and short shelf-life. Defining the physiologically relevant active conformation of insulin and understanding the molecular mechanism of insulin action are essential for the development of improved insulin mimetics. We have developed a series of novel rapid- and long-acting insulin analogues with a modified A6-A11 cysteine framework. Structural and functional studies of these analogues provide us a unique position in defining the active conformation of insulin that has never been described before.
Materials and methods: Insulin analogues with an unsaturated carbon-carbon bond (dicarba insulins) were generated using olefin metathesis. The functional capabilities were measured in receptor binding, activation and downstream signaling assays (Western Blot). Biological activities of the analogues were analyzed in glucose uptake (metabolic) and DNA synthesis (mitogenic) assays. Biophysical analysis (Circular Dicroism, X-ray crystallography) investigated the structural and stability properties.
Results: Our studies using two non-interconvertible dicarba stereoisomers (Z and E) revealed surprising observations that point to the critical role of A6-A11 disulfide bond in the biological properties of insulin, including receptor potency, downstream signaling and insulin structural stability. Insulin receptor binding and activation assays showed that 'Z' is active and 'E' is inactive revealing the importance of A6-A11 disulfide bond topology for insulin efficacy. Astoundingly, while the active dicarba analogues effectively lower blood glucose levels in mice (high-fat diet), they possess significantly lower mitogenic activity in vitro through IGF-1 receptor (IGF-1R) and insulin receptor (IR). Downstream signaling of the active dicarba insulin analogues also revealed a dose-dependent lower activation ($0.001 \leq p \leq 0.05$) of the mitogenic Ras-MAPK pathway. Not only is the intrachain disulfide bond critical for insulin action but it can also influence signaling outcomes. Biophysical analysis also suggested that the active dicarba analogues might acquire a more open, less flexible conformation than insulin. Our findings point to the crucial role of the A6-A11 disulfide bond in maintaining insulin stability and acts as a "toggle" controlling the transition of insulin between inactive and active states.
Conclusion: We conclude that dicarba insulins effectively lower blood glucose levels while having minimal mitogenic effect and thus represent potential exciting novel diabetic therapies. Most importantly, our findings also provide us valuable insight in redesigning insulin analogues with improved stability.
Supported by: NHMRC: APP1069328; ARC: LP120200792
Disclosure: **S.C. Ong:** None.

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Efficacy differences of long acting insulin analogues on glucose oxidation and cardiac efficiency in healthy and diabetic mouse hearts

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Background and aims: Cardiovascular safety is important for the long-term use of insulin and insulin analogs. Diabetes results in cardiac insulin resistance, increased cardiac fatty acid oxidation, and decreased cardiac glucose oxidation. These changes lead to a decrease in cardiac efficiency. Consequently, insulin or insulin analogs should promote a more pronounced glucose oxidation with benefits for the diabetic heart. Here we investigated the influence of human insulin (INS) and insulin analogs insulin glargine (GLA) and insulin degludec (DEG) on cardiac energy metabolism and signaling in diabetic mouse hearts. To address the fact that in vivo the metabolite M1 (GLA-M1) of insulin glargine is active, we used GLA-M1. We also used a high dose of DEG because of its potential for decreased availability due to albumin binding.
Materials and methods: Isolated working hearts from 10 wk old C57bl/6 and db/db mice were treated with either vehicle, insulin (C57bl/6, 100 μU/ml; db/db, 500 μU/ml), DEG (C57bl6, 1000 μU/ml; db/db 1000 μU/ml), or GLA-M1 (C57bl/6, 100 μU/ml; db/db, 500 μU/ml). Glycolysis and glucose, palmitate, and lactate oxidation were continuously measured. The phosphorylation status of cardiac Akt and GSK3β was also investigated.
Results: GLA-M1 was more effective than INS and DEG at stimulating glucose oxidation in healthy and diabetic mouse hearts. GLA-M1 increased glucose-derived ATP production in healthy hearts to 30% (control: 13%), INS treatment stimulated to 18% followed by DEG (12%). In db/db mouse hearts, glucose oxidation was significantly reduced. Under this condition, both INS and GLA-M1 increased glucose oxidation to a similar extent (control: 5%; INS: 13%; GLA-M1: 14%), whereas DEG had no effect. When measuring ATP production from palmitate oxidation, GLA-M1 treatment resulted in a reduced rate in healthy (GLA-M1: 37%; control: 64%) and db/db mouse hearts (GLA-M1, 56%; control: 74%) followed by INS (healthy mouse hearts: 55%; db/db mouse hearts: 53%), whereas DEG was not effective (healthy mouse hearts: 62%; db/db mouse hearts: 75%). Further, the metabolic switch induced by the GLA-M1 was associated with improved cardiac efficiency (control: 412.8±48.5; INS: 515.7±110.1; GLA-M1: 617.0±55.4; DEG: 435.6±62.6 joules*nmol-1). All insulins stimulated AKT and GSK3β phosphorylation to a similar extent indicating no difference in their insulin receptor mediated potency.
Conclusion: In summary, our data suggest that, compared to INS and DEG, GLA-M1 is more effective at improving cardiac energy metabolism in healthy and diabetic mouse hearts, which results in improved cardiac efficiency. Whether these differences translate to in vivo conditions needs further investigation.
Supported by: Sanofi
Disclosure: **N. Fillmore:** Grants; Sanofi.

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Achieving near euglycaemia using long-term insulin pump therapy differentially improves C-peptide level or disposition index in type 2 diabetes

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Background and aims: Through insulin pump (CSII) therapy, hyperglycemia can be controlled to near normal level in patients with type 2 diabetes (T2D). We wanted to find out whether the changes in beta cell function, insulin sensitivity, and disposition function would be observed in T2D patients during long-term CSII therapy.
Materials and methods: We discontinued oral antidiabetic drugs and applied CSII therapy to T2D patients (number, 163 with 56.4% of male; age, 59.7 ± 9.7 years; duration, 11.1 ± 6.9 years; HbA1c 8.9 ± 1.9%; BMI 24.4 ± 3.1 kg/m2). Blood samplings were performed yearly for 4 years at overnight fasting and 120 minutes after ingestion of a standard mixed meal (500 kcal). Serum C-peptide, glucose, and HbA1c were measured and C-peptidogenic Index (CI), Matsuda Index (MI), and disposition Index (DI) were calculated.
Results: Patients were grouped into high MI [insulin sensitive (IS) group; age 58.0 ± 10.2 years; BMI 23.7 ± 3.1 kg/m2] and low MI [insulin resistant (IR) group; age 61.1 ± 9.1 years; BMI 25.0 ± 3.0 kg/m2] by the mean value of baseline MIs. HbA1c decreased significantly from 8.9% to 6.6% in both groups with no difference between the two groups. In the insulin sensitive group, serum C-peptide increased significantly but DI did not change during the 4-years CSII therapy. Whereas, in the insulin resistant group, serum C-peptide did not increase but the DI increased significantly during the same period of CSII therapy (Figure 1).
Conclusion: Glycaemic control to near normal level was associated with increase in C-peptide level only in IS group but not in IR group, while it was associated with increased DI level in IR group but not in IS group. Therefore, achieving near euglycaemia using long-term insulin pump therapy seems to restore the original defect in each diabetic group.

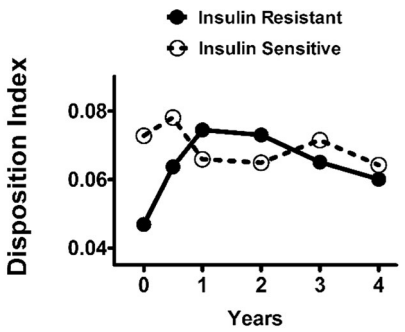

Fig. 1. Disposition Index change for 4 years by insulin pump therapy in type 2 diabetics

Disclosure: **S. Choi:** None.

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Role of intracellular DPP-4 in hepatic insulin signalling

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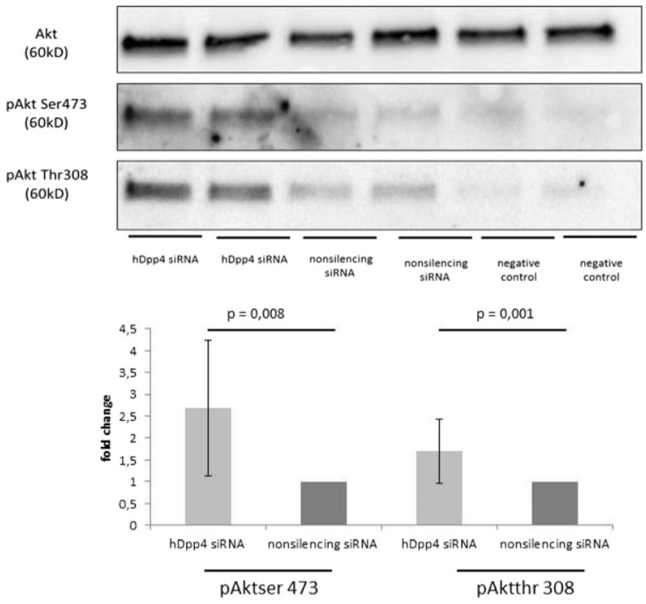
Background and aims: Systemic dipeptidylpeptidase - 4 (DPP - 4) cleaves incretins such as glucagon-like peptide - 1 (GLP - 1) on the N-terminal end. Incretins are critically involved in glucose homeostasis by regulating glucagon and insulin secretion. In contrast to its systemic effects, intrahepatic function of DPP - 4 are still unknown. We examined to determine the role of DPP - 4 in hepatic insulin metabolism.
Materials and methods: hDPP - 4 knockdown was induced in HepG2 cells by siRNA transfection. Protein levels of hDPP - 4, Akt, pAKT, ERK, pERK, IR, IRS-1 and pIRS - 1 were determined by Western blot analysis. Glycogen content was quantified enzymatically.
Results: In HepG2 cells, hDPP - 4 knockdown was associated with a significant increase in basal glycogen content. Insulin stimulated glycogen content tended to be higher in hDPP - 4 knockdown cells without reaching statistical significance. Additionally, phosphorylation of Akt, ERK and IRS - 1 on the tyrosine 612 site were significantly increased in hDPP - 4 suppressed cells compared to control cells. In parallel,

phosphorylation of IRS - 1 on the serine 307 site which exerts inhibiting properties on the IRS - 1/PI3K/Akt signal cascade was significantly decreased in hDPP - 4 knockdown cells compared to control cells.
Conclusion: In summary, hDPP - 4 knockdown in hepatocytes is associated with activation of the IRS - 1/PI3K/Akt signaling suggesting that besides its well - known systemic effects DPP - 4 also directly modulates hepatic insulin signaling by intracellular mechanism.



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Disclosure: **K.H. Rufinatscha:** Grants; Austrian Federal Ministry of Science, Research and Economy and the National Foundation for Research, Technology and Development.

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Metformin ameliorates peripheral insulin resistance by inhibiting the activity of SHIP2

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Background and aims: Type 2 diabetes mellitus (T2DM) with its complications, including diabetic diabetic kidney disease (DKD), is a major personal and public health care concern. Insulin resistance, the pathogenetic hallmark mechanism of T2DM, is also an independent risk factor for DKD. The need for safe and effective hyperglycemic control to prevent the development and progression of DKD presents a challenge to discover new drug targets and drug candidates. The expression of lipid phosphatase SHIP2 (SH2 domain-containing inositol 5´-phoshatase 2), a negative regulator of the insulin signaling pathway, is elevated in kidney, adipose and muscle tissue in experimental models of diabetes. Thus, SHIP2 is a potential therapeutic target to treat insulin resistance. To date only a few chemical compounds possessing an inhibitory effect on SHIP2 are known. All of them have poor bioavailability and none have reached clinical use. This study aimed to identify novel SHIP2 inhibitors with improved characteristics.
Materials and methods: To identify novel small molecules that inhibit SHIP2 we performed virtual screening of chemical libraries followed by validation by biological screening and analyses using cultured cells, diabetic db/db mice and kidney tissue from patients with or without T2DM.
Results: Virtual screening of chemical libraries containing 88680 molecules, confirmed by biological screening, revealed among other molecules metformin as a potential SHIP2 inhibitor. Although metformin has been used to treat T2DM for a long time, the exact molecular mechanism by which it enhances peripheral insulin sensitivity has remained elusive. We found that metformin inhibits the catalytic activity of the in vitro produced and purified SHIP2 phosphatase domain. Metformin also inhibited the activity of SHIP2 immunoprecipitated from cultured rat skeletal muscle cells and glomerular epithelial cells or podocytes, and protected podocytes against SHIP2 overexpression-induced apoptosis. Furthermore, metformin enhanced glucose uptake which was reduced by SHIP2 overexpression in these cells. In vivo, metformin reduced the activity of SHIP2 immunoprecipitated from skeletal muscle and kidney tissue of metformin-treated diabetic db/db mice compared to control db/+ mice. Furthermore, we observed that T2DM patients on insulin medication showed increased SHIP2 activity in the kidney compared to patients without T2DM. In patients receiving metformin the activity of SHIP2 in the kidney was similar to that observed in patients without T2DM.
Conclusion: Our data indicate that metformin inhibits the activity of SHIP2, providing a mechanism by which metformin ameliorates peripheral insulin resistance. Identification of SHIP2 as a target of metformin highlights the potential of SHIP2 as a drug target and provides an avenue to identify and design novel molecules, such as the ones identified in our virtual screening, which can be used to develop new insulin sensitizers for future clinical trials.
Supported by: ERC, Academy of Finland, Diabetes Research Found., Sigrid Jusélius Found.
Disclosure: **S. Lehtonen:** Grants; ERC, Academy of Finland, Diabetes Research Foundation, Sigrid Juselius Foundation, Medical Faculty, University of Helsinki.

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Metabolic effects of combined glucose-fructose supplementation versus glucose alone in exercising individuals with type 1 diabetes

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Background and aims: Exercise-associated glycemic imbalance remains a challenge in patients with type 1 diabetes (T1D). In practice, two approaches may be considered to prevent exercise-related hypoglycemia: adaptation of insulin therapy and/or ingestion of carbohydrates (CHO). In this context, CHO types and combinations of differing CHO may have an important impact on exercise-related fuel metabolism. The aim of the present study was to investigate the effect of fructose co-ingested with glucose compared to glucose alone on the metabolic and hormonal response in exercising individuals with T1D without prior insulin reduction.
Materials and methods: Eleven individuals with well-controlled T1D (aged 26±4y, diabetes for 14±7y, HbA1C 7.0±0.6%, VO2max 47.0 ±7.0ml*kg-1*min-1) were randomly assigned to a 90 min cycling session at 50% VO2max with regular ingestion of a 1:1 mixture of glucose and fructose (GLUFRU) or glucose (GLU) alone. CHO supply was based on regular blood glucose measurements with the aim of keeping patients euglycemic during exercise following a pre-specified algorithm. Hormones, metabolites and substrate oxidation were measured at regular intervals. Glucose and fructose kinetics were investigated using a dual tracer approach (oral [U-13C] fructose/glucose and intravenous [6,6-2H2] glucose).

Results: Blood glucose (GLUFRU: 7.9±0.3 mM; GLU: 7.7±0.3 mM, p=0.7) and insulin levels (20.5±0.2 and 20.7±0.2 mU/l, p=1.0) did not differ between interventions. The total amount of ingested CHO immediately before and during exercise was similar in GLUFRU and GLU (31±3 and 34±4 g, p=0.49) and the exercise interventions were iso-energetic (867 vs. 825 kcal, p=0.34). Counter-regulatory hormones did not differ between interventions. Lactate levels were significantly higher in GLUFRU (2.5±0.2 and 1.7±0.2mM, p=0.02). Rates of glucose appearance (Ra) and disappearance (Rd) during exercise were comparable (Ra 7.51 vs. 7.74 mg kg-1 min-1, p=0.69; Rd 8.13 vs. 8.33 mg kg-1 min-1, p=0.41). Fat oxidation was significantly higher (5.6±0.3 vs 2.5±0.2 mg kg-1 min-1) and CHO oxidation was significantly lower (16.9±1.0 vs 23.9±0.9 mg kg-1 min-1) in GLUFRU when compared to GLU (p<0.001 for both). Based on a combined analysis of whole body substrate oxidation and glucose kinetics myocellular glycogen oxidation was significantly lower in GLUFRU compared to GLU (myocellular glycogen oxidation: 9.26 vs. 16.76 mg kg-1 min-1, p=0.001).

Conclusion: Co-ingestion of glucose and fructose in exercising individuals with T1D without prior insulin reduction increased fat oxidation when compared to glucose alone. Conversely, the addition of fructose was associated with lower peripheral glycogen oxidation. In conclusion, co-ingestion of glucose and fructose may be promising strategy to optimize exercise-related fuel metabolism in individuals with T1D.

Clinical Trial Registration Number: NCT02068638

Supported by: Swiss National Science Foundation (#320030-149321)

Disclosure: **T. Züger:** None.

PS 032 Determinants of insulin sensitivity in adipose and skeletal muscle tissues

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Plasmalogens regulate macrophage polarisation and protect against adipose tissue inflammation in high fat diet-fed mice

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Background and aims: Low grade inflammation in adipose tissue is a major contributing factor for the development of insulin resistance in obesity. It was proposed that high fat diet (HFD)-feeding leads to a shift of M2 macrophages to M1 macrophages in adipose tissue to contribute to insulin resistance. Plasmalogens, a key structural component of the cell membrane, are highly distributed in nervous tissues and defects in plasmalogen biosynthesis are related to several inherited neurologic diseases in human. In addition, recent studies have found that alterations in plasmalogen levels may be related to metabolic diseases and inflammatory diseases. This study examined the possible protective role of plasmalogens against adipose tissue inflammation and whole body insulin resistance.

Materials and methods: To evaluate whether plasmalogen depletion can affect systemic energy metabolism, we generated KO mice of glyceronephosphate O-acyltransferase (Gnpat), the rate-limiting enzyme for plasmalogen biosynthesis.

Results: Body weight gain of Gnpat+/- mice fed a HFD for 8 weeks was comparable to WT mice. However, HFD-fed Gnpat+/- mice exhibited significantly higher fasting plasma insulin levels, despite comparable fasting plasma glucose levels. In addition, HFD-induced glucose intolerance and insulin resistance measured by IPGTT and ITT, respectively, were exacerbated in Gnpat+/- mice. Conversely, administration of alkyl glycerol, a precursor of plasmalogen biosynthesis, ameliorated HFD-induced adipose inflammation and insulin resistance. Alkyl glycerol dose-dependently decreased LPS-induced increases in the expression of inflammatory markers in RAW 264.7 macrophage cell line.

Conclusion: These results suggest that plasmalogens are an important regulator of obesity-associated adipose tissue inflammation and systemic insulin resistance.

Supported by: NRF-2006-2005412; 2009-0091988: K.-U.L.

Disclosure: **K. Lee:** None.

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Role of cannabinoid receptor type 1 in glucocorticoid-induced insulin resistance (IR) and lipolysis regulation in human adipose tissue (AT)

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Background and aims: Glucocorticoids and the endocannabinoid system are both involved in the regulation of energy balance. Cannabinoid receptor type 1 (CNR1) is highly expressed in the central nervous system, but it is also expressed in AT. We recently showed that CNR1 is upregulated in human AT by the synthetic glucocorticoid dexamethasone. Here, we study the involvement of CNR1 in glucocorticoid-induced lipolysis and IR in human AT.

Materials and methods: Human subcutaneous (sc) and omental (om) AT, obtained from non-diabetic volunteers (13M/30F, 24-66 yr, BMI

20.7-55.5 kg/m^2) undergoing kidney donation or bariatric surgery, was incubated with or without dexamethasone (0.003-3 μM, 24h) to measure CNR1 gene and protein expression levels using real-time PCR and immunohistochemistry, respectively. In addition, sc AT acquired from non-diabetic volunteers (5M/17F, 23-72 yrs, BMI 21.3-32.8 kg/m^2) by needle biopsies was incubated with or without dexamethasone (0.3 μM, 24 h) in the presence or absence of the CNR1 antagonist/inverse agonist AM281 (3 μM) during the last 4 h of incubation to measure adipocyte lipolysis and glucose uptake. Furthermore, a separate cohort with 20 type 2 diabetic subjects (10 males and 10 females) was group-wise matched by gender, age and BMI with 20 non-diabetic subjects; and CNR1 gene expression was assessed in fresh sc AT explants.

Results: Dexamethasone (0.003-3 μM) increased CNR1 mRNA expression dose-dependently in AT ($p<0.001$, sc: by 14-fold, om: by 29-fold). Dexamethasone (0.3 μM) increased CNR1 protein expression by about 2-fold ($p<0.05$) compared with control. In multivariate regression analyses, HOMA-IR was a significant predictor of the CNR1 gene expression of 24 h-incubated non-treated AT from both depots (sc: β standardized coefficient (std β) =0.45, $p<0.001$; om: std β =0.65, $p<0.001$), suggesting that IR is associated with higher CNR1 expression in AT. Furthermore, waist circumference (std β =0.83, $p<0.001$) and om adipocyte diameter (std β =-0.45, $p<0.001$) were significantly associated with CNR1 gene expression in sc incubated AT. CNR1 gene expression in freshly harvested AT was 2-fold elevated in type 2 diabetic subjects compared to healthy controls. In sc AT, pre-treatment with dexamethasone (0.3 μM) for 24 h increased the rate of isoprenaline-stimulated lipolysis by about 50%, compared with control ($p<0.01$), whereas AM281 prevented this effect ($p<0.01$). Moreover, incubation of sc AT for 24 h with only the CNR1-specific agonist ACEA (1 μM), increased isoproterenol-stimulated lipolysis in adipocytes by about 17% ($p<0.01$). Dexamethasone also reduced the rate of basal glucose uptake in sc by about 40% ($p<0.001$) and insulin-stimulated glucose uptake by about 30% ($p<0.01$). However, treatment with AM281 did not prevent these effects.

Conclusion: Our findings suggest that CNR1 is upregulated in states of IR and type 2 diabetes. Furthermore, CNR1 is involved in glucocorticoid-regulated lipolysis in sc human AT. This study gives further support of a role of the peripheral endocannabinoid system in glucocorticoid-induced IR. It supports the potential of peripherally restricted CNR1 antagonists for future treatment of type 2 diabetes.

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Disclosure: **C.O. Sidibeh:** None.

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The central effect of galanin receptor 1 agonist M617 on insulin sensitivity in adipocytes of diabetic rats

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Background and aims: To explore whether central galanin receptor 1 (GALR1) mediates the benefiting effect of galanin on insulin sensitivity and glucose uptake.

Materials and methods: This study was conducted to evaluate glucose transporter 4 (GLUT4) levels in plasma membranes of adipocytesand insulin sensitivity of diabetic rats,as well as associated signaling molecules after intracerebroventricular administration of M617, a GALR1 agonist.

Results: The central M617 treatment significantly increased body weight and food intake of rats, as well as the plasma adiponectin contents, the glucose infusion rates in hyperinsulinemic euglycemic clamp tests, the GLUT4 mRNA expression levels and GLUT4 concentration in plasma and total cell membranes, but attenuated the plasma C-reactive protein concentration in adipocytes. The ratios of GLUT4 contents in plasma membranes to total cell membranes in M617 group were higher than diabetic controls. In addition, central M617 enhanced pAkt and pAS160 levels, but not phosphorylation of cAMP response element-binding protein content in adipocytes.

Conclusion: These results suggest that the GALR1-triggered activation of Akt/AS160 signaling pathways mediate the central facilitating effect of galanin on insulin sensitivity and GLUT4 translocation from intracellular pools to plasma membranes. Therefore, galanin may enhance insulin sensitivity and glucose uptake via activating central GALR1 in adipocytes of diabetic rats.

Disclosure: **Z. Zhenwen:** None.

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Independent role of circulating glucose, NEFA and insulin on glucose and lipid metabolism in skeletal muscle and adipose tissue

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Background and aims: High plasma glucose and non-esterified fatty acid (NEFA) levels occur in obesity and type 2 diabetes. Skeletal muscle and adipose tissue play distinct roles in substrate handling. Glucose and NEFA utilization by skeletal muscle and effective storage by adipose tissue are considered protective against insulin resistance, excessive lipolysis, and skeletal muscle steatosis. However, active substrate storage by adipose tissue may also promote obesity. The aim of the study was to investigate the independent effect of circulating glucose, insulin, and NEFA levels on glucose uptake, triglyceride synthesis and *de novo* lipogenesis in subcutaneous, visceral, and epicardial adipose tissue and in skeletal muscle.

Materials and methods: Twenty-two weight-matched pigs were stratified according to four protocols: low NEFA and low insulin (nicotinic acid, NA), high NEFA and low insulin (prolonged fasting), low NEFA and high insulin (euglycaemic-hyperinsulinaemic clamp), low NEFA and high insulin and glucose (hyperglycaemic-hyperinsulinaemic clamp). Positron emission tomography with [18F]fluorodeoxyglucose and [11C]acetate, was combined with [U-13C]palmitate enrichment techniques, and tissue biopsies to assess glucose and lipid metabolism.

Results: High insulin levels increased glucose extraction, and hyperglycaemia enhanced glucose uptake in skeletal muscle, subcutaneous and visceral adipose tissues compared to fasting. Furthermore, data confirmed a higher glucose avidity in visceral compared to subcutaneous adipose tissue ($p<0.001$). In hyperglycaemia-hyperinsulinaemia, *de novo* lipogensis was increased by 70% systemically and by 39% in subcutaneous adipose tissue compared to baseline. In the latter, hyperglycaemia also resulted in greater [U-13C]palmitate and triglyceride (TG) contents (112.4 ±11.7 vs 23.4±5.9, 27.6±11.0, 15.8±7.2 μmol/100 mg in euglycemic-hyperinsulinaemia, fasting and NA groups, $p<0.05$ vs all groups), which were independently associated with plasma glucose levels ($r=0.685$, $p=0.001$ and $r=0.772$, $p<0.001$, respectively). Similar trends were observed in visceral fat, while no differences were found in the epicardial depot. Conversely, in skeletal muscle TG accumulation was greater after prolonged fasting (21.3±11.1 μmol/100 mg vs 3.5±1.4 in eugylcaemic and 3.9±2.2 in hyperglycaemic clamps, $p<0.05$ and $p<0.08$ respectively), consistent with a higher systemic lipolysis. Skeletal muscle [U-13C]palmitate uptake was inversely associated with tissue glucose clearance ($r=-0.582$, $p=0.007$) and plasma insulin levels ($r=-0.541$, $p=0.031$).

Conclusion: Our data show that skeletal muscle TG accumulation is primarily driven by circulating NEFA levels, and not affected by hyperinsulinaemia or hyperglycaemia. Hyperinsulinaemia inhibits adipose tissue lipolysis, but does not elevate lipid deposition in

adipose tissue. Hyperglycemia is required to increase TG stores in adipose tissue, via the uptake of circulating NEFA and promotion of *de* novo lipogenesis. These results suggest that the suppression of lipolysis and the expansion of adipose tissue are differently regulated, and that post-prandial hyperglycaemia may play a role in the pathogenesis of obesity.
Disclosure: **M.A. Guzzardi:** None.

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Identifying microRNAs involved in the regulation of skeletal muscle mitochondrial function

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Background and aims: Type 2 diabetes mellitus is characterized by a reduced peripheral insulin sensitivity and a diminished mitochondrial capacity in skeletal muscle. Importantly, strategies that increase muscle mitochondrial capacity, such as caloric restriction and exercise, have been repeatedly demonstrated to parallel improvements in insulin resistance. Interestingly, previous research in cardiac tissue demonstrated that specific microRNAs could regulate PPARδ, a major transcription factor involved in regulating mitochondrial oxidative capacity. Based on these findings, we hypothesize that - also in skeletal muscle - specific microRNAs exist that regulate mitochondrial function.
Materials and methods: We conducted an unbiased, hypothesis free, high-throughput microRNA-silencing screen in C2C12 myoblasts, using a library containing >700 specific microRNA inhibitors. After 24 hours of microRNA silencing we investigated several read-out parameters for mitochondrial capacity, including ATP levels, redox potential, COX4, HSP60 and mitochondrial membrane potential. Subsequently, we aimed to confirm the identified candidate miRNAs in a validation screen in C2C12 myotubes, measuring the same 5 read-out parameters plus oxygen consumption rates, at both 24 and 48 hours post-microRNA silencing. The validation screen was followed by investigation of individual microRNAs.
Results: The screening in myoblasts resulted in 62 microRNA hits that changed at least one of the mitochondrial parameters. Subsequently, we aimed to validate these 62 candidates in differentiated C2C12 myotubes, which resemble the post-mitotic adult skeletal muscle more closely. This validation screen confirmed 45 candidate microRNAs that changed at least one of the read-outs in at least one of the timepoints. Next, we excluded microRNAs without a human homologue and investigated the 20 most prominent hits in more detail. To this end, we measured gene expression of 27 major mitochondrial genes, 24 and 48 hours post-silencing. This analysis revealed that silencing of miRNA-382 increased the expression of several genes involved in mitochondrial dynamics and -biogenesis. However, conventional microarray analysis in C2C12 myotubes with silenced microRNA-382, revealed a collective downregulation of mitochondrial ribosomal proteins and respiratory chain proteins. Interestingly, this effect was accompanied by an imbalance between mitochondrial proteins encoded by the nuclear and mitochondrial DNA ($p<0,01$). This so-called mitonuclear protein imbalance was accompanied by activation of the mitochondrial unfolded protein response (mtUPR), indicated by an induction of HSP60 protein ($p<0,05$).
Conclusion: In the present study, we identified 20 specific microRNAs as modulators of skeletal muscle mitochondrial metabolism. Furthermore, we highlighted microRNA-382 and showed that silencing of this microRNA leads to a collective downregulation of mitochondrial ribosomal protein expression, induces a mitonuclear protein imbalance and activates the mtUPR, previously associated with improved longevity.
Supported by: Diabetes fonds
Disclosure: **D. Dahlmans:** None.

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New targets to control skeletal muscle inflammation: microRNAs regulated by adiponectin

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Background and aims: Low-grade pro-inflammatory state contributes to the metabolic syndrome (MS). Adiponectin (ApN), which is reduced in the MS, has emerged as a master regulator of inflammation/immunity. We wished to identify whether microRNAs (miRNAs) may mediate the anti-inflammatory action of ApN on skeletal muscle.
Materials and methods: miRNA expression profiling was performed in tibialis anterior muscles of ApN-knockout (ApN-KO) mice: one leg was electrotransferred with a plasmid coding for the ApN gene, while the contralateral leg received an empty plasmid and served as control. Mice were next challenged by lipopolysaccharide (LPS) to induce inflammation. Role of specific miRNAs was analyzed by gain-of or loss-of function approaches in C2C12 myotubes and in vivo by muscle electrotransfer. miRNA expression was also studied in human myotubes.
Results: Expression of miR-711 was up-regulated by muscle electrotransfer of ApN, which concomitantly reduced inflammation (TNFα, IL-1β) and oxidative stress (peroxiredoxin-3) markers. Likewise, in C2C12 cells, ApN treatment upregulated miR-711 expression. Transfection of miR-711 mimic reproduced the anti-inflammatory effects of ApN, while miR-711 blockade attenuated its protective effects. We found that miR-711 repressed the expression of 4 genes belonging to the Toll-like receptor-4 (TLR4) pathway. This pathway is activated by LPS and ultimately leads to stimulation of NF-kB, a pro-inflammatory transcription factor. As expected, NF-kB activity, measured via a luciferase reporter plasmid, was reduced by the miR mimic and enhanced by miR silencing. This protection against inflammation was recapitulated in ApN-KO mice by in vivo muscle electrotransfer of a plasmid coding for miR-711. Eventually, miR-711 expression was also upregulated in human myotubes after ApN treatment.
Conclusion: miR-711, which is up-regulated by ApN, represses TLR4 signaling and NF-kB, acting therefore as a major mediator of the anti-inflammatory action of ApN on muscle. This novel miRNA may open new therapeutic perspectives for the MS or other inflamed muscle conditions.
Supported by: ARC, FRSM
Disclosure: **R. Boursereau:** None.

PS 033 Determinants of insulin sensitivity in skeletal muscle

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Decreased TCF7L2 expression enhances insulin action in primary human skeletal muscle cell cultures

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Background and aims: Variants within transcription factor 7-like 2 (TCF7L2) are the strongest genetic predictor of type 2 diabetes risk. Expression of TCF7L2 is ubiquitous but while many studies have examined the role of TCF7L2 in beta cells, few have examined TCF7L2 function in relation to glucose metabolism in peripheral tissues. The aim of the current study is to explore if TCF7L2 has a role in glucose metabolism in primary human skeletal muscle cells.
Materials and methods: Primary muscle cell cultures were derived from insulin-resistant type 2 diabetic subjects and age- and BMI-matched non-diabetic subjects. These were genotyped for the TCF7L2 risk allele at rs7903146. Gene expression was measured using real-time PCR while knockdown of TCF7L2 expression was achieved using siRNA. The effects of TCF7L2 knockdown on insulin action were assessed by measuring insulin-stimulated glucose uptake and glycogen synthesis. Insulin signalling was assessed by Western blot and PCR arrays were used to examine changes in gene expression between control and TCF7L2 knockdown cells.
Results: Expression of TCF7L2 was increased in heterozygous carriers of the diabetes risk T allele compared with those homozygous for the wild-type C allele at rs7903146 ($p<0.01$). TCF7L2 expression was knocked down in cultured human skeletal muscle cells from non-diabetic heterozygous carriers of the risk allele. A 72% reduction in TCF7L2 gene expression resulted in a significant increase in both insulin-stimulated glucose uptake and glycogen synthesis compared with cells transfected with a scrambled control. Basal levels of glucose uptake were similar between the scrambled control and TCF7L2 knockdown cells (43.99±4.253 vs 44.60±5.315 pmol/min/mg, respectively) while insulin-stimulated glucose uptake increased from 54.77±4.708pmol/min/mg in cells transfected with the scrambled control to 90.12±15.34pmol/min/mg in TCF7L2 knockdown cells ($p<0.001$). Basal glycogen synthesis levels increased from 123.3 ±23.2pmol/min/mg in the scrambled control to 169.7±28.2pmol/min/mg in TCF7L2 knockdown cells and insulin-stimulated glycogen synthesis also increased from 341.5±45.6pmol/min/mg to 515.1±51.5 (vs scrambled control, $p<0.05$). However, there was no change in the phosphorylation status of the insulin signalling molecules, Akt or GSK-3. Gene expression analysis using a pathway-focussed PCR array indicated that protein kinase C zeta (PRKCZ) was the top regulated gene in TCF7L2 knockdown cells compared to control. PRKCZ expression was significantly upregulated ($p<0.005$) in TCF7L2 knockdown cells. In order to examine the effects of PRKCZ inhibition on insulin action siRNA was used to inhibit expression of both TCF7L2 and PRKCZ in the same cells. Double knockdown of TCF7L2 and PRKCZ was confirmed at the mRNA level and insulin-stimulated glycogen synthesis assessed as a measure of both uptake of glucose into the cell and its storage as glycogen. Insulin-stimulated glycogen synthesis was increased in TCF7L2 knockdown cells ($p<0.05$) as observed previously, however, knockdown of PRKCZ had no effect on insulin-stimulated glycogen synthesis.
Conclusion: This study confirms that carriers of the risk T allele at rs7903146 have an increased mRNA expression of TCF7L2. TCF7L2 appears to play a role in insulin action in cultured human skeletal muscle cells but this is not mediated via PRKCZ. This study indicates a role for TCF7L2 in glucose metabolism in skeletal muscle cells independent of the insulin signalling pathway.
Supported by: DRWF, NIHR BRC
Disclosure: **A.E. Brown:** None.

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Regulation of fatty acid oxidation and glucose metabolism by miR-29 in human and mouse skeletal muscle

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Background and aims: MicroRNAs (miRs) have emerged as key regulators of metabolic processes, consequently, dysregulation of miRs may contribute to metabolic abnormalities. Preliminary evidence from our laboratory suggests that miR-29a and c expression is increased in skeletal muscle from type 2 diabetes patients. Moreover, skeletal muscle miR-29a and c expression is reduced in rodents following one week exercise training. Based on these findings, we tested the hypothesis that miR-29a and miR-29c alter lipid or glucose metabolism using cultured human primary myotubes and intact mouse skeletal muscle.
Materials and methods: Cultured human muscle cells were transfected either with pre-miRs or with a specific miR inhibitor to determine the role of miR-29 on glucose and fatty acid metabolism. Additionally, miR-29 was overexpressed in tibialis anterior muscle of C57Bl/6J mice by electroporation and in vivo glucose uptake was determined.
Results: Overexpression of miR-29a or c in both cultured human primary myotubes and in intact mouse muscle decreased glucose uptake and insulin responsiveness. Overexpression of miR-29a or miR-29c in human primary myotubes decreased basal and AICAR-stimulated palmitic acid oxidation, whereas triglyceride synthesis remained unchanged. Inhibition of miR-29a or miR-29c in cultured human primary myotubes increased fatty acid oxidation, whereas mitochondrial abundance was unaffected. Expression of PGC1alpha and PGC1alpha target genes were reduced after miR-29c overexpression, and increased following miR inhibition. Overexpression of miR-29a and miR-29c in intact mouse skeletal muscle reduced PGC1alpha mRNA expression by 30%.
Conclusion: We provide evidence that miR-29 plays a direct role in glucose and lipid metabolism in skeletal muscle. Modulation of miR-29 family member abundance in skeletal muscle may contribute to lipid oxidation, and may explain altered lipid metabolism in the context of type 2 diabetes or exercise training.
Supported by: ERC, SI, SRC
Disclosure: **J. Massart:** None.

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WISP1 associates with insulin resistance and impairs insulin action in skeletal muscle cells but not in hepatocytes

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Background and aims: The secreted extracellular matrix-associated protein WISP1 (Wnt1-inducible signaling pathway protein-1) has recently been identified as a novel adipokine. Increases in the expression of WISP1 have been linked to insulin resistance, and WISP1 expression levels are decreased after weight loss. This study examined whether WISP1 expression and circulating levels are altered in patients with type 2 diabetes. Furthermore, we analyzed whether recombinant WISP1 affects insulin signaling in primary human skeletal muscle cells (hSkMC) and murine AML12 hepatocytes.

Materials and methods: Serum and visceral adipose tissue biopsies for analysis of circulating WISP1 levels by ELISA and WISP1 expression by real-time PCR were collected from normal-weight control men (n=34), and morbidly obese men with (n=52) and without type 2 diabetes (n=47) undergoing abdominal surgery. The effects of WISP1 on insulin action were examined by Western blotting in hSkMC and AML12 hepatocytes that were exposed for 24 h to WISP1 prior to insulin stimulation.
Results: Circulating WISP1 levels were 44.3 pg/ml in morbidly obese men independent of type 2 diabetes versus 17.3 pg/ml in controls ($p<0.07$). Also, WISP1 mRNA levels in VAT were 1.8-fold higher in morbidly obese men versus normal weight men ($p<0.05$), and associated positively with HOMA2-IR (age- and BMI-adjusted $\beta=0.23$, $p<0.05$) and insulin levels (age- and BMI-adjusted $\beta=0.24$, $p<0.05$). In vitro, WISP1 induced a trend to lower insulin-mediated Akt-Thr308 phosphorylation by 18% in hSkMC, whereas a 37% reduction in insulin-mediated phosphorylation of GSK3β-Ser9 was significant ($p<0.001$).No effect of WISP1 on Akt phosphorylation was observed in AML12 hepatocytes.
Conclusion: Collectively, both circulating WISP1 levels and WISP1 expression in visceral adipose tissue are elevated in obesity. Furthermore, our data suggest that WISP1 associates with impaired insulin sensitivity in muscle but not in liver cells.
Supported by: DZD
Disclosure: **T. Hörbelt:** None.

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Ketoacidosis and insulin resistance in muscle in subjects with type 1 diabetes: a human, randomised, controlled, cross over study
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Background and aims: Ketoacidosis is a life-threatening condition for patients with type 1 diabetes (DM1). Reduced insulin together with the stress metabolic and inflammatory response contributes to impaired glucose metabolism. This results in increased glucose output from the liver and reduced oxidation in muscle. Several lines of evidence suggest an important role of skeletal muscle metabolism during early stages of ketoacidosis. We therefore established a human model for ketoacidosis using lipopolysaccharide (LPS - endotoxin) and relative hypoinsulinaemia to define metabolic events in muscle.
Materials and methods: Nine DM1 were included in a randomized, controlled, crossover study. All subjects were investigated twice using a hyperinsulinaemic euglucaemic clamp(clamp) after an overnight fast during 1) euglycaemic conditions (CTR) or 2) hyperglycaemic, ketotic conditions (KET). KET was induced using an intravenous bolus of endotoxin/lipopolysaccharide (LPS) combined with a reduced insulin treatment.
Results: Energy expenditure (EE) under clamp conditions was similar during KET and CTR, but glucose oxidation was remarkably lower during KET than CTR (434 vs. 737 kcal/day, $p<0.05$). Respiratory exchange rate (RER) tended to be lower on KET day (0.81±0.01) compared with CTR (0.87±0.03). Arteriovenous glucose difference during clamp was negative (-0.2±0.1 mmol/l) during KET compared with CTR (1.2±0.3 mmol/l, $p<0.01$).
Conclusion: We conclude that glucose oxidation is markedly reduced during KET despite similar EE. This is associated with profound insulin-resistance in muscle evident by arteriovenous glucose difference. This underscores the presence of muscle insulin-resistance patients with DM1 during early phases of ketoacidosis.
Clinical Trial Registration Number: NCT02157155
Supported by: Danish Council for Strategic Research
Disclosure: **M. Svart:** None.

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Sarcopenia is associated with insulin resistance and the early phase of type 2 diabetes in Korea
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Background and aims: Recent studies have focused on the potential impacts of sarcopenia and SO on metabolic disorders beyond physical disability. It has been postulated that sarcopenia with or without obesity may contribute to the risk of metabolic syndrome and type 2 diabetes. However, the definition of sarcopenia and SO in older persons is still controversial, and its use in clinical practice is debated.The aim of this study was to determine appropriate criteria for age-related sarcopenia and sarcopenic obesity (SO), and investigate relationships among sarcopenia, insulin resistance and risk of type 2 diabetes.
Materials and methods: Our analyses included 1285 men and 1724 women who had complete data available for body composition analysis and the 75 g oral glucose tolerance test. Sarcopenia was assessed by the appendicular skeletal muscle mass (ASM)/height2, and the ASM/weight (%) or the skeletal muscle mass index (SMI). Obesity was identified based on the total body fat percentage or the BMI.
Results: ASM/weight and SMI, but not ASM/height2, were inversely associated with the homeostasis model assessment of insulin resistance (HOMA-IR). The prevalence of sarcopenia and SO, defined as ASM/weight less than the one standard deviation below the sex-specific normal mean of a younger reference group and a BMI of over 25 kg/m2, tended to be higher with increased HOMA-IR tertile. Compared to either sarcopenia or obesity alone, SO was associated with a higher risk of pre-diabetes and type 2 diabetes in those 60 years or older after adjusting for confounding factors. Subjects with sarcopenia were at especially high risk of newly diagnosed diabetes in older age groups.
Conclusion: Sarcopenia and SO, assessed by the ASM/weight and BMI, were strongly associated with the degree of insulin resistance and the early phase of type 2 diabetes. These findings suggest that sarcopenia could be an important role in the progression from pre-diabetes to type 2 diabetes.
Disclosure: **S. Kim:** None.

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The effect of high intensity training on insulin sensitivity in overweight, inactive elderly subjects
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Background and aims: Reduced insulin sensitivity, muscle mass and strength and increased abdominal fat are common changes seen with aging. Whether these changes can be explained by aging alone or they occur in combination with an unfavourable life style changes is not clear. Nonetheless, regular exercise improves insulin sensitivity and has beneficial effects on body composition, regardless of age, strongly advocating for the importance of staying physical active during aging. High intensity training (HIT) is a time efficient alternative to endurance training but the knowledge on the health related effects of HIT in elderly is sparse. This study aimed to investigate if HIT training can be applied to inactive, overweight elderly subjects and to study the effect on health related parameters with a focus on body composition, whole body insulin sensitivity and insulin signaling in muscle.
Materials and methods: 11 males and 11 females aged 55-75 years with BMI > 27 kg/m2 performed 6 weeks supervised high intensity training three times a week on a bicycle ergometer. The training consisted of 5 HIT intervals of 60 sec at cadence > 50 RPM interspersed by 90 sec. breaks.

Before and after (>48 hrs after cessation) training a hyperinsulinemic-euglycemic clamp was performed to measure insulin sensitivity. On a separate day DXA scanning (Visceral fat estimated by Core Scan software) and a VO2max test were carried out and muscle biopsies obtained. Proteins relevant for glucose metabolism and insulin signaling were quantified in muscle tissue by western blotting.
Results: 6 weeks HIT induced an increase in VO2max (pre: 2234 ± 106, post: 2361 ± 134 ml O2 /min, $p<0.05$). HIT also induced a reduction in fat% (pre: 39.8 ± 2, post: 39.1 ± 2%, $p<0.05$) and visceral fat content (pre: 1.9 ± 0.2, post: 1.8 ± 0.2 kg, $p<0.05$) and an improvement in insulin sensitivity (GIR: pre: 7.5 ± 0.4, post: 8.0 ± 0.5 mg/min/kg, $p<0.05$). In addition muscle protein content of GLUT4 (delta: 19.9%, $p<0.05$), glycogen synthase (delta: 21.5% , $p<0.01$), hexokinase II (delta: 35.4, $p<0.05$) and AKT (delta: 26.4%, $p<0.001$) were all increased. Statistical analysis: Two-way ANOVA with repeated measures.
Conclusion: This study demonstrates that HIT can be applied to inactive, overweight elderly subjects with clear beneficial training effects on body composition, insulin sensitivity and insulin signaling.
Supported by: Grant from Danish Diabetes Academy supported by Novo Nordisk Foundation
Disclosure: **D. Søgaard:** Grants; This work was supported by a research grant from the Danish Diabetes Academy supported by the Novo Nordisk Foundation.

PS 034 Insulin action: focus on liver

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Regulation of hypothalamic JAZF1 in hepatic glucose production and insulin signalling by triggering a brain-liver neuronal circuit in high-fat fed rats

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Background and aims: Juxtaposed with another zinc finger gene 1 (JAZF1), an inhibitor of the nuclear receptor subfamily 2, group C, member 2signaling pathway, has been reported to be involved in glucose and lipid metabolism, and insulin sensitivity in the peripheral tissues. However, the role of central JAZF1 signaling on glucose homeostasis and insulin action remains unknown. The aim of this study was to determine whether the changes of hypothalamic JAZF1 expression alter hepatic glucose production (HGP) and insulin signaling in vivo.
Materials and methods: We selectively upregulated the expression of hypothalamic JAZF1 in high-fat diet (HFD) fed rats via delivering an adenovirus expressing vector (Ad-JAZF1) into the mediobasal hypothalamus(MBH). And the changes in glucose kinetics and hepatic insulin signaling were evaluated with the hyperinsulinemic-euglycemic clamp technique combined with [3-3H]glucose as a tracer. We further enhanced hypothalamic JAZF1 in the presence or absence of KATP channel inhibition(Glibenclamide), hepatic branch vagotomy (HVAG) or the AMPK activator(AICAR) to assess the effect of hypothalamic Ad-JAZF1 administration on glucose metabolism and insulin signaling in vivo.
Results: In HFD-fed rats, overexpression of JAZF1 in the MBH led to a significant suppression of glucose production with enhancing hepatic insulin signaling during the clamps compared to MBH Ad-GFP-injected control rats. These changes were associated with decreased hepatic expression of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase). Importantly, MBH Ad-JAZF1 administration augmented phosphorylation of insulin receptor substrate1 (IRS1), insulin receptors(InsR) and Akt kinase. Hypothalamic JAZF1's action was blunted by HVG or the delivery of the KATP channel blocker in these animals. In addition, the metabolic effects of hypothalamic JAZF1 were negated by MBH co-injection with both Ad-JAZF1 and the AMPK activator.
Conclusion: These data reveal a vital role for hypothalamic JAZF1 that modulates both HGP and hepatic insulin signaling at least in part through both KATP-chanel and AMPK pathway in central neurons, and further through a brain-liver neuronal circuit.
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Disclosure: **L. He:** None.

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Increased coagulation factor activities and expression in 'Metabolic NAFLD' and 'PNPLA3 NAFLD'

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Background and aims: The I148M gene variant in PNPLA3 increases the risk of non-alcoholic liver disease (NAFLD) but not insulin resistance (IR) or adipose tissue (AT) inflammation ('PNPLA3 NAFLD'). In contrast, NAFLD due to IR ('Metabolic NAFLD') is associated with an

increased risk of atherothrombosis. We hypothesized that increased coagulation factors may characterize 'Metabolic NAFLD' but not 'PNPLA3 NAFLD' and i) measured plasma coagulation variables in 'Metabolic NAFLD' and 'PNPLA3 NAFLD', ii) measured coagulation factor expression in the human liver, and iii) determined whether coagulation factor activities are associated with AT inflammation in 'Metabolic NAFLD' without the I148M gene variant.

Materials and methods: We measured liver fat content (1H-MRS), PNPLA3 genotype at rs738409, plasma activated partial thromboplastin time (APTT), activities of vWF:RCo, FVII, FVIII, FIX, FXI, FXII, FXIII and fibrinogen, and D-dimer concentration in 98 subjects divided into 2 groups based on median homeostatic model assessment (HOMA) of IR ('Low HOMA' and 'High HOMA'; a model for 'Metabolic NAFLD'), and based on PNPLA3 genotype ('PNPLA3-148II' and 'PNPLA3-148MM/MI'; a model for 'PNPLA3 NAFLD'). Liver biopsies were obtained from 26 bariatric surgery non-carriers of the I148M gene variant divided into 2 groups based on liver fat content (NAFLD and non-NAFLD group). In addition, we obtained needle biopsies of AT from 26 subjects non-carriers of the I148M gene variant to quantify mRNA expression of pro-inflammatory (TNFα, MCP-1, macrophage marker CD68) and anti-inflammatory (adiponectin, Twist1) markers by RT-PCR. Serum MCP-1 and adiponectin were also measured.

Results: Liver fat content was similarly increased in 'High HOMA' (13±1 vs. 'Low HOMA' 6±1%, $p<0.05$) and in 'PNPLA3-148MM/MI' (12±2 vs. PNPLA3-148II 8±1%, $p<0.05$) groups. Plasma FVIII, FIX, FXI, FXIII, fibrinogen and vWF:RCo activities were increased and APTT was shortened ($p<0.05$ or less for all) in 'High HOMA' vs. 'Low HOMA'. All coagulation factors were comparable between 'PNPLA3-148MM/MI' and 'PNPLA3-148II'. FVIII, FIX and fibrinogen γ-chain gene expression in the liver were significantly increased in NAFLD as compared to non-NAFLD group ($p<0.05$). AT expression of CD68 correlated with active plasma FIX, FXI, VWF:RCo and fibrinogen ($p<0.05$) and of adiponectin correlated inversely with plasma FIX and fibrinogen ($p<0.05$). Plasma fibrinogen correlated inversely with serum adiponectin ($p<0.05$).

Conclusion: Patients with 'Metabolic NAFLD' but not 'PNPLA3 NAFLD' have increased activities of coagulation factors VIII, IX, XI, XIII and fibrinogen, and shortened APTT. The increase in coagulation factor activities reflect increased production in the liver possibly as a consequence of inflamed adipose tissue.

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Involvement of GRK2/CRTC2 in hepatic gluconeogenesis regulation by follicle-stimulating hormone in ovariectomised mice

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Background and aims: It has been reported that Follicle-Stimulating Hormone (FSH) level was correlated with abnormal glucose level. Dysregulation of hepatic gluconeogenesis is believed to be one of the major factors that contribute to hyperglycemia in metabolic disorders such as type 2 diabetes. While, FSH receptor (FSHR), belonging to GPCR, was identified in hepatocyte recently. GRK2 (GPCR kinases 2) has the ability to induce desensitization of GPCR. However, it shows its magical effect that it could take actions independent of receptor desensitization once impinging into intracellular signal pathways. To date, it is unclear if FSH is associated with hepatic gluconeogenesis and if GRK2 is involved.

Materials and methods: Ovariectomized mice supplemented with estradiol (E2) were injected with and without FSH. Thus we obtained the characteristic mouse model that FSH level was dependent on the dose of FSH injection, maintaining a relatively constant E2 level. We examined fasting glucose and insulin levels, performed pyruvate tolerance test (PTT), oral glucose tolerance test (OGTT), insulin tolerance test (ITT), as well as determined the expression of gluconeogenic genes and related GRK2/CRTC2 signaling in liver. In vitro, HepG2 cells treated with different doses of FSH were also employed. Moreover, we explored the role of GRK2 by application of a specific GRK2 inhibitor methyl ([5-nitro-2-furyl]vinyl)-2-furoate.

Results: 1. Ovariectomized mice injected with FSH exhibited increased fasting glucose level (8.95±0.42 vs. 7.43±0.79 mmol/L; $p<0.05$), increased PTT as well as impaired OGTT and ITT, compared to those without FSH. Fasting insulin level was not altered obviously (185.01 ±31.92 vs. 184.68±32.79 nIU/mL; $p>0.05$). 2. G6Pase and PEPCK, key enzymes of hepatic gluconeogenesis, whose mRNA expressions increased about 2.6 times and about 1.9 times respectively, in liver of mice receiving FSH (both $p<0.05$). 3. However, after silencing FSHR by tail injection of adenoviral siRNA, FSH lost its effect on G6Pase and PEPCK. 4. Increased GRK2 protein level by western blotting and confocal image exhibited activated GRK2 in ovariectomized mice injected with FSH. Oppositely, GRK2 inhibitor could abolish the effect of FSH on gluconeogenesis. 5. CRTC2, a major transcription factor of gluconeogenesis key enzymes, was activated after injecting FSH. When all ovariectomized, supplemented with E2 and then injected with FSH, Crtc2 -/- mice showed reduced fasting glucose level and hepatic gluconeogenesis in comparison with its littermate Crtc2 +/+ and Crtc2 +/- mice (Crtc2 -/- vs. Crtc2 +/+, $p<0.05$). 6. In vitro, FSH consistently up regulated PEPCK and G6Pase mRNA expressions as well as promoter luciferase activities in HepG2 cells. FSH increased glucose production to more than 2 times compared to the control ($p<0.05$). 7. Meantime, no obvious alterations of glycogen synthesis, mRNA and protein levels of GLUT2/4 were observed.

Conclusion: FSH increased hepatic gluconeogenesis via GRK2/CRTC2, indicating an essential role of FSH in the pathogenesis of hyperglycemia.

Disclosure: **Q. Xiaoyi:** None.

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Beneficial effects of TM-25659 on insulin-resistance and hepatic steatosis induced by a high-fat diet in C57BL/6J mice

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Background and aims: Obesity triggers insulin-resistance, inflammation, and hepatic steatosis. 2-butyl-5-methyl-6-(pyridine-3-yl)-3-[2'-(1H-tetrazole-5-yl)-biphenyl-4-ylmethyl]-3H-imidazo[4,5-b]pyridine] (TM-25659), a known transcriptional coactivator with PDZ-binding motif (TAZ) activator, inhibits adipocyte differentiation by interacting with peroxisome proliferator-activated receptor gamma. Our aim was to explore the therapeutic effects of TM-25659 in the context of obesity, obesity-related inflammation, insulin-resistance, and hepatic steatosis.

Materials and methods: C57BL/6J mice were fed a normal diet (ND) or a high-fat (HF) diet for 6 weeks. The ND group continued to consume a normal diet for a further 8 weeks, whereas the HF-diet animals were randomly assigned into two subgroups who consumed an HF-diet or an HF+TM-25659 diet for a further 8 weeks. Body weight and food intake were monitored, as were glucose homeostasis and insulin-sensitivity. Fasting insulin and serum lipid levels were measured at the end of the study. Also, the transcriptional levels of genes involved in inflammation and lipid metabolism were analyzed by real- time PCR. We also investigated the molecular mechanisms of TM-25659-mediated palmitate (PA)-

induced insulin-resistance in HepG2 cells. We used PA and HF diet-induced insulin-resistance models to this end, and measured both the extent of insulin signaling and the expression levels of inflammatory cytokines to explain the therapeutic effects of TM-25659.
Results: Mice consuming the HF+TM-25659 diet were less obese than controls, exhibited less insulin-resistance, and had lower plasma levels of inflammatory cytokines. TM-25659 also inhibited hepatic steatosis induced by the HF diet. Immunoblotting revealed that TM-25659 increased hepatic AMP-activated protein kinase (AMPK) levels. TM-25659 also reduced the liver levels of inflammatory cytokines and reduced the fall in insulin-stimulated (protein kinase B) (AKT) phosphorylation by AMPK evident in mice on the HF diet.
Conclusion: Thus, TM-25659 may be useful to treat insulin-resistance and hepatic steatosis. The drug activated AMPK in a high fat diet-induced model of obesity.
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Elevated plasma branched-chain amino acids are associated with insulin resistance, hepatic fat content and mitochondrial function
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Background and aims: Recent studies implicate that circulating branched-chain amino acids (BCAA) are involved in the development of insulin resistance, likewise BCAA are elevated in pre-diabetic subjects. In the present study we sought to investigate whether systemic BCAA levels in humans are associated with key metabolic parameters like insulin sensitivity, mitochondrial function and intrahepatic lipid (IHL) content. Profiling of amino acids was performed in i) an observational study including T2DM, first-degree relatives (FDR) of T2DM, and control participants (CONo) and ii) an intervention study in which 12 young, healthy participants (CONy) were transiently rendered insulin resistant by 60h of fasting.
Materials and methods: In the observational study 16 patients with T2DM, 6 FDR and 10 CONo (age within 50-65 years) were matched for BMI (31±1, 30±1 and 32±1 kg/m2, respectively T2DM, FDR and CONo). A hyperinsulinemic-euglycemic clamp was performed to measure whole body insulin sensitivity (M-value: μmol/kg/min), proton magnetic resonance spectroscopy (1H-MRS) to determine intrahepatic lipid (IHL) content of the liver (%) and high-resolution respirometry in vastus lateralis muscle tissue to examine ex vivo mitochondrial function (oxygen flux: pmol/mg wet weight/s).
Results: Circulating BCAA were higher in T2DM vs. CONo (531 ± 67 vs. 428 ± 62 μmol/l; $p<0.05$), but similar to FDR (447 ± 69 μmol/l; p=n.s.). We also examined if insulin infusion resulted in a suppression of BCAA levels. Indeed, during the hyperinsulinemic-euglycemic clamp BCAA levels dropped, but the reduction of valine and isoleucine was less pronounced in T2DM compared to CON (28% vs 38%, $p<0.05$ for valine and 48% vs. 59% for isoleucine, $p<0.05$). Strong negative correlations were found between baseline circulating BCAA levels and M-value ($r=-0.49$; $p<0.05$) or mitochondrial function ($r=-0.41$; $p<0.05$). In addition, a strong positive correlation was found between BCAA and IHL ($r=0.52$; $p<0.01$). Interestingly, these associations of BCAA were opposite in young volunteers (CONy: association with M-value ($r=+0.69$;$p<0.01$) and mitochondrial function ($r=+0.64$;$p<0.05$)). Upon 60h fasting, M-value in CONy decreased from 7.1±1.6 to 3.2±0.6 μmol/kg/min ($p<0.05$) and mitochondrial oxidative capacity was reduced by ~19% ($p<0.05$) paralleled by strongly elevated circulating free-fatty acids and BCAA.
Conclusion: BCAA negatively associates with key metabolic parameters in older, obese and T2DM subjects. These associations, however, are not found in young, lean participants. In patients with T2DM a blunted insulin-suppressed proteolysis of BCAA could partly underlie elevated fasting BCAA levels. Our results suggest a pivotal role for a defective BCAA metabolism in insulin resistant, older people.
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Disclosure: **E. Phielix:** Grants; EFSD/Lilly Programme and the NWO VENI grant 016.136.132.

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Mitochondrial ND2 gene mutation in complex I of the respiratory chain - ROS generation, liver steatosis and induction of mitoprotective effects in the course of aging
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Background and aims: The mitochondrial genome of the diabetes-resistant B6-mtALR mouse strain is characterized by a mtDNA encoded Nd2 mutation in complex I of the respiratory chain. OXPHOS mutations in liver and beta cells are linked to obesity, liver steatosis and diabetes. Therefore, we investigated ROS production, antioxidative defense and metabolic key regulators in correlation with fat accumulation in liver and beta cell mass adaptation in conplastic B6-mtALR mice compared to B6-mtAKR control strain.
Materials and methods: Conplastic B6-mtAKR (AKR) and B6-mtALR (ALR) mice were monitored over a period of 2 years. Liver and pancreas samples were collected at the age of 3, 6, 9, 12, 18 and 24 months. Gene expression of antioxidative enzymes and UCP2 were quantified by real-time RT-PCR analysis and protein expression by Western blot analysis. ROS production was measured by the MitoSOX assay. Liver steatosis was scored by staining with OilRedO and HE. The beta cell mass was quantified by computer-assisted fluorescence microscopy.
Results: Blood glucose levels of ALR mice were significantly increased at the age of 3 months compared to the AKR control strain (7.3 vs. 5.6 mmol/l, $p<0.01$). The ALR strain showed lower fat accumulation in liver tissue and decreased beta cell mass in comparison to the AKR control strain in the course of aging. After a constant decline of ROS production in liver until the age of 9 month significantly higher amounts of ROS (8 fold, $p<0.05$) could be detected in ALR mice with progressive aging. Expression of cytoprotective proteins SOD1, SOD2, catalase, glutathione peroxidase in liver were higher in ALR mice compared to the control strain. In particular at juvenile age 3 and 6 months SOD1 and SOD2 were significantly increased in ALR mice. Also UCP2 expression was elevated in early and late life in the ALR strain. Interestingly, UCP2 expression showed a correlation with blood glucose levels in both mouse strains.
Conclusion: The mtND2 mutation in complex I of the respiratory chain favored ROS production in liver with mitohormetic adaptation of antioxidative enzymes and UCP2. This adaptation protected liver against inflammatory steatosis and diabetes until middle age. Thus, mitohormetic adaption proved to be age-dependent and mitoprotective strategies are required at later age to counteract liver steatosis and development of type 2 diabetes.
Disclosure: **M. Wietzke:** None.

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Substrate-specific impairment of liver gluconeogenesis and altered feeding behavior in liver glutamate dehydrogenase knockout mice

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Background and aims: Glutamate dehydrogenase (GDH, encoded by *Glud1* gene) is mainly expressed in the liver, kidney, beta-cells, and the brain. This mitochondrial enzyme catalyzes the reversible oxidative deamination of glutamate to alpha-ketoglutarate and ammonia, although direction of the predominant flux is tissue-dependent, reflecting its organ-specificity. In the liver, GDH is of major importance for ammonia detoxification through nitrogen metabolism and urea synthesis. Hepatic GDH is also involved in the metabolism of most amino acids, in particular glutamine (Gln) and alanine (Ala) being transported from skeletal muscles during periods of active gluconeogenesis. The importance of GDH activity is witnessed by the severity of disorders where GDH function is inappropriate, such as hyperinsulinemia/hyperammonemia syndrome. Here, we have generated inducible liver-specific GDH knockout mice (Hep-*Glud1*$^{-/-}$) to investigate consequences of the lack of hepatic GDH on metabolic homeostasis in basal and energy challenging conditions.
Materials and methods: The *in vivo* deletion of GDH in hepatocytes was induced at 8 weeks of age by subcutaneous implantation of tamoxifen pellets in *Glud1*$^{lox/lox}$ mice carrying the Albumin-Cre-ER^{T2} construct. Metabolic responses were investigated by *ip* challenges (glucose, pyruvate (Pyr), Gln, Ala), by monitoring in metabolic cages and EchoMRI, by measurements of amino acids by HPLC and metabolites by commercially available kits.
Results: Three weeks after *in vivo* induction of recombination by tamoxifen, deletion was successful and specific for the liver in Hep-*Glud1*$^{-/-}$ mice (<5% remaining of immunoreactive GDH), GDH being preserved in non-hepatic tissues. We also observed abrogation of GDH enzymatic activity in liver homogenates (-67%, $p<0.01$). Immunohistochemical analyses of Hep-*Glud1*$^{-/-}$ livers did not show morphological abnormalities. IP glucose tolerance tests revealed normal glucose homeostasis in Hep-*Glud1*$^{-/-}$ mice upon standard conditions and both body weight and fat/lean mass ratio were similar between control and Hep-*Glud1*$^{-/-}$ mice. Remarkably, the food intake profile was changed by the absence of liver GDH, knockout animals exhibiting a shift in the circadian rhythm of feeding. Moreover, lipids were favored over carbohydrates as energy fuel in Hep-*Glud1*$^{-/-}$ mice. Finally, we tested the gluconeogenic capacity of Hep-*Glud1*$^{-/-}$ mice in response to substrates specific for situation of energy imbalance, such as fasting, i.e. Gln and Ala. Control mice increased glycemia in response to both Gln and Ala challenge. However, the Ala response was abrogated in Hep-*Glud1*$^{-/-}$ mice, while the gluconeogenic capacity of its deaminated product Pyr was preserved. These data were validated *in vitro* on isolated primary hepatocytes. Indeed, contrary to controls, knockout hepatocytes were not able to increase neither ammonia production nor glucose output in response to Ala, while the Gln and Pyr responses were preserved. Moreover, plasma amino acids quantification revealed increased Gln and decreased Ala levels in fed Hep-*Glud1*$^{-/-}$ mice when compared with controls.
Conclusion: Liver-specific GDH deletion induced substrate-specific impairment of liver gluconeogenesis, higher lipid consumption, and a shift in the circadian rhythm of feeding. These results highlight the central role of hepatic GDH in energy metabolism.
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Diets high in glucose and fructose alter intestinal short-chain fatty acid in mice: implications for fatty liver and type 2 diabetes development

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Background and aims: High consumption of refined sugar is implicated in the current epidemics of non-alcoholic fatty liver disease (NAFLD) and Type 2 diabetes (T2D). The fructose component of sugar is considered to be more harmful to the liver than its glucose counterpart since its metabolism is poorly controlled resulting in possible energy deficits as well as high carbon inflows into glycolysis and lipogenesis. Fructose and glucose can also be metabolized by both intestinal microflora and enterocytes, but the effects of high intake of either sugar on microbial fermentation activity and microflora-enterocyte dysbiosis - processes that may also play significant roles in NAFLD and T2D pathogenesis, are not known.
Materials and methods: To assess the effects of high dietary levels of refined sugars on intestinal microbial activity and populations and to determine if fructose and glucose promoted different effects on these parameters, we compared three groups of mice over a 10 week feeding period: the first group fed with a diet where fructose was the sole carbohydrate supplying 60% of total calories (FRU), the second group fed a corresponding diet with glucose as the sole carbohydrate (GLU), and the third fed with normal chow (NC). Intestinal metabolites and bacterial species were assessed from periodic 1H NMR and qPCR analyses of feces.
Results: The ratio of Enterobacteria/Lactobacillus is a marker of intestinal health and we observed that there was no significant alteration in this parameter between the three diets. However, we observed significant differences in fecal metabolites between FRU, GLU and CTRL. Fecal short chain fatty acids (SCFA), which are generated by bacterial fermentation and are important substrates and signalling molecules for both intestine and liver, showed significant changes between FRU, GLU and NC. Specifically, both FRU and GLU had lower levels of butyrate and higher amounts of acetate and formate compared to NC. Feces of FRU mice also had high levels of unmetabolized fructose indicative of fructose malabsorption.
Conclusion: Mice that were fed with diets where glucose and fructose were the sole carbohydrates did not show significant alterations in intestinal microflora composition relative to normal-chow fed animals, but did reveal significant differences in SCFA fermentation metabolites. Most notably, the observed depletion of butyrate relative to other SCFA in fructose and glucose-fed mice may promote a reduction in intestinal barrier function and increased exposure of liver and visceral fat to proinflammatory agents. Diet-induced changes in gut fermentation products can occur in the absence of significant changes in microflora species distributions.
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Disclosure: **J.C.P. Silva:** None.

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A chemically modified GIP-Xenin hybrid peptide restores GIP sensitivity, enhances beta cell function and improves glucose homeostasis in high fat fed mice

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Background and aims: GIP and xenin are regulatory gut hormones secreted from enteroendocrine K-cells that exert important effects on glucose homeostasis, insulin secretion and energy regulation. In addition, xenin potentiates biological actions of GIP, which are known to be diminished in type 2 diabetes mellitus. In the present study we evaluated the biological efficacy and therapeutic utility of a novel GIP-Xenin hybrid peptide (GIP-XEN). GIP-XEN was engineered to combine insulin-releasing actions of GIP with satiety and GIP-potentiating effects of xenin within a single molecule and tested against parent peptides.
Materials and methods: GIP-XEN was synthesised by linking aa residues 1-14 of GIP with aa residues 18-25 of xenin (xenin-8). Additionally, L-Ala at position 2 in GIP was substituted with D-Ala and Lys and Arg residues at positions 21 and 22, respectively in xenin-8 were substituted with Gln aa residues to confer stability. GIP-XEN was incubated (0-12 h) with DPP4 (5 mU; n=3) to confirm enzyme stability and BRIN-BD11 cells to evaluate acute insulin secretion (n=8). Acute and persistent effects of GIP-XEN on cumulative food intake, glucose and insulin concentrations were examined in lean mice (n=8). For long-term in vivo studies, groups of HF mice (n=8) received twice-daily injections (09:30 and 17:30 hr) of saline vehicle or test peptides (each at 25 nmol/kg; ip) for 21 days. Energy intake, body weight, circulating glucose and insulin concentrations were measured at regular intervals. Glucose tolerance (18 mmol/kg; ip), biological response to GIP (25 nmol/kg; ip) and insulin sensitivity (15 U/kg; ip) together with pancreatic islet histology were performed at the end of the study.
Results: GIP-XEN was resistant to DPP4 for up to 12 hr and significantly ($P<0.05$-$P<0.001$) enhanced insulin secretion from BRIN-BD11 cells at 5.6 and 16.7 mmol/l glucose concentrations. Acute injection of GIP-XEN in combination with glucose significantly ($P<0.05$) lowered glucose and increased insulin concentrations in lean mice with a protracted response for up to 4 hours ($P<0.05$). GIP-XEN elicited appetite suppressive effects ($P<0.05$ to $P<0.01$) particularly at elevated doses. Twice-daily administration of GIP-XEN for 21 days to HF mice returned circulating glucose concentrations to levels similar to lean controls ($P<0.05$-$P<0.01$). There was no significant effect of GIP-XEN on body weight, energy intake or circulating insulin concentrations. However, glucose tolerance ($P<0.05$) and insulin sensitivity ($P<0.001$) were improved by GIP-XEN treatment. Interestingly, GIP-mediated glucose-lowering and insulin-releasing effects were substantially ($P<0.05$-$P<0.001$) improved by GIP-XEN treatment. Pancreatic islet ($P<0.001$) and beta-cell ($P<0.001$) area, as well as pancreatic insulin content ($P<0.01$) were augmented in GIP-XEN treated mice.
Conclusion: In summary, GIP-XEN has an impressive profile of beneficial metabolic effects, including improvements of glucose tolerance, insulin sensitivity, pancreatic islet morphology and augmentation of biological action of GIP in HF mice. This highlights clear potential of GIP-Xenin hybrid based approach to treatment of type 2 diabetes mellitus.
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Disclosure: **V.A. Gault:** None.

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Duodenal GLP-1 signalling regulates hepatic glucose production through PKC-δ-dependent neurocircuitry

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Background and aims: Intestinal glucagon-like peptide-1 (GLP-1) is a hormone that stimulates insulin secretion and acts as a neuropeptide to control glucose homeostasis, but little is known whether intestinal GLP-1 has any effect in the control of hepatic glucose production(HGP). The present study was designed to explore it.
Materials and methods: We first established the model of intraduodenal GLP-1 infusion in normal-chow-diet rat to determined its role on HGP and detected with GLP-1R-immunoreactivity whether GLP-1 Receptors is expressed in rat duodenum. We then performed pharmacological inhibition of GLP-1 Receptors to disrupt the effect of duodenal GLP-1 on HGP. We further examine whether gut GLP-1 lowers glucose production through a neuronal network, we co-infused the GLP-1 with the local anesthetic tetracaine into the duodenum or co-administration of MK-801, a NMDA receptor blocker to inhibited the NMDA receptors-mediated neuronal transmission in the NTS, or surgical transection of the hepatic vagus nerve and evaluated the impact on changes in glucose kinetics during pancreatic-euglycemic clamps in rats in vivo. Moreover, in order to explore duodenal GLP-1 -dependent mechanisms, we evaluated the role of duodenal PKC-δ. We first administered GLP-1 into the gut of rats and examined PKC-δ activation in the mucosal layer of the duodenum. Then we inhibited duodenal mucosal PKC-δ via intraduodenal coinfusion of GLP-1 with the PKC-δ inhibitor rottlerin to investigated whether duodenal mucosal PKC-δ activation is required for duodenal GLP-1 signaling to regulate glucose metabolism.
Results: Here we found that intraduodenal infusion of GLP-1 activated duodenal PKC-δ, lowered HGP, and was accompanied by a decrease in hepatic expression of gluconeogenic enzymes and an increase in hepatic insulin signaling in rats. However, gut coinfusion of either the GLP-1 receptor antagonist Ex-9, or the PKC-δ inhibitor rottlerin with GLP-1 negated the ability of gut GLP-1 to lower HGP and to increase hepatic insulin signaling during clamps. The metabolic and molecular signal effects of duodenal GLP-1 were also negated by coinfusion with tetracaine, pharmacologic inhibition of N-methyl-D-aspartate receptors within the dorsalvagal complex, or hepatic vagotomy in rats.
Conclusion: Our data show that a rise in intestinal GLP-1 activates GLP-1 receptors and stimulates duodenal mucosal PKC-δ activation triggering a gut-brain-liver neuronal axis which lowers glucose production in normal rodents. There is a neural glucoregulatory function of gut GLP-1 signaling.
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Disclosure: **Y. Lai:** None.

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A novel exendin-xenin fusion peptide substantially improves weight loss and metabolic control in high fat fed mice

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Background and aims: Clinical improvements in weight loss and metabolic control are not as impressive as first hoped with receptor specific GLP-1 mimetics. Xenin-8 is the C-terminal octapeptide of xenin-25 which is released from intestinal K-cells and exhibits antihyperglycaemic and satiety actions. The present study evaluated the biological efficacy and therapeutic utility of a novel Exendin-xenin-8 fusion peptide (EXE-

XEN). EXE-XEN was engineered to enhance the recognised antidiabetic actions of GLP-1, using a single molecule.
Materials and methods: EXE-XEN was synthesised by linking residues 1-28 of exendin-4 with residues of xenin-8. Additionally, Lys and Arg residues at positions 31 and 32, in the xenin-8 region of EXE-XEN, were substituted with Gln. EXE-XEN was incubated (0-6 h) with fasted plasma (n=4) to confirm enzyme stability and BRIN-BD11 cells to evaluate insulinotropic activity (n=8). Acute and persistent effects of EXE-XEN on cumulative food intake, glucose and insulin concentrations were examined in lean mice (n=8). High fat fed (HFF) mice (n=8) were used to evaluate chronic effects of peptides (25 nmol/kg; *ip*) using a twice-daily injection regimen (09:30 and 17:30 hr) over a 21 day period. Energy intake, body weight, circulating glucose and insulin concentrations were measured at regular intervals. Oral glucose tolerance (18 mmol/kg), metabolic response to GIP (25 nmol/kg; *ip*), insulin sensitivity (15 U/kg; *ip*) and aspects of metabolic rate were determined. Terminal plasma lipid, glucagon and amylase activity were also assessed.
Results: EXE-XEN was resistant to plasma degradation and significantly ($P<0.001$) enhanced insulin secretion from BRIN-BD11 cells at 5.6 mmol/l glucose. Acute injection of EXE-XEN in combination with glucose significantly ($P<0.001$) lowered glucose and increased insulin concentrations in mice. Prominent ($P<0.05$) antihyperglycaemic effects were still evident 8 hours post-injection. EXE-XEN also possessed impressive ($P<0.05$) appetite suppressive effects at a dose of 25 nmol/kg. Twice-daily administration of exendin-4 or EXE-XEN for 21 days to HFF mice significantly ($P<0.05$) enhanced percentage body weight loss. Both treatments returned non-fasting glucose concentrations similar levels as lean controls, with EXE-XEN possessing clear superiority. Unlike exendin-4, EXE-XEN significantly ($P<0.05$ to $P<0.01$) increased insulin concentrations during the entire 21 day period. There was no effect of either treatment regimen on energy intake. However, oral glucose tolerance was improved ($P<0.05$) with EXE-XEN, but not exendin-4, treatment. Similarly, GIP-mediated glucose-lowering and insulin-releasing effects were substantially ($P<0.05$-$P<0.01$; respectively) augmented only by EXE-XEN. Insulin sensitivity was enhanced ($P<0.05$) in both groups, and neither treatment altered metabolic rate or amylase activity. Plasma glucagon concentrations were reduced ($P<0.05$) by exendin-4 and EXE-XEN, and total cholesterol levels were lower ($P<0.05$) in the EXE-XEN treated group of mice.
Conclusion: EXE-XEN displays an impressive array of beneficial antidiabetic effects in HFF mice, which was superior to exendin-4. This highlights clear potential of fusion peptides based on GLP-1 and xenin as a suitable approach for the treatment of type 2 diabetes.
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Disclosure: **N. Irwin:** None.

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Exaggerated postprandial GLP-1 secretion after Roux-en-Y gastric bypass is highest after glucose compared with fat and protein ingestion

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Background and aims: Roux-en-Y gastric bypass (RYGB) leads to exaggerated postprandial secretion of glucagon-like peptide-1 (GLP-1). The mechanisms behind this enhanced GLP-1 secretion remain poorly understood, including the relative contribution from the individual macronutrients. Our primary aim was to investigate GLP-1 secretion following isocaloric amounts of glucose, fat and protein in RYGB-operated subjects and secondly to compare with responses in matched non-surgical controls (CON).
Materials and methods: 10 RYGB-operated subjects (3.7 ± 0.4, mean ± SEM, years post-RYGB) and 7 controls (CON) were included and matched on age (RYGB 46 ± 2.0 vs CON 44 ± 3.4 yrs, p=0.60), BMI (RYGB: 33.6 ± 1.5 vs CON: 32.0 ± 0.7 kg/m2, p=0.39) and sex (M:F) (RYGB 3:7 vs CON 2:5, P = 0.95). In randomized order, on 3 separate experimental days, subjects underwent 4-h isocaloric liquid meal-tests containing >90E% of either protein (53 g whey protein), fat (26 g butter) or glucose (50 g) dispersed in 200ml water with 4 g of bouillon. The GLP-1 response was evaluated as incremental area above baseline (iAUC), 0-240 minutes.
Results: In the RYGB subjects, GLP-1 secretion was comparable after fat and protein ingestion (iAUC GLP-1: fat 1819.0 ± 272.9 vs protein 1972.6 ± 394.6 pmol/l*min, p = 0.97), while glucose ingestion increased GLP-1 secretion more than 2-fold (iAUC GLP-1: glucose 4425.8 ± 930.1, $p < 0.01$ compared with both fat and protein). In CON subjects, GLP-1 secretion was also similar after fat and protein ingestion (iAUC GLP-1: fat 1144.4 ± 196.2 vs protein 1005.2 ± 200.9 pmol/l*min, p=0.77), but in contrast, glucose resulted in a lower secretion (iAUC GLP-1: glucose: 460.2 ± 197.4 pmol/l*min, $p<0.05$ compared with both fat and protein). Thus, RYGB subjects displayed an almost 10-fold higher GLP-1 secretion in response to glucose compared with CON ($p<0.01$), while protein (p=0.06) and fat (p=0.05) elicited 1.5-2 fold higher GLP-1 responses in RYGB compared with CON subjects.
Conclusion: Postprandial GLP-1 secretion after RYGB depends on the ingested macronutrients and is markedly higher after glucose compared with fat and protein ingestion. Compared to non-surgical controls, postprandial GLP-1 secretion is slightly enhanced after fat and protein ingestion, but almost 10-fold higher in response to glucose. The results support the importance of the direct delivery of especially glucose to the intestinal L cells for the exaggerated GLP-1 response after RYGB surgery.
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Disclosure: **C.Z. Jensen:** Grants; The Danish Council for Independent Research.

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Estrogens protect against type 2 diabetes by increasing glucagon-like peptide-1 secretion from L cells and alpha cells

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Background and aims: Over the last years, estrogens and their associated signaling pathways have known a growing interest, and in particular regarding their role in metabolism. In fact, clinical and experimental data highlight their beneficial impact on energy and glucose homeostasis. Indeed, their administration has been shown to reduce the risk of type 2 diabetes in several animal models but also in humans. This benefit is partly linked to protective effects on the endocrine pancreas. The aim of our study is to investigate the impact of estrogen on proglucagon producing cells, the alpha and L cells.
Materials and methods: In order to characterize the direct impact of estrogens on proglucagon producing cells, we will use the transgenic "Gcg-Venus /Rip-Cherry" mouse model, characterized by the specific expression of Venus in proglucagon producing cells and Cherry in the insulin producing cells, allowing the isolation of pure cells after sorting by flow cytometry. To free ourselves from the estrogenic impregnation, "Venus / Cherry" adult female mice are ovariectomized (ovx), or sham operated. Next, to identify the direct effects of estrogens on proglucagon producing cells, α and L cells from one week ovx mice are isolated and purified by FACS and treated or not during 48h with 17β-estradiol (E2, 10-8M). Then, to confirm this effect in vivo, "Venus / Cherry" adult female ovx mice receive an administration of 17β-estradiol (E2, 80μg/kg) for 48h.

Results: As expected, the estrogenic deprivation of one week has an effect in vivo on glucose homeostasis after 6 hours of fasting. Indeed, the mice exhibit an altered response to glucose during an OGTT (2g/kg), with a significant increase in the glycemia's AUC (+80%, sham vs ovx, p< 0,05) a week after ovx. This effect is correlated to a decrease in insulin levels (-23%, p< 0,05) and an increase in glucagon (+37%, p< 0,05). Furthermore, ovx mice presented a lower GLP-1 secretion in response to glucose than sham mice (-50%, p< 0,05). Then to determine if E2 has a direct impact on proglucagon producing cells, we isolated pure α cells, or realised mixed intestinal cells cultures from the small intestine and treated them in vitro with E2 10-8M for 48h. The purified α cells showed no differences in terms of proglucagon expression (mRNA), but we noticed that estradiol influences de prohormone convertase expressions (pcsk-2 -40% and pcsk-1 +100%). This is correlated with a decrease in glucagon biosynthesis, and an increase in GLP-1 . Concordantly we also observed an increased capacity of α cells to secrete GLP-1, and a decrease in glucagon's secretion. Furthermore, E2 treatment in small intestine explants, raised their GLP-1 secretary capacity (+75%, p< 0,05). Next in vivo administration of E2 (E2, 80μg/kg/2days) increases pancreatic insulin content and decreases pancreatic glucagon content. We also determined on the purified L cells from these mice that E2 increases GLP-1 biosynthesis (+52%, p< 0,05).

Conclusion: Our results demonstrate that the favorable action of estrogen on the carbohydrate homeostasis is partly due to its direct effects on proglucagon cells, by increasing GLP-1 biosynthesis and secretion. These results open the way to a better understanding of the mechanisms underlying the protective effects of estrogen for the prevention and treatment of diabetes.

Disclosure: **S. Handgraaf:** None.

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Up-regulation of *selenoprotein P* and *HIP/PAP* mRNAs in hepatocytes by intermittent hypoxia via down-regulation of miR-203

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Background and aims: Sleep apnea syndrome (SAS) is characterized by upper airway narrowing or collapse during sleep that leads to a cessation of airflow. An apnea and a hypopnea are often accompanied by a drop in oxygen saturation. Accumulating evidence suggests that recurrent episodes of oxygen desaturation and reoxygenation (intermittent hypoxia (IH)), which are typical features of SAS, contribute to the development of β-cell dysfunction and impaired glucose tolerance. Epidemiological and clinical evidence postulates that SAS may be a causal factor of type 2 diabetes. We have been investigated the mechanism by which IH induces insulin resistance. We have reported that the mRNA levels of *selenoprotein P (SEPP1)*, encoding a diabetes-associated hepatokine, and *hepatocarcinoma-intestine-pancreas/pancreatitis-associated protein (HIP/PAP)*, encoding a hepatic growth factor for regeneration and proliferation, were significantly increased in human hepatocytes (JHH5, JHH7, and HepG2 cells) by IH. In the present study, we researched the mechanism by which IH up-regulates the mRNA levels of *SEPP1* and *HIP/PAP*.

Materials and methods: Reporter plasmids were prepared by inserting the promoter fragments of human *SEPP1* (-2989~+10) and *HIP/PAP* (-4030~+27) upstream of a firefly luciferase reporter gene in pGL4.17 vector. The reporter plasmids were transfected into human HepG2 hepatocytes and the cells were exposed either to 71 cycles/24 h of IH (5 min hypoxia (5% CO_2•1% O_2)/10 min normoxia (5% CO_2•21% O_2)), mimicking hepatocytes of SAS patients, or normoxia for 24 h. After cells were exposed to IH, cells were lysed and promoter activities were measured. The levels of microRNA (miR)-203 and mRNAs for *DROSHA*, *DICER*, *MCPIP1* and *TP63* were measured by real-time RT-PCR. To clarify the role of miR-203 in *SEPP1* and *HIP/PAP* expression in IH, miR-203 inhibitor and non-specific control RNA (miR-203 inhibitor NC) were introduced into HepG2 cells just before IH/normoxia exposure, and the mRNA levels of *SEPP1* and *HIP/PAP* were measured by real-time RT-PCR.

Results: (1) The promoter activities of *SEPP1* and *HIP/PAP* were not increased by IH. (2) Target mRNA search of microRNAs using MicroRNA.org program revealed that both *SEPP1* and *HIP/PAP* mRNAs have a potential target sequence for miR-203. (3) In fact, the miR-203 level of IH-treated cells was significantly decreased than that of normoxia (0.0270±0.02058 fold vs normoxia, P=0.0391). (4) The mRNA levels of *DROSHA*, *DICER*, *MCPIP1* and *TP63*, which are involved in biosynthesis and degradation of microRNAs, were not increased by IH. (5) The IH-induced expression of *SEPP1* and *HIP/PAP* was abolished by introduction of miR-203 inhibitor but not by miR-203 inhibitor NC.

Conclusion: These results indicate that IH stress down-regulates the miR-203 level in human hepatocytes and that the levels of *SEPP1* and *HIP/PAP* mRNAs are increased via the miR-203 mediated mechanism, by which the decrease in miR-203 results in up-regulation of *SEPP1* and *HIP/PAP* mRNAs in IH-exposed hepatocytes, leading SAS patients to insulin resistance/type 2 diabetes.

Disclosure: **T. Uchiyama:** None.

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Bile acids stimulate secretion of GLP-1 and PYY through activation of basolateral L-cell GPBAR1 receptors but have no direct effect on glucagon or insulin secretion

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Background and aims: Recent studies show that bile acids affect the secretion of appetite- and metabolism-regulating hormones, but details are lacking. We investigated the effects of bile acids (BAs) on the secretion of glucagon-like peptide-1 (GLP-1) polypeptide YY (PYY), glucagon and insulin from isolated perfused rat small intestine or pancreas.

Materials and methods: Wistar rat small intestine/pancreas were isolated and perfused with a modified Krebs-Ringer buffer (n=6). Venous effluent was collected every minute from vena portae and peptide concentrations measured by radioimmunoassays.

Results: Intra-luminal (1mM) administration of taurodeoxycholate (TDCA) increased secretion (GLP-1: from 23±1 to 43±3 pM, PYY: 17 ±1 to 55±3 pM, both P<0.0001) and luminal instillation of unconjugated BAs (cholic acid (CA), deoxyCA (DCA) and chenoDCA (CDCA), all 1mM) resulted in comparable GLP-1 and PYY responses (P<0.001 compared to baseline). The bile acid sequestrant cholesevelam eliminated the GLP-1 and PYY response to luminal BAs (CA, DCA and CDCA in both unconjugated, glycine- and taurine-conjugated forms: 1 mM each). Conjugated BAs (TDCA) depended on absorption via the apical-bile acid transporter (ASBT) to stimulate GLP-1 and PYY secretion, whereas specific inhibition of ASBT neither affected absorption of nor the GLP-1/PYY response to the mixture of (lipophilic) unconjugated BAs. Depolarization generated by sodium-coupling of ASBT-mediated uptake of conjugated BAs did not contribute to the secretory GLP-1 and PYY response to TDCA, as blockage of voltage-gated calcium channels had no effect. Therefore, BAs stimulate GLP-1 and PYY secretion secondary to

intestinal absorption, supposedly by direct activation of the Gαs-linked G-protein-bile-acid receptor 1 (GPBAR1), which we here show immunhistochemically to localize basolaterally in GLP-1 producing cells in mouse and rat small intestine and to be expressed in isolated murine L-cells. Supporting this, intra-arterial infusion of a poorly absorbable GPBAR1 agonist (1μM) robustly stimulated the secretion of GLP-1/PYY, whereas intra-luminal infusion (10μM) was ineffective. In contrast to intestinal L-cells, GPBAR1 mRNA was only expressed at very low levels in murine α- and β-cells. While both DCA and CDCA activated human and rat GPBAR1 (cAMP) heterologously expressed in COS7-cells independent of conjugation, all tested bile acids (CA, DCA, CDCA un- or glycine-/taurine conjugated at 0.1 mM) failed to illicit glucagon or insulin secretory responses in perfused rat pancreas either at 3.5 or 10 mM glucose.

Conclusion: BAs stimulate GLP-1 and PYY secretion through basolateral GPBAR1 activation, but have no direct effect on glucagon or insulin secretion. Thus, GPBAR1 is an attractive pharmacologic target for enhancing the secretion of GLP-1 and PYY with a view to treat type-2-diabetes and obesity.

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Disclosure: **R.E. Kuhre:** None.

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Beta cell deficient mouse models reveal that glucose and K_{ATP} channel blockers control glucagon secretion independently of beta cell derived paracrine factor

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Background and aims: The mechanisms by which glucose (G) controls glucagon release by pancreatic α-cells are still unclear. In particular, it is highly disputed whether glucagon secretion is inhibited by a paracrine factor released by β-cells. We therefore assessed this issue using transgenic mouse models with major β-cell ablation.

Materials and methods: The effect of G, tolbutamide (Tolb) and insulin were tested on insulin and glucagon secretion of the *in situ* perfused pancreas of several mouse models. Two mouse models with major β-cell ablation were generated. The RipCre/DTR model, characterized by conditional ablation, was obtained by crossing RIP-Cre mice (RIP: rat insulin promoter) with Rosa26-LoxP-STOP-LoxP-DTR mice. These mice harbor the diphtheria toxin receptor (DTR) specifically in β-cells. Ablation of β-cells was induced at adult stage by injection of diphtheria toxin (DT, 500 ng/mouse, 2 injections 2 consecutive days). Overt hyperglycemia (400 to ≥600 mg/dl) was induced 2 days after the first injection. Seven days after the first injection (5 to 6 days of hyperglycemia), one group of mice was used for perfusion. The other group was transplanted with normal islets to normalize their glycemia (≤200 mg/dl) and perfusion of their pancreas was performed 3 weeks later. A model of constitutive β-cell ablation was also used (RIPCre/DTA). It was obtained by crossing RIP-Cre mice with Rosa26-LoxP-STOP-LoxP-DTA mice. Because the A subunit of diphtheria toxin (DTA) is expressed as soon as RIP is activated, these mice were diabetic from birth (glycemia ≥400 mg/dl). They were made normoglycemic (glycemia ≤200 mg/dl) by transplantation of normal islets at time of weaning (1.5-month-old) and the perfusion experiments were performed 1.5 months later. Three control mouse models were used which have normal β-cells: RipCre/DTR mice not injected with DT, Rosa26-LoxP-STOP-LoxP-DTA mice, and C57Bl6 mice.

Results: In control mice, insulin secretion was stimulated by a rise of [G] of the medium from 1 (G1) to 7 mM (G7), or by the addition of the K_{ATP} channel blocker Tolb (500 μM) to a medium containing G1. By contrast, glucagon release was inhibited by 65-80% by G7 and by 70-80% by Tolb. In all models of β-cell ablation, insulin secretion was undetectable. However, glucagon release was inhibited by G7 and Tolb to the same extent as in control models. No major difference of glucagon secretion was observed in normoglycemic or hyperglycemic mice. These results suggest that glucose and Tolb can control glucagon release independently of a paracrine factor released from β-cells, and that the control of glucagon secretion is not altered by hyperglycemia lasting 5 to 6 days. We also tested the effect of insulin (100 nM) on glucagon release. The acute application of insulin inhibited glucagon release by 25-35% in control mice and by 15-35% in β-cell deficient models. The inhibition was thus smaller than that elicited by G7 and Tolb. Other experiments showed that the continuous presence of insulin did not affect the amplitude of the glucagonostatic effect of G7 and Tolb, suggesting that both agents act independently of insulin.

Conclusion: The control of glucagon release by G and K_{ATP} channel closers does not involve a paracrine factor released by β-cells. It is not altered by short-term hyperglycemia. High insulin concentrations induce however a slight glucagonostatic effect.

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Disclosure: **H. Chae:** None.

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Free fatty acids amplify basal secretion of both glucagon and insulin from isolated human islets at normoglycaemia via metabolic and FFAR1 dependent mechanism

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Background and aims: In obesity the elevation of basal insulin secretion has been linked to high plasma concentrations of free fatty acids (FFAs). In addition, glucagon levels are often elevated in these subjects, which is counterproductive and detrimental for glucose control. Long chain FFAs interact with the free fatty acid receptor 1 (FFAR1) on alpha and beta-cells and influence both insulin and glucagon secretion. However, less is known about how it affects glucagon secretion.
Materials and methods: Human islets were cultured for different time periods at 5,5 mM glucose in CMRL media. Islets were treated with long-chain fatty acid (0,5 mM palmitate, 0,5% bovine serum albumin), with and without pharmacological (ANT203, ANT825) and transcript inhibition (lentiviral particle shRNA) of FFAR1. Insulin, glucagon and somatostatin secretion were measured at different time intervals during 48 hour culture. To assess the metabolic effects of the fatty acid, islet oxygen consumption was also measured during palmitate exposure with and without pharmacological inhibition of FFAR1 and fatty acid beta-oxidation (etomoxir).
Results: Insulin secretion was enhanced during fatty acid treatment at 5.5 mM glucose and increased further with culture time. FFAR1 inhibition lowered insulin secretion by up to 50%. Glucagon and somatostatin secretion was also elevated during culture and the increase was in part dependent on FFAR1 and in part on fatty acid metabolism. In the absence of fatty acid palmitate, both agonist and antagonists to the receptor had no statistically significant effect on hormone secretion. Mitochondrial oxygen consumption was amplified in islets during palmitate exposure and was reduced by the FFAR1 antagonist as well as by inhibitor of fatty acid beta-oxidation etomoxir.
Conclusion: We conclude that FFAs are regulators of not only insulin but also glucagon and somatostatin secretion at fasting glucose concentrations and that FFAR1 is crucial in this effect. This implies that the effect of FFAR1 on hormone secretion is not glucose dependent as previously reported but rather fuel substrate dependent. We propose that the natural ligands of FFAR1 can via their dual role as receptor activators and fuel metabolites amplify basal pancreatic islet hormone release.
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Disclosure: **H. Kristinsson:** None.

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Endoplasmatic reticulum stress and beta cells functionality: the role of ERO1LB

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Background and aims: Mammals have two genes encoding homologues of the endoplasmic reticulum (ER) disulfide oxidase ERO1 (ER oxidoreductin 1), ERO1a and ERO1b (ERO1LB). ERO1LB, whose expression is highly enriched in pancreatic beta-cells, contributes to proinsulin oxidative folding by reoxidizing PDI (protein disulfide isomerase) to sustain disulfide bond formation. . Chronic ER stress conditions, chemical or physiological, can induce severe loss of ER folding capacity and lead to an accumulation of misfolded/unfolded proteins, and eventually compromise beta cell function, including insulin production and secretion, contributing to type 2 diabetes development. Here we investigated the potential role of ERO1LB in responding to ER-stress and its capacity to protect against beta-cells loss of function.
Materials and methods: By using a combination of biochemical, molecular biology and immunofluorescent techniques we explored the subcellular distribution and the functional role of ERO1LB protein in a mouse beta-cell line (MIN6) by using overexpression and silencing approaches.
Results: We characterized ERO1LB subcellular localization in basal and under ER-stress induced by high palmitate and observed a change in ERO1LB clustering treatment that clearly show the formation of ER-subcompartment enriched in proteins with folding activity, including ERO1LB. We assayed ERO1LB effects on ER calcium levels and ER protein folding capacity in order to elucidate its effect on subcellular functions. We also investigated at functional level how ERO1LB affects glucose-stimulated insulin secretion. Upon palmitate induced ER-stress we observed a decreased insulin folding capacity resulting in an increased proinsulin/insulin ratio, an effect typical of stressed beta cells. Interestingly, we showed the ability of ERO1LB to rescue insulin folding capacity in the palmitate induced ER-stress model. Moreover, we looked at the molecular mechanisms that allow ERO1LB to regulate the UPR (unfolded protein response) necessary to prevent ER-stress driven beta-cell death, elucidating the signaling pathways involved in the UPR response triggered by ERO1LB.
Conclusion: Our data strongly indicate the involvement of ERO1LB in preventing unresolvable ER stress in beta cells and, remarkably, its ability to rescue beta-cells from ER-stress induced loss of function.These findings suggest ERO1LB as an attractive novel candidate target for the treatment of type 2 diabetes.
Disclosure: **D. Amadio:** None.

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Pancreatic stellate cells express insulin receptor (IR) and IGF-1 receptor (IGF-1R) on the cell surface: implication for pancreatic fibrogenesis in type 2 diabetes

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Background and aims: In type 2 diabetes, a large number of studies have focused on the impact of the elevated levels of glucose and insulin on pancreatic islet cells. However, very few studies have addressed effects of high glucose and insulin on activation of pancreatic stellate cells (PSCs), which plays a central role in fibrogenesis associated with pancreatitis and pancreatic cancer. The aim of this study was to investigate whether the islet-specific environment represented by hyperglycemia and hyperinsulinemia had additive effects on PSCs activation and the insulin/insulin-like growth factor 1(IGF-1) receptor signaling pathways.
Materials and methods: In primary mouse PSC and a novel immortalized mouse pancreatic stellate cell line (imPSC), cells were stimulated with high glucose and insulin, either individually or concomitantly. We determined the expression of insulin/IGF-1 receptors and signaling pathway components, analyzed their operation as profibrotic and/or proliferative pathways and assessed the potential contributions of PSCs to a fibrogenic microenvironment.
Results: Immunofluorescence showed PSCs express insulin receptor (IR) and IGF-1 receptor (IGF-1R) on the cell surface of PSC. The amount of IR/IGF-1R expression on the cell surface is different from cell to cell. Insulin dose dependently enhanced tyrosine phosphorylation of IR/IGF-1R at specific autophosphorylation sites, and lead to the activation of its downstream MAPK, PI3K/AKT/mTOR/p70S6K and AKT/FOXO1 signaling pathway in PSC. The stimulation of high glucose (25 mM) and/or insulin (100 nM) induced PSC activation, ECM production and galectin-3 expression, insulin stimulation was more potent. Treatment with high glucose combined with insulin was more effective than high glucose or insulin treatments by themselves. Results in immortalized mouse PSC were in accordance with primary mouse PSC.

Conclusion: Our data show that in type 2 diabetes, hyperglycemia and hyperinsulinmia are two crucial mitogenic factors to activate PSCs. Abnormalities in insulin/IGF receptor signaling network and increased ECM production may be involved in development and progression of pancreatic fibrosis.
Disclosure: **X. Zhu:** None.

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Role of micu2 in metabolite induced stimulus secretion coupling in pancreatic beta cells

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Background and aims: One of the main challenges in understanding the progression of type 2 diabetes (T2D) is to decipher the exact role of mitochondrial calcium in regulating stimulus-secretion coupling in the pancreatic β-cells. Mitochondrial Calcium Uptake 2 (MICU2) was recently identified as a subunit of mitochondrial calcium uptake (MCU)-complex in mouse liver, the function of which in pancreatic β-cells has not yet been investigated. Given the important role of mitochondrial calcium in β-cell stimulus-secretion coupling, metabolic characterization of MICU2 should therefore lead to better appreciation of the role the MCU-complex and mitochondrial calcium signaling play in β-cells. We specifically aimed to functionally characterize MICU2 in rodent insulin-producing cell lines (832/12).
Materials and methods: Standard assays of insulin secretion, mitochondrial respiration measurement and live cell imaging tools and techniques were used in these studies.
Results: We have previously identified an expression quantitative trait locus (eQTL) associated with the *MICU2* gene, which correlated with β-cell function (HOMA-β) and fasting glucose in humans (unpublished results). Interestingly, there was 50% decrease in insulin secretion (n=3, $p<0.05$) and ~50% reduction in mitochondrial respiration (n=3, $p<0.0055$) when mitochondrial malate-aspartate shuttle dependent metabolite such as 16.7 mM glucose was used for stimulation upon *Micu2* knock down (KD). In contrast, insulin secretion (n=3) and mitochondrial respiration (n=2) upon *Micu2* KD were not perturbed when provoked by shuttle-independent metabolites, such as 10 mM pyruvate. These experiments gave indication of probable cellular communication between MICU2 and malate-aspartate shuttle. Our live cell imaging experiments further revealed that mitochondrial calcium uptake was 25% reduced upon *Micu2* KD when stimulated with 16.7 mM glucose (NC, n=23 cells, *Micu2* KD, n=47 cells, $p<0.0001$) as compared to 8.73% decrease with 10 mM pyruvate (NC, n=54 cells, *Micu2* KD, n=44 cells, $p<0.0033$). Furthermore, imaging experiments also revealed a 62% decrease in mitochondrial calcium uptake upon 36 mM K^+ treatment (NC, n=11 cells, *Micu2* KD, n=36 cells, $p<0.001$). Importantly, dual imaging of plasma membrane depolarization and cytosolic calcium revealed a 17.5% decrease in plasma membrane depolarization and 22% reduction in cytosolic calcium elevation in response to 36 mM K^+ upon *Micu2* KD (NC, n=24 cells, *Micu2* KD, n= 52 cells, $p<0.0001$), indicating an energetic crisis in these cells. This was further substantiated in live cell measurements of cytosolic ATP, which showed that 16.7 mM glucose-stimulated ATP levels was 8% reduced upon *Micu2* KD (NC, n=23, *Micu2* KD, n=47, $p<0.0001$). In contrast, the cytosolic ATP rise after stimulation with 10 mM pyruvate was largely unaffected by *Micu2* KD (NC, n=23, *Micu2* KD, n=44). Taken together, our results suggest that the mitochondrial malate-asparate shuttle interacts with MICU2 and that metabolites, which bypass this shuttle system, could overcome the limitation of the bioenergetic deficit induced by *Micu2* KD in β-cells.
Conclusion: Our findings indicate that KD of *Micu2* leads to cellular bioenergetic failure, which could be overcome by addition of malate-aspartate shuttle-independent metabolites. This suggests a molecular interaction between Micu2 and the malate-aspartate shuttle in pancreatic β-cells.
Supported by: Swedish Diabetes Foundation
Disclosure: **N. Vishnu:** None.

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GPR43 receptor expression, insulin sensitivity and beta cell function in patients with newly diagnosed type 2 diabetes

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Background and aims: Studies in knockout mice have shown that the G-protein-coupled receptor GPR43 protect from insulin resistance during high-fat diet (HFD). In line, HFD-fed GPR43 knockout mice develop glucose intolerance due to abnormal insulin secretion. However, no data are available on the role of GPR43 expression for whole-body insulin sensitivity and pancreatic beta-cell function in humans with type 2 diabetes (T2DM).
Materials and methods: We studied 25 T2DM and 25 obese but glucose tolerant persons, matched for sex, age and BMI (OBESE). Fasting insulin sensitivity was assessed by QUICKI and fasting beta-cell function by HOMA-BETA. Furthermore, T2DM underwent euglycemic-hyperinsulinemic clamp test for assessing insulin sensitivity (M), while OBESE underwent 75-g oral glucose tolerance tests (OGTT) for assessing insulin sensitivity by OGIS, which was transformed into M value according to a formula used in previous studies (M=0.023 x OGIS - 4.1). All participants also underwent a mixed meal test (MMT) for quantification of insulin secretion and beta-cell function. Insulin secretion/beta-cell function was assessed by the Insulinogenic Index (IGI), and by the corresponding C-peptide-based index (Δcp30/Δglu30). Further indices were the ratio of suprabasal AUC of insulin to that of glucose (ΔAUCins/ΔAUCglu) and similar with C-peptide (ΔAUCcp/ΔAUCglu). Also, shape indices, previously shown to be related to beta-cell function, were computed. Quantitative rt-PCR was used to measure mRNA expression levels of GPR43 in biopsies of adipose tissue (AT).
Results: We found that mRNA expression of GPR43 (see Table) was not different between T2DM and OBESE. Insulin sensitivity was similar in both groups. In contrast, insulin secretion/beta-cell function as well as insulin and C-peptide shape indices were lower in T2DM. Serum lipid and incretin kinetics were not different between the groups.
Conclusion: Humans with different glucose tolerance, but comparable insulin resistance exhibit different beta-cell function in the face of similar GPR43 expression levels, suggesting that GPR43 may mainly protect from insulin resistance rather than beta-cell dysfunction. One might therefore speculate that GPR43 may serve as a novel treatment target to improve insulin sensitivity in humans with T2DM.

Table: Main characteristics, GPR43 receptor expression, insulin sensitivity and beta-cell function, all as mean±SE in T2DM and OBESE.

	OBESE (n=25)	T2DM (n=25)	p-value (ANOVA or Mann-Whitney as appropriate)
MAIN CHARACTERISTICS			
Sex (M/F)	19/6	19/6	-
Age(years)	49.9±2.3	50.3±2.1	NS
BMI (Kg/m^2)	34.2±1.8	32.9±1.4	NS
Fasting glycemia (mmol/l)	4.48±0.07	5.83±0.24	<0.0001
HbA1c (%)	5.24±0.06	6.25±0.17	<0.0001
GPR43 RECEPTOR			
mRNA expression in AT (non-dim.)	58±21	38±7	NS
INSULIN SENSITIVITY			
QUICKI (non-dim.)	0.26±0.01	0.24±0.01	NS
M (mg min^{-1} kg^{-1})	6.08±0.31	6.32±0.53	NS
INSULIN SECRETION/BETA-CELL FUNCTION			
HOMA-BETA (non-dim.)	294±43	190±44	0.014
IGI (pmol/mmol)	604±140	292±128	0.003
Δcp30/Δglu30 (pmol/mmol)	2270±581	1545±788	0.003
ΔAUCins/ΔAUCglu (pmol/mmol)	958±208	209±48	0.0003
ΔAUCcp/ΔAUCglu (pmol/mmol)	4087±652	1299±194	0.0001
SHAPE INDICES			
Glucose shape (10^{-3} mmol/l min^{-2})	1.39±0.14	1.33±0.15	NS
Insulin shape (10^{-3} μU/ml min^{-2})	54.2±9.4	27.0±5.8	0.022
C-peptide shape (10^{-3} ng/ml min^{-2})	3.28±0.33	2.03±0.31	0.008

Disclosure: **G. Pacini:** None.

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Mid51 is not only important for mitochondrial morphology but also for glucose-stimulated insulin secretion in pancreatic beta cells

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Background and aims: Mitochondria form a cell-type specific dynamic network that continuously cycle between fusion and fission events. Fission is initiated by the fission protein 1 (Fis1), but only the dynamin-related protein 1 (Drp1) is able to finally complete the mitochondrial division. Unlike in yeast, Drp1 cannot directly interact with Fis1, but recent studies indicate that Mid51 may work as an important anchor protein between both key players of mitochondrial fission in mammalian cells. We have previously shown that alterations in the expression of Fis1 and Drp1 affect glucose-stimulated insulin secretion in pancreatic beta cells. Therefore, the aim of this study was to investigate the role of Mid51 in pancreatic beta cells.
Materials and methods: Mid51 was overexpressed in insulin-secreting MIN6 cells and mouse pancreatic beta cells. Quantitative real-time PCR and western blot analyses were used to determine the gene and protein level of Mid51. Glucose-stimulated insulin secretion was analysed using ELISA. Mitochondrial activity was invesigated by measuring the mitochondrial membrane potential with TMRE. Mitochondria morphology was characterized by fluorescence microscopy after MitoTracker Green or MitoTracker DeepRed staining.
Results: Mid 51 is expressed in insulin-secreting MIN6 cells and mouse pancreatic beta cells. Overexpression in MIN6 cells resulted in significantly higher gene and protein expression of Mid51 compared to control transfected cells. The mitochondrial membrane potential was significantly reduced in cells overexpressing Mid51. A homogenous mitochondrial network with a high degree of interconnections was observed in MIN6 control cells and mouse pancreatic beta cells. Overexpression of Mid51 resulted in fragmentation of mitochondria with cluster formation. Mitochondrial aggregation appeared primarily perinuclear. Mitochondrial loop structures, which we have found after Drp1 overexpression, were less detectable. Drp1 overexpression resulted in significantly reduced glucose-stimulated insulin secretion in MIN6 cells compared to control transfected cells. The effect of Mid51 overexpression was even more pronounced and resulted in a total loss of glucose-stimulated insulin secretion.
Conclusion: Mid51 plays a pivotal role in regulation of mitochondrial morphology and function in pancreatic beta cells. Our results in insulin-secreting MIN6 cells suggest that Mid51 is important for glucose-stimulated insulin secretion, but future work is required to elucidate endogenous regulation of Mid51 in pancreatic beta cells.

Disclosure: **J. Schultz:** None.

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Systemic metabolic effects exerted by a point mutation in the RED subdomain of PAX6

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Background and aims: The paired box protein 6 (PAX6) is a major transcription factor involved in eye development. Additionally, its role in the development of the pancreas has previously been documented, although the underlying mechanisms in the homeostasis of the adult pancreas remain largely unknown.
Materials and methods: We investigated the *in vivo* effects of the mutation on the overall metabolism of the mice by carrying out glucose tolerance tests, indirect calorimetry and euglycemic-hyperinsulinemic clamps. Furthermore, transcriptome analysis of isolated islets, liver and hypothalamus was undertaken along with histological examinations.
Results: The ENU-generated $Pax6^{Leca2}$ mouse line with a point mutation (R128C) in the RED subdomain of the PAX6 protein, recapitulates a human mutation causing foveal hypoplasia. In addition to the retinal defects, the mutation translates into numerous observable abnormalities in the pancreas. Islet distortion was discernible from the age of 4 weeks including various dysregulated genes. More specifically, up-regulation of the endocrinal progenitor marker *Neurog3* and down-regulation of several β-cell specific markers in addition to increased proliferation within the islet suggested the prevailing phenotype to be most consistent with β-cell dedifferentiation. Moreover, glucose stimulation of isolated islets *in vitro* and an intraperitoneal glucose challenge *in vivo* indicated reduced insulin content and secretion. However, this reduction was accompanied by a decreased fasting blood glucose level and a normal glucose clearance, possibly explained through the evident increased insulin sensitivity and decreased hepatic glucose production. Additionally, increase in locomotor activity and energy expenditure indicated an effect mediated via the hypothalamus, as a consequence of the mutation.
Conclusion: Taken together, the data suggests, as yet, unknown systemic function of PAX6 affecting the overall metabolism and consequently protecting the organism from developing hyperglycemia in absence of insulin increment

Disclosure: **N.F. Chhabra:** None.

PS 037 Dissecting islet biology in vivo and in vitro

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Whole organism chemical screening to identify modulators of beta cell function

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Background and aims: Pancreatic β cells play a central role for glucose homeostasis through insulin production. Destruction and dysfunction of β cells result in type 1 and type 2 diabetes, respectively. Identifying novel molecules and/or novel molecular pathways to generate and/or to improve β cell function will contribute significantly to developing new diabetes therapies. Zebrafish is a highly productive model organism to study mechanisms of pancreas development and β-cell differentiation, being amenable to perform high throughput chemical screening in vivo. So far, zebrafish chemical screening has revealed novel insights into complex biological pathways and discovered new uses or new targets for existing bioactive compounds. Our research aim is to identify new molecules and/or new molecular pathways which promote β cell functional maturation and/or enhancement, by zebrafish chemical screening.
Materials and methods: We screened 4640 small molecules using a zebrafish line expressing firefly luciferase under the insulin promoter (ins:Luc2). Following hits selection, we measured free glucose levels to identify enhancers or repressors of insulin expression which have a functional effect on glucose levels. Furthermore, we characterized our insulin enhancers and repressors by conducting time course studies and by assaying their effects on gluconeogenesis using a zebrafish line expressing firefly luciferase under the promoter of phospho¬enolpyruvate carboxykinase 1 (pck1:Luc2), which encodes a key enzyme for gluconeogenesis
Results: 232 enhancers and 30 repressors of insulin promoter activity were identified by screening 4640 small molecules with the ins:luc2 line. Interestingly, subsequent glucose measurements revealed that 84 of the 232 insulin enhancers reduced glucose levels and that 7 of the 30 insulin repressors increased glucose levels in zebrafish larvae. Therefore we focused on these 91 compounds and characterized them by additional screening using shorter time courses and with the pck1:luc2 line.
Conclusion: The resulting data suggest that 13 insulin enhancers have a direct effect on β cells and that these 13 compounds regulate glucose homeostasis through stimulating β cell function and/or insulin signaling in zebrafish. Currently we are working with 5 of these 13 compounds (OPRK antagonist; GNTI, auxin; 2,4-DB, Kv1.3 blocker; Psora-4, LTD4/LTE4 antagonist; SR-2640 and flavonoid ; Karanjin) in order to understand their mechanism of action and test their effect in the mammalian models.
Disclosure: **H. Matsuda:** None.

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ERK7: a novel regulator of insulin secretion and lipid metabolism in Drosophila

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Background and aims: Mitogen Activated Protein Kinases (MAPKs) are Ser/Thr kinases which regulate a wide variety of cellular processes. Although this family is very well studied, some of the members like ERK7 (ERK8/MAPK15) are poorly characterized. ERK7 is an atypical MAP kinase which was shown to be activated by amino acid and serum starvation in Drosophila S2 cells. We have developed in vivo Drosophila models to further characterize its physiological functions.
Materials and methods: dILP2-Gal4; UAS-GFP driver line was used for Insulin Producing Cells (IPC) specific genetic manipulations. CG-Gal4 was used for fat body specific experiments. RNAi lines and p53 overexpression lines were obtained from VDRC, Austria and Bloomington Stock Center, USA. qRT-PCR assays were used to measure mRNA levels of insulin like peptides and lipogenic genes. Immunostainings using anti-dILP2 antibody were used to assess secretion of insulin like peptides. Gas chromatography was used for quantitative lipidomics.
Results: We hereby show that ERK7 is an important regulator of growth and lipid metabolism in Drosophila. We observed that upon starvation, ERK7 is expressed in median neurosecretory cells (IPCs) which regulate larval growth by secreting insulin-like peptides (dILPs) in diet-dependent manner. We demonstrate that overexpression of ERK7 in IPCs strongly inhibits dILP secretion which consequently leads to reduced body size and a delay in larval development. We also identify p53 as an upstream activator of ERK7 in IPCs. Further, we establish that ERK7 function in IPCs is necessary for efficient starvation response. Finally, we also show that ERK7 has important metabolic functions outside IPCs. We show that ERK7 function in lipogenic tissues of Drosophila is important for normal lipid homeostasis. Overexpression of ERK7 in these tissues caused qualitative and quantitative changes in lipid levels. We have also generated ERK7 mutants using CRISPR/Cas9 technology. We are currently utilizing the mutants to decipher the role of ERK7 in animal physiology.
Conclusion: ERK7 regulates starvation response by inhibiting insulin secretion from insulin producing cells. It also has a role outside IPCs in regulation of lipid homeostasis.
Disclosure: **K. Hasygar:** None.

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The role of GRP94 chaperone in proinsulin processing

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Background and aims: Insulin availability is a determinant of glucose homeostasis and insufficient insulin production is the underlying cause of diabetes. Insulin, like any other secreted protein, needs folding in the endoplasmic reticulum (ER) to attain its proper three-dimensional structure. Folding failure leads to insulin degradation and beta cell stress. While insulin biosynthesis and secretion have been extensively studied, surprisingly little is known about the specific folding requirements of proinsulin and the necessary ER chaperones. Glucose Regulated Protein 94 (GRP94), a resident chaperone of the ER, is essential in folding of insulin-like growth factors 1 and 2 (IGF). Insulin and IGFs are evolutionarily linked and share structural homology. Recent publications indicate that GRP94 is part of a complex required for preproinsulin transport to the ER and subsequent insulin secretion. Thus, we hypothesize that proinsulin folding is crucially dependent on the function of GRP94. Aim 1: To define functional impact of GRP94 on insulin production and secretion. Aim 2: To outline structural characteristics of the GRP94/proinsulin complex.
Materials and methods: Cellular models of beta cells, freshly isolated rat and human islets will be subjected to shRNA and pharmacological treatment to diminish expression and activity of GRP94. Results are presented as mean ± s.e.m. with statistical differences determined using two-tailed Student's t test or ANOVA.
Results: 1. >95% GRP94 knock-down (KD) in cell lines and dispersed human islets was achieved by shRNA targeting GRP94. These models were tested for glucose induced insulin secretion. Control cells increased (in response to 20mM glucose concentration) 3-10 fold their insulin secretion while GRP94 KD cells failed to do so. 2. Similar results were obtained when cells, rat and human islets were pre-treated with GRP94 specific inhibitor. 3. We analyzed the ability of GRP94 KD cells to restore

insulin content after a period of forced secretion (high to low glucose concentration shift). Insulin content was inversely correlated to the degree of KD of GRP94 with control cells able to build up its insulin content 4 fold within 1h while GRP94 KD cells showed no such an increase. 4. Analysis of GRINCH cells by confocal microscopy revealed: a) In 3mM glucose conditions insulin secretory granules aligned close to the cellular membrane in control cells, while GRP94 KD cells failed to produce similar pattern exhibiting distorted cellular morphology with expanded ER and accumulation of proinsulin. b) In 20mM glucose, control cells secreted membrane localized granules while GRP94 KD cells failed in that respect. In addition, control cells showed strong proinsulin signal resembling Golgi apparatus while GRP94 KD cells showed much more dispersed localization. 5. Analysis by SDS-PAGE for the formation of configurational isomers of proinsulin under reducing and non-reducing conditions discovered in GRP94 KD a) increased presence of disulfide isomers of proinsulin; b) lower amount of pro- and mature insulin (Insulin gene transcription was only minimally lower in GRP94 KD). 6. Co-immunoprecipitation experiment shows GFP-tagged proinsulin precipitating GRP94.

Conclusion: Our preliminary results indicate that GRP94 is necessary for proinsulin processing into mature insulin and that it directly interacts with it or at least is a part of proinsulin folding complex in ER.

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Disclosure: **M.T. Marzec:** None.

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Consideration of incretin response acquisition organisation by formation of pseudoislets

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Background and aims: Cell-cell contacts and interactions between pancreatic beta-cells and/or other cell population within islets are essential for cell survival, insulin secretion, and functional synchronization. It has been shown that insulin secretion from intact pancreatic islets is greater than that from dispersed beta-cells. We have previously reported that incretin responsiveness was drastically induced in the incretin unresponsive MIN6-K20 β-cells by formation of pseudoislets (PIs). In this study, we tried to elucidate the mechanism of incretin responsiveness by formation of PIs.

Materials and methods: PIs were formed from clonal beta-cells (designated MIN6-K20 cells) by culture on Ultra-Low Attachment dishes for seven days. Morphology of monolayer-cultured clonal beta-cells (MCCs), PIs and mouse islet was observed by electron microscope. Insulin secretion was measured by batch incubation method with stimulation of incretins such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) At the condition of MCCs and PIs. Metabolome analysis was performed by capillary electrophoresis/mass spectrometry (CE/MS) and Liquid Chromatography - tandem Mass Spectrometry (LC-Ms/Ms).

Results: MIN6-K20 cells respond to glucose but not to incretins in MCCs, but, incretin responsiveness is drastically induced by formation of PIs. In the analysis by electron microscope, insulin secretion granules was increased, mitochondria were also well developed in PIs compared to the MCCs. In the presence of GLP-1, glucose- dependent insulin secretory response was observed in PIs. And, in the presence of high concentration of glucose, GLP-1 concentration- dependent insulin secretory response was also observed in PIs. Insulin contents in the PIs were drastically increased to levels similar to those of native islets. Metabolites of glycolysis, TCA cycle, amino acids and nucleic acids were increased. In addition, intracellular glutamic acid content was increased in high glucose conditions in PIs. Recently, we have reported that glutamic acid derived from malate-aspartate shuttle contribute to the incretin responsiveness (Cell Rep, 2014). Therefore, the increase of intracellular glutamic acid content by PIs formation maybe involved of incretin responsiveness.

Conclusion: Our results suggest that, by formation of PIs, production of glutamic acid through malate-aspartate shuttle is increased, which leads to the induction of incretin responsiveness.

Disclosure: **A. Arman:** None.

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Partial inhibition of PERK enhanced glucose-stimulated insulin secretion through BiP-dependent modulation of cytosolic calcium transit

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Background and aims: PERK is a sensor of ER stress, and has an implication on the development and function of pancreatic β cells. Complete deletion of *Perk* gene and abrogation of PERK function induced β cell failure, but effects of partial reduction of *Perk* have been inconsistent. Recently, attenuation of IRE1α, another sensor of ER stress, was reported to preserve β cell viability and function during ER stress. Therefore, we investigated how partial inhibition of PERK affected glucose homeostasis and insulin secretion.

Materials and methods: A specific PERK inhibitors (PERKi), GSK2606414 was treated to INS1 cells and islets at various concentrations for 24 h. Glucose-stimulated insulin secretion (GSIS), calcium transit, and some UPR markers were measured. Because of the significant induction in the expression of BiP by low-dose PERKi, islets cells were dissociated into single cell and transfected with siBiP to examine the role of BiP in the low-dose PERKi effects on GSIS. Hyperglycemia was induced in B6 mice by high-fat diet and low-dose streptozotocin, and low-dose PERKi, GSK2656157 was administered *in vivo* orally. Blood glucose and insulin levels were measured.

Results: *In vitro* PERKi at low concentrations significantly enhanced GSIS and calcium transit in INS1 cells and islets. Although the low-dose PERKi did not affect the phosphorylation of eIF2α, BiP expression significantly increased. Inhibition of BiP expression abolished PERKi-induced increase of calcium concentration and insulin secretion, associated with reduction of ER calcium. Administration of low-dose PERKi to diabetic mice significantly increased GSIS and improved hyperglycemia.

Conclusion: In contrast to PERK shutdown, partial inhibition of PERK enhanced GSIS through BiP-dependent modulation of cytosolic calcium transit, suggesting that low-dose PERKi could be a new therapeutic strategy for diabetes.

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Disclosure: **H. Jung:** None.

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A mathematical modelling of the beta cell explaining in vitro and in vivo findings with consistent mechanisms

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Background and aims: Mathematical models of the β cell have contributed to explain the mechanisms of biphasic insulin secretion (ISR). Newer generation models have focussed on the role of calcium (Ca^{2+}) in exocytosis, but important aspects are still unresolved: the interplay of Ca^{2+} and glucose is incompletely described; fasting ISR is often poorly predicted; in vivo data are rarely considered; and the model complexity sometimes hampers the understanding of the key factors regulating ISR. We present a

β cell model that represents Ca^{2+} signaling and granule trafficking in an essential way, able to fit a variety of in vitro and in vivo data.

Materials and methods: Literature data from mouse islets were used: a test in which Ca^{2+} and glucose were decoupled; a hyperglycemic clamp (HGC) at multiple glucose levels; and a stepped HGC. *In vivo* data on human subjects from our previous studies included: a stepped HGC; an IVGTT; and an i.v. glucose infusion test simulating an OGTT. The model features an immediately releasable pool (IRP, representing the "triggering pathway") the content of which depends on the Ca^{2+}-mediated exocytosis, and a refilling flux (the "amplifying pathway"). The exocytotic rate is represented as a function of Ca^{2+}, while refilling depends on both glucose and Ca^{2+} in a delayed fashion. Ca^{2+} was available experimentally *in vitro*, while in humans it was predicted as a function of glucose levels as derived from the mouse studies. The initial Ca^{2+} peak observed *in vitro* in HGC was recreated in humans with a simple model. The model was also used to simulate the increase in 1st-phase ISR with insulin resistance and the loss of response in type 2 diabetes.

Results: The figure (Panel A) shows the response to 16.7 mmol/L HGC *in vitro*. In the stepped HGC *in vivo*, the predicted Ca^{2+} peaks (Panel B, *left*) were higher than in mice and necessary to describe the sharp ISR peaks (Panel B, *right*). The model ascribes the reduction of the 1st-phase peaks to a decrease of the IRP. The simulated ISR during the IVGTT was reasonably well described, although 1st-phase ISR was somewhat lower than the experimental data. The gradual ISR changes during the OGTT-like test were well reproduced. To simulate the increase in 1st-phase ISR observed in insulin resistance, it was sufficient to increase fasting ISR: this condition produced an increase in the IRP. The diabetic response, with marked 1st-phase ISR impairment and blunted 2nd-phase ISR, was reproduced by hyperglycaemia, which determined IRP depletion, and impairment of the refilling process, with no need to postulate defects in Ca^{2+} effects.

Conclusion: A parsimonious mathematical model in which the role of the triggering and amplifying pathways are clearly appreciable is able to reproduce a variety of *in vitro* and *in vivo* insulin secretory responses and stresses the importance of calcium-independent amplifying pathway as the possible key defect in type 2 diabetes.

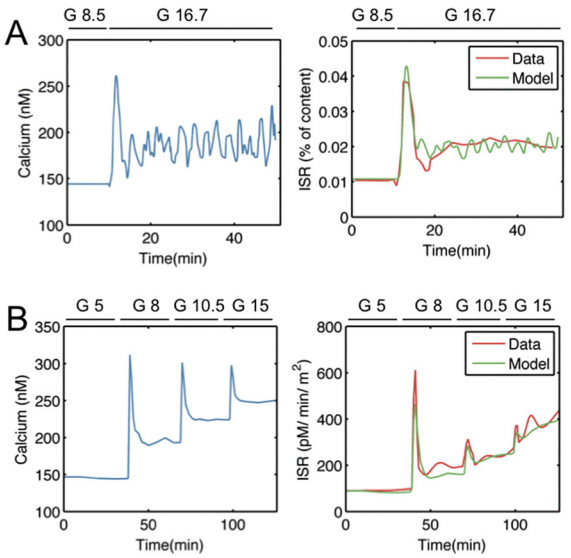

Fig 1: A) Simulation of *in vitro* test. Measured calcium in response to glucose step (*left*). Measured and simulated insulin secretion in response to glucose step (*right*). B) Simulation of *in vivo* test. Simulated calcium in response to staircase glucose (*left*). Measured and simulated insulin secretion in response to staircase glucose (*right*).

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Disclosure: **E. Grespan:** None.

PS 038 Determinants of beta cell function in humans and in non-human primates

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Mechanisms of glucose intolerance in circadian disruption caused by shift work

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Background and aims: Epidemiological studies have shown that disruption of circadian rhythms by shift work is associated with an increased risk of obesity and type 2 diabetes mellitus. Acute circadian disruption in rodents and humans induces impaired glucose tolerance. However, the underlying mechanisms have not been clearly delineated. Therefore we measured insulin, C-peptide, glucagon concentrations and postprandial glucose turnover in healthcare providers following either 12 hour day shifts or 12 hour night shifts to determine the mechanism of circadian disruption on glucose homeostasis.

Materials and methods: Twelve nurses who worked rotating day and night 12 hour shifts were studied on two occasions in random order immediately after either 2 days of day-shift work or two days of night-shift work. On both occasions they were studied during conditions mimicking a day shift (7am-7pm) or night shift (7pm-7am). The studies were performed 2 weeks apart. On each occasion subjects ingested a mixed meal labeled with [1-13C]-glucose. [6-3H]-glucose and [6,6-2H2]-glucose infused intravenously enabled measurement of the rate of meal appearance (MRa), endogenous glucose production (EGP) and glucose disappearance (Rd).

Results: Subjects were young (25±1 years) and overweight (BMI 26.9±1 kg/m2) but otherwise healthy. Despite comparable fasting glucose concentrations (4.9±0.1 vs 4.8±0.1 mmol/l, p=0.13), fasting insulin (36 ± 5 vs. 23 ± 3 pmol/l, p<0.01), C-peptide (0.6 ± 0.06 vs. 0.5 ± 0.04 nmol/l, p=0.01), and EGP (16.5±0.7 vs. 14.4±0.6 μmol/kg/min, p=0.02) were lower during the night shift. Glucagon concentrations did not differ. Following meal ingestion the glycemic excursion was higher during the night shift (381±33 vs. 580±48 Mol per 5hr, p<0.01) despite comparable C-peptide, insulin and glucagon concentrations. However, the time to peak postprandial insulin (61 ±9 vs. 76±10 min, p=0.02) and peak C-peptide (71±9 vs. 96±7 min, p=0.01) concentrations as well as time to maximal suppression of glucagon (85±8 vs. 123±11 min, p=0.01) was also delayed during the night shift. Integrated MRa, EGP and Rd did not differ between study days. Insulin action (Si - 12±3 vs. 10±2 10-4 dl/kg/min per μU/ml, p=0.45) was also unchanged. However, β-cell responsivity to glucose (ϕs) was impaired (50±4 vs. 39±3 10-9 min-1, p=0.002) during the night shift.

Conclusion: Working rotational night shifts causes glucose intolerance by delaying insulin secretion and glucagon suppression. Although insulin action (Si) was unchanged, β-cell responsivity to glucose was impaired by night shift work.

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Disclosure: **A. Sharma:** None.

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Role of GLP-1 variability in modulating insulin secretion across glucose tolerance: a DIRECT study

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Background and aims: From numerous mechanistic studies GLP-1 is known to potentiate insulin secretion. Recent large-scale studies have evaluated whether the GLP-1 response after a glucose load is altered in impaired glucose regulation and type 2 diabetes (T2D); less explored in large cohorts is the impact of GLP-1 on beta-cell function. We assessed in the DIRECT study the relationships between the GLP-1 response and beta-cell function across the spectrum of glucose tolerance.
Materials and methods: We performed a 2-hour OGTT in 2182 nondiabetic subjects (881 with normal glucose tolerance, NGT; 1012 with isolated impaired fasting glucose, iIFG; 83 with isolated impaired glucose tolerance, iIGT; 532 female/1650 male) and a meal test in 806 T2D patients (341 female/465 male), with measures of glucose, insulin and C-peptide. GLP-1 was assayed at fasting (intact and total forms) and 60 min (total). Insulin secretion (ISR) and beta-cell function were evaluated by mathematical modeling. From the individual relationships expressing ISR as a function of glucose concentration, ISR at 5.6 mmol/L glucose (ISR5.6) and glucose sensitivity (the slope) was determined. The relationships between the GLP-1 response and beta-cell function were assessed by linear regression on log-transformed data, with adjustment for sex, age and study center, plus the classical predictors of ISR, BMI and insulin sensitivity (as the OGIS index).
Results: In NGT subjects, fasting total GLP-1 was directly correlated with both fasting ISR (r^2=0.45, p<0.0001) and ISR5.6 (r^2=0.33, p<0.0001), independently of the classical predictors. When the analysis was extended to include iIFG, the correlation with ISR5.6 remained significant (r^2=0.39, p<0.0001), but iIFG had lower ISR5.6 values (89 [39] vs 110 [53] pmol $min^{-1}m^{-2}$, median [interquartile range], p<0.0001). Extension to iIGT confirmed the model (r^2=0.40, p<0.0001), with no different relationships between NGT and iIGT. In these relationships, although significant, the standardized effect estimate for GLP-1 was lower than that for BMI and OGIS. Fasting intact GLP-1 did not contribute significantly to the final model. Analogous relationships held in T2D (r^2=0.17, p<0.0001 for ISR at 7.5 mmol/L glucose and fasting total GLP-1). In NGT subjects, 60-min GLP-1 was weakly (r^2=0.10) but significantly (p<0.05) associated with glucose sensitivity, after adjustment for BMI and OGIS and fasting total GLP-1. In the whole nondiabetic group, the relationship remained similar (r^2=0.14, p<0.02), but with lower glucose sensitivity in the IFG/IGT groups. In T2D subjects, glucose sensitivity, which was impaired compared to NGT (70 [55] vs 109 [67] pmol $min^{-1}m^{-2}L$ $mmol^{-1}$, p<0.0001), was not significantly related to the 60-min GLP-1 response.
Conclusion: Fasting GLP-1 secretion appears to be clearly related with fasting ISR across the spectrum of glucose tolerance. However, the impairment in ISR observed in isolated IFG was not attributable to a GLP-1 deficit. The correlation between beta-cell glucose sensitivity and the post-load GLP-1 response was weaker in nondiabetic subjects and lost in T2D. This suggests that the interindividual variability in GLP-1 levels has a more important effect on fasting ISR than on beta-cell glucose sensitivity.
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Disclosure: **A. Mari:** None.

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Physiologic determinants of islet of Langerhans beta, alpha, and δ-cell mass in normoglycaemic non-human primates

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Background and aims: The unique composition and architecture of the islet of Langerhans is essential to ensure its normal function. α (glucagon), β (insulin), and δ (somatostatin)cells play an important role in glucose metabolism and islet of Langerhans remodelling is an important feature of type 2 diabetes mellitus physiopathology. The aim of this work was to evaluate the correlation between beta, alpha and delta cell mass with anthropometric and biochemical parameters in non-human primates.
Materials and methods: In this cross-sectional study, islet cell physiology and insulin sensitivity were assessed by the 2-step hyperglicemic clamp followed by a L-Arginine bolus (1st step: glucose +125mg/dl over baseline for 90 min; 2nd step: 225mg/dl over baseline for 90 min; arginine bolus 0,5gr/kg BW; β-cell function was measured as the Disposition Index during the first 90 min. Anthropometric and biochemical parameters, as well as the α-, β-, and δ-Islet of Langerhans cell mass were also evaluated by immunocytochemistry with Morphometric analysis using the Computer Assisted Stereology Toolbox (CAST) 2.0 system from Olympus on pancreatic sections randomly collected from the pancreas body and tail in 28 normoglycemic baboons.The operator was blinded to the metabolic status of the baboon and the reproducibility of the measurements was estimated twice in 5 specimens with coefficient of variation less than 5%. Correlation analysis between the studied variables was performed using the Pearson correlation test.
Results: β-cell mass showed a positive correlation with β-cell function (r=0.422, p 0.045), weight (r=0.645, p 0.001), waist circumference (r=0.527, p 0.010), trunk fat (r=0.572, p 0.004), α-cell mass (r=0.540, p 0.008), and δ-cell mass (r=0.522, p 0.011); on the other hand, α-cell mass positively correlated with weight (r=0.456, p 0.025), waist circumference (r=0.458, p0.024), trunk fat (r=0.439, p 0.032) and total fat (r= 0.450, p 0.024), β-cell mass (r=0.540, p 0.008), δ-cell mass (r=0.467, p 0.022), and total cholesterol (r=0.435, p 0.038); and negatively correlated with insulin sensitivity (r= - 0.483, p 0.017). Finally, δ-cell mass positively correlated with fasting glucose and insulin levels (r=0.447 and 0.461, respectively, p 0.023), weight (r=0.581, p 0.003), trunk fat (r=0.432, p 0.035), and β- and α-cell mass (r=0.522 and 0.467, respectively, p 0.011 and 0.022)
Conclusion: In baboons, β-cell mass is directly related to β-cell function (Disposition Index), anthropometrical parameters, including visceral fat, and α and δ cell mass; α-cell mass is directly related to anthropometrical parameters and β- and δ-cell mass, and indirectly related to whole body insulin sensitivity; on the other hand, δ-cell mass seems to be more related to fasting glucose and insulin levels as well as to visceral fat, β- and α-cell mass.
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Disclosure: **F. Folli:** None.

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Increments of C-peptide in glucagon stimulation test in patients with new-onset diabetes and healthy control group

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Background and aims: The glucagon stimulation test (GST) allows to evaluate residual beta cell secretory capacity and helps in the choice of a therapeutic approach in patients with new-onset diabetes. It has been demonstrated recently that increments of C-peptide (Δ) in GST is the best method of predicting the human relative beta cell area in comparison to other indicators e.g. HOMA%β. Some studies have shown that GST is also helpful in diagnosing LADA. The aims of our study was to evaluate C-peptide concentration levels in GST in patients with new-onset type 1 diabetes (T1D), LADA and type 2 diabetes (T2D) in comparison to the healthy control group (HC).
Materials and methods: The study included 300 patients with newly diagnosed diabetes (36 patients with T1D, 52 with LADA and 212 with T2D) and 95 healthy subjects. Median±lower-upper quartile of age was respectively: T1D=24±21-26.5; LADA=40.5±34-55; T2D=55.5±44.5-63; HC=39±27-50. Median±lower-upper quartile of BMI was respectively: T1D=21.8±19.9-22.8; LADA=24±21.6-26.5; T2D=30.4±26.7-33.6; HC=24.6±22-28.1. Subjects who were qualified for HC had normal values of OGTT, CRP, creatinine, AST, ALT, GADA, IA2, IAA and a negative history of autoimmune diseases. C-peptide was measured by EASIA method, glucose - enzymatic method with hexokinase, insulin - RIA, HbA1c - HPLC, lipid profile - Cobas analyser. We calculated HOMA-IR, HOMA%β and ΔC-peptide.
Results: Fasting C-peptide concentration levels were respectively: T1D=0.33±0.2-0.46 pmol/ml; LADA=0.30±0.19-0.57 pmol/ml; T2D=0.93±0.65-1.24 pmol/ml; HC=0.55±0.42-0.7 pmol/ml. Six minutes after glucagon stimulation C-peptide concentration levels were respectively: T1D=0.47±0.29-0.67 pmol/ml; LADA=0.49 ±0.31-0.96 pmol/ml; T2D=1.62±1.18-2.27 pmol/ml; HC=1.41 ±1.04-1.79 pmol/ml. ΔC-peptide were respectively: T1D=0.14 ±0.07-0.18; LADA=0.17±0.08-0.49; T2D=0.68±0.43-1.04; HC=0.84±0.56-1.2. A significantly lower concentration of fasting C-peptide was established between T1D, LADA and HC and a significantly higher concentration was found between T2D and HC ($p<0.01$ between respective groups). ΔC-peptide was found to be significantly higher in T2D in comparison with T1D and LADA and significantly lower in comparison with HC ($p<0.05$ between respective groups). No statistically significant differences in C-peptide concentration levels were established between T1D and LADA.
Conclusion: Significantly lower concentration levels of fasting C-peptide and ΔC-peptide in T1D and LADA in comparison with T2D indicates a smaller number of active beta cells and a higher reduction in residual beta cell secretory capacity at the time of diagnosing autoimmune diabetes in comparison with T2D.
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Disclosure: **R. Maciulewski:** Grants; Polish Society Diabetology Grant.

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Association of haemoglobin and serum iron levels with beta cell function in non-diabetic subjects

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Background and aims: Recent studies have revealed a link between elevated hemoglobin levels and increased insulin resistance in non-diabetic subjects, suggesting that iron overload has a role to play in the development of diabetes. However, little is known regarding the association between hemoglobin levels and pancreatic β-cell function. Furthermore, there is no current data available that assesses the possible association between serum iron levels and β-cell function. Therefore, using a cross-sectional study on both sexes, we aimed to investigate the association of hemoglobin and serum iron levels with insulin resistance and early-phase insulin secretion in non-diabetic subjects.
Materials and methods: We recruited 804 non-diabetic Japanese subjects (482 males and 322 females), aged over 30, who underwent a 75-g oral glucose tolerance test (OGTT) as part of a routine health examination. Subjects identified as having chronic kidney disease, anemia (defined as hemoglobin < 130 g/L for male subjects and hemoglobin < 120 g/L for female subjects), being pregnant, or taking iron supplements were excluded. Basal insulin resistance was evaluated according to the homeostatic model assessment of insulin resistance (HOMA-IR). Early-phase insulin secretion was estimated using the insulinogenic index (IGI) [ΔInsulin(30-0 min)/ΔGlucose(30-0 min)] during a 75-g OGTT.
Results: In both sexes, HOMA-IR and other indicators of insulin resistance, such as body mass index, systolic/diastolic blood pressure, uric acid, and lipid profile, positively correlated with hemoglobin levels. Simple linear regression analysis showed that IGI negatively correlated with hemoglobin levels in male subjects ($r = -0.197$, $P < 0.001$) but not in female subjects. In male subjects, multivariate linear regression analyses, adjusted for relevant potential confounding factors, detected a significant association between hemoglobin levels and IGI ($\beta = -0.164$, $P = 0.008$) and revealed that hemoglobin levels were a predictor of IGI and were responsible for 3.0% of IGI variation ($P = 0.008$). In addition, neither HOMA-IR nor IGI correlated with serum iron levels in male and female subjects.
Conclusion: In non-diabetic Japanese subjects, hemoglobin levels negatively correlated with early-phase insulin secretion to a significant extent only in male subjects. Furthermore, in contrast to hemoglobin, there was no association between serum iron levels and insulin resistance and secretion. These findings suggest that elevated hemoglobin levels may have a sex-specific effect on β-cell function and could therefore be an independent predictor of β-cell dysfunction.
Disclosure: **M. Shimodaira:** None.

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Morphology of the pancreas in type 2 diabetes: effect of return of normal insulin secretion over 6 months

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Background and aims: Beta cell function can be normalized by hypocaloric dieting in short duration type 2 diabetes, and this allows study of the transition between normal glucose tolerance and type 2 diabetes. Specifically, the effect of restoration of insulin secretion on pancreas morphology can now be determined. 3 Tesla magnetic resonance imaging has shown two abnormalities of pancreas morphology: decreased pancreas volume and irregularity of its shape.
Materials and methods: To assess shape of the pancreas by magnetic resonance imaging, a novel quantitative method based on 3D volume rendering and fractal analysis has been developed. The previously described semi-quantitative scoring scheme was also used. Individuals

(n=29) with a wide range of duration of type 2 diabetes (0.5-23 years) were studied on a very low calorie diet for 8 weeks and weight maintenance for 6 months.

Results: Reversal of diabetes and normalisation of first phase insulin secretion was achieved in those with shorter duration diabetes (12/29). In this group there was a paradoxical increase in irregularity of the pancreas borders between baseline to 8 weeks (fractal dimension 1.143 ±0.013 vs. 1.169±0.006; p=0.05), followed by a greater decrease over 6 months (1.130±0.012), p=0.006). In the group with no recovery of first phase insulin response there was no change in fractal dimension (1.175 ±0.006, 1.176± 0.005 and 1.167± 0.008 at baseline, 8 weeks, and 6 months respectively). These changes were consistent with the semi-quantitative method. Pancreas volume did not change over 6 months (responders 52.0±4.9 to 51.4±4.5 cm ^{3} , p=0.69; non-responders 39.7 ±2.7 to 41.3±2.7 cm ^{3} , p=0.13). There was a strong inverse relationship between volume and fat content of the pancreas in the whole population of the study (r=-0.50, p=0.006), but no correlation between pancreas volume and subcutaneous or visceral fat.

Conclusion: Following weight loss, smoothing of the pancreas borders occurred only with return of beta cell function, suggesting a trophic paracrine effect. There was no change in pancreas volume over 6 months. Pancreas morphology in type 2 diabetes may be prognostically important.

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Disclosure: **A. Al-Mrabeh:** None.

PS 039 The alpha cell and glucagon

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Glucose potently inhibits glucagon secretion in mice with alpha cell-specific deletion of K_{ATP} channels

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Background and aims: The role of K_{ATP} channels in the control of glucagon secretion is hotly debated and difficult to assess because β-, α- and δ-cells express K_{ATP} channels and can influence each other. In this study, we created mouse models lacking K_{ATP} channels specifically in α-cells to evaluate their role in the control of glucagon release.

Materials and methods: Several global transgenic mouse models were used: mice without K_{ATP} channels (Kir6.2$^{-/-}$), mice with functional K_{ATP} channels and harboring two floxed Kir6.2 alleles (Kir6.2$^{loxP/loxP}$) and mice with a single functional Kir6.2 allele (Kir6.2$^{loxP/-}$). By multiple crossing of the above strains with Glu-cre mice, two α-cell-specific K_{ATP} channel KO models were generated: GluCre/Kir6.2$^{loxP/loxP}$ and GluCre/Kir6.2$^{loxP/-}$ mice. The percentage of cells without K_{ATP} channels was evaluated by measuring $[Ca^{2+}]_c$ in dispersed islet cells and testing their sensitivity to K_{ATP} channel modulators. α-cells were identified by their specific response to adrenaline and arginine in the presence of 3 mM glucose (G). The effects of G and K_{ATP} channel modulators were tested on glucagon and insulin secretion of perifused isolated islets.

Results: We first compared glucagon and insulin secretion of islets from Kir6.2$^{-/-}$, Kir6.2$^{loxP/-}$ and Kir6.2$^{loxP/loxP}$ mice. Kir6.2$^{-/-}$ islets were totally insensitive to K_{ATP} channel modulators. By contrast, Kir6.2$^{loxP/-}$ and Kir6.2$^{loxP/loxP}$ islets responded similarly to glucose, tolbutamide and diazoxide, demonstrating that a single functional Kir6.2 allele is sufficient for normal secretion. To evaluate the role of α-cell K_{ATP} channels in the control of glucagon release, we first evaluated by $[Ca^{2+}]_c$ measurements the percentage of α-cells without K_{ATP} channels. It was 7% (3/40) in Kir6.2$^{loxP/-}$, 100% in Kir6.2$^{-/-}$, 56% (40/71) in GluCre/Kir6.2$^{loxP/loxP}$ and 88% (44/50) in GluCre/Kir6.2$^{loxP/-}$ α-cells. The last model displays thus a robust α-cell specific K_{ATP} channel ablation. Increasing the G concentration of the medium from 1 to 7 mM similarly inhibited glucagon secretion of GluCre/Kir6.2$^{loxP/-}$ and Kir6.2$^{loxP/-}$ islets (75 versus 77%). Interestingly, addition of diazoxide (100 μM) at 1 mM G strongly inhibited glucagon release of Kir6.2$^{loxP/-}$ islets but increased that of GluCre/Kir6.2$^{loxP/-}$ islets. The increase was abolished by pretreatement with pertussis toxin (24h, 200ng/ml), which inactivates $G_{i/o}$ proteins. This is compatible with an alleviation of the glucagonostatic effect of somatostatin released upon δ-cell K_{ATP} channel closure. Increasing the G concentration of the medium from 1 to 7 mM in the continuous presence of diazoxide markedly inhibited the already elevated glucagon release of GluCre/Kir6.2$^{loxP/-}$ islets. Since, in these conditions, the K_{ATP} channels of β- and δ-cells were kept opened by diazoxide, this suggests that glucose can inhibit glucagon release independently of α-, β- and δ-cell K_{ATP} channels. Moreover, since glucagon secretion was more sustained in GluCre/Kir6.2$^{loxP/-}$ islets than in Kir6.2$^{loxP/-}$ islets, it appears that a closure of α-cell K_{ATP} channels exerts a glucagonotropic rather than a glucagonostatic effect.

Conclusion: Glucose inhibits glucagon secretion independently of a closure of α-, β- and δ-cell K_{ATP} channels. Closure of δ-cell K_{ATP} channels exerts a strong tonic glucagonostatic effect.

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Disclosure: **B.K. Lai:** None.

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Regulation of glucagon secretion and alpha cell proliferation by a neutral amino acid transporter Slc38a5

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Background and aims: Amino acids are potent and physiologically important secretagogues of glucagon secretion, yet mechanisms underlying amino acid stimulated glucagon release remain poorly understood. To identify key molecular players involved in the nutrient stimulated glucagon secretion, we used glucagon receptor knockout mouse (Gcgr(-/-)) as a model. Compared to wild type mice expressing Gcgr, Gcgr(-/-) mice displayed glucagon hypersecretion and alpha cell hyperplasia. We hypothesized that genes upregulated in alpha cells of Gcgr(-/-) mouse may play important roles in glucagon secretion and alpha cell proliferation.
Materials and methods: We sorted islet alpha cells from Gcgr(-/-) and wild type (C57Bl6) mice and analyzed their gene expression by RNA-Seq. Differential gene expression analysis revealed a number of genes that are significantly upregulated in Gcgr(-/-) islet cells (>4-fold increase, padj < 0.01). Among these candidate genes, Slc38a5 (Solute Carrier Family 38, Member 5) was found to be the highest up-regulated gene in Gcgr(-/-) islet alpha cells and represented the only amino acid transporter showing a significant difference in the expression level between wild type and Gcgr(-/-) islet cells.
Results: Slc38a5 encodes a sodium-coupled neutral amino acid transporter, SNAT5. Using qRT-PCR and immunofluorescence, we confirmed that Slc38a5 was highly upregulated at both mRNA and protein levels in Gcgr(-/-) islets. In mouse pancreatic islets, SNAT5 is selectively expressed in alpha cells but not beta cells. To investigate the role of SNAT5 in glucagon secretion and glucose homeostasis, we generated SNAT5 knockout mouse (SNAT5-KO). Compared to wild type controls (C57/Bl6), SNAT5-KO mouse maintained the same metabolic parameters including blood glucose, insulin, islet cell mass, and IPGTT. In perfused mouse pancreas, both alanine and arginine reliably caused biphasic glucagon secretion in SNAT5-KO and control animals. However, the second phase of alanine stimulated, but not arginine stimulated, glucagon secretion was reduced in SNAT5-KO islets, suggesting a specific role of SNAT5 in mediating neutral amino acid stimulated glucagon release. To test if SNAT5 upregulation is sufficient to enhance alanine stimulated glucagon secretion, we overexpressed SNAT5 in mouse islets by adenovirus. Glucagon perifusion assay of infected islets showed the same glucagon release as the islets infected with a control virus, arguing that upregulation of SNAT5 per se was not enough to increase glucagon secretion. Ongoing experiments include assaying amino acid importing activity of SNAT5 in alpha cells, and crossing SNAT5-KO mice with Gcgr(-/-) mice to generate SNAT5/Gcgr double knockout mice to study the role of SNAT5 in alpha cell hyperplasia and glucagon hypersecretion in vivo when glucagon signaling is disrupted.
Conclusion: Our study showed that Slc38a5 is a key player regulating amino acid stimulated glucagon secretion in mouse islet alpha cells. In addition to mechanistic understanding of stimulus-secretion coupling in islet alpha cells, our studies may also offer insights to assist pharmaceutical industry to develop more effective and safer therapeutics against diabetes based on the rational of glucagon antagonism, as both alpha cell hyperplasia and glucagon hypersecretion have been encountered when glucagon signaling is disrupted in vivo by different approaches including using glucagon receptor antibodies.
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Disclosure: **W. Li:** None.

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Glucagon receptor gene deletion modestly reduces blood glucose and ketones but does not promote survival in insulin knockout mice

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Background and aims: It has been thought that the depletion of insulin is responsible for the catabolic consequences of diabetes; however, evidence suggests that glucagon also plays a role in diabetes pathogenesis. Glucagon suppression by glucagon receptor (Gcgr) gene deletion, glucagon immunoneutralization, or Gcgr antagonist can reverse or prevent type 1 diabetes in mice suggesting that dysregulated glucagon is also required for development of diabetic symptoms. However, the models used in these studies were rendered diabetic by chemical- or immune-mediated β-cell destruction where insulin depletion is incomplete. Therefore, it is unclear whether glucagon suppression could overcome the consequence of the complete lack of insulin.
Materials and methods: To directly test this we characterized $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$, $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$, $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ mice to better elucidate the role of glucagon receptor signalling in the absence of insulin. Mice were characterized at postnatal day 1 and at 4 weeks of age. To generate young adult $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ mice, pups were injected with insulin twice daily until 2 weeks of age when the mice underwent an islet transplant into the eye. At 4 weeks of age the eye was enucleated to render the mice completely insulin deficient.
Results: During the first week of life, both $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ pups failed to gain mass, surviving no longer than 6 days, while $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ pups survived and gained weight. Blood glucose was modestly reduced in $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ compared to $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ pups, but was not normalized to that of $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ pups (19±1, 26±1, 4±0, and 4±0 mM $p<0.05$). Similarly plasma ketones were modestly reduced in $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ pups compared to $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ pups, yet were still elevated compared to $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ pups (4±1, 8±2, 1±0, and 1±0 mM, $p<0.05$). Similar to the pups, following enucleation in young adults, both $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ mice rapidly lost weight compared to $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ mice (-14±2, -19±2, -4±2, and -2±2% at day 1.25 post-enucleation, $p<0.05$) and reached humane endpoint within 6 days. At day 1.25 post-enucleation blood glucose was slightly reduced in $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ compared to $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ mice, but not normalized to $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ mice (21±2, 28±2, 6±1, and 5±0 mM, $p<0.05$). $Gcgr^{KO}Ins1^{KO}Ins2^{KO}$ mice trended towards improved ketone levels compared to $Gcgr^{Het}Ins1^{KO}Ins2^{KO}$ mice ($P=0.09$) but remained higher than $Gcgr^{KO}Ins1^{KO}Ins2^{Het}$ and $Gcgr^{Het}Ins1^{KO}Ins2^{Het}$ mice (1.2±0.2, 3.0±1.1, 0.3±0.0, and 0.3±0.1 mM).
Conclusion: Glucagon receptor gene deletion can modestly lower blood glucose and ketones in mice with congenital insulin deficiency; however, survival was not extended. This suggests that glucagon signaling does not play a substantial role in diabetes pathogenesis in the absence of insulin.
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Disclosure: **U.H. Neumann:** None.

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Glucose lowers cAMP to inhibit glucagon secretion by a direct effect on alpha cells

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Background and aims: Cyclic AMP (cAMP) is a positive modulator of exocytosis in α-cells that has been implicated in glucose-regulated

paracrine control of glucagon secretion by insulin and somatostatin. Since α-cell-intrinsic, non-paracrine mechanisms probably are most important for sensing glucose and adjusting the physiologically important glucagon response to hypoglycemia, we now studied whether glucose can control glucagon release by direct effects on α-cell cAMP.

Materials and methods: Changes of the cAMP and Ca^{2+} concentrations in the sub-plasma membrane space ($[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$) of cells within intact mouse and human islets were measured with total internal reflection fluorescence microscopy using a protein-based cAMP reporter and the Ca^{2+} indicator Fluo-4. α-cells were identified by their small size and characteristic $[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$ responses to adrenaline and glutamate stimulation. Glucagon secretion from mouse islets was measured with ELISA.

Results: In mouse α-cells exposed to 1-3 mM glucose, $[cAMP]_{pm}$ was stable or slightly fluctuating. Increase of the glucose concentration to 7 and 20 mM induced marked lowerings of $[cAMP]_{pm}$. Also human α-cells reacted to glucose elevation with $[cAMP]_{pm}$ reduction. Simultaneous recordings of $[cAMP]_{pm}$ and $[Ca^{2+}]_{pm}$ in mouse islets did not reveal any obvious relationship between the glucose-induced $[cAMP]_{pm}$-lowering and $[Ca^{2+}]_{pm}$, which often showed irregular oscillations both at 1 and 7 mM of the sugar. To investigate if the $[cAMP]_{pm}$ changes were due to paracrine effects of somatostatin and/or insulin, we inhibited SSTR2 somatostatin receptors with PRL 2903 or CYN 154806 and insulin receptors with S961. The insulin receptor antagonist (1 μM) lacked effects on $[cAMP]_{pm}$ at both 1 and 20 mM glucose. In contrast, 5 μM PRL 2903 often increased $[cAMP]_{pm}$, but nevertheless failed to prevent the glucose-induced $[cAMP]_{pm}$-lowering. Glucagon secretion was inhibited by >80% in response to elevation of the glucose concentration from 1 to 7 or 20 mM and this effect was prevented by 5 mM of the membrane permeable cAMP analogue 8-Br-cAMP. SSTR2 antagonism with CYN 154806 did not diminish the inhibitory effect of glucose on glucagon secretion, but $[cAMP]_{pm}$-elevation by 100 μM of the phosphodiesterase inhibitor IBMX prevented the glucose effect in the presence of the SSTR2-antagonist. Inhibition of protein kinase A with 100 μM Rp-8-Br-cAMPS reduced glucagon secretion at 1 mM glucose by 65±9%, and there was further reduction at 7 mM of the sugar. Glucagon secretion at 1 mM glucose was also markedly reduced (>80%) by Epac inhibition with 10 μM ESI-09.

Conclusion: Glucagon secretion during hypoglycemia depends on elevated $[cAMP]_{pm}$ and signalling via protein kinase A and Epac. Glucose inhibits glucagon secretion by a direct $[cAMP]_{pm}$-lowering effect in α-cells independent of paracrine influence from somatostatin and insulin.

Disclosure: **Q. Yu:** None.

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RAMP2 influences glucagon receptor pharmacology via trafficking and signalling

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Background and aims: Endogenous satiety hormones provide an attractive target for drugs combating diabetes and obesity. Glucagon causes weight loss by reducing food intake and increasing energy expenditure, but induces hyperglycaemia. To further understand the cellular mechanisms by which glucagon and related ligands activate the glucagon receptor (GCGR), we have investigated the interaction of the GCGR with RAMP2, a member of the family of Receptor Activity Modifying Proteins.

Materials and methods: CHO and HEK cells expressing the human GCGR were stably transfected with human RAMP2. A combination of saturation binding experiments, functional assays to assess the Gαs and Gq pathways and β-arrestin recruitment, and siRNA knockdown was used to examine the effect of RAMP2 on the GCGR. Ligands tested were glucagon, glucagon-like peptide-1 (GLP-1), oxyntomodulin and analogue G(X), a GLP-1/glucagon co-agonist developed in-house. Confocal microscopy was employed to assess whether RAMP2 affected the subcellular distribution of GCGR.

Results: Co-expression of RAMP2 and the GCGR resulted in a ten-fold reduction in GCGR binding sites ($p<0.0001$) ($n = 4$ separate experiments). This was confirmed by confocal microscopy, which demonstrated that RAMP2 co-localises with the GCGR and causes significant GCGR internalisation ($P<0.0001$) ($n = 14$ cells per group, paired observations). Furthermore, the presence of RAMP2 reduced potency of glucagon signalling through the Gαs pathway (EC_{50} 0.161± 0.063 *versus* 1.263 ± 0.289 nM; control *versus* RAMP2 positive cells; $P<0.05$) (n = 4 separate experiments). RAMP2 also reduced efficacy of glucagon signalling through the Gq pathway (E_{max} 109.0 ± 2.215 versus 57.7 ± 1.313%; control *versus* RAMP2 positive cells; $P<0.01$) (n = 4 separate experiments). SiRNA knockdown of RAMP2 restored glucagon signalling via Gαs and Gq to levels observed in cells not expressing RAMP2. Finally, the presence of RAMP2 completely abolished recruitment of β-arrestin ($P<0.0001$) (n = 4 separate experiments).

Conclusion: This work suggests that RAMP2 can modify the agonist activity and trafficking of the GCGR. It may inform the construction of new peptide analogues with selective agonist activities.

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Hyperglucagonaemia is associated with elevated plasma triglycerides and increased visceral fat in children and adolescents

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Background and aims: Despite the importance of glucagon in diabetes pathophysiology, the phenotype associated with hyperglucagonemia in children and adolescents with obesity is not well known. Fatty acids are known to stimulate glucagon secretion from rodent islets, a mechanism possibly of importance in obesity where plasma lipids are often elevated. The aim was to study the association between lipids and glucagon by firstly, investigating the association between fasting plasma glucagon, blood lipids and adipose tissue compartments in children and adolescents and secondly to study the effect of fatty acids on glucagon secretion from human islets.

Materials and methods: A two-centre cross-sectional study was carried out at a university hospital in Sweden and a medical university hospital in Austria. Children and adolescents age 5-18 years old with overweight or obesity (n=119) were recruited from the childhood obesity clinic at respective hospital and lean controls (n=42) were recruited through advertisement and local schools in Austria and Sweden. Fasting plasma glucagon, triglycerides, HDL cholesterol and LDL cholesterol were quantified. A subset of subjects (n=65) underwent magnetic resonance imaging to quantify visceral, subcutaneous, hepatic and pancreatic fat. Human islets were cultured with and without 0.5 mM palmitate (0.5% bovine serum albumin) for 48 hours and glucagon was subsequently quantified from culture medium and after culture during normoglycemic (5.5 mM) perifusion with the fatty acid no longer present (n=4 donors). Linear regression models were used to analyse the association between fasting glucagon and blood lipids and fat compartments. In case of outliers or not normalized data, linear regression analyses were performed after log-transformation. Differences in glucagon secretion between treatments of human islets were tested by paired t-test.

Results: Fasting glucagon was positively associated with plasma insulin (R^2=0.40, p<0.001) and plasma triglycerides (R^2=0.18, p<0.001). These associations were significant after adjusting for age and BMI. Among quantified fat compartments fasting plasma glucagon was positively associated with visceral adipose tissue volume (R^2=0.32, p<0.001), subcutaneous adipose tissue volume (R^2=0.12, p<0.01), liver fat (R^2=0.10, p<0.01) and pancreatic fat (R^2=0.07, p<0.05). However, when adjusting for age and BMI, only the association with visceral fat was significant. Palmitate treatment of human islets increased glucagon secretion on average 52.5% during culture (p<0.05) and 101.7% during perifusion (p<0.01) vs control.
Conclusion: High circulating triglycerides and visceral adiposity is associated with high fasting plasma glucagon in children and adolescents. The glucagonotropic effect of palmitate on human islets provides a potential mechanism that links high circulating lipids to high plasma glucagon.
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Disclosure: **H. Manell:** None.

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Novel tracer approach to estimate postprandial complex carbohydrate metabolism
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Background and aims: The tenet of measuring postprandial (PP) carbohydrate metabolism by isotope dilution method is that both tracer and tracee possess similar biochemical backbone to ensure identical metabolic handling. Meeting this proviso has impeded complex carbohydrate (CC) tracer research until now. The aim of this study was to develop a novel tracer approach for the estimation of postprandial complex carbohydrate metabolism in humans.
Materials and methods: We established a method exploiting the natural abundance of [13C]polysaccharide (PS) in Sorghum (S, -11.6‰) and Madagascar Rice (R, -28.9‰) [δ13C relative to VPDB] enabling detection of plasma enrichment of [13C]glucose by GC/C/IRMS after ingestion of S and R containing mixed meals. This technique, of labelling the complex carbohydrate content of a solid meal, was coupled with the infusion of [6,6-2H2]glucose and [6-3H]glucose from -180 min and 0 min, respectively, until 360 min after meal ingestion at time 0, thus recreating the triple tracer protocol conditions already used to assess PP glucose turnover after a simple carbohydrate ingestion. Sixteen healthy subjects were divided equally into 2 matched groups: S (5M, age 34.8 ±10.5 y, BMI 22.9±2.1 kg/m2) and R (4M, age 30.9±7.0 y, BMI 23.9±2.7 kg/m2). Each subject was studied in random order: glucose (G) as Jell-O (labeled with [13C]glucose) and [13C]PS containing mixed meals with either S or R meals. All meals had ~50g carbohydrates and similar macronutrients. Plasma glucose, insulin, glucagon and glucose enrichments were measured for 6 hours after the meal and glucose fluxes, i.e. rates of meal glucose appearance (Rameal), endogenous glucose production (EGP) and glucose disappearance (Rd), were calculated.
Results: iAUC (0-120 min) of glucose and insulin were lower with polysaccharide (PS) than glucose (G) meals (p<0.04) in both groups with no differences in iAUC (0-360 min). [13C]glucose concentrations were measurable and patterns differed (p<0.05) between G and PS meals in both. Both peak and iAUC (0-120 min) of Rameal and Rd were significantly lower with PS and G meal (p<10-3); while baseline and nadir of EGP were similar between groups, iAUC (0-120 min) of EGP was significantly higher with PS than G meal (p<0.01).
Conclusion: A new isotopic method to measure postprandial (PP) complex carbohydrate metabolism in humans has been developed and successfully validated. Moreover, the method was exploited in the standard triple tracer approach allowing the calculation of PP glucose fluxes with real-world complex carbohydrate containing mixed meals.
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Disclosure: **M. Schiavon:** None.

PS 040 Changes of insulin action in response to weight loss and nutritional intervention

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Association between changes in body weight and glycaemic control following insulin initiation in people with type 2 diabetes: a UK primary care study
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Background and aims: Treatment intensification with insulin in people with type 2 diabetes is recognised to be associated with greater weight gain compared to other glucose-lowering therapies (GLTs) but the association between insulin induced weight-gain with glycaemic control among patients undergoing routine clinical care is not known. We therefore aimed to establish the association between changes in weight at 1 year post-insulin initiation and glycaemic control (HbA1c reduction) and to clarify if any association is independent of baseline HbA1c and GLT.
Materials and methods: In a large Primary care cohort of patients at 1-year post-insulin initiation, the differences in weight and HbA1c from baseline were determined and patients were classified into 3 groups: (1) weight loss, (2) no change in weight and (3) weight gain. Descriptive statistical analyses were used to characterise the baseline variables. Time-varying variables were compared at one year using paired t-test and chi-square tests. Point estimates with 95% confidence intervals at conventional statistical significance level of 0.05 were used in the Spearman's regression coefficient model which was fitted to determine the relationship between change in HbA1c and the different weight groups, adjusting for significant covariates.
Results: There were 18,227 patients in our cohort (53.2% male; mean age 61.5±13.6 yrs; body weight 91.3±18.7 Kg; and HbA1c 8.7±1.8%). There was a significant association between the mean changes in body weight and HbA1c (0.28kg vs -0.32%; p <0.00001) 1 year post-insulin initiation. Compared to the weight loss group; there was a 0.07% (95% CI: -0.12 to -0.03) greater reduction in Hba1c in the weight-gain group (p = 0.003) but a non-significant increase of 0.05% (95% CI: -0.02 to -0.12) in HbA1c in the group that had no change in body weight (p=0.155) after adjusting for confounders. Age, gender, BMI, baseline HbA1c, GFR, total cholesterol and low-density lipoprotein (LDL) levels were found to be the independent predictors of the association between HbA1c reduction and change in weight.
Conclusion: Among insulin naïve patients who received treatment intensification with insulin, a reduction in HbA1c was associated with significant weight gain. The favourable effect on Hba1c levels was not observed among those who lost weight or whose weight remained unchanged
Disclosure: **I. Idris:** None.

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Metabolic effects of short-term caloric restriction in wildtype mice and mice with reduced insulin gene dosage
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Background and aims: The prevalence of obesity and age-related diseases are increasing worldwide as a result of patterns of overeating and inactivity. Caloric restriction (CR) is the only environmental intervention

known to extend lifespan and delay the symptoms of ageing, but mechanisms behind it are incompletely understood. Based on knockout mouse models for growth hormone, IGF-1 or IRS with prolonged longevity, it was hypothesized that the insulin-IGF pathway could be targeted as a CR mimic. The aim of this study was to test whether CR has additive and distinct effects on glucose homeostasis and beta-cell function in the context of reduced insulin dosage.

Materials and methods: C57BL/6J (wildtype) and Ins2 null mice, a mouse model with reduced basal insulin levels, either homozygous of heterozygous for the Ins1 allele, were put on 8-weeks of 40% CR and physiological parameters were measured before and after caloric restriction.

Results: Both male and female mice had lost a significantly amount of weight due to a reduced WAT mass. Interestingly, absolute BAT mass was increased only in female Ins2 null mice but not in either wildtype mice or male Ins2 null mice, suggesting an increased thermogenic efficiency only in females. Glucose homeostasis was improved by CR in both wildtype and Ins2 null mice, which was associated with decreased fasting glucose levels. However, mainly females seemed to develop insulin resistance in order to maintain glucose levels after fasting or insulin injection.

Conclusion: Collectively, these results suggest that even a short-term CR intervention can be beneficial in the context of reduced insulin production. However, no additional improvement was seen in heterozygous Ins1 animals after CR, suggesting that compensation mechanisms for the loss of insulin alleles are activated under normal conditions and CR challenges.

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Disclosure: **M.B. Dommerholt:** None.

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Surrogate indices of insulin sensitivity after Roux-en-Y gastric bypass: a comparison with results from the hyperinsulinaemic clamp

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Background and aims: Roux-en-Y gastric bypass (RYGB) induces extensive weight loss and improvements in insulin sensitivity when evaluated by the hyperinsulinaemic clamp. Surrogate indices of insulin sensitivity calculated from insulin and glucose concentrations at fasting or after an oral glucose tolerance test (OGTT) are frequently used in diabetes research, but have not been systematically validated after RYGB.

Materials and methods: Twenty obese subjects (10 preoperative type 2 diabetes, 10 normal glucose tolerance) were investigated before, 1 week (wk), 3 months (mo) and 1 year (y) after RYGB using a 4 h hyperinsulinaemic (40 mU/m^2/min) euglycaemic (5.5 mmol/L) clamp combined with [6,6-$^2H_2$]-glucose tracer infusion for estimation of peripheral insulin sensitivity (Rd: Rate of glucose disappearance, mg/kg ffm/min) and hepatic insulin sensitivity index (HISI: 10^6/[basal rate of glucose appearance × basal C-peptide). Fat free mass (ffm) was assessed by DXA. At all study visits, 4 fasting surrogate indices (inverse HOMA-IR, HOMA2-%S, QUICKI, revised QUICKI) were calculated, while OGTT-derived indices (OGIS, Matsuda, Gutt) were obtained at all but 1 wk. Preoperative correlations between surrogate indices and Rd or HISI were evaluated by Pearsons tests. Postoperative trajectories for surrogate indices were compared to Rd or HISI trajectories after log-transformation by use of repeated measurements ANOVA in a linear mixed effects model.

Results: Before RYGB, all surrogate indices correlated with Rd (r-values: 0.56-0.81, all $p<0.05$), whereas only fasting indices and Matsuda correlated with HISI (r: 0.63-0.85, all $p<0.01$). Postoperative fasting surrogate indices: Changes in inverse HOMA-IR and HOMA2-%S differed ($p<0.01$) from changes in peripheral insulin sensitivity at all study visits, whilst relative changes in QUICKI and revised QUICKI were comparable to changes in Rd at 3 mo and 1 y, but not at 1 week. For hepatic insulin sensitivity, inverse HOMA-IR and HOMA2-%S changed comparably to HISI at 1 wk after RYGB, whereas changes at 3 mo and 1 y differed significantly ($p<0.01$ for inverse HOMA-IR, $p<0.05$ for HOMA2-%S). Changes in QUICKI and revised QUICKI differed from HISI-changes at all postoperative study visits (all $p<0.01$).Postoperative OGTT surrogate indices: Changes in Matsuda and Gutt differed from changes in Rd ($p<0.05$ and $p<0.01$, respectively), but changes in OGIS and Rd were comparable both at 3 mo ($p=0.93$) and 1y ($p=0.81$).

Conclusion: Surrogate indices of insulin sensitivity may be inaccurate after RYGB. When compared with the gold standard hyperinsulinaemic clamp, HOMA-IR, HOMA2-%S, Matsuda and Gutt may not be valid for the estimation of post-RYGB changes in peripheral insulin sensitivity, while QUICKI, revised QUICKI and OGIS perform better. HOMA-IR and HOMA2-%S seem to provide reliable estimates of early, but not late, changes in hepatic insulin sensitivity.

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Disclosure: **K.N. Bojsen-Møller:** None.

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Vitamin D improves insulin sensitivity and myosteatosis in a model of diet-induced insulin resistance

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Background and aims: Human studies indicate a strong association between vitamin D deficiency and type 2 diabetes. In particular epidemiological evidence show that a poor vitamin D status increases the risk of insulin resistance, however the mechanisms underlying this effect are still not completely understood. In addition, early clinical description reported that severe vitamin D deficiency is associated to myopathy, thus suggesting a potential association between vitamin D and muscle. Aim of this study is to evaluate the effect of vitamin D administration in a murine model of diet induced insulin-resistance focusing on the skeletal muscle.

Materials and methods: Male C57DL/6 mice (n=40) were provided with a standard diet or High Fat-High Sugar Diet (HFHS) for 4 months. Subsets of animals were treated with Vitamin D (7 μg•kg-1, i.p. three times/week) for the last 2 months. Body weight and food intake were recorded weekly. At the end of the treatment, glucose tolerance test was performed. The expression of markers of lipogenesis and phosphorylation of insulin signalling intermediates were evaluated by western blot on gastrocnemius specimens. The expression of RAGE receptor and carboxymethyl lysine (CML), one of the main AGEs, were also evaluated by immunoblotting. Gastrocnemius fat accumulation was detected by oil red staining. Data were assessed by Kruskal-Wallis with Dunns post-test for non-parametric distribution and ANOVA with Bonferroni post-test for parametric distribution and a P value <0.05 was considered significant.

Results: In comparison to standard diet, HFHS diet induced body weight increase (24.84±1.05 g vs 31.82±1.95 g), hyperglycemia (107.8±29.58 vs 144.8±26.1 mg/dl) and impaired glucose tolerance. Neither the diet manipulation nor the drug treatment affected lipid profile. At the muscle level HFHS animals showed fat accumulation and a significant increase of triglycerides level ($p<0.01$) and these effects were correlated to an impaired insulin responsiveness. Vitamin D administration reduced body

weight and improved the oral glucose tolerance test. Interestingly, vitamin D reverted myosteatosis induced by the diet and this effect was correlated to a reduction of SCAP/SREBP1 activation ($p<0.01$). In addition, animals treated with vitamin D showed an improved muscle insulin responsiveness. HFHS diet increased CML concentration ($p<0.05$) and it was associated to overexpression of RAGE receptor ($p<0.001$). Most notably, these effects were significantly reduced by vitamin D treatment.
Conclusion: Our data clearly demonstrate that vitamin D administration improves insulin resistance due a chronic exposure to an high fat high sugar diet. Reduction of myosteatosis and muscle CML/RAGE expression can significantly contribute to the beneficial effects of vitamin D.
Disclosure: **E. Benetti:** None.

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Effect of Momordica charantia administration on insulin secretion and insulin sensitivity in type 2 diabetes

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Background and aims: Momordica charantia (MCH), also known as bitter melon, is a popular plant used for the treatment of diabetes. A decrease in glucose levels, glycated hemoglobin A1c (A1C) and fructosamine has been observed with MCH in patients with type 2 diabetes mellitus (T2DM). It is unknown whether the improvement observed is through a modification on insulin secretion, insulin sensitivity, or both. To date, the effect of MCH on these pathophysiological alterations in T2DM has not been investigated. The aim of this study was to evaluate the effect of MCH administration on insulin secretion and insulin sensitivity in patients with T2DM.
Materials and methods: A randomized, double blind, placebo controlled clinical trial was carried out in 24 patients (aged 35-60 years) with newly diagnosed T2DM, according to the criteria of the American Diabetes Association (ADA), without pharmacological treatment. Patients received MCH (1000 mg orally, twice daily) or homologated placebo for 3 months. All patients received medical nutritional therapy and were instructed to continue with their normal physical activity. An oral glucose tolerance test (OGTT) of 2-h was done before and after the intervention. A1C, blood pressure, weight, body mass index (BMI), waist circumference, fat % and fat mass were evaluated. Areas under the curve (AUC) of glucose and insulin were calculated. Total insulin secretion (Insulinogenic index), first phase of insulin secretion (Stumvoll index) and insulin sensitivity (Matsuda index) were assessed. Statistical analyses were performed with Wilcoxon and Mann-Whitney U tests. An Ethic Committee approved the protocol and a written informed consent was obtained from all volunteers.
Results: In the MCH group, there were significant decreases in weight (79.4±9.2 vs. 78.0±9.2 kg, $p=0.007$), BMI (29.1±2.4 vs. 28.3±1.9 kg/m2, $p=0.007$), fat % (36.7±7.8 vs. 36.3±7.6%, $p= 0.021$), fat mass (31.3±8.7 vs. 30.7±7.9 kg, $p=0.037$), waist circumference (106±12 vs. 104±11 cm, $p=0.013$), A1C (7.8±0.8 vs. 7.1±1.3%, $p=0.039$), 2-h glucose in OGTT (17.1±3.7 vs. 13.2±4.3 mmol/l, $p=0.008$), and AUC of glucose (61±9 vs. 52 ±13 mmol/l/min, $p=0.047$). A significant increase in AUC of insulin (1,885±1,202 vs. 2,174±1,422 pmol/l/min, $p=0.093$), in total insulin secretion (0.29±0.18 vs. 0.41±0.29, $p=0.028$) and first phase of insulin secretion (557.8±645.6 vs. 1,135.7±725.0, $p=0.093$) was observed after MCH administration. Insulin sensitivity was not modified with any intervention. The placebo group showed no changes.
Conclusion: MCH administration reduced A1C, 2-h glucose, AUC of glucose, weight, BMI, fat %, fat mass and waist circumference, with an increment of AUC of insulin, first phase and total insulin secretion.
Clinical Trial Registration Number: NCT02397447
Disclosure: **E. Martínez-Abundis:** None.

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Differences in lipid content in standard diets lead to disbalance in energy homeostasis during aging

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Background and aims: Aging associates with increased adiposity and insulin resistance. Adiposity signals work together with hunger and satiety signals in modulating energy homeostasis. We have previously reported that aged Wistar rats show hyperleptinemia and central leptin resistance that contribute to maintain a state of insulin resistance in aged animals. Cholecystokinin (CCK) and leptin are two anorexigenic hormones which work in short and long time respectively. Here we explore the effect of two standard diets differing in lipid content in the development of resistance to these hormones during aging, as well as the influence on weight and adiposity changes, and the accumulation of triglyceride and cholesterol in different tissues.
Materials and methods: 3 and 24 month-old male Wistar rats fed with standard diet 1 (SD1) (2.79kcal/g; 3.1%fat) or standard diet 2 (SD2) (2.9kcal/g; 4% fat) throughout their lifespan (8 animals per diet and age) were administered intraperitoneal with CCK (10μg/kg) and short-term food intake was determined. After recovering from CCK, animals were infused intracerebroventricular with leptin (0.2 μg/day) or vehicle with an osmotic pump and body weight and food intake were assessed daily. Animals were sacrificed 7 days after surgery. Experimental procedures were approved by the institutional Ethics Committee.
Results: Young rats responsiveness to both hormones was similar independently of the diet. In contrast, significant differences were observed in aged animals. Thus, SD2 fed rats treated with CCK show similar short time food intake than those treated with saline ($p>0.05$); in contrast, a marked satiating effect of CCK was observed in rats fed with SD1 ($p<0.001$). Similarly, aged animals fed with SD2 diet showed resistance to icv leptin whereas those under SD1 diet showed body weight loss and decrease of daily and total food intake similar to young animals. Content of triglyceride was 33.5% and 27.4% higher in liver and cardiac muscle respectively, in aged rats under SD2 diet compared to SD1 fed ones ($p<0.01$; $p<0.05$). Likewise, an increment of 29.8% in cholesterol content was observed in liver in SD2 versus SD1 fed rats ($p<0.05$). Surprisingly, no differences in body fat were detected by nuclear magnetic resonance analysis between SD1 and SD2 fed aged rats. In contrast, young rats under SD2 diet present higher body fat.
Conclusion: Our results suggest that fat content in diet is a key factor determining the responsiveness to anorexigenic signals in aged animals. Higher fat in the diet results also in a major infiltration of fat in cardiac tissue and liver. Young animals under SD2 diet present higher levels of body fat than SD1 fed young rats. Nevertheless, fat mass accretion along aging is similar independently of the fat content of the diet. Fat promotes CCK release from enteroendocrine cells in gut; hence elevated diet fat might result in long-term hypercholecystokinemia and posterior resistance to this hormone in SD2 fed animals. Regarding leptin, its secretion correlates with triglyceride content in adipose tissue. Thus, it seems likely that SD2 fed rats develop early hyperleptinemia that leads later to leptin resistance in aged animals, whereas SD1 fed animals seem to be protected. These data emphasize the relevance of diet fat content along life-span in preserving hormonal action.
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PS 041 Novel treatment concepts in metabolic disease

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Liraglutide upregulates adipose tissue angiogenic pathways and sprout formation, contributing to a better metabolic outcome

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Background and aims: Adipose tissue storage, metabolic and secretory functions are highly dependent of its ability to expand when necessary. Accordingly, correct vascularization is a crucial process in adipose tissue expansion and metabolic regulation. It has been shown that incorrect angiogenesis is a trigger to insulin resistance and metabolic dysregulation and increased capillarization improves insulin sensitivity and the metabolic outcome. Thus, our aim was to assess the pro-angiogenic effects of liraglutide.
Materials and methods: For in vivo studies 14 week male Wistar and non-obese type 2 diabetic Goto-Kakizaki (GK) rats were injected twice a day with liraglutide (200μg/Kg s.c.) during 14 days. Epididymal adipose tissue was collected, homogenized and used for Western Blot analysis. For ex vivo studies (adipose tissue angiogenic assay), adipose tissue from 4 week old Wistar rats was collected and cut into very small pieces (~1mm3). Explants were then embedded in collagen matrix and incubated for 5 days in EGM- 2 MV, BulletKit (Lonza, Switzerland) with or without liraglutide (50nM).
Results: In rats, liraglutide treatment upregulated adipose tissue angiogenic pathways by increasing the levels of VEGFR2, Tie-2 and FGFR, as well as HIF2alpha levels, which are involved in activation of angiogenic pathways. Interestingly, liraglutide-treated rats significantly lost body weight, but not epididymal adipose tissue weight. This was associated with increased PPARgamma levels after liraglutide treatment, showing loss of non-adipose fat and better lipid storage in adipose tissue. Improved insulin signalling and glycaemic outcome was observed in GK rats after liraglutide treatment, possibly resulting from better adipose tissue function. In the adipose tissue angiogenic assay, liraglutide did not result in significantly higher area of vascularization, but dramatically increased vessel density in a PI3K-dependent manner.
Conclusion: Liraglutide improves adipose tissue angiogenesis and adipose tissue function, contributing to better lipid storage and metabolic outcome.

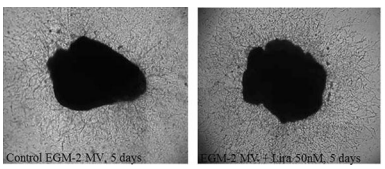

Figure 1: Example of the adipose tissue angiogenic assay showing a control explant (left) and a Liraglutide-treated explant (right). Images show similar area of capillarization but increased vessel density in Liraglutide-treated explant.

Disclosure: **P. Matafome:** None.

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Teneligliptin decreases the uric acids levels by xanthine dehydrogenage expression in white adipose tissue of the male Wistar rats

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Background and aims: The uric acid is also one of risk factor in the cardiovascular diseases. Recently it has reported adipose tissue produces and secretes uric acid through xanthine oxidoreductase (XOR) and that the production is enhanced in obesity. On the other hand, it is also reported that DPP-4 is released from adipose tissue correlated positively with an increasing risk score for the metabolic syndrome. Teneligliptin, a novel dipeptidyl peptidase-4 (DPP-4) inhibitor, exhibits a unique structure characterized by five consecutive rings, which produce a potent and long-lasting effect. In this study, we investigated the effects of teneligliptin on the uric acids metabolism in the male Wister rats and 3T-3L1 addipocytes.
Materials and methods: Male Wistar rats were fed normal chow diet (NCD), or 60% high fat diet (HFD) containing with either teneligliptin (~4.0 mg/kg/day) or not, for 4 weeks. Plasma uric acid levels and the expression level of xanthine dehydrogenase (Xdh), the major form of XOR in tissues, in either liver or WAT of NCD-fed rats were examined. All procedures were performed in accordance with the Guide for Care and Use of Laboratory Animals of the NIH and were approved by the Animal Subjects Committee of our University. Mouse 3T3-L1 preadipocytes were cultured and differentiated into adipocytes and, it were used 14 days after differentiation. 3T3-L1 adipocytes were incubated with the indicated concentration of teneligliptin (0, 1, 5, 10 μM) or/and DPP-4 (200ng/ml) for the indicated period before the experiments. After the process, Xdh mRNA expression were analyzed by qRT-PCR.
Results: Body weight was not significantly different between the control and teneligliptin groups, while body weight of the HFD-fed rats was significantly greater than in the NCD-fed rats. The plasma uric acid level did not significantly differ between the control and teneligliptin groups under the NCD condition. However, the plasma uric acid level was significantly decreased by 21% (from 0.34 ± 0.02 to 0.27 ± 0.02; $P < 0.05$) in the HFD-fed teneligliptin treated rats compared to the HFD-fed control rats. To investigate the molecular mechanisms of this effect of teneligliptin, we performed quantitative real-time PCR analysis. The expression level of Xdh in either liver or epididymal adipose tissue of NCD-fed rats was not altered by teneligliptin treatment. On the other hand, the Xdh expression was reduced significantly by 32% ($P < 0.01$) in the epididymal adipose tissue of the HFD-fed teneligliptin treated rats compared that of HFD-fed control rats, whereas the Xdh expression in liver did not change significantly in either group. Furthermore, teneligliptin significantly decreased Xdh expression by 45% ($P < 0.01$), 35% ($P < 0.01$), and 34% ($P < 0.01$) at 1, 5, and 10 μM concentration, respectively, in 3T3-L1 adipocytes. The treatment of DPP-4 (200 ng/ml), which was a novel adipokine that impaired insulin sensitivity in an autocrine and paracrine fashion, significantly increased Xdh expression by 49% ($P < 0.01$) in 3T3-L1 adipocytes. Under condition with pretreatment of DPP-4 for 12 hours, 10 μM teneligliptin significantly decreased Xdh mRNA expression by 26% ($P < 0.01$) compare to the DPP-4 treated 3T3-L1 adipocytes.
Conclusion: These data suggest that teneligliptin has the effect of reducing the uric acid level by suppressing the Xdh expression in epididymal adipose tissue of obesity.
Disclosure: **C. Tsukagoshi Moriya:** Non-financial support; Tanabe Mitsubishi Pharma. Other; Eli Lilly Japan K.K.

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One year efficacy, safety and tolerability of duodenal exclusion using Endobarrier as an adjunct to glucagon-like-peptide-1 (GLP-1) therapy: a randomised controlled trial

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Background and aims: New, effective treatments are needed to combat the global diabesity pandemic. 75% of UK patients commencing GLP-1 receptor agonist (GLP-1RA) therapy fail to achieve national targets for continuation. To investigate the efficacy, safety and tolerability of adding endoscopic duodenal exclusion to GLP-1RA therapy not achieving targets, compared to either treatment alone.

Materials and methods: Adults with suboptimally controlled type 2 diabetes (HbA1c≥58mmol/mol, ≥7.5%) and obesity (BMI≥35kg/m2) despite GLP-1RA therapy (liraglutide 1.2mg daily) were randomised to (1) additional duodenal exclusion using a novel endoscopic device, Endobarrier (2) Endobarrier without GLP-1RA; (3) escalated dose liraglutide (1.8 mg daily). All groups underwent the same initial 2-week diet. Those randomised to endobarrier were implanted with the device for 1-year. Participants were seen 3-monthly. This 1-year analysis was by modified intention to treat. Patient satisfaction was assessed using the NHS friends and family test.

Results: Groups were matched for age, sex, ethnicity, duration of diabetes, BMI and HbA1c (Table 1). In groups 1, 2 and 3 respectively, weight fell by 12.4±6.5kg, 11.4±7.6kg and 2.6±6.5kg; HbA1c fell by 22.8±16.1mmol/mol, 13.6±17.6mmol/mol and 13.8 ±15.5mmol/mol over 1-year (Table 1). Group 1 achieved this whilst reducing other diabetes medications whereas groups 2 and 3 added them (-3, 13, 5 new diabetes drugs respectively, P=0.01). 5/48 (10.4%) Endobarrier-treated patients had serious device-related adverse events (gastrointestinal bleed, obstruction, complicated removal, liver abscess; 1 from group 1) with resolution in all cases. There were 5/42 (11.9%) early device removals related to gastrointestinal symptoms (3 from group 1). Patient satisfaction levels were high with all treatment groups at 1 year: 89.5, 85.7 and 88.9% would be very likely or likely to recommend the treatment they received to family and friends. There were no significant differences in fasting glucose, hunger or satiety levels but by 1 week fasting and post-prandial satiety levels were significantly increased in group 1.

Conclusion: At 1 year, both Endobarrier groups produced similar clinically significant reductions in weight. Group 1 showed a trend towards superior effect on HbA1c reduction, achieved despite reduction of other diabetes medications compared to groups 2 and 3, in which there was intensification of other diabetes medications. These data suggest adding duodenal exclusion to suboptimally performing GLP1-RA therapy has clinical benefit and advantage over converting to duodenal exclusion or increasing GLP-1RA dose. The Endobarrier safety and tolerability profile up to 1 year was acceptable. Combination endobarrier-GLP-1RA therapy was well tolerated, with high levels of patient satisfaction in all groups.

Table 1 shows baseline characteristics of patients by treatment group along with 1-year outcomes as mean±SD or median(interquartile range). P-values shown in the 1-year columns reflect change from baseline within each group. P-values shown in the right columns reflect differences between treatment groups for the given parameter. P<0.0001 = ***, P<0.001 = **, P<0.01 = *

	Endobarrier+liraglutide (n24)		Endobarrier (n24)		Liraglutide (n22)		P-value
	Baseline	1-year	Baseline	1-year	Baseline	1-year	
Age (years)	52.0 ±11.7		50.7 ±8.4		54.0 ±10.1		
Sex (%male)	41.7		29.2		36.4		
Ethnicity (%Caucasian)	66.7		70.8		72.7		
Duration of Diabetes (years)	10.8 (6.6-15.6)		10.0 (7.8-12.6)		13.3 (9.0-18.4)		
Weight (Kg)	112.8 ±20.4	100.4 ±22.2 ***	115.6 ±19.4	104.1 ±21.0 ***	113.9 ±14.9	111.3 ±15.2	***
BMI (Kg/m²)	40.3 ±4.8	35.7 ±5.6 ***	41.7 ±4.9	37.4 ±4.6 ***	40.6 ±4.4	39.6 ±3.5	***
HbA1c (%)	9.6 ±1.4	7.5 ±1.3 ***	9.3 ±1.7	8.0 ±1.7 *	9.7 ±1.7	8.4 ±1.6 **	0.09
HbA1c (mmol/mol)	81.5 ±14.9	58.7 ±14.4 ***	78.1 ±19.0	64.5 ±18.9 *	82.5 ±18.8	68.7 ±17.1 **	

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Comprehensive metabolomic analysis in plasma, skeletal muscle, adipose tissue and urine in men with metabolic syndrome; with focus on the effects of resveratrol

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Background and aims: Resveratrol is a polyphenolic compound naturally present in many food items, especially berries, nuts and red wine. Resveratrol has been intensively studied in recent years as it has shown promising effects in cell culture and animal studies on various pathways involved in insulin sensitivity, low-grade inflammation, and consequences of obesity. The recently developed Metabolomic approach profile small-molecule metabolites using non-targeted approaches and has the advantage that it integrates gene-expression, transcript and protein levels, in addition to posttranslational modifications. It directly assesses the metabolic changes in a biological sample and offers the possibility to identify metabolic signatures of cellular metabolism involved in different pathways. The aim of the study is to provide a comprehensive Metabolomic analysis of the changes following resveratrol treatment in men with metabolic syndrome.

Materials and methods: The study was a randomized, placebo-controlled, double-blinded, single centre study. A total of 44 males with metabolic syndrome were randomized to treatment for four months with tablets containing placebo or 500 mg trans-resveratrol twice daily. Metabolomic profiling was performed on plasma, urine and biopsies from adipose tissue and skeletal muscle using ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS) and gas chromatography-mass spectroscopy (GC-MS).

Results: A total of 405, 282, 446 and 604 named biochemicals were detected in plasma, adipose tissue, skeletal muscle and urine. When comparing the resveratrol group to the placebo group a total number of compounds changed significantly ($p \leq 0.05$) in plasma, adipose tissue, muscle tissue, and urine with 38, 45, 24 and 43, respectively. Changes in plasma steroid hormones were one of the most pronounced alterations with reduction of many of the cholesterol-derived steroid hormones, including dehydroisoandrosterone sulfate and epiandrosterone sulfate among

others. Urine analysis demonstrated elevation of sulphated steroid hormone metabolites suggesting increased renal excretion. Accumulation of long-chain saturated, monounsaturated and polyunsaturated (n3 and n6) free fatty acids was observed in adipose tissue along with elevated levels of glycerol, phospholipids and lysolipids which indicate greater lipolysis and increased release of free fatty acids after resveratrol treatment. Also, trending ($0.05<p<0.10$) elevations in glycolytic and pentose phosphate pathway intermediates; glucose 6-phosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, and sedoheptulose 7-phosphate in adipose tissue indicates greater glucose uptake and glucose utilization through both glycolysis and alternative pathways. Finally, changes in metabolites derived from aromatic amino acids predominantly produced by the gut microbiome were observed in urine and may represent an important effect of resveratrol on the metabolome.

Conclusion: The metabolomic profiling revealed interesting metabolic changes following treatment with resveratrol to males with metabolic syndrome. Multiple pathways including steroid hormone metabolism, adipose tissue lipolysis and glucose utilization, and the gut microbiome were affected.

Clinical Trial Registration Number: NCT01412645

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Leptolide improves insulin resistance in diet-induced obese mice

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Background and aims: Insulin resistance is a hallmark of type 2 diabetes mellitus (DM2), a devastating disease worldwide. Current pharmacological therapies to treat DM2 are focused countering insulin resistance. In this work, we aimed to investigate the potential therapeutic use of Leptolide, a natural compound member of the cembranolide family.

Materials and methods: Three months-old C57BL/6J male mice fed regular diet (SD) or high fat-diet (HFD; 60% kcal fat) were treated with daily i.p. injections of Leptolide (100μg/kg) or vehicle for one month. Intraperitoneal glucose tolerance and insulin sensitivity were assessed during and at the end of the treatment. Plasma insulin, triglycerides (TG) and cholesterol (CHL) were measured after treatment. HepG2 cells were treated with 0.1 μM Leptolide and insulin signaling was studied by PKB activation.

Results: Mice fed SD and treated with Leptolide showed improved glucose tolerance and insulin sensitivity compared to control mice. Concomitant, plasma insulin levels were reduced by 40% and plasma TG levels by 25% in Leptolide treated mice. Likewise, diet-induced obese mice exhibited improved whole glucose tolerance and insulin sensitivity when treated with Leptolide. In parallel, plasma insulin levels were reduced by ~30% and plasma TG levels by ~12%. Importantly, these beneficial effects on glucose homeostasis, lipid levels and insulin sensitivity were not accompanied by toxicity. To further investigate the molecular mechanism by which Leptolide improves insulin sensitivity, we have shown that Leptolide augmented ~2-fold PKB phosphorylation levels in liver cells in a dose- and time-dependent manner.

Conclusion: We identified Leptolide as a new potential compound for the treatment of insulin resistance in DM2.

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Disclosure: **P. Villa-Perez:** None.

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New-onset diabetes as a target for pancreatic cancer screening

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Background and aims: New-onset diabetes mellitus/prediabetes with duration of less than 2 years (T3cDM) can be the earliest symptom of pancreatic cancer (PAC), especially when significant weight loss is present. On the contrary, long-term diabetes (T2DM) is a risk factor for developing PAC. Our aim was to determine the sensitivity and specificity of the current biochemical marker CA 19-9 alone or together with promising new markers microRNA-196 and -200 in distinguishing PAC patients from non-cancer patients.

Materials and methods: Sixty PAC patients with DM (35 men/25 women, age 67±8 yrs), 34 Type 2 DM patients without PAC (27 M/7 W, age 63±6 yrs) and 30 controls (22M/8W, 63±7 yrs) were enrolled in our study. Diagnosis of the cancer was confirmed either by needle biopsy or by surgical resection of the tumour later on. DM/prediabetes diagnosis was made according to ADA criteria. CA 19-9 was performed routinely in a laboratory, serum samples were used for microRNA isolation. Expressions of microRNAs were determined after reverse transcription and real-time PCR. ANOVA test was performed to evaluate the results. Tests of sensitivity and specificity of the markers were determined.

Results: In our group more patients with PAC were associated with new-onset diabetes than with long-term diabetes. All three markers were significantly elevated in PAC patients, with no difference in the subgroups according to the duration of diabetes. Data are shown in the table. While sensitivity and specificity of CA 19-9 alone to detect the cancer was 85% and 73%, respectively, a combination of CA 19-9 and microRNA-196 and -200 improved sensitivity to 95% and specificity to 77%.

Conclusion: Higher detection of new-onset DM/prediabetes (T3cDM) in PAC could play an important role in the early diagnosis of pancreatic cancer. Other signs like weight loss and/or gastrointestinal symptoms may initiate further examination. Thanks to high sensitivity a combination of modern molecular markers microRNA-196 and -200 together with CA 19-9 could be used in the first line of non-invasive PAC screening in patients with new-onset DM. It would diminish a delay in the diagnosis of pancreatic cancer and improve the prognosis of diabetic patients with this malignant disease.

	T3cDM/PAC (n=44)	T2DM/PAC (n=16)	T2DM (n=34)	Controls (n=30)
CA 19-9 [kIU/l]	120,03 (9,3-1549,83)^{dz}	165,36 (21,87-1250,43)^{dz}	8,08 (3,69-17,72)	4,64 (1,85-11,59)
miR-196 [U]	1,17 (0,45-3,02)^{by}	1,26 (0,62-2,54)^{ax}	0,63 (0,31-1,28)	0,71 (0,40-1,24)
miR-200 [U]	1,66 (0,39-6,99)^{dz}	1,52 (0,36-6,34)^{cv}	0,45 (0,21-0,95)	0,39 (0,18-0,84)
Results are expressed as means with SD ranges after logarithmic transformation. Statistical significance as compared to controls ($^{a}p<0,05$, $^{b}p<0,01$, $^{c}p<0,001$, $^{d}p<0,0001$) and to type 2 diabetics ($^{w}p<0,05$, $^{x}p<0,01$, $^{y}p<0,001$, $^{z}p<0,0001$)				

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Increased dietary AGEs and diabetes: possible inducers of colon cancer

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Background and aims: Advanced glycation end products (AGEs) dietary intake is increased as a consequence of processed foods consumption and is also high in diabetes patients. Recent evidence has suggested there may be an association between AGEs and colorectal cancer risk. We investigate the contribution of glycated casein in the modulation of signaling pathways involved in the biology of human cancer.
Materials and methods: Casein was subjected to an in vitro glycation process, in the same conditions as those used in the production of sweetened UHT milk drinks: 20 mg/ml total casein were UHT treated (30 min at 70°C followed by 4 sec at 140°C) in the presence of 116 mM lactose - 55 mM glucose-fructose or without sugars (for control casein). Long term storage effects were simulated by storage at room temperature for 60 days). Total AGEs content in glycated casein was 17.5 μg/mg protein while in control casein, AGEs were 10 times lower. Furosine content was 2 μg/mg protein in glycated casein and 0.17 μg/mg protein in control. C2BBe1 (expressing brush border) colorectal adenocarcinoma cells were treated with 50, 100 and 200 μg/ml glycated or control casein for 3, 6, 9, 12 and 24 h, in serum-free conditions.
Results: Cell proliferation was slightly encouraged by glycated casein in a time and dose-dependent manner and increased by 1.4 fold after 24 h ($p<0.05$). Compared to control cells, RAC-alpha serine/threonine-protein kinase (AKT1) expression increased 1.7 fold after 24 h of exposure to 200 μg/ml glycated casein. AKT1 is known to inhibit apoptosis and to activate the NF-kB pathway and antioxidant enzymes. This could enhance cancer cells ability to cope with stressful stimuli and promote survival. NF-kB inhibitor alpha (IkB) expression was high at early intervals after glycated casein exposure, which suggested NF-kB is initially inhibited. However, as IkB decreased, the expression of p50 subunit of NF-kB began to increase after 12 h of exposure to 200 μg/ml glycated casein, which suggested NF-kB signaling became activated. AKT1 expression appeared to be inversely associated with H2O2 levels, which were high (4 fold increase, $p<0.001$) after 3 h of incubation with glycated casein. The generation of reactive oxygen species is known to be induced as a primary result of AGE-receptor binding. After the H2O2 surge, catalase activity increased promptly, and was maximal after 6 h of exposure to 200 μg/ml glycated casein (1.6 fold increase, $p<0.05$). Conseqent to this catalase activation, H2O2 levels returned to control levels. N(epsilon)-(carboxymethyl)lysine (CML) - protein adducts significantly increased after 12 h and 24 h of exposure to 200 μg/ml glycated casein, by over 2 fold at both intervals ($p<0.01$). Phosphorylated extracellular signal-regulated kinases 1/2 (Erk1/2) expression also increased at the longer exposure intervals. This aspect might be particularly significant for cancer cell types, as Erk1/2 is a known inducer of matrix metalloproteinase 2, which is involved in the metastatic process.
Conclusion: Glycated casein benefited the cellular proliferation of adenocarcinoma cells C2BBe1 and activated signaling mechanisms which may be involved in cancer metastasis. High AGEs levels, either from diet or due to diabetes may be key signaling molecules involved in the initiation and development of colon cancers.
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Mitogenic activity of the metabolically relevant hepatokine FGF21 in human carcinoma cells

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Background and aims: Type 2 diabetes mellitus (T2DM) is associated with increased incidence and progression of various types of cancer, among others those of liver and breast. In contrast, inverse correlation between T2DM and risk of prostate carcinoma (PCa) has been demonstrated. However, diabetic men have more aggressive PCa with worse prognosis than non-diabetic patients. Which factors may link metabolic abnormalities to enhanced cancer risk is not fully understood yet. Serum FGF21 levels are significantly increased in situations of metabolic imbalance in humans, as in obesity, metabolic syndrome and T2DM. We asked whether a link may exist between high FGF21 serum concentrations and tumor cell proliferation.
Materials and methods: Breast adenocarcinoma cells (MCF7), prostate adenocarcinoma cells (PC3), hepatocellular carcinoma cells (HepG2) and, for control, non-transformed breast epithelial cells (MCF10a) were treated with increasing concentrations of recombinant human FGF21 (rhFGF21) over 4 days. Cell viability/cell number, DNA content, cellular protein and RNA were analyzed.
Results: Treatment of MCF7, PC3, and MCF10a cells with rhFGF21 at increasing concentrations over 4 days induced a modest but significant increase in cell viability and/or cell number ($p<0.001$, $p<0.01$ and $p<0.05$) as measured by XTT assay. No effect was measured in HepG2 cells. In parallel, DNA amount was significantly higher in all cell lines. In HepG2 cells, the effect was present only at pharmacological rhFGF21 concentrations. All cell lines expressed the genes FGFR1, FGFR4 and FRS2 encoding for surface receptors and receptor substrate/adapter protein involved in FGF21 signaling. A clear dose-response was seen in PC3 cells. In these cells, acute stimulation with rhFGF21 at physiological doses induced phosphorylation of ERK1/2. Interestingly, expression of the immediate early genes FOS and JUN, but not MYC, and of IRS1 was significantly increased In PC3 cells within 4h after addition of rhFGF21. Furthermore, a trend for increase in SLC2A1 and HK2 expression within 24h of treatment with rhFGF21 was observed.
Conclusion: Altogether, our data show that rhFGF21 treatment of human carcinoma cells increases cell viability and proliferation probably by activating the MAPK pathway and up-regulating expression of mitogenic and trophic genes. Thus, the hepatokine FGF21 that is known to circulate at elevated concentrations in obesity and obesity-associated diseases may represent a new player in the connection between metabolic diseases and certain tumors.
Disclosure: **L. Berti:** None.

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The classical nuclear estrogen receptors ERα and ERβ, and the G-protein-coupled receptor-30 are differently expressed in prostate cancer depending on diabetes status

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Background and aims: In contrast to many other malignancies, incidence of prostate cancer (PCa) is not elevated in patients with diabetes mellitus. However, PCa survival is markedly reduced in diabetic men.

Prostate cell growth and prostate carcinogenesis are not only mediated by androgens, they are also depending on functional estrogen signaling. To address potential underlying mechanisms for the reduced survival in diabetic men we looked for gene expression patterns of the classical nuclear estrogen receptors ERα (ESR1) and ERβ (ESR2) both in diabetic and nondiabetic patients. Moreover, expression of the G-protein-coupled receptor-30 (GPR30) with estrogen-binding affinity was also investigated, which was shown as well as ERα and ERβ to be involved in prostate tumorigenesis.

Materials and methods: 12 prostate tissue samples of diabetic patients (6 cancer, 6 tumor-adjacent benign tissue, mean HbA1c 6.4±0.1%) and 12 samples of patients without diabetes (6 cancer, 6 tumor-adjacent benign tissue, mean HbA1c 5.6±0.1%), matched for age and BMI, who underwent a radical prostatectomy, were included in the study. mRNA expression of target genes was analyzed by RT-qPCR and normalized to RPS13 mRNA in duplicate. Insulin sensitivity index was calculated as proposed by Matsuda and deFronzo during a 5-point oral glucose tolerance test.

Results: Absolute ESR1, ESR2, and GPR30 gene expressions were not different between diabetic and nondiabetic men. While ESR1 and GPR30 gene expressions were unaltered in tumor in comparison with benign tissue, absolute ESR2 gene expression, which was already very low, was further reduced in tumor ($p=0.0008$). The study group was then stratified according to their diabetes status. Now, however, when correlated to the tumor content in the biopsies, only in diabetic patients we found an impressive reduction in ESR1 gene expression with increasing tumor content ($p=0.007$). Moreover, ESR2 and GPR30 gene expression analyses showed only in diabetic patients a difference between tumor and benign samples, where the gene expressions were clearly reduced in tumor tissue ($p=0.006$ and $p=0.02$, respectively). These associations were independent of age, BMI and insulin sensitivity in multivariate model analyzes.

Conclusion: In this study we could demonstrate an impressive reduction in ESR2 and GPR30 gene expression in prostate tumor tissues only in diabetic patients. As both receptors are described to play protective roles in PCa development, and because their reduction in PCa seems to be dependent on a diabetic environment, they might represent one mechanistic way for the understanding the markedly reduced survival in diabetic men with PCa. Because a suppressive role in prostate cancer invasion was recently attributed to ERα, a reduction in ESR1 gene expression with increasing tumor content may also contribute to the reduced survival of diabetic men with PCa.

Disclosure: **S.Z. Lutz:** None.

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The risk of epithelial ovarian cancer in obesity before and after marked weight loss obtained by bariatric surgery

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Background and aims: Epithelial ovarian cancer (EOC) is a tumor associated to a high mortality. Obesity has been shown to be an established risk factor for EOC. The current biomarker for ovarian cancer is CA-125. Recently, Human Epididymis Protein 4 (HE4) has been proposed as a biomarker in EOC differential diagnosis. An algorithm (ROMA, Risk Of Malignancy Algorithm) has been developed for pre-menopausal and post-menopausal women to combine the diagnostic power of plasma CA125 and HE4 to predict the EOC risk. Aims of the study are to evaluate whether obesity could represent a risk factor for EOC and could increase the ROMA index and if this risk could decrease after marked weight loss by bariatric surgery.

Materials and methods: 163 obese women with body mass index (BMI) 42.4±10.8kg/m^2 age 33±15.9 years (Group 1), and 130 normal weight women with BMI 22.8±3.6kg/m^2, age 30±4.6 (Group 2) underwent CA-125 and HE4 plasma determinations that have been incorporated in ROMA algorithm. Patients of Group 1 underwent Sleeve Gastrectomy (SG) and evaluated 1 year after surgery. We selected a population of premenopausal women to exclude menopausal status as a confounding factor.

Results: HE4 levels above the normal range were detected in 8.6% (14/163) of Group 1. and only in 3% (4/130) of Group 2 ($p<0.007$). CA125 plasma concentrations above the normal range were observed in 2.5% (4/163) of Group 1 and in 6.1% (8/130) of Group 2 ($p<0.004$). ROMA score above the cut-off (>13%) was detected in 24.5% (40/163) of Group 1, whereas in the normal-weight women group a high ROMA score was identified only in 5.3% (7/130) ($p<0.009$). After SG and decrease of BMI (from 42.4 to 32.5 kg/m^2, p 0.001), we observed a decrease of ROMA score from 17.8±2.6 to 14.1±3.6 ($p<0.05$) and it was in the normal range in 62% of the high risk for EOC obese women.

Conclusion: Our data suggest that obesity in premenopausal women is a risk factor for EOC. It seems to have enough evidence to speculate the possible function of ROME score as a simple, non-invasive test able to screen obese women at risk of developing EOC. This risk decreased after SG.

Disclosure: **F. Coccia:** None.

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The impact of diabetes and antidiabetic treatment on the survival of patients with multiple myeloma

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Background and aims: Multiple myeloma (MM) is the second most common hematological malignancy. The coexisting diabetes mellitus (DM) is associated with poor clinical outcomes of MM. In addition, high-dose glucocorticoids used to treat MM can worsen the preexisting diabetes or provoke steroid induced diabetes. In vitro studies have shown that insulin stimulates the growth of MM cells via the activation of insulin/insulin-like growth factor-1 hybrid receptor. The aim of our study was to investigate the survival rate of MM with and without diabetes and its association with the antidiabetic treatment.

Materials and methods: In our retrospective study data were collected between 2007-2013 from 135 consecutive patients (68 male/67 female) with newly diagnosed multiple myeloma. The diagnosis of diabetes mellitus was based on WHO criteria. At baseline there was no significant difference regarding age and staging of MM between the patient groups with and without diabetes (MM+DM vs MM). 18/135 patients had diabetes in our study population; 14 of them received metformin alone or in combination and 7 patients were treated with insulin alone or in combination. The statistical analysis was carried out using SPSS version 20.0.

Results: The median fasting and maximum blood glucose level were higher in MM+DM group compared with MM group ($p<0.0001$, $p<0.0001$). The progression free survival was 21.3 months in the MM+DM group and it was 26.1 months in the MM group ($p=0.039$). The overall survival was 44.8 vs 58.8 months in the diabetic vs non-diabetic MM group. The overall survival of metformin treated diabetic patients was 50.4 months compared to 15.2 months in non-metformin treated diabetic patients ($p=0.018$). The overall survival did not differ significantly between the MM group and the MM+DM treated with metformin. All patients treated with insulin (7/7) died during the study period however, 7/14 are still alive from the metformin-treated group.

Conclusion: In our study the coexisting diabetes decreased the progression free survival in multiple myeloma. The antidiabetic treatment seemed to influence the survival rate which was significantly lower in insulin treated group and higher in metformin treated patients. Based on our results the treatment of multiple myeloma requires multidiscipline approach involving a diabetologist.

Disclosure: **E. Sipter:** None.

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Increased lipotoxicity and insulin resistance in non diabetic patients with hepatocellular carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the main type of primary liver cancer and although viral infections (HCV and HBV) and excess alcohol intake have been considered the major risk factors for HCC development, recent data showed that subjects with metabolic alterations, i.e., insulin resistance (IR) and type 2 diabetes (T2DM), are at higher risk for HCC. Hepatocarcinogenesis is a multistep process, which starts mostly by a chronic disease although it can develop independently of cirrhosis. The mechanisms for the development of HCC are still unknown but it has been hypothesized that derangement in lipid metabolism might lead to HCC through lipotoxicity, inflammation and apoptosis. Thus, we have studied if adipose tissue IR and hepatic lipid metabolism were altered in liver and plasma of patients with HCC undergoing liver transplant (LT).
Materials and methods: In 31 patients with HCC and 21 healthy subjects (CT) we have evaluated plasma metabolites involved in lipotoxicity i.e., lipid profile by LCMS and FFAs composition by GCMS. We calculated indexes of insulin resistance, as Adipo-IR= FFA x Insulin and HOMA = glucose x Insulin/22.5, palmitic/linoleic acid (16:0/18:2) as a surrogate of DNL index, unsaturated to saturated fat ratio (PUFA/SFA), and a score of fibrosis (APRI = AST/platelet count). In addition, we have collected non tumor liver tissues of 13 patients with HCC undergoing LT and 17 donors and measured the expression of Stearoyl-CoA Desaturase-1 (SCD1), Sterol Regulatory Element-Binding Protein 1 (SREBP-1), Carbohydrate-Responsive Element-Binding Protein (ChREBP), Notch Homolog 1 Translocation-Associated (NOTCH1) and B-cell lymphoma (Bcl-2) by Real-Time PCR analysis. We also evaluated hepatic content of lysophosphocholines (LPC) and ceramides (CER) by LC-MS. All patients were positive to HCV genotype 1.
Results: HCC patients compared to CT had higher IR (Adipo-IR 14.5 ±2.5 vs 5.6±0.9; HOMA 7.4±0.94 vs 2.04±0.32, all $p<0.05$); increased lipotoxicity, as shown by reduced PUFA/SFA ratio (0.43±0.036 vs 0.9 ±0.029, $p<0.0001$) and increased palmitic/linoleic ratio (1.05±0.05 vs 1.8 ±0.08, $p<0.0001$). Lipotoxicity increased in parallel to IR as shown by the correlation between palmitic/linoleic ratio with Adipo-IR ($R=0.40$, $p=0.0017$) and HOMA ($R=0.62$, $p<0.0001$). Similarly, the increase in APRI score in HCC (3.3±0.5 vs 0.21±0.023) was related to the increase in Adipo-IR ($R=0.41$, $p=0.01$), HOMA, palmitic/linoleic, PUFA/SFA ; $R=0.53$, $p=0.001$; $R=0.61$, $p<0.0002$; $R=-0.55$, $p<0.0009$). Thus, we evaluated if hepatic expression of genes of lipid metabolism and apoptosis were altered in HCC. In non tumor liver tissues of patients with HCC we found a higher expression of SCD1 ($p=0.02$, NOTCH1 ($p=0.05$) and Bcl-2 ($p=0.006$) compared to donors. Also SREBP-1 and ChREBP expression tended to be higher in HCC ($p=0.06$). Lipidomic analysis by LCMS showed a lipotoxic profile in HCC patients with decreased liver content of LPC (LPC-16:0 (54.3±10.3 vs 81.4±6.8); LPC 18:2 (vs 24.2±6.6 vs 45.4 ±5.1); LPC 24:0 (3.8±1.4 vs 6.5±0.78) all $p<0.05$, while we did not find differences in ceramides.
Conclusion: Patients with hepatic cancer have an altered lipid profile associated to increased IR and lipotoxicity, not only in plasma but also in non tumor liver tissue, thus predisposing to type 2 diabetes.
Supported by: Funding from project, CNR-Interomics
Disclosure: **M. Gaggini:** None.

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Anti-inflammatory protection of pancreatic islets during prolonged High Fat Diet (HFD) by NUPR1 through enhanced local secretion of IL-1RA

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Background and aims: Metabolically unhealthy obesity confers a state of subclinical low grade inflammation that contributes to insulin resistance and beta cell dysfunction in susceptive individuals. Previously, we demonstrated that mice with beta cell-specific overexpression of the intracellular protein NUPR1 under the control the rat insulin gene 1 promoter (RIP1/NUPR1-Tg) maintained normal glycemia during prolonged HFD (60% energy from fat) while wild type (WT) controls became diabetic. Here we investigated insulin secretion, apoptosis, and secretion of inflammatory IL-1beta and its physiologic receptor antagonist IL-1RA in isolated islets from normal and 20 weeks HFD-fed WT and RIP1/NUPR1-Tg mice.
Materials and methods: Isolated islets were cultured in inserts with 8 μm pores and exposed for 24 h to 0.33 mM STZ or 10 ng/ml IL-1beta. Glucose-induced insulin secretion and apoptotic cleavage of the DNA repair enzyme PARP was measured after 24 h. For measurement of secreted IL-1beta and IL-1RA, stress medium was exchanged after 24 h to normal culture medium and supernatants were collected after another 24 h.
Results: Islets from normal diet-fed WT mice demonstrated significantly increased apoptotic cleavage of the DNA repair enzyme PARP over time in culture and after 24 h exposure to STZ or IL-1beta. In contrast, PARP cleavage was not much affected in RIP1/NUPR1-Tg islets. Under same stress conditions, basal and stimulated insulin secretion was significantly reduced in WT but maintained in RIP1/NUPR1-Tg islets. Secretion of IL-1beta was hardly detectable in islets from normal-fed mice but significantly upregulated in islets from HFD-fed mice. In RIP1/NUPR1-Tg islets, HFD-mediated induction of endogenous IL-1beta was reduced and less further inducible by STZ or IL-1beta. The reduced secretion of IL-1beta from RIP1/NUPR1-Tg islets was associated with significant upregulation of IL-1RA which was not observed in WT islets.
Conclusion: NUPR1 overexpression efficiently preserves insulin secretory functions during HFD and cellular stress in vitro. During prolonged HFD, protective anti-inflammatory mechanisms involve upregulation of IL-1RA. NUPR1 may therefore represent an endogenous molecular target for the preservation of islet function during chronic low grade inflammation in metabolically unhealthy obesity.
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Disclosure: **G. Päth:** None.

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Skeletal muscle expression profile of selected pro-inflammatory myokines in obesity and type 2 diabetes

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Background and aims: Myokines are secretory products of skeletal muscle involved in energy metabolism, muscle remodeling, angiogenesis

or inflammation. Muscle secretory profile can be modulated by physical (in)activity, inflammation, lipids, insulin resistance or hyperglycemia. The aim of this study was to study the expression of myokines IL8, NAP2, GROα, MCP1 in human skeletal muscle & primary muscle cells in (i) obesity, prediabetes and type 2 diabetes, as well as in response to (ii) glucose and saturated fatty acid.

Materials and methods: Sedentary middle-aged men were divided into 4 groups: lean (n=28; 37±9 years) and obese with normal glucose tolerance (n=29; 38±8 years), obese with impaired glucose tolerance (prediabetes; n=25; 44±10 years) and newly-diagnosed type 2 diabetics (T2D; n=15; 50 ±8 years). Phenotyping included the assesment of glucose tolerance by oral glucose-tolerance test, insulin sensitivity by euglycemic hyperinsulinemic clamp, adipose tissue content and distribution by bioimpendance and/or MRI, skeletal muscle lipids by 1H-MRS, physical activity by accelerometers. Samples of m. vastus lateralis were taken by percutaneous needle biopsy and used to establish primary skeletal muscle cell cultures. Differentiating muscle cells (n=6-8/group) were exposed to saturated fatty acid (palmitate, 100μM) or glucose (5,5/10/20mM). Myokines in the conditioned media were screened by cytokine-antibody array. Gene expression in muscle & muscle cells was assesed by real-time qPCR and protein content in the conditioned media by Multiplex assay.

Results: Prediabetes was associated with increased expression of IL8 ($p<0,05$), GROα and NAP2 ($p<0,01$) in skeletal muscle. Muscle from T2D expressed the highest levels of MCP1 mRNA ($p<0,01$), which were positively associated with fasting glycemia ($R=0,26$; $p=0,01$). Muscle cells from prediabetic and diabetic donors tended to express and release lower levels of myokines, with MCP1 secretion being significantly reduced from cells of obese and T2D donors compared to the lean ($p<0,05$), while simultaneously increasing IL8 expression ($p<0,05$). Palmitate and glucose treatment did not induce any other changes of selected myokines in human primary muscle cells.

Conclusion: Increased skeletal muscle expression of specific myokines in (pre)diabetes suggests the activation of inflammatory response with progressive metabolic disease. Myokines in human skeletal muscle and primary muscle cells are distinctly regulated by obesity and (pre)diabetes, suggesting the existence of the whole body factors, other than glucose or fatty acids, that are involved in the development of metabolic disease.

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Disclosure: **D. Maderova:** None.

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Targeted disruption of inducible nitric oxide synthase in adipocytes improves insulin sensitivity and dyslipidaemia in diet-induced obese mice

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Background and aims: Obesity and insulin resistance are well known to be associated with a low-grade but chronic proinflammatory state. Expression of the inducible nitric oxide synthase (iNOS) is increased in insulin target organs including adipose tissue in diet-induced and genetic models of obesity. We have further shown that mice lacking iNOS through whole-body genetic disruption are protected from obesity-linked insulin resistance. iNOS is overexpressed in both adipocytes and myeloid cells in adipose tissue of obese mice and previous studies have shown that myeloid-specific ablation of iNOS failed to improve insulin resistance suggesting that iNOS induction in adipocytes is required for the development of inflammation-linked insulin resistance. The aim of the present study was to determine whether targeted disruption of iNOS in adipocytes could alleviate insulin resistance in mice fed high-fat high-sucrose (HFHS) diet.

Materials and methods: Using the CRE-lox approach (adipo-Cre), the nos2 gene (encoding iNOS) was selectively deleted in adipocytes of mice (ADPnos2-KO). ADPnos2-KO mice and their wildtype littermates (ADPnos2-WT) were kept either on a standard chow diet or a HFHS-diet (65% fat, 15% protein, 20% carbohydrates) for 10 weeks. Insulin and glucose tolerance tests were then performed and mice were sacrificed for further plasma and tissue analyses.

Results: Validation of the ADPnos2-KO mouse model was first achieved by testing the effect of lipopolysaccharide (LPS) a well-known inducer of iNOS expression. LPS challenge induced a robust iNOS gene expression and NO production in adipocytes from ADPnos2-WT, which was markedly reduced in adipocytes from ADPnos2-KO mice. When challenged with a HFHS diet, no difference in food intake or body weight gain was observed between ADPnos2-KO mice and their ADPnos2-WT littermates. Tissue weights (adipose tissues, muscle, heart, liver, pancreas), fasting insulinemia and glycemia were similar between ADPnos2-KO and ADPnos2-WT mice. HFHS diet-induced glucose intolerance was not improved in ADPnos2-KO as compared to ADPnos2-WT mice. However, insulin tolerance tests revealed that insulin sensitivity was improved in the HFHS fed ADPnos2-KO mice compared to their HFHS-fed ADPnos2-WT counterparts ($P<0.05$). Interestingly, plasma triglyceride levels were also significantly reduced in HFHS-fed ADPnos2-KO mice as compared to their WT controls.

Conclusion: We conclude that selected disruption of iNOS in adipocytes confers some protection against obesity-linked metabolic syndrome as shown by improvement of insulin sensitivity and reduction of hypertriglyceridemia in obese mice. Further analyses are underway to examine the selective impact of iNOS disruption in adipocytes on levels of insulin signalling and inflammatory mediators in adipose tissue as well as other insulin target tissues of obese mice.

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Disclosure: **K. Bellmann:** None.

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Activation of heat shock response provided by mild electrical stimulation with heat shock improves metabolic abnormalities in obese subjects with type 2 diabetes

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Background and aims: This study was designed to identify the optimal intervention strategy of the activation of heat shock response provided by mild electrical stimulation with heat shock (MES+HS) in obese type 2 diabetes patients. We have previously reported that MES+HS improved HbA1c by -0.43% in male subjects with obese type diabetes.

Materials and methods: This study was a prospective, frequency-escalating, randomized, open-label, triple-arm trial. A total of 60 obese type 2 diabetes patients were randomized into three groups of two, four, or seven times treatments per week for 12 weeks.

Results: No adverse events were identified. In comparison to the baseline, MES+HS treatment over time significantly improved visceral adiposity (- 11.69 cm2. $p<0.001$), glycemic control (HbA1c: - 0.36%: from 7.64% to 7.28%. $p<0.001$), insulin resistance (HOMA-IR: - 1.09. $p<0.001$), systemic inflammation (TNF-α: - 0.40 pg/mL. $p<0.001$. CRP: - 663.6 ng/mL. $p=0.008$), renal function (eGFR: + 2.96 mL/min/1.73m2. $p<0.001$), hepatic steatosis (AST/ALT: + 0.06. $p=0.007$) and lipid profiles (triglyceride: - 30.02 mg/dL. $p=0.015$). The clinical target of HbA1c less than 7.0% was achieved by 38.3% (n=23) of particpants after MES+HS treatment. The reduction in HbA1c was significantly greater in 4 per week (- 0.36%. $p=0.036$) or 7 per week (- 0.65%. $p=0.001$) than that in 2 per week (- 0.10%) of treatment. The decrease in the visceral fat area showed similar trend of changes (- 5.37, - 14.24, - 16.45 cm2 by two, four, seven

per week, respectively), indicating that the beneficial effects depend on its frequency. More pronounced effects were observed in males (HbA1c: - 0.44%. from 7.70% to 7.25%. p<0.001) than those in females (HbA1c: - 0.17%. from 7.50% to 7.33%. p=0.140).
Conclusion: This research provides additional lines of evidence to support the positive impacts of MES+HS in improving metabolic outcomes in obese type 2 diabetes patients. Those who do not reach the glycemic control goal of HbA1c less than 7.0% could be offered additional personalized medical care including MES+HS treatment.
Clinical Trial Registration Number: UMIN 000016309
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Disclosure: **T. Kondo:** None.

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Toll like receptor 4 mediates the anti-inflammatory effect of metformin in human peripheral blood mononuclear cells

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Background and aims: Chronic inflammation has been identified as pivotal component of both insulin resistance and Type 2 Diabetes (T2D). Chronic inflammation is characterized by cytokines secretion, as well as the activation of Toll-Like receptor (TLR) 2 and 4. Early stage no treated T2D patients have shown an increase in TLR2 and TLR4 activation on Peripheral Blood Mononuclear Cells (PBMCs). Metformin is the first line drug for treatment of TD2 and it has been shown to reduce proinflammatory cytokines blood levels; in the present study we sought to evaluate the effect of Metformin on the modulation of innate immune system activation in PBMCs to better define its anti inflammatory mechanism.
Materials and methods: PBMCs were isolated from healthy donors by Ficoll gradient, stimulated with different doses of Metformin (1mM, 5mM, 10mM, 20mM and 30mM) and incubated for 18h at +37°C. At the end of each treatment, TLR2 and TLR4 expression were evaluated by flow cytometry. To assess the anti-inflammatory effect of Metformin, IL-1β levels were determined by intracellular staining whereas Lipopolysaccharide (LPS) binding on PBMCs were evaluated by flow cytometry after LPS-Alexafluor488 conjugated stimulation. All the experiments were performed at least in triplicate. Results were analyzed with t-test and p<0.05 were considered statistically significant.
Results: Metformin resulted able to decrease both TLR4 and TLR2 expression in a dose dependent fashion. Metformin treatment at dosage form 5mM to 30 mM induced a significant reduction of TLR4 expression respect to the control (p<0.05). No significant effect has been shown at 1mM of Metformin treatment. Conversely, Metformin at 1mM, 5mM and 10mM doses did not induce decrease of TLR2 expression, while a significant reduction in TLR2 expression was observed only at 20mM and 30Mm. These data suggest that the effect of Metformin could be more specific for TLR4 than for TLR2. Since TLR4 is the LPS receptor, we evaluated the effect of LPS treatment after Metformin incubation on PBMCs. We observed that incubation with high dose of Metformin (30Mm) significantly decreased the LPS induced IL-1β production (p<0.05). By confirming its anti-inflammatory mechanism, we also showed that Metformin treatment blunted LPS binding on TLR4.
Conclusion: Our data suggest that Metformin can reduce TLR4 expression in PBMCs and subsequently can modulate cytokine secretion associated with TLR4. Metformin can contribute to reduce the inflammatory state associated with T2D. Further studies are needed to better understand the pleiotropic mechanisms of this drug.
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Disclosure: **A. Coppola:** None.

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Il-1β signalling in macrophages is required for normal glucose homeostasis in the obese state

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Background and aims: It is widely accepted that type 2 diabetes is associated with chronic systemic inflammation and elevated levels of circulating lipids. Increases in both resident and recruited macrophages to both white adipose tissue and pancreatic islets have been seen in all models of type 2 diabetes. Interleukin-1beta (Il-1β) has been implicated as a major contributor to macrophage recruitment, as well as beta cell damage and secretory dysfunction, and the IL-1receptor is highly expressed on beta-cells. However, our understanding of underlying mechanisms, the actual roles that macrophages play, and the timing of when those impacts occur, are still relatively unknown. It is therefore the aim of this project to examine how IL-1β signalling in macrophages, (through myeloid differentiation factor 88 (MyD88)) impacts on glucose-tolerance in vivo in the context of obesity.
Materials and methods: C57Bl/6 mice with the floxed transgene for MyD88 (MyD88fl/fl) were crossed with C57Bl/6 LycM cre (LycMcre+/-) mice to create a myeloid specific knockout mouse (MyD88KO). Mice were placed on a high fat diet (HFD) at 8weeks of age, and glucose and insulin tolerance tests were performed at 4, 8, and 12 weeks diet. Islets were taken after 12 weeks, and glucose stimulated insulin secretion (GSIS) was performed. Peritoneal macrophages were also taken from HFD mice and co-cultured with min6 cells for 24hrs before GSIS was performed.
Results: MyD88KO mice exhibit basal hyperinsulinemia, irrespective of diet (p<0.05 2wayANOVA). MyD88KO mice are also glucose intolerant (p0.005 1way ANOVA) and liver triglyceride levels (p<0.05 1way ANOVA). In contrast however, MyD88KO islets taken at 12wk HFD show a significant defect in GSIS (p<0.05 2way ANOVA). Peritoneal macrophages from 12wk HFD control mice, co-cultured with min6 cells, inhibited glucose stimulated insulin secretion (p<0.01 2way ANOVA), but this was not affected by 12wk HFD MyD88KO macrophages
Conclusion: Il-1 signalling in macrophages plays a key role in regulating glucose homeostasis in the obese mouse. Mice without macrophage Il-1 signalling are hyperinsulinemic which is consistent with an increase in whole body weight, increased fat deposition in the epididymal fat pad at 12wk, and increased liver triglyceride levels at 12wk. While these macrophages may appear beneficial to beta cells in an *ex vivo* setting, they are in fact detrimental when observed *in vivo* in the obese state.
Supported by: NHMRC
Disclosure: **P.T. Whitworth:** Grants; NHMRC.

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Clinical implication of CD163 on flow mediated dilatation in type 2 diabetes

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Background and aims: CD163 was identified as the endocytic receptor binding hemoglobin (Hb)- haptoglobin (Hp) complex and participates in haeme metabolism. Recent studies showed CD163 is expressed on macrophages as well as scavenger receptors and is associated with inflammation. Mononuclear cell linage, including macrophages and monocytes, are believed to be central immune cells for the development of atherosclerosis

through the vascular chronic inflammation. It is accumulating evidences that monocytes with CD163 are involved in the regulation of micro-inflammation process on atherosclerosis. Serum soluble form of CD163 (sCD163), which is shed by monocyte cell surface, has been reported to be elevated in obesity and insulin resistance, suggesting that shedding CD163 from mononuclear cells by disordered glucose metabolism may enhance atherosclerosis. Since, several studies on sCD163 have been made on atherosclerosis, little is known about the relationship between monocyte CD163 levels and atherosclerosis in type2 diabetes. The aim of this study is to examine the association of monocyte CD163 levels with the index of atherosclerosis.

Materials and methods: In total 160 type2 diabetes patients who were admitted to our university diabetes center for diabetes educational and/or complication check-up programs, were enrolled in this cross-sectional study (sex: 92 male, 68 female, age: 63.5 ± 11 years, duration:14 (7 - 20) years, BMI: 26 ± 5.4 kg/m^2). Patients with CKD stage 5, malignancy and acute inflammation were excluded. Circulating leukocytes were collected from the patients and labeled with fluorescently conjugated CD163 antibodies. Then, patient's leukocyte were analyzed by flow cytometry. (FACS Canto®, BD Bioscience, Japan). The population of monocyte was gated by forward scatter and side scatter and mean fluorescent intensity (MFI) of CD163 on monocyte were quantified. As an index of atherosclerosis, endothelium-dependent flow-mediated dilatation (FMD), which is widely used as assessing endothelial function is measured by high resolution ultrasound (UNEX EF 18G®, UNEX, Japan).

Results: Mean leukocyte number was 5905 ± 1767 /μL. Mean distribution of monocyte was 9.3 ± 3.6%. MFI levels of monocyte CD163 was 6702 ± 3218. Median of FMD was 5.9 (3.4 - 8.5)%. In simple regression analysis, FMD was correlated with systolic blood pressure (r=-0.170, p=0.044), serum creatinine (r=-0.260, p=0.002), leukocyte counts (r=-0.229, p=0.006) and monocyte CD163 levels (r=0.229, p=0.006). On multiple regression analysis including age, BMI, systolic blood pressure, FPG, HbA1c, LDL cholesterol levels, serum creatinine, leukocyte counts and monocyte CD163 levels as independent variables revealed that serum creatinine (β=-0.264, p=0.003), monocyte CD163 levels (β=0.203, p=0.020) were the significant independent contributors to FMD (R^2=0.168, p=0.004).

Conclusion: In conclusion, monocyte CD163 levels are associated with FMD in patients with type 2 diabetes. This results suggest monocyte CD163 plays protective role on developing atherosclerosis in type2 diabetes.

Disclosure: **R. Kawarabayashi:** None.

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Hepatic insulin clearance governs primary hyperinsulinaemia in pre-diabetes progression: a perverse paradox mediated by nitric oxide

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Background and aims: We have put forward the hypothesis that suppression of hepatic insulin clearance firstly determines primary hyperinsulinemia constituting an early event in prediabetes and subsequent development of type 2 diabetes. Hepatic insulin clearance determined primary hyperinsulinemia precedes and potentiates glucose dysmetabolism by fostering peripheral insulin resistance, which results in mild hyperglycemia and added insulin secretion, creating a vicious cycle towards diabetes. We propose that this results from compromised hepatic insulin clearance that is controlled by increased nitric oxide levels, in consequence of a subclinical inflammatory process, through inhibition of the insulin-degrading enzyme (IDE).

Materials and methods: Animal studies: Male Wistar rats and C57BL/6 mice were submitted to a high caloric diet. Postprandial glucose excursions, Insulin and C-peptide levels were evaluated after a Meal Tolerance Test (MTT). Blood samples were taken at time 2, 5, 10, 20, 30, 45, 60, 90 and 120 minutes. Insulin clearance was calculated by the molar ratio of C-peptide to insulin using the incremental area under the curve (trapezoid method). Expression of hepatic inducible nitric oxide synthase expression and nitric oxide levels were evaluated. Kupffer cells from the C57BL/6 mice were isolated and gene expression associated with inflammation was evaluated (IL1b, ARG1, TLR4, Myd88, Tgfb, TLR9). Kupffer cells and peritoneal macrophages were isolated, stimulated with LPS and nitric oxide levels were evaluated. Human studies: A cohort of 1100 individuals with normoglycemia or prediabetes was evaluated for insulin clearance through an oral glucose tolerance test and genetic variants at the IDE locus were accessed.

Results: High caloric diet, as its early effects, resulted in 56.6% reduction in insulin clearance, mainly due to increased plasma insulin levels, while the C-peptide levels remained unchanged. Hepatic iNOS expression was increased in both prandial states resulting in increased nitric oxide levels. ARG1 expression in isolated Kupffer cells was decreased suggesting higher availability of arginine for nitric oxide production in Kupffer cells. Moreover, we show that in vitro exposure of Kupffer cells to LPS leads to nitric oxide production. This corroborates our findings that decrease of insulin clearance in prediabetic individuals with impaired glucose tolerance is controlled by genetic variants at the IDE locus.

Conclusion: Together, these results suggest that high caloric diet results in activation of Kupffer cells and exhibit decreased ARG1 expression enabling a higher capacity of the Kupffer cells to produce nitric oxide. We propose that the nitric oxide produced by the inducible nitric oxide synthase act in a paracrine fashion to inhibit the IDE activity in hepatocytes resulting in decrease insulin clearance originating primary hyperinsulinemia, which might precede and potentiate glucose dysmetabolism by fostering insulin resistance.

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Disclosure: **M. Macedo:** None.

PS 044 Experimental treatments of type 2 diabetes

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Treatment with chemical chaperone 4-phenylbutyrate restores glucose metabolism in mice overexpressing human islet amyloid polypeptide

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Background and aims: Amyloid deposition in pancreatic β-cells is considered one of the main causes of β-cell loss and dysfunction in Type 2 Diabetes (T2D). Overexpression of human islet amyloid polypeptide (hIAPP), the main peptide of amyloid deposits, is associated with both impaired β-cell function and reduced β-cell mass as a result of endoplasmic reticulum (ER) stress-mediated apoptosis. We have previously shown that chemical chaperone 4-phenylbutyrate (PBA) relieves ER stress and ameliorates β-cell dysfunction in vitro. The aim of the present work is to determine whether in vivo administration of PBA is able to counteract hIAPP-induced β-cell dysfunction in hIAPP expressing transgenic mice.
Materials and methods: For in vivo studies, 8-week-old Wild Type (WT) and hIAPP transgenic (hIAPP-Tg) mice were treated for 12 weeks with 300 mg/mL of PBA dissolved in water. Glucose and insulin tolerance tests were performed before and after treatment. Insulin plasma levels were determined by ELISA. Global gene expression analysis of isolated islets and morphologic studies of pancreas were performed at sacrifice. PBA in vitro effect on hIAPP aggregation was monitored by thioflavin-T fluorescence assay and transmission electron microscopy (TEM). In silico analysis of PBA's binding capacity to hIAPP was performed using docking calculations, Molecular Dynamics (MD) simulations and Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) calculations.
Results: After 12 weeks of treatment, PBA administration in hIAPP-Tg mice was able to completely prevent the impaired glucose tolerance observed in the non-treated animals, decreasing the area under the curve of glucose, from 41354 ± 2257 to 23368 ± 3055 ($p<0.05$, arbitrary units). Insulin tolerance test showed no differences between WT and hIAPP-Tg mice regardless of PBA treatment. hIAPP-Tg mice presented an increase in β-cell area compared to WT, but PBA treatment was able to revert this phenotype. Moreover, global gene expression analysis revealed that PBA administration was able to ameliorate islet inflammation associated with hIAPP overexpression. mRNA expression levels were significantly reduced ($p<0.05$) in hIAPP-Tg islets after PBA treatment for Ccl2 (2.67 ± 0.06 vs. 0,53 ± 0.06, fold induction in hIAPP respect to WT), Lyz1 (11.16 ± 1.56 vs. 3.78 ± 0.97) and Txnip (6.96 ± 0.87 vs. 2.21 ± 0.48). In vitro thioflavin-T assay showed that treatment with growing concentrations of PBA prevented hIAPP fibril formation in a dose-dependent manner. Clusters of amyloid fibrils were visualized by TEM, which were much fewer in number and more dispersed when hIAPP was incubated with PBA. Furthermore, MD simulations and MM/GBSA calculations confirmed that PBA could bind not only to monomeric and dimeric structures, but also to pentameric fibrillar structures.
Conclusion: PBA administration is able to improve glucose tolerance in hIAPP-Tg mice and may play an important role in preventing β-cell dysfunction and amyloid formation. This approach demonstrates the feasibility of small chemical chaperones, such as PBA, as drug candidates for treating T2D.
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Disclosure: **S. de Pablo:** None.

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The protective effect of luseogliflozin on pancreatic beta cell mass

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Background and aims: Sodium glucose co-transporter 2 (SGLT2) inhibitors improve glucose tolerance by suppressing renal glucose reabsorption without direct pharmacological action on pancreatic beta cells. Although a number of studies have shown that SGLT2 inhibitors exert protective effects on pancreatic beta cells, the mechanism by which this occurs remains unknown. In the present study, we examined the effects of the SGLT2 inhibitor Luseogliflozin on pancreatic beta cell mass and gene expression in db/db mice.
Materials and methods: Six-week-old db/db mice were exposed to either standard chow (Control group) or standard chow containing 0.01% Luseogliflozin (Luseo group). After 4 weeks on the above diets, we examined body weight, blood glucose, visceral and subcutaneous fat, and liver weight. Oral glucose tolerance test (OGTT) and immunohistochemical analysis were used to assess beta cell mass and proliferation. Furthermore, we evaluated gene expression in isolated islets using microarray analysis.
Results: Body weight was higher and blood glucose levels lower in the Luseo group than in the Control group (Control vs Luseo body weight: 39.8 ± 0.9 g vs. 41.3 ± 0.4 g, respectively, $p < 0.05$; Control vs. Luseo fed blood glucose: 537.9 ± 28.3 mg/dL vs. 233.4 ± 20.9 mg/dL, respectively, $p < 0.01$). Although liver weight was significantly lower in the Luseo mice than in the Control mice, there were no differences in visceral or subcutaneous fat weight between the two groups. In addition, OGTT results showed that Luseo glucose tolerance was improved compared with Controls. Immunohistochemical analysis revealed a significant increase in beta cell mass and proliferation in Luseo mice compared with Control mice (Control vs. Luseo beta cell mass: 2.96 ± 0.34 mg vs. 6.56 ± 0.87 mg, respectively, $p < 0.01$. Proportion of Control vs. Luseo 5-bromo-2-deoxyuridine positive beta cells: 0.62 ± 0.16% vs. 1.94 ± 0.33%, respectively, $p < 0.01$). Microarray analysis revealed 358 genes to be differentially expressed by +/− 1.5 fold in Luseo vs Control islets by a two-tailed Fisher's exact test ($p < 0.05$). Among these, 142 were upregulated and 216 were downregulated. Top ranking gene ontology (GO) term analysis of upregulated Luseo genes included cell cycle (23%) and cell division (18%). Moreover, Luseo MafA expression was elevated relative to Controls, whereas Ngn3 expression was lower.
Conclusion: SGLT2 inhibitor Luseogliflozin improves glucose tolerance and maintaisns beta cell mass. Our results suggest that the protective effect of Luseogliflozin on beta cell mass mainly results from increasing beta cell proliferation.
Disclosure: **K. Takahashi:** Grants; Taisho Pharmaceutical Co., Ltd.

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Mechanism for anti-diabetic action of eicosapentaenoic acid in the high-fat diet-sensitive diabetes mouse model

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Background and aims: Eicosapentaenoic acid (EPA) exhibits therapeutic effects on metabolic disorders, such as atherosclerosis and dyslipidemia, through various mechanisms in multiple tissues. However, its anti-diabetic effect has not been elucidated. We recently established a mouse model of high-fat diet (HFD)-sensitive diabetes mellitus. We administered EPA to the mice and found that EPA supplementation significantly improved the hyperglycemia. In the present study, the mechanism of its anti-diabetic effects was examined.

Materials and methods: HFD-sensitive diabetes mice were generated by HFD feeding of InsrP1195L/+ mice (InsrP1195L/+/HFD mice) starting at 8 weeks of age. For EPA supplementation, EPA (5% wt/wt) mixed in HFD was given to InsrP1195L/+ and wild-type (WT) mice starting at the same age. The changes in metabolic parameters in vivo, gluconeogenesis in liver, and inflammatory response in white adipose tissue (WAT) were studied.
Results: Hyperglycemia in InsrP1195L/+/HFD mice was significantly improved by EPA supplementation. Glucose intolerance and severe insulin resistance in InsrP1195L/+/HFD mice was significantly ameliorated by EPA supplementation. While glucose-6-phosphatase (G6Pase) expression, the critical determinant of gluconeogenesis in liver, was paradoxically increased by re-feeding in InsrP1195L/+/HFD mice, this paradoxical response was not induced in EPA-supplemented InsrP1195L/+/HFD mice. Histological analysis of WAT revealed that HFD feeding significantly increased the number of crown like structure (CLS), the morphological indicator of chronic inflammation in WAT. However, their abundance was not different between InsrP1195L/+/HFD and WT/HFD mice, and was not changed by EPA supplementation. By contrast, EPA supplementation significantly reduced the cellular size of adipocytes in WAT in both InsrP1195L/+/HFD and WT/HFD mice. We measured serum levels of adiponectin, the anti-diabetic adipokine known to be decreased in obesity. However, although the adipocyte size of InsrP1195L/+/HFD mice was smaller than that of WT/HFD mice, serum adiponectin levels in the former were significantly lower than those in the latter. Intriguingly, EPA supplementation ameliorated hypoadiponectinemia along with a decrease in body weight in InsrP1195L/+/HFD mice, but not in WT/HFD mice. In addition, when the entire data from all animal groups (InsrP1195L/+ and WT mice irrespective of HFD and/or EPA treatment) were combined, there was an inverse correlation between blood glucose and serum adiponectin levels.
Conclusion: Our data suggests that InsrP1195L/+ mice are prone to hypoadiponectinemia in response to HFD-induced obesity and that EPA improved hyperglycemia of InsrP1195L/+/HFD mice, possibly through its action against hypoadiponectinemia. EPA supplementation might be a novel therapeutic option for a certain type of type 2 diabetes mellitus in human.
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Disclosure: **E. Lee:** None.

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Experiment study of renal denervation in treatment of canine with type 2 diabetes mellitus

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Background and aims: Recently, the efficacy of renal denervation (RDN) has been debated, not only for resistant hypertension, but also for insulin resistance and type 2 diabetes mellitus (T2DM). It is discussed whether RDN is able to adequately target the renal nerves and reduce sympathetic nerve activity and insulin resistance, and then improve glycaemic control. In this study, we sought to determine the effects of RDN on insulin resistance and glycaemic control in canines with T2DM.
Materials and methods: Ten beagles were randomly divided into a SHAM group (n = 5) and RDN group (n = 5). At 16-week after high-fat diet feeding, animals received one dose of streptozotocin (STZ) by intravenous injection and were fed a high-fat diet for an additional 4 weeks. Then RDN group underwent bilateral renal artery ablation while SHAM group just underwent bilateral renal arteriography. After that, beagles were fed a high-fat diet for another 12 weeks. Blood samples collected from renal arteries and veins as well as renal arteriography were performed on all animals at 20-week and 32-week to evaluate the effects of RDN on the levels of fasting glucose, fasting insulin, renal function and homeostasis model assessment-insulin resistance (HOMA-IR) as well as the pathological changes of renal arteries.
Results: High-fat diet feeding and STZ injection succeeded leading to canine models of T2DM (4.21±0.87 mmol/L vs 9.98±2.53 mmol/L for fasting glucose, $p < 0.001$; 2.69±0.53 mmol/L vs 18.43±3.62 mmol/L for fasting insulin, $p < 0.001$; 0.54±0.21 vs 8.74±2.02 for HOMA-IR, $p < 0.001$) at 20-week. Compared with SHAM group, fasting glucose, fasting insulin and HOMA-IR in RDN group had significantly decreased at 3 months post-surgery (5.02±0.98 mmol/L vs 7.64±1.01 mmol/L for fasting glucose, $p < 0.001$; 3.43±0.69 mmol/L vs 9.76±2.14 mmol/L for fasting insulin, $p < 0.001$; 0.79±0.32 vs 2.76±0.53 for HOMA-IR, $p < 0.001$), while in terms of renal function the two groups showed no statistical significance. Renal arteriography showed no renal artery stenosis or renal atrophy in both group during the follow-up. Histopathological analysis showed significantly peripheral sympathetic nerve damage in the renal arteries and no reinnervation of renal nerves at 3-month follow-up.
Conclusion: The results suggest that RDN could effectively remove peripheral renal sympathetic nerves and improve insulin resistance in canines with T2DM, thus facilitating glycaemic control in canines.
Disclosure: **T. Pan:** None.

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Salsalate improves metabolic disorders associated with metabolic syndrome in hereditary hypertriglyceridaemic rats

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Background and aims: There is a growing evidence that low-grade chronic inflammation plays an important role in pathogenesis of metabolic syndrome, type 2 diabetes and their organ complications and therefore it represents a potential subject for treatment. Recently, some small clinical studies have shown that salsalate, which is a nonacetylated form of salicylate, was effective in improving glycaemic control in patients with type 2 diabetes. However, the underlying mechanisms and other effects of salsalate are not completely established. In this study, we investigated the effect of salsalate treatment on glucose and lipid metabolism, liver steatosis, glutathione system, inflammatory parameters and dicarbonyl stress in an animal model of metabolic syndrome - a strain of non-obese hereditary hypertriglyceridaemic (HHTg) rats, which exhibits all symptoms of metabolic syndrome.
Materials and methods: Adult male HHTg rats were fed a standard diet with or without salsalate in a dose of 200 mg/kg of b.wt. for 6 weeks. The concentration of methylglyoxal and glutathione were determined using the HPLC-method. Gene expression was measured by quantitative RT-PCR. Tissue sensitivity to insulin action was measured ex vivo according to basal and insulin-stimulated ^{14}C-U-glucose incorporation into muscle glycogen or adipose tissue lipids.
Results: Salsalate treatment was associated with lowered visceral adipose tissue weight (-16%, p 0,01), reduced serum triglycerides (2.24±0.12 vs 5.56±0.24mmol/l, p 0.001), cholesterol (-18%, p 0.01), fasting glycaemia (6.53±0.1 vs 7.5±0.1mmol/l, p 0,001), MCP-1 (165±14 vs 231±26pg/ml, p 0.05) and IL-6 (9.7±0.9 vs 12.7±1.5pg/ml, p 0.05). Salsalate administration markedly reduced hepatic triglycerides (- 29%, $p<0.001$), cholesterol (-14%, $p<0.01$) and methyglyoxal (-38%, $p<0.01$). To the reduction of methylglyoxal can contribute increased relative expression of glyoxalase 1 ($p<0.001$), which participates in methylglyoxal degradation. In the liver, salsalate improved glutathione system (GSH/GSSG, $p<0.01$) which may ameliorate hepatic oxidative and dicarbonyl stress. Salsalate-treatment improved insulin sensitivity in white adipose tissue ($p<0.05$) and insulin stimulated incorporation of glucose into muscle glycogen ($p<0.05$).
Conclusion: These findings demonstrate important hypolipidemic, antidiabetogenic, antiinflammatory, and antioxidative effects of salsalate in the non-obese model of metabolic syndrome. These effects were associated with reduced dicarbonyl stress in the liver, and amelioration of

tissue insulin sensitivity. Our results contribute to understanding of the mechanism which in turn may explain the beneficial metabolic effects of salsalate in patients with type 2 diabetes.

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Antidiabetic action of esculentin-2CHa-GA30 and its synthetic analogues [D-Arg7, D-Lys15, D-Lys23] - and [Lys15-Octanoate]-esculentin-2CHa-GA30 in high fat fed mice

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Background and aims: We previously demonstrated that modified analogues of esculentin-2CHa-GA30 (GFSSIFRGVAKFASKGLGKDLAK LGVDLVA) (peptide 2) exhibited impressive characteristics in terms of enzymatic stability, insulinotropic effects, cellular mechanisms of action and acute antihyperglycaemic effects in vivo. The present study investigated the long-term actions of esculentin-2CHa-GA30 and its synthetic analogues [D-Arg7, D-Lys15, D-Lys23] - (peptide 7) and [Lys15-Octanoate]-esculentin-2Cha-GA30 (peptide 10) in obese, insulin resistant, glucose intolerant high fat fed mice.

Materials and methods: Male mice fed a high-fat diet for 3 months were treated with saline or esculentin-2CHa-GA30 or its synthetic analogues [D-Arg7, D-Lys15, D-Lys23] - and [Lys15-Octanoate]-esculentin-2CHa-GA30 (75nmol/kg body weight, i.p.) for 28 days. A further group similarly received exendin-4 (25nmol/kg). Body weight, food intake, glucose, insulin, glucose tolerance (18mmol/kg i.p), insulin sensitivity (25units/kg), body composition, plasma lipid profile were examined. Immunohistochemical analysis was performed to assess changes in islet morphology and both beta cell proliferation and apoptosis. Collagenase isolated islets were used to assess insulin secretory responses to known secretagogues in acute tests.

Results: Administration of peptides 2, 7 and 10 over 28 days significantly reduced body weight ($p<0.01$, $p< 0.001$), without appreciably altering cumulative energy intake. All peptides reduced blood glucose levels by 6-12mmol/l ($p<0.01$) with significance evident from day 6. This was associated with appreciably lower plasma insulin levels from day 6 ($p<0.01$). All peptides improved glucose tolerance, insulin response to glucose, insulin sensitivity and blood glucose profile over 24 h ($P<0.01$) and resulted in a 2% drop of HbA1C similar to exendin-4 ($P<0.01$). Peptides also reduced high fat diet induced increases in plasma GLP-1 and glucagon ($P<0.05$). None of the peptides tested altered bone mineral density/content or lean mass but decreased fat mass. Islets isolated from peptide-treated mice exhibited improved insulin secretory responses ($P<0.05$) to 20mmol/l glucose, 10mmol/l alanine and 10-6mol/l GLP-1 compared to high fat controls. Islet morphometric analyses revealed that Exendin-4 and peptides 2, 7 and 10 significantly reduced ($p<0.001$) islet, beta and alpha cell areas compared to high fat controls. High fat diet or peptide treatment was not associated with islet lymphocyte infiltration, confirming that peptides did not induce insulitis. High fat diet significantly increased ($p<0.001$) beta cell proliferation and apoptosis, which was markedly reduced by long term 28 day administration of peptides 2, 7 and 10 ($p<0.05$-$p<0.001$). High fat diet significantly increased LDL cholesterol which was reduced ($p<0.01$) by the acylated analogue (peptide 10). High fat diet or peptide treatments did not alter plasma amylase activity and plasma ALT, AST and ALP levels, indicating a lack of toxicity.

Conclusion: These data indicate that esculentin-2CHa-GA30 and its analogues may be useful for improvement of blood glucose control and weight loss in type 2 diabetes.

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The dipeptidyl peptidase-4 inhibitor linagliptin exerts neurotrophic effects in the olfactory cortex of type 2 diabetic rats

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Background and aims: Type 2 Diabetes (T2D) is strongly associated with decreased cognitive function and Alzheimer's disease. T2D patients also often present with olfactory dysfunction, which is an early symptom of neurodegenerative disease. Therefore, it is important to determine the potential causal relationship between impaired olfaction and neurodegenerative disease in T2D patients and, if it exists, to determine whether olfactory problems can be treated pharmacologically. Our group has previously shown that T2D induces specific interneuronal pathology in the rat piriform cortex, which is the main brain area responsible for the identification of odours. The aim of this study was to determine the potential efficacy of the dipeptidyl peptidase-4 inhibitor (DPP-4i) linagliptin to counteract T2D-induced interneuronal pathology in the piriform cortex of T2D rats. Linagliptin is a well-tolerated and efficacious treatment for T2D that has also shown neuroprotective properties in preclinical studies.

Materials and methods: Five-month-old T2D Goto-Kakizaki (GK) rats were treated with vehicle (n=8) or linagliptin [(n=8), included in food mixture (83 mg/kg food; approx. 5 mg/kg body weight)] for 4 weeks before sacrifice. To determine the potential efficacy of linagliptin, we quantified the total number of NeuN-positive neurons (NeuN is a specific marker of mature neurons) as well as of interneurons positive for Calbindin-D28k (a marker for a subpopulation of GABAergic interneurons), using immunohistochemistry and stereology methods. We also assessed neurite arborisation (i.e., number of neurites leaving the neuronal body). Data were analysed using an unpaired two-tailed t-test and expressed as mean ± SEM.

Results: The results showed no significant difference in the total number of NeuN-positive neurons between the linagliptin-treated rats and the control animals (p=0.8). However, we observed a significant 45% increase in the number (1849 ± 124 vs 1278 ± 86; p=0.002) and 14% increase in the size (1567 ± 56 μm^3 vs 1373 ± 49 μm^3; p=0.02) of Calbindin-D28k-positive interneurons in linagliptin-treated rats compared with control animals. In addition, linagliptin increased neurite arborisation of the Calbindin-D28k-positive interneurons compared with control animals (0.4 ± 0.06 vs 0.1 ± 0.04; p=0.001).

Conclusion: GABAergic inhibitory interneurons play a crucial role in the functioning of the central nervous system (CNS), and their impairment has been associated with some neurological disorders. We recently showed that decreased neurite arborisation and downregulation of Calbindin-D28k expression in the interneurons present in the piriform cortex of GK rats may be the impaired basic mechanisms responsible for olfactory dysfunction in T2D. The results of this study show that linagliptin can strongly normalise the pathological effects of T2D in the piriform cortex, suggesting a potential role for DPP-4i in preventing T2D-induced complications in the CNS, based on the normalisation of olfactory functions.

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Linagliptin partially reverses striatal neuropathology in type 2 diabetic mice

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Background and aims: Type 2 diabetes (T2D) is associated with Alzheimer's disease (AD). However, T2D patients present a broad range of cognitive/sensorimotor problems which do not progress towards AD and do not necessarily involve the same brain areas as in AD. The pathogenic mechanisms at the basis of these central nervous system (CNS) impairments need to be identified. Studies have shown that impairment of fast-spiking GABAergic interneurons positive for parvalbumin (PV) is involved in the development of brain diseases. However, the potential role in T2D-induced CNS complications is unknown. Dipeptidyl peptidase-4 inhibitors (DPP-4i), which are in clinical use for the treatment of T2D, have shown neuroprotective effects in preclinical studies. However, their potential efficacy in preventing T2D-induced CNS complications has not been studied thoroughly. We aimed to determine whether: 1) T2D affects PV-positive interneurons in the striatum (an important brain area involved in sensorimotor functions); 2) Chronic treatment with the DPP-4i linagliptin prevents the development of striatal pathology.
Materials and methods: In Study 1, we analysed 2-month-old young adult C57BL/6 mice (n=10) and middle-aged C57BL/6 mice receiving standard (n=6) or high-fat diet (HFD; n=7) for 48 weeks. We quantified the number of cells (per 100,000 μm^2) and the average cell volume of PV-immunoreactive (-ir) neurons in the striatum. We also assessed potential signs of gliosis and inflammation by counting glial fibrillary acidic protein (GFAP)-ir astrocytes and measuring ionised calcium binding adaptor molecule 1 (Iba-1)-ir microglia cell volume, respectively. In Study 2, young adult C57BL/6 mice received HFD for 36 weeks before being treated with vehicle (n=10) or linagliptin (5 mg/kg b.w./day; n=8) for 12 weeks. The same assessments as in Study 1 were performed. Data were analysed using one-way ANOVA (Study 1) or unpaired t-test (Study 2), and expressed as mean ± SEM.
Results: Aging induced a significant decrease in the number (6.646 ± 0.297 vs 3.431 ± 0.537; p<0.0001) and volume (1182 ± 27.0 μm^3 vs 733.6 ± 55.7 μm^3; p<0.0001) of PV-ir interneurons. Interestingly, HFD-treated mice showed a further 51% decrease in PV-ir cells versus their age-matched controls (1.737 ± 0.156 vs 3.431 ± 0.537; p<0.01). Aging also increased the number of GFAP-ir cells (28.66 ± 2.29 vs 61.02 ± 4.59; p<0.001) and volume of Iba-1-ir cells (174.1 ± 12.7 μm^3 vs 274.3 ± 12.8 μm^3; p<0.0001), with no effect of HFD. Interestingly, linagliptin partially reversed the HFD-induced effect on PV-ir interneurons (cell number: 14.51 ± 0.63 vs 18.63 ± 1.01; p=0.0023), but also the gliotic (73.15 ± 4.03 vs 53.89 ± 3.80; p=0.0036) and inflammatory (246.1 ± 7.3 vs 217.0 ± 8.8; p=0.0210) effects induced by aging.
Conclusion: We show that T2D significantly accelerates the aging-induced decrease of PV-ir interneurons in striatum. The impairment of these striatal cells may be one of the pathogenic mechanisms at the basis of decreased sensorimotor functions in T2D. We show that linagliptin exerts pleiotropic normalising effects against the impact of T2D and aging. Taken together, these results may have implications for the identification of T2D-induced pathogenic mechanisms in the brain as well as for the development of suitable treatment strategies.
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PS 045 Prediction of outcome after bariatric surgery

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Genetic and circulating predictors of weight loss after bariatric surgery in severely obese individuals

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Background and aims: Rouen-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are the most frequently performed bariatric surgery procedures. However, several patients encompass a lack of weight loss or a substantial weight regain. As these events are not related to complications of the surgical procedure, it is important to find markers able to identify "well-responders" patients and to predict the amount of weight loss after surgery. Ghrelin regulates appetite and energy balance. Common polymorphisms in its encoding genes (receptor expression or peptide release) have been associated with body weight regulation. Other peptides involved in satiety modulation, like the CD40/CD40L complex, have been less explored.
Materials and methods: One hundred severely obese (mean age 45.2±11.4 years, 65 females, BMI 47.1±7.0 Kg/m2) otherwise healthy candidates for bariatric surgery (RYGB, n=74 and SG, n=26), were sequentially enrolled in 2013-2014. SNP rs696217 (in the coding region of the preproghrelin gene), rs2241766 (the most common SNP of the adiponectin gene) and rs1126535 (regulator of sCD40L synthesis) were determined on DNA extracted from circulating lymphomonocytes; metabolic profile was assessed by routine methods and serum levels of ghrelin, adiponectin and sCD40L were measured by ELISA. Patients were followed after surgery, evaluating body weight at 6, 26 and 52 weeks after the procedure.
Results: At baseline, mean BMI of subjects carrying the T allele of the ghrelin gene did not differ from that of non-carriers (G-G: 47.3 ±7.2, G-T: 46.7±7.1 Kg/m2; p=ns); however, T subjects showed a significantly greater reduction in BMI 52 weeks after surgery (G-G: -30.7±9.3% vs G-T: -38.6±6.1%; p<0.001). This was associated with a significant difference in their BMI at 52 week follow up (G-G: 32.6 ±5.6, G-T: 28.5 ±4.3 Kg/m2; p=0.021), while difference was not significant at 6 (G-G: 41.6 ±6.5, G-T: 39.8±6.0 Kg/m2) and 26 weeks (G-G: 36.3 ±5.8, G-T: 34.5±5.4 Kg/m2). In the whole study group, baseline plasma ghrelin levels inversely correlated with baseline BMI (R: -0.215, p=0.04) and post-surgery BMI (R: -0.360, p=0.02), and directly with percent of weight loss at 1 year (R: 0.261, p=0.04). To carry the C allele of the rs1126535 in the sCD40L gene, even in the absence of difference in BMI at baseline, was associated with a significantly lower BMI reduction at week 52 (T-T: -33.6±9.5% vs C-T: -27.2±6.9%; p<0.03); as a consequence, at follow-up BMI differed (T-T: 30.7±5.2, C-T: 35.9 ±5.6 Kg/m2; p=0.003). 52 weeks after surgery, no difference in weight loss was found among patients carrying the G allele for adiponectin gene (T-T: -32.5±8.76% vs G-T: -32.0±10.35%; p=ns).
Conclusion: Carrying a G to T substitution at position 72 in exon 2 of the preproghrelin gene seems to predict a successful weight loss after bariatric surgery in morbidly obese subjects; we also report for the first time as the C allele (rs1126535 in the sCD40L gene) may predict a worse response to bariatric surgery.
Disclosure: **E. Vitolo:** None.

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Prebariatric screening for diabetes and cardiovascular risk in an interdisciplinary obesity center discloses unexpected gender differences

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Background and aims: Type 2 diabetes prevalence is higher in men, but diabetic women lose more years of life. Whereas obesity prevalence is only slightly higher, the number of bariatric operations performed is much higher in women. We therefore performed a gender-specific evaluation of both systematic diabetes screening and atherosclerotic cardiovascular risk assessment in an obese cohort qualifying for bariatric surgery.
Materials and methods: 300 consecutive patients (66% women) who qualified for bariatric surgery according to current guidelines and a local modification of the Edmonton obesity staging system (EOSS) were analyzed. In addition to HbA1c and fasting plasma glucose (FPG), a 75 g oral glucose tolerance test was performed in all non-diabetic subjects. In patients with known or newly diagnosed diabetes, 10-year atherosclerotic cardiovascular disease risk was assessed using established tools (ASCVD, UKPDS-risk score, ARRIBA).
Results: [mean±SEM; f vs. m] Among the 131 (72f/59m) patients with known or newly diagnosed diabetes, age (50.0±1.5 vs. 49.5 ±1.8 y), BMI (47.7±1.5 vs. 47.7±1.6 kg/m2) and diabetes duration (7.1±1.3 vs. 8.2±1.3 y) were comparable between men and women. In contrast, there was a pronounced gender difference in the prevalence of known diabetes (27.9 vs. 49.5%), rate of newly diagnosed diabetes cases (21 vs.10%) and HbA1c (7.39±0.3 vs. 7.91 ±0.4%; p=0.01). Clinical atherosclerosis was 3.1 times more prevalent (9.8 vs. 30.6%) and atherosclerotic cardiovascular 10-year risk was significantly higher (factor 2.5/2.7/3.6 - ASCVD/UKPDS/ARRIBA) in the male cohort.
Conclusion: Systematic prebariatric screening discloses both higher than expected diabetes prevalence in men and higher than expected numbers of newly diagnosed diabetes cases in women. The notion, that diabetes would neutralize the apparent gender difference in atherosclerotic cardiovascular disease is apparently inapplicable in the context of very high BMI. In fact, obese diabetic men have three times more clinical atherosclerosis and three times higher 10-year atherosclerotic cardiovascular disease risk, than women. In contrast to widespread perception, female preponderance among qualifyers for bariatric surgery may be a misallocation of resources and a waste of bariatric risk reduction potential. Obese diabetic men may therefore deserve more selective attention. ASCVD, atherosclerotic cardiovascular disease. ARRIBA, absolute and relative risk reduction in general practice

	women (n=72)	men (n=59)	p (2-sided t-test)
Age [years]	50.1 (1.5)	49.6 (1.8)	0.95
BMI [kg/m²]	47.4 (1.5)	47.6 (1.7)	0.78
Diabetes duration [years]	7.1 (1.3)	8.1 (1.3)	0.49
HbA1c [%]	7.39 (0.3)	7.91 (0.4)	0.01
Clinical atherosclerotic vascular disease [%]	9.8	30.6	<0.05
ARRIBA	3.9 (0.7)	14.3 (3.6)	<0.05
ASCVD	8.7 (1.8)	21.6 (2.6)	<0.05
UKPDS	7.4 (1.1)	19.9 (2.4)	<0.05

131 obese subjects with type 2 diabetes (prevalent or newly diagnosed).
Clinical characteristics, clinical atheroslerosis prevalence and estimated atherosclerotic cardiovascular 10-year risk (±SEM).
Estimated atherosclerotic cardiovascular 10-year risk [% (±SEM)]

Disclosure: **K. Rett:** None.

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Comparable weight loss and improvement in insulin sensitivity early after Roux-en-Y gastric bypass or sleeve gastrectomy for morbid obesity

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Background and aims: Bariatric surgery leads not only to substantial and sustained weight loss, but also to improvement in glycemia. Whether this last phenomenon is a result only of improvement in insulin sensitivity because of weight reduction, or a function of additional specific aspects of each surgical modality is a matter of research. The aim of the present study was to compare the effect of Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) on weight loss, insulin sensitivity, as well as postprandial glycemia and insulinemia.
Materials and methods: Twelve morbidly obese patients undergoing RYGB and 15 undergoing SG, matched for age (37.3 ± 8.1 vs 40.7 ± 8.3 years, p=NS) and BMI (48.7 ± 6.1 vs 50.8 ± 7.2 kg/m2, p=NS) were consecutively recruited. They were examined preoperatively, as well as 3 and 6 months postoperatively. In each session, all subjects consumed a 450 kcal standard test meal, and serum samples were taken at baseline and every 30 minutes up to 3 hours after meal consumption for the measurement of glucose and insulin. Insulin resistance was assessed using the HOMA-IR index (Glucose*Insulin/405). Postprandial insulin and glucose responses were calculated as area under the curve (AUC) using the trapezoid rule. Differences between preoperative and postoperative values were assessed as percentage change (Δ%).
Results: At baseline, both groups were comparably hyperinsulinemic (fasting insulin RYBG: 29.3 ± 21.5 vs SG: 26.2 ± 12.4 mU/l, p=NS) and insulin resistant (HOMA-IR RYGB: 6.7 ± 5.6 vs SG: 8.4 ± 6.6, p=NS). Both experienced significant and comparable weight loss at 3 (ΔBMI% RYGB: -19.0 ± 3.9% vs SG: -17.8 ± 5.1%, p=NS), and 6 months (ΔBMI% RYGB: -27.2 ± 4.6% vs SG: -25.9 ± 5.1%, p=NS). Glucose and insulin postprandial AUC decreased similarly at 3 (ΔGlucose AUC% RYGB: -8.6 ± 12.3% vs SG: -13.8 ± 20.8%, p=NS, and ΔInsulin AUC% RYGB: -51.5 ± 13.2% vs SG: -39.2 ± 28.3%, p=NS) and 6 months (ΔGlucose AUC% RYGB: -12.8 ± 12.9% vs SG: -21.4 ± 17.4%, p=NS, and ΔInsulin AUC% RYGB: -57.1 ± 18.1% vs SG: -54.3 ± 18.3%, p=NS). Moreover, the improvement in fasting insulin and HOMA-IR did not differ between groups at 3 (ΔInsulin% RYGB: -52.1 ± 23.3% vs SG: -54.9 ± 15.5%, p=NS, and ΔHOMA-IR% RYGB: -53.1 ± 32.1% vs SG: -62.1 ± 19.0%, p=NS), and 6 months postoperatively (ΔInsulin% RYGB: -57.1 ± 18.1% vs SG: -54.3 ± 18.3%, p=NS, and ΔHOMA-IR% RYGB: -60.2 ± 20.8% vs SG: -64.6 ± 19.0%, p=NS).
Conclusion: Both Roux-en-Y gastric bypass and sleeve gastrectomy lead to substantial decreases in body weight, postprandial glycemia, insulinemia and insulin resistance. The main drive behind these metabolic improvements seems to be the amount of weight loss per se, and not the differences between the two surgical modalities.
Disclosure: **A. Kokkinos:** None.

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Type 2 diabetes and laparoscopic sleeve gastrectomy: long-term follow up, review of the criteria for diabetes remission and relationship with weight "regain"

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Background and aims: Sleeve Gastrectomy (SG) has demonstrated to be effective for weight loss and glucose control in obese and diabetic

patients, over a short follow-up time. Actually, data on long term follow up after this bariatric procedure are missing. Aims of the study are to evaluate the long-term effects of SG on obesity and Type 2 Diabetes (T2D) 7 years after surgery, re-evaluate the criteria for T2DM remission, study the incidence of weight regain and his relationship with T2DM.

Materials and methods: 195 obese patients (43M) aged 43.9±10.6 years, 78 with T2DM, underwent SG and followed for 7 years. The patients in complete remission from T2DM (fasting blood glucose FBG<100 mg/dl and HbA1c<6%) and partial remission (FBG<110 mg/ dl and HbA1c<6.5%) according to international guidelines underwent Oral Glucose Tolerance Test (2h OGTT with BG determination every 30'). Regain was defined as weight recovered (in%) after reaching the minimum weight.

Results: Before surgery body weight and BMI were 123±21Kg and 44.6±6.8Kg/m^2 respectively; seven years after surgery 104.9±18. Kg and 37±6 kg/m^2. Minimum weight and BMI (79.2±16.1 Kg, BMI 28.6±5.3 kg/m^2) were reached after two years, when a modest regain (22±6.7%) started in 47% of patients operated. The highest incidence of weight regain occurred in patients with T2D before SG (52% of patients with diabetes vs 37% of non-diabetics). The T2D resolution was similar between the group without regain (56%) and the regain group (60%); no recurrence of T2D in no regain group, one in regain group; no new diagnosis of T2D. Partial and complete resolution of T2D according to guidelines was observed in 72% (56/78) of patients. However, using OGTT only 40% had normal glycaemic curve, 46% showed alterations (IGT or BG>200 mg/dl at the intermediate times) and 14% showed overt diabetes. All patients with abnormal OGTT had HbA1c>5.5%.

Conclusion: SG leads to weight loss comparable to other bariatric procedures. After 24 months of follow-up less than half of patients has a modest regain, far from returning to preoperative levels, more pronounced in diabetic patients. The resolution of T2D according to international guidelines is not always confirmed by OGTT which is therefore indicated in cured patients with HbA1c>5.5%.

Disclosure: **D. Capoccia:** None.

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Prediction of diabetes remission after gastric bypass, a direct study

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Background and aims: Roux-en-Y gastric bypass (RYGB) leads to a high Type 2 diabetes (T2D) remission rate but the results are heterogeneous. We systematically compare previously published models for prediction of diabetes remission to identify the best model and build, based on the proposed parameters, an optimized model. Finally, our results are integrated in a simple tool that can be used to identify patients who can safely stop all anti-diabetic medications immediately after RYGB.

Materials and methods: Glycemic control was assessed in 170 consecutive T2D patients undergoing RYGB at baseline, after 3 month (M3) and 12 month (M12). The parameters used in nine logistic regression approaches and the models that are published for predicting T2D remission after RY were systematically compared. We then applied stepwise forward selection to identify from all baseline parameters that have been used in these published models a model that includes few variables but warrants high accuracy. This new model was validated in an independent cohort of 80 patients with RYGB.

Results: M3 remission was observed in 67 patients (39%) and persisted at M12 in 66 (96%). M3 remission was accurately predicted by 8 out of 9 published models (AUC=0.76-0.88). Stepwise forward selection for M3 & M12 remission revealed a model including HbA1c, fasting C-Peptide, insulin treatment and number of antidiabetic treatments (excluding insulin) (0/1/>1). The AUC of this model (0.89 [0.89-0.9]) was even better than the full model including all parameters (0.87 [0.86-0.88]). The model predicted in the replication cohort diabetes remission with a false positive rate of 15.8% and a false negative rate of 16.7%. The false positive turned out to be in majority patients on metformin with an HbA1c below 6.0%.

Conclusion: Guidelines for antidiabetic drug withdrawal after RYGB are missing. Based on only four preoperative parameters, the proposed model can identify T2D patients that will be in remission at 3 and 12 months and who can safely stop all antidiabetic drugs.

Clinical Trial Registration Number: NCT01129297
Supported by: IMI DIRECT

Disclosure: **H. Gassenhuber:** None.

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Changes in skeletal muscle oxidative capacity after bariatric surgery are driven by changes in free fatty acids, baseline BMI and type 2 diabetes status

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Background and aims: Bariatric surgery has been shown to improve muscle insulin sensitivity, but the time course of improvement, the underlying mechanism and the impact of type of surgery remain unclear.

Materials and methods: Obese patients (OBE, n=36, age 40±11yrs, BMI 51±7 kg/m^2) underwent hyperinsulinemic-euglycemic clamps to assess peripheral insulin sensitivity (M-value) and muscle biopsies to determine mitochondrial capacity by high resolution respirometry. Measurements were performed before, 2, 12, 24 and 52 weeks after gastric sleeve (GS) or gastric bypass (GB) surgery. Covariance pattern models were used for statistical analysis. Normal-weight humans were studied at baseline (CON; n=7, age 38±4 yrs, BMI 24.9±1.1 kg/m^2).

Results: OBE had lower M-values at baseline (2.7±1.6 vs 7.9±2.2 mg*kg^{-1}*min^{-1} in CON, p<0.001), which started to improve already at 3 months after surgery (Effect Estimate, EE 0.94 mg*kg^{-1}*min^{-1} CI[0.41;1.47] at 3 months vs baseline; EE 1.95 mg*kg^{-1}*min^{-1} CI[1.25;2.65] at 6 months vs baseline; EE 3.35 mg*kg^{-1}*min^{-1} CI[2.58;4.11] at 12 months vs baseline; p<0.01). Surprisingly, maximal uncoupled respiration decreased by 17% at 2 weeks, paralleled by reduction in respiratory control ratio and increased circulating free fatty acids (FFA) and only returned to baseline at 6 and 12 months, independent of changes in M-value. Time course of changes in mitochondrial respiration was influenced by BMI and presence of type 2 diabetes at baseline (p=0.02 and p=0.001, respectively). Of note, type of surgery neither affected the time course of changes in M-value (p=0.51) nor in mitochondrial function (p=0.4).

Conclusion: Bariatric surgery leads to a transient decrease in muscle mitochondrial oxidative capacity and efficiency probably due to uncoupling effects of lipotoxicity. Body mass and glucometabolic control but not insulin sensitivity, may determine the changes in mitochondrial respiration over one year after both bariatric procedures.

Clinical Trial Registration Number: NCT01477957

Disclosure: **S. Gancheva:** None.

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Corneal confocal microscopy detects an improvement in small fibre neuropathy after bariatric surgery in obese subjects with type 2 diabetes

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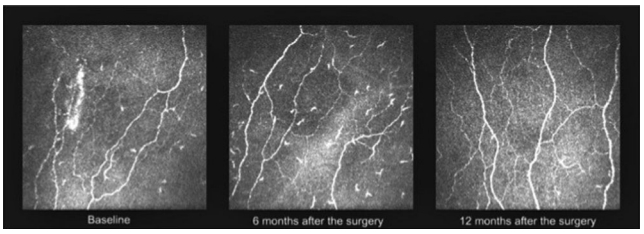
Background and aims: Bariatric surgery can rapidly improve metabolic risk factors and cure Type 2 Diabetes Mellitus (T2DM) in obese subjects. However, it has shown limited benefits on microvascular complications, in particular neuropathy.

Materials and methods: 32 morbidly obese subjects underwent detailed assessment of small and large fibre neuropathy at baseline and 12 months after bariatric surgery.

Results: Post-bariatric surgery, there was a significant improvement in BMI (52.5±1.9 v 35.9±1.7, P<0.0001), HbA1c (50.7±3.1 v 36.7±1.1, P<0.0001), Systolic BP (133.9±3.7 v 123.2±3.0, P=0.002) with no change in other metabolic factors. There was a significant improvement in the Neuropathy Symptom Profile (5.2±1.1 v 0.7±0.2, P<0.0001), Neuropathy Disability Score (2.2±0.5 v 0.9±0.5, P=0.006), Corneal nerve fibre density (CNFD) (23.0±1.5 v 26.5±1.4, P=0.03), fibre length (CNFL) (14.2±0.6 v 15.8±0.8, P=0.04) and branch density (CNBD) (31.6±3.7 v 39.0±3.3, P=0.04) with no change in cold (23.5±1.5 v 24.4±1.1, p=0.9) and warm (40.1±0.7 v 41.3±0.9, p=0.1) perception thresholds, sural nerve conduction velocity (46.2±3.6 v 46.2±1.7, p=0.7), amplitude (8.7±3.4 v 8.8±1.8, p=0.7) and peroneal nerve conduction velocity (44.1±1.2 v 45.9 ±0.8, p=0.2) and amplitude (3.1±0.7 v 3.3±0.6, p=0.3). Obese subjects were further divided into those with (n=19) and without (n=13) T2DM and had comparable CNFD (23.1±2.2 v 22.8 1.6, P=0.6), CNBD (35.9 ±5.2 v 24.4 ± 3.9, P=0.8) and CNFL (14.4 ± 0.9 v 13.9±0.8, P=0.6) at baseline. Whilst there was a significant improvement in CNFD (23.1±1.0 v 28.0±1.8, P=0.03), CNBD (35.9±5.2 v 45.5±3.9, P=0.04) and CNFL (14.4±0.9 v 16.9±1.1, P=0.007) in those with T2DM, there was no change in those without DM.

Conclusion: Obese subjects with and without T2DM have a comparable small fibre neuropathy (SFN). Bariatric surgery results in a significant improvement in small but not large fibre neuropathy in patients with T2DM. Corneal confocal microscopy detects early small nerve fibre repair following bariatric surgery.



Disclosure: **S. Azmi:** None.

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Correlation between food-evoked signal change in the dorsolateral frontal cortex and ad libitum food consumption: a human neuroimaging study

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Background and aims: Mechanisms causing weight loss after Roux-en-Y gastric bypass (RYGB) are unclear. Modulation of inhibitory control of food intake may contribute. Aim: To explore correlations between brain responses to food ingestion and ad libitum food intake in normal weight (NW), obese (Ob) and post-RYGB people.

Materials and methods: Twelve NW (age 32.3±9.3 years, BMI 22.3±1.4 kg/m^2), 21 Ob (age 31.1±10.5 years, BMI 34.1±2.6 kg/m^2) and 9 RYGB (age 45.1±10.7 years, BMI 34.0±3.3 kg/m^2) subjects underwent [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET) neuroimaging twice after overnight fasting: once FED (400 kcal mixed meal before scanning), once FASTED (water only) in random order. Subjects underwent an ad libitum meal after scanning. Brain FDG uptake, a marker of neuronal activation, was compared using Statistical Parametric Mapping. In clusters showing an interaction between fed state and group (voxel level p<0.01, cluster size threshold 100 voxels), post hoc Spearman's correlational analyses between food-evoked FDG signal change (FESC, FED minus FASTED) and ad libitum consumption at the FASTED visit were performed.

Results: There were 10 clusters where the response to food ingestion was different between groups (previously reported). Correlations within each group between FESC in each cluster and ad libitum consumption are shown in the table. Mean±SD FESCs in cluster C were: NW -4.02 ±2.48%, Ob -0.11±1.22%, RYGB -4.18±3.07%.

Conclusion: The highly significant, strong, positive correlation in NW between FESC in cluster C (DLFC) and ad libitum consumption, with greater deactivation associated with lower ad libitum consumption, supports an association between DLFC deactivation (in this FDG-PET neuroimaging paradigm) and increased inhibitory control of food intake. There were no such correlations in Ob or RYGB. The mean FESCs in cluster C are consistent with low inhibitory control of food intake in the obese which is increased after RYGB and suggest altered DLFC activity after RYGB may contribute to weight loss.

Cluster		Cluster size (voxels)	NW		Ob		RYGB	
			r_s	p	r_s	p	r_s	p
A	Anterior dorsolateral frontal cortex (DLFC), medial frontal cortex, medial orbital cortex (R)	686	0.548	0.065	0.213	0.354	-0.335	0.379
B	Medial orbital cortex (L)	119	0.387	0.201	0.008	0.973	-0.008	0.983
C	Anterior and posterior DLFC (R)	1036	0.910	<0.001 ***	-0.078	0.737	0.126	0.748
D	Lateral orbital cortex, anterior and posterior DLFC, frontal operculum, insula (R)	310	0.137	0.671	0.182	0.429	0.059	0.881
E	Hypothalamus	154	-0.699	0.011*	-0.239	0.298	-0.176	0.651
F	Pituitary	130	-0.541	0.069	-0.080	0.729	-0.025	0.949
G	Posterior cingulate, precuneus (R & L), cuneus (R)	1692	0.144	0.655	0.188	0.414	-0.100	0.797
H	Angular gyrus, superior & middle temporal gyri posterior, occipital pole (R)	1103	0.534	0.074	0.530	0.014*	-0.285	0.458
I	Angular gyrus, occipital pole, parietal lobule (L)	813	0.478	0.116	0.544	0.011*	-0.008	0.983
J	Lingual gyrus (L)	163	-0.179	0.577	-0.225	0.327	-0.251	0.515

Supported by: The Diabetes Foundation, UK
Disclosure: **K.F. Hunt:** None.

PS 046 Adipose growing and hypoxia

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Small Proliferative Adipocytes (SPA): a newly identified adipocyte progenitor expressing adipocyte specific proteins

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Background and aims: Adipocyte differentiation has been intensively studied in cultured cells, however its differentiation in vivo has not been fully understood. We identified a novel adipocyte population in adipose tissue, which exhibited a proliferating activity and expressed adipocyte specific proteins. We named them small proliferative adipocytes (SPA). In this study, we further investigated the characteristics of SPA.
Materials and methods: Mouse epididymal adipose tissue was digested by collagenase, and centrifuged at 9 ×g for 1 sec. Cells in the sedimentary fraction were regarded as stromal vascular cells (SVC). Floating cell fraction was separated and further centrifuged at 226 ×g for 3 min. The resultant sedimentary cells were regarded as half-floating cells, while the floating cells as mature adipocytes (MA). Since half-floating cells were small but expressed adiponectin and leptin, they were considered as SPA. We studied the gene expression and their ability to differentiate into MA.
Results: Incubation of SPA with differentiation medium containing insulin, dexamethasone and IBMX for 2 days converted them to small round cells containing lipid droplets. In contrast, very few lipid droplets ware detected in SVC cultured in the differentiation medium. Furthermore, incubation of SPA but not SVC with 10 μM pioglitazone caused accumulation of lipid. These results indicate that SPA are able to differentiate into MA more easily than SVC. Incorporation of EdU, an analog of thymidine, was detected in SPA, however it was not detected in lipid-laden SPA appeared after the induction of differentiation. Next, to compare the gene expression among SVC, SPA and MA, microarrays were performed. Expression of several adipocyte-specific genes including adiponectin, fatty acid binding protein 4, resistin and aquaporin 7, increased in the order of SVF<SPA<<MA. The expression levels of adipocyte-related genes, such as growth hormone receptor and klotho-B, increased in MA compared with SVC and SPA. These results suggest that SPA may be the cells in the differentiation process from SVC into MA. On the other hand, greater amount of neural cell-related genes, including axon guidance, synaptic vesicle, GABA-A receptor (Gabrg2), serotonin receptor 1F (Htr1F) and glutamate receptor 7 (Grm7) were expressed in SPA than in SVC, most of which were downregulated in MA. Real-time PCR confirmed these results. Furthermore, an immunohistological study revealed that Grm7 is predominantly expressed in SPA, suggesting that Grm7 is a candidate of marker protein for SPA in adipose tissue.
Conclusion: Our results supported the idea that SPA are adipocyte progenitor cells. SPA are able to differentiate into mature adipocytes more easily than SVC. SPA may play an important role in adipocyte differentiation.
Disclosure: **K. Kajita:** None.

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Introduction of NFIA into myoblasts drives brown adipocyte differentiation via controls of brown fat gene programme by co-localising with PPARγ at cell type-specific enhancers

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Background and aims: Brown fat dissipates energy in the form of heat, and is a promising target for treatment of obesity. However, global landscape of brown fat development is only partially understood.
Materials and methods: We performed ChIP-seq and FAIRE-seq on murine brown and white fat tissues. We analysed brown fat of NFIA knockout mice and human perirenal brown fat of patients with pheochromocytoma.
Results: Here we performed FAIRE-seq on murine brown and white fat tissues and found that the binding motif for NFI transcription factor is enriched within brown-fat specific open chromatin regions. Of the four isoforms of NFI family, NFIA is highly expressed in brown fat compared to white fat or muscle. Introduction of NFIA into myoblasts results in brown adipocyte differentiation such as lipid accumulation, activation of the brown-fat-specific gene program and suppression of muscle genes. Conversely, knockdown of NFIA in brown adipocytes suppressed the brown-fat-specific genes. ChIP-seq and FAIRE-seq demonstrate that NFIA binds to and increases chromatin accessibility of brown fat-specific enhancers. Further, NFIA and PPARγ selectively co-localize at the brown fat-specific enhancers and co-localization of NFIA facilitates binding of PPARγ to these enhancers. Brown fat of NFIA knockout mouse neonates show impaired expression of brown fat genes and reciprocal elevation of muscle genes. Finally, human perirenal brown fat of patients with pheochromocytoma show concurrent increase in NFIA and UCP1 expression.
Conclusion: Collectively, these results indicate that NFIA is a novel key transcription factor that co-localizes with PPARγ and activates the brown-fat-specific gene program.
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The effect of miR-21 on beige adipose tissue increase and its relationship with obesity

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Background and aims: Adipose tissue plays an important role as a pathogenic site of obesity-related insulin resistance and type 2 Diabetes. MicroRNAs are small non coding RNA known to play an important role in the regulation of adipose tissue functionality. MiR-21 is known to play a pivotal role in the regulation of several physiological processes controlling tissue growth and cell proliferation. However, until the date the main role of this microRNA in adipose tissue regulation in relation to obesity and type 2 Diabetes is still not well defined. The aim of our study was to analyse the role of miR-21 on obesity and adipose tissue functionality.
Materials and methods: MiR-21 levels were measured by qPCR from subcutaneous adipose tissue (SAT) of normoweight subjects (NW n=10), healthy morbid obese (HMO n=10), diabetic morbid obese (DMO n=10), and from mice (C57BL/6J) fed a standard diet (SD; 10% kcal fat) (n=10),

obese mice fed a high-fat diet (HFD; 45% kcal fat) (n=10), and diabetic mice fed a high-fat diet (HFD diab; 45% kcal fat) (n=10). For in vivo study, obese C57BL/6J mice were treated during 8 weeks with mimic miR-21 (0,5 μg). mRNA and miR-21 were extracted from SAT and interscapular fat depot (white (WAT), beige and brown adipose tissue (BAT)). Thermoregulatory genes such as Pgc1a, Ucp1 and Tmem26 were measured by qPCR. Comparaisons were made using student t-test analysis.

Results: In this study, we observed that in both human and mice SAT miR-21 expression increased significantly with obesity, and decreased in diabetic mice compared with obese mice. The in vivo study showed that treatment with mimic miR-21 decreased body weight gain and improved insulin sensitivity compared with control without showing any differences in food intake and locomotor activity. Also, miR-21 expression increased significantly in SAT and BAT in mimic-treated mice compared to control. Moreover, thermoregulatory genes such as Pgc1a and Ucp1 showed an up-regulation in BAT from mimic-treated mice compared to control, while Tmem26, a specific beige fat marker, showed a significant increase in mimic-treated mice in both WAT and beige fat compared to control.

Conclusion: Our data highlight the potential role that could play miR-21 on beige increase and browning through inducing Ucp1, Pgc1a and Tmem26 genes, thus decreasing body weight gain and obesity onset.

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Irisin improves perivascular adipose tissue dysfunction via regulation of the heme oxygenase-1/adiponectin axis in diet-induced obese mice

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Background and aims: Perivascular adipose tissue (PVAT) modulates vascular function by attenuating vasoconstriction response. However, this anti-contractile effect is lost in obesity. Irisin is secreted by myocytes and has been proposed to improve endothelial function in obesity. Upregulation of the heme oxygenase-1 (HO-1)/adiponectin axis has a vascular protective role by preventing endothelial dysfunction through its anti-oxidative and anti-inflammatory effects. Considering that in obesity both irisin and upregulation of the HO-1/adiponectin axis could improve endothelial function, and that PVAT dysfunction is mainly associated with an endothelium-dependent pathway, it is tempting for us to know whether irisin also has a protective effect on PVAT dysfunction in obesity and whether this beneficial effect is related to upregulation of the HO-1/adiponectin axis in PVAT. Therefore, the objective of this study was to explore whether irisin could improve PVAT dysfunction via regulation of the HO-1/adiponectin axis in obesity.

Materials and methods: C57BL/6 mice were given chow or a high fat diet with or without treatment with irisin. The responses of thoracic aorta with or without PVAT (PVAT+ or PVAT−) to phenylephrine were studied in an organ bath. Protein levels of HO-1 and adiponectin were determined by western blot. UCP-1, Cidea and TNF-α gene expression in PVAT were analyzed by real-time PCR.

Results: Treatment of obese mice with irisin improved glucose and lipid metabolism (glucose, 97.0 ± 4.8 mg/dl vs. 105.7 ± 6.1 mg/dl; triglycerides, 95.79 ± 12.92 mg/dl vs. 127.76 ± 13.00 mg/dl; NEFA 0.89 ± 0.17 mmol/L vs. 1.21 ± 0.25 mmol/L; $P < 0.01$), reduced plasma levels of TNF-α (29.25 ± 4.43 pg/ml vs. 35.36 ± 4.18 pg/ml, $P < 0.01$), malondialdehyde (5.17 ± 0.60 umol/L vs. 6.27 ± 0.66 umol/L, $P < 0.01$) and enhanced plasma adiponectin levels (17.34 ± 1.78 ug/ml vs. 13.87 ± 2.41 ug/ml, $P < 0.01$). PVAT had a significant anti-contractile effect on the aorta from control mice. The anti-contractile effects of PVAT were attenuated in obese mice (maximum attenuation rate: 4.5% vs. 17.3%; $P < 0.05$). This attenuation of the anti-contractile effects of PVAT was restored in obese mice treated with irisin (maximum attenuation rate: 15.5% vs. 4.5%; $P < 0.05$). Transferring the solution from aortas (PVAT+) in control or irisin-treated obese mice to aortas (PVAT-) in obese mice caused a 14.5% or 13.3% attenuation of the anti-contractile response, respectively, compared with 3.5% when transferred from aortas (PVAT+) in obese mice ($P < 0.05$). Incubation of aortas (PVAT+) with SnPP (HO-1 inhibitor), ACRP-30 N-20 (adiponectin receptor blocking peptide), or L-NAME in irisin-treated obese mice abolished the beneficial effects of irisin on PVAT function ($P < 0.05$). The same results were also observed in obese mice treated with irisin in vitro. Treatment of obese mice with irisin significantly enhanced protein levels of HO-1 and adiponectin in PVAT, increased brown adipocyte marker UCP1 and Cidea expression in PVAT, and reduced superoxide production and TNF-α expression in PVAT. ($P < 0.05$)

Conclusion: Irisin improved the anticontractile properties of PVAT from thoraic aorta in diet-induced obese mice. The mechanism for this protective effect of irisin appeared to be related to upregulation of HO-1/adiponectin axis in PVAT and browning of PVAT.

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Impaired adipose histone deacetylases 5 and 6 expression in obesity mimics the adverse effect of hypoxia on adipocytes

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Background and aims: Abnormal expression of histone deacetylases (HDAC) contributes to epigenetic mechanisms involved in cancer and inflammatory diseases. In obesity, the expression of numerous insulin resistance-associated adipokines is impaired, but the putative role of epigenetic modifiers in the adipocyte dysfunction is unknown. The goal of this study was to investigate whether adipose HDAC activity and expression contribute to the epigenetic mechanisms that may modulate adipokines expression impairing glucose metabolism in obesity

Materials and methods: Mice C57bl6 were fed a high fat diet (HFD) for 12 weeks. Biopsies of visceral adipose tissues (VAT) were prepared from normal weight (body mass index [BMI] lower than 25 Kg/m2) or severely obese human subjects (BMI higher than 35 Kg/m2). Total RNA was prepared from white adipose tissues (WAT) of chow- or HFD-fed mice and VAT of normal weight and obese subjects. Total HDAC activity was measured using a kit manufacturer instruction. The role of hypoxia and HDAC on the expression of inducible cAMP early repressor (Icer) was confirmed in 3T3-L1 adipocytes cells using hypoxic chamber and HDAC siRNA. Icer and HDAC inhibition was performed by electroporation of specific siRNA. Gene expression was quantified by quantitative real-time PCR

Results: Total activity of HDAC is reduced in WAT of obese mice fed a HFD. Measurement of 11 HDACs by qRT-PCR unveiled the reduction of Hdac5/HDAC5 and Hdac6/HDAC6 expression in WAT and adipocytes fraction of obese mice when compared to controls. The decrease of the two HDACs was confirmed in VAT and adipocytes fraction of human obese subjects, and was associated with a reduced total HDAC activity. The loss of Hdac5/HDAC5 and Hdac6/HDAC6 was further associated with the drop of ICER/Icer. Suppression of Icer by interference RNA

alters insulin-induced glucose uptake and adipokine expression in 3T3-L1 adipocytes. We found that silencing of Hdac5 and Hdac6 levels in 3T3-L1 adipocytes reduced the expression of Icer and insulin-induced glucose uptake. Hypoxia decreased the expression of the two HDACs, resulting in the loss of Icer mRNA level and the reduced expression of Adipoq, Agt, Lep, Nampt, Rtn, and increased mRNA level of Serpin1, Il6 and Lcn2. Silencing of Icer mimicked the effects of hypoxia on the expression of adipokines.

Conclusion: In human and mice obesity, there is an impaired adipose expression of HDAC 5 and 6. It may contribute to abnormal production of adipokines through decreased ICER expression, mimicking hypoxia deleterious effects on adipocyte function.

Disclosure: **J. Bricambert:** None.

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Energy expenditure of human cervico-thoracic muscles during mild cold stress: a positron emission tomography study

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Background and aims: Mild cold stress has been shown to increase whole-body energy expenditure (EE) in humans via the activation of sympathetic nervous system. Conventionally, brown fat has been considered an underlying responsible tissue for this increase in whole-body EE; however, recent studies have shown it to be a minor contributor towards EE. The present study is an extension of our previous efforts to study human cervico-thoracic muscles as a major contributor to cold induced thermogenesis (CIT), using $[^{15}O]O_2$ PET imaging. We aimed to determine the factors that may affect the thermogenic ability of these muscles during a mild cold stress.

Materials and methods: Twenty two (22) healthy study subjects of both genders were scanned with $[^{15}O]O_2$ and $[^{15}O]H_2O$ PET imaging under mild non-shivering cold stress to estimate tissue-specific EE of cervico-thoracic muscles. Oxygen consumption was measured in levator scapulae, splenius cervicis, deltoid, trapezius, and pectoralis major muscles. Whole-body energy expenditure was measured using indirect calorimetry, performed simultaneously with the scanning.

Results: Oxygen consumption in pectoralis major muscle (0.9 ± 0.9 ml/100g/min) was higher than that of deltoid (0.3 ± 0.4 ml/100g/min, p = 0.004), trapezius (0.3 ± 0.3 ml/100g/min, p = 0.005) and splenius cervicis muscle (0.6 ± 0.5 ml/100g/min, p = 0.06); however it was not statistically different compared to levator scapulae muscle (0.7 ± 0.6 ml/100g/min, p = 0.49). Mean oxygen consumption of these cervico-thoracic muscles was 0.5 ± 0.4 ml/100g/min. Mean EE of cervico-thoracic muscle was estimated to be 91 ± 60 kcal/day; while the mean whole-body EE, measured with indirect calorimeter, was 1909 ± 378 kcal/day. There was a significant direct relationship between whole-body EE and EE of cervico-thoracic muscles (r = 0.54, p = 0.016). We did not observe any significant relationships between cold-induced EE of cervico-thoracic muscles with plasma insulin, free T3, free T4, adrenaline or noradrenaline, which possibly points toward an unknown underlying metabolic mechanism.

Conclusion: The present results demonstrate the potentials to look into the skeletal muscles mitochondrial uncoupling proteins, as a basis for elevating whole-body energy expenditure, which resultantly could be a therapeutic target for obesity, and its related metabolic disorders, i.e. type 2 diabetes mellitus.

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Disclosure: **M. U Din:** None.

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Oxygen uptake in subcutaneous adipose tissue in type 2 diabetes: relation to perfusion and insulin-mediated glucose uptake and response to exercise

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Background and aims: Adipose tissue (AT) hypoxia and hypoperfusion may be involved in the modulation of AT growth and in the pathogenesis of AT insulin resistance. The aim of the present study was to examine the effect of type 2 diabetes (T2DM) on subcutaneous AT oxygen uptake, and its relation to AT blood flow and insulin sensitivity.

Materials and methods: We examined 28 patients with newly diagnosed T2DM and 12 BMI-matched (27.3±0.8 vs 28.0±0.4 kg/m^2) non-diabetic control subjects. Positron emission tomography in combination with labelled oxygen ($^{15}O_2$, n=17 T2DM, n=11 controls), water ($H_2{}^{15}O$), and ^{18}fluorodeoxyglucose (^{18}FDG) were used to assess oxygen uptake, blood flow and insulin-mediated glucose uptake in subcutaneous AT, in a resting thigh and in an exercising thigh. Body composition was measured by bioimpedance.

Results: Patients with T2DM had a larger body fat mass compared to controls (24±2 vs 18±1 kg, p=0.018). In the resting thigh, patients with T2DM showed significant impairments in AT blood flow (0.94 ±0.11 vs 1.27±0.15 $ml \cdot min^{-1} \cdot 100g^{-1}$, p=0.036) and oxygen uptake (3.34±0.19 vs 5.10±0.51 $\mu mol \cdot min^{-1} \cdot 100g^{-1}$, p=0.003). AT insulin-mediated glucose uptake was also reduced in T2DM (1.42±0.11 vs 2.21±0.26 $\mu mol \cdot min^{-1} \cdot 100g^{-1}$ per 100 mU/L, p=0.008). Exercise increased AT oxygen uptake to a similar extent in the two groups (7.59 ±1.45 vs 6.18±0.72 $\mu mol \cdot min^{-1} \cdot 100g^{-1}$) without affecting AT blood flow or glucose uptake. AT oxygen uptake was positively related to AT glucose uptake only in control subjects in the resting thigh, and did not correlate with blood flow. Blood flow was tightly correlated with glucose uptake only in T2DM patients (rest r=0.66, p=0.0002, exercise r=0.74, p<0.0001).

Conclusion: In T2DM, oxygen uptake in AT is impaired, but responds to exercise normally, whereas AT hypoperfusion and insulin resistance do not improve with exercise. The defect in resting oxygen uptake in T2DM may depend on the fat mass expansion, but does not explain AT insulin resistance. Our data also suggest that a severe reduction in AT blood flow may become rate limiting for glucose uptake in T2DM.

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Effect of vitamin D on human mesenchymal stem cells adipogenesis and cytokine secretion in hypoxia

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Background and aims: Obesity and pandemic vitamin D (vitD) deficiency both share similar pathological associations with

adipose tissue chronic inflammation leading to well-known conditions such as insulin resistance and hypertension. Understanding adipogenesis is of major relevance for human health, as increased adiposity leads to cellular hypoxia followed by inflammation, oxidative damage and adipocyte dysfunction resulting in metabolic diseases. We have shown earlier that vitD supplementation has significant beneficial effects on adipocyte inflammation and improved insulin sensitivity in vitD deficient mice. The modulation of adipogenesis under these circumstances might be beneficial in improving its metabolic functioning. Therefore, we hypothesize that vitD modulates adipogenesis in pathological conditions like hypoxia.

Materials and methods: Human mesenchymal stem cells (hMSCs) were differentiated into adipocytes ± vitD (1nM to 100nM) containing adipogenic induction and maintenance medium under either hypoxic (1% O2) or normoxic (21% O2) conditions. At the end of differentiation cycles the process was assessed by the oil red O staining of the adipocytes. Fully differentiated adipocytes were stimulated with lipopolysaccharide (LPS 10 ng/ml) ± vitD (1nM to 100nM) for 24 hrs and cells and cell culture supernatants were collected from both hypoxic and normoxic basal ± vitD (1nM to 100nM) as well LPS stimulated ± vitD (1nM to 100nM). The inflammatory marker interleukin-6 (IL-6) levels were measured by ELISA. RNA and cell lysate were prepared and analyzed for adipogenic, hypoxic and inflammation regulating proteins.

Results: The adipogenic differentiation of hMSCs under hypoxia was significantly inhibited in comparison to the normoxia. VitD (1nM to 100nM) significantly enhanced adipogenic differentiations dose dependently under both hypoxic and normoxic culture conditions. VitD (100nM) inhibited basal IL-6 secretion by 83% in hypoxic and 66% in normoxic hMSCs differentiated adipocytes in comparison to their respective controls ($p<0.05$). In addition, vitD dose dependently reduced LPS induced IL-6 secretion from hMSCs differentiated adipocytes under both hypoxic (100nM:10nM:1nM; 66%:40%:19%) and normoxic (100nM:10nM:1nM; 56%:44%:2%) condition in comparison with the LPS 10 ng/ml alone. VitD significantly reduced the hypoxia-inducible factor 1 alpha (HIF1-α) expression under hypoxia and enhanced the expression of adipogenic transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ) in both normoxic and hypoxic condition.

Conclusion: Our data demonstrates that vitD enhances the adipogensis of hMSCs under hypoxic culture condition; it inhibits basal IL-6 release as well LPS induced IL-6 release under both hypoxia and normoxia. This suggests that sufficient vitD levels would be able to modulate the hypoxia induced inflammation and adipogenic differentiation potential in obese subjects. These vitD actions might therefore partially mediate the beneficial effect on insulin sensitivity of adipose tissue and reduce the risk of known co-morbidities of obesity.

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Disclosure: **S.J. Mutt:** None.

PS 047 Macrophages and other blood cells in inflammation

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Depletion of macrophages in mice fed a high-fat diet leads to a systemic inflammatory state and improved beta cell function

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Background and aims: Macrophages are increasingly recognised as contributors to islet inflammation and β-cell dysfunction in Type 2 Diabetes. Chronic inflammation and elevated pro-inflammatory cytokines lead to β-cell dysfunction and death, while short term exposure to certain cytokines (IL-1β, IL-6) can potentiate insulin secretion. Here we induced macrophage-specific cell death in vivo in mice fed a high fat diet (HFD). The role of acute as well as long term inflammation was investigated in relation to β-cell function.

Materials and methods: Mice were fed a normal chow or HFD for up to 8 weeks. To deplete macrophages we used CD11b diphtheria toxin receptor (DTR) transgenic mice. Transgenic mice express the simian diphtheria toxin (DT) receptor under the control of the CD11b promoter, thus limiting expression to myeloid cells that are now susceptible to apoptosis following injection of DT. DTR mice and wild-type (WT) littermates were injected with a single dose of DT and studies were performed 24 hours following unless otherwise stated. Mice were subjected to i.p. glucose tolerance test (GTT) and i.p. insulin tolerance test (ITT). Insulin and c-peptide excursions during GTTs were monitored by ELISA. Islet gene expression and resident immune cell populations were analysed by quantitative RT-PCR and flow cytometry. Serum cytokines were analysed by cytokine bead array.

Results: GTTs performed at 4 and 8 weeks of chow or HFD feeding showed impaired glucose tolerance in HFD-fed mice. Following DT injection, HFD-fed DTR mice showed a complete protection from HFD-induced glucose intolerance (incremental area under the curve (IAUC), $p<0.05$, one-way ANOVA)) compared to WT HFD mice. Insulin and c-peptide excursions in the DTR HFD group were also elevated during these GTTs (IAUC, $p<0.05$, one-way ANOVA), compared to WT HFD controls. Insulin sensitivity as measured by ITT was not different between DTR and WT HFD-fed mice. These parameters were not altered in chow-fed DTR mice. Macrophage numbers were reduced in the peritoneum, and stromal vascular fraction (SVF) of adipose tissue ($p<0.05$, student's t-test), but were not different in the blood, or surprisingly, islets. All tissues above showed elevated neutrophil and monocyte populations in DTR mice compared to WT controls ($p<0.05$, student's t-test). Gene expression analysis of islets showed increased IL-1β and chemokine (C-C motif) ligand 2 ($p<0.05$, student's t-test) expression, with IL-6 and TNFα expression tending towards an increase compared to WT controls. Systemic inflammation as measured by levels of serum IL-6 and TNFα was increased in DTR mice compared to WT controls ($p<0.05$, student's t-test) up to 1 week post DT administration. When GTTs were performed 1 week after DT administration there was no longer any benefit on glucose tolerance in the HFD-fed mice, and insulin secretion was now actually impaired during the GTT.

Conclusion: Short-term depletion of macrophages in mice fed a HFD leads to a marked but transient improvement in β-cell function, with no alteration in peripheral insulin sensitivity. This is most likely due to macrophage cell death leading to a systemic inflammatory state and recruitment of neutrophils and monocytes to islets. 1 week after DT administration, short-term improvements in glucose tolerance are dissipated, and β-cell function is potentially impaired. This study suggests that inflammation initially serves as a beneficial adaptation to a HFD, but over time can contribute to β-cell dysfunction.

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Involvement of membrane remodelling induced by fatty acids in the regulation of the NLRP3 inflammasome activity in human macrophages

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Background and aims: A chronic low-grade inflammation and an activation of the immune system are involved in the pathogenesis of type 2 diabetes in obese people. Macrophages are recruited in adipose tissue of these patients and secrete pro-inflammatory cytokines including interleukin-1 beta (IL-1β) involved in insulin resistance. The NLR family pyrin domain-containing-3 (NLRP3) inflammasome, required for the maturation of the pro-IL-1β to IL-1β mature, was shown to instigate obesity-induced inflammation and insulin resistance. The two most important saturated fatty acids, stearate (C18:0) and palmitate (C16:0), unlike the unsaturated fatty acids oleate (C18:1) and linoleate (C18:2), are able to activate the NLRP3 inflammasome in human macrophages. Interestingly, addition of unsatured to saturated fatty acid stop this activation. However, the underlying molecular mechanisms are still not elucidated.
Materials and methods: Lipidomic analyses were performed on PMA-differentiated THP-1 cells after 8 hours of treatment with BSA (control), BSA/C18:0 alone or combined with BSA/C18:1 to assess the phospholipid profile of cellular membranes. Membranes fluidity and lipid droplets formation were also observed by confocal microscopy with fluorescent probes Laurdan and HCS LipidTOX.
Results: We have shown that neither the ROS, ER stress nor the lysosomes are involved in saturated fatty acids-mediated NLRP3 inflammasome activation. Inhibition of the long-chain fatty acyl-CoA synthetase with triacsin C prevents the NLRP3 inflammasome activation mediated by C18:0, suggesting that C18:0 has to be activated into fatty acyl-CoA to be metabolized. Indeed, C18:0 leads to a rigidification of the plasma membrane without stimulating lipid droplets formation, and induces a dramatic change in phospholipid profile with an increase of saturated (L18:0) lysophosphatidylcholines (LysoPC) (5,1% vs 56,9% of total LysoPC; $p<0,0001$) and saturated (L18:0) lysophosphatidylethanolamines (LysoPE) (15,4% vs 60,9% of total LysoPE; p=0,0001). Interestingly, addition of C18:1 simultaneously with C18:0 reverses the situation, restores the membrane fluidity and stimulates lipid droplets formation with very little effect on C18:0 uptake. Moreover, the co-treatment decreases the saturated phospholipid species (L18:0) LysoPC (56,9% vs 14,5% of total LysoPC; $p<0,0001$) and LysoPE (60,9% vs 20,9% of total LysoPE; p=0,0002), and increases unsaturated phospholipid species (L18:1) LysoPC (14,8% vs 54,3% of total LysoPC; $p<0,0001$) and LysoPE (13,3% vs 36,6% of total LysoPE; p=0,0006).
Conclusion: The increase of the phospholipid saturation in the presence of saturated fatty acids induces a rigidity of the plasma membrane and could lead to the NLRP3 inflammasome activation. The protective effect of C18:1 could be explained by a reduction of the membrane saturated phospholipid species with a re-establishment of its fluidity due to the increase of unsaturated membrane phospholipid species combined with lipid droplets biogenesis.
Disclosure: **M.A. Gianfrancesco:** None.

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Glucocorticoid-induced insulin resistance is related to macrophage visceral adipose tissue infiltration

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Background and aims: Insulin resistance is frequently present in patients with glucocorticoid (GC) excess (Cushing's syndrome) or treated with high doses of GCs. Furthermore, others similarities between metabolic syndrome (visceral obesity, elevated blood glucose level, dyslipidemia) and Cushing's syndrome suggest that GC could play a role in obesity-linked complications.
Materials and methods: In this study, we investigated the effects of a long-term GC exposure in C57/Bl6 male mice. Corticosterone was given in drinking water (100 μg/mL) for 8 weeks. For macrophage ablation, mice were intraperitoneally injected with 100 mg/kg of clodronate liposomes (5 mg/ml) or equal volume of PBS liposomes twice weekly. Insulinemia, insulin sensitivity, pancreatic insulin content, gene expression in adipocytes and macrophage phenotype were studied.
Results: Long-term corticosterone exposure in mice induced weight gain, dyslipidemia as well as hyperglycaemia and systemic insulin resistance. Treated mice exhibited an increased 11β-HSD1 expression in all fat depots but a specific upregulation of glucocorticoid receptor only in visceral adipose tissue an a downregulation of mineralocorticoid receptor only in subcutaneous depot, suggesting that GC could act differentially on various fat depots. Despite fat accumulation in all depots, an increased expression of adipogenic key genes (PPARγ, C/EBPα) was restricted to visceral adipose tissue while hypertrophied adipocytes were observed in both visceral and subcutaneous depots. Surprisingly, GC treatment promoted macrophage infiltration (F4/80, CD68) within all adipose tissues along with increased MCP1 adipose tissue expression and plasma levels. Of note, a M1/inflammatory macrophage phenotype (IL-6, TNF-α) was restricted to visceral adipose tissue, compared to subcutaneous fat depot, suggesting a substantial damaging effect on insulin sensitivity. Accordingly, specific macrophage depletion in visceral fat partially restored insulin sensitivity in mice with GC-induced obesity and insulin resistance. Interestingly, visceral macrophage recruitment preceded fat mass expansion and adipocyte hypertrophy, indicating an intrinsic GC effect.
Conclusion: These data provide evidence that GCs act on adipose tissue in a depot-dependent manner and that visceral adipose macrophages are key effectors of GC-associated insulin resistance.
Disclosure: **T.T.H. Do:** None.

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Autophagy deficiency in myeloid cells increases susceptibility to diabetes by enhancing inflammasome activation

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Background and aims: Autophagy that is critical for turnover of organelles such as endoplasmic reticulum and mitochondria, affects diverse aspects of body metabolism. Autophagy also controls innate and adaptive immunity. Autophagy deficiency is a proinflammatory condition particularly in relation to inflammasome activation. Since inflammasome activation is a mechanism leading to metabolic inflammation in diabetes, autophagy insufficiency can be a factor in the development of metabolic syndrome and diabetes. Although the role of autophagy of diverse tissues in body metabolism has been intensively investigated, the role of myeloid cell autophagy in the control of insulin sensitivity and in the development of diabetes has not been clearly elucidated.
Materials and methods: Myeloid-specific Atg7-null (Atg7ΔLys) mice were generated by breeding Atg7F/F mice with Lys-Cre mice. Serum

insulin and IL-1β levels were determined by ELISA. Real-time RT-PCR was conducted using SYBR Green I dye and ABI Prism 7000. Infiltration of cells expressing IL-1βor F4/80 was studied by confocal microscopy after immunofluorescent staining. NAD+/NADH ratio was determined using a commercial. Stromal vascular fraction was isolated by digesting cut adipose tissue in a collagenase solution. To isolate Treg cells, stromal vascular fraction was suspended in FACS buffer and then incubated with a mixture of anti-CD3, -CD4 and -CD25 antibodies. After permeabilization, cells were incubated with Foxp3 antibody, and triple-color flow cytometry was performed.

Results: While Atg7ΔLys mice were metabolically indistinguishable from control mice, they developed diabetes when bred to ob/w mice (Atg7ΔLys-ob/ob mice), accompanied by increases in the crown-like structure, inflammatory cytokine expression and inflammasome activation in adipose tissue. Primary macrophages from Atg7ΔLys mice showed significantly higher IL-1β release and inflammasome activation in response to a palmitic acid plus lipopolysaccharide combination. Moreover, a decrease in NAD+/NADH ratio and increase in intracellular ROS content after treatment with palmitic acid in combination with lipopolysaccharide were more pronounced in macrophages from Atg7ΔLys mice, suggesting that mitochondrial dysfunction in autophagy-deficient macrophages leads to an increase in lipid-induced inflammasome and metabolic deterioration in Atg7ΔLys-ob/ob mice. In the stromal vascular fraction of white adipose tissue from Atg7F/F-ob/ob mice, the percentage of Foxp3+CD4+ Treg cells among CD3+ T cells was significantly suppressed compared to Atg7F/F-ob/w mice. In the stromal vascular fraction of Atg7ΔLys-ob/ob mice, the percentage of Foxp3+CD4+ Treg cells among CD3+ T cells was further suppressed compared to Atg7F/F-ob/ob mice. The percentage of Foxp3+CD25+ Treg cells among CD3+ T cells was also further suppressed in the stromal vascular fraction of white adipose tissue from Atg7ΔLys-ob/ob mice compared to Atg7F/F-ob/ob mice that was already lower than that in lean Atg7F/F-ob/w mice.

Conclusion: These results suggest that autophagy of macrophages is important for the control of inflammasome activation in response to metabolic stress. Autophagy deficiency in macrophages may contribute to the progression from obesity to diabetes associated with lipid injury.

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Disclosure: **M. Lee:** None.

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Neutrophil elastase and myeloperoxidase genes are overexpressed in overweight and obese subjects and are associated with body mass index and atherogenic index of plasma

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Background and aims: Chronic low-grade inflammation contributes to obesity-related disorders such as insulin resistance (IR) and atherosclerosis. However, neutrophils have been shown to be the first immune cells that infiltrate adipose tissue at the very early stage of obesity. Neutrophil elastase (NE) and myeloperoxidase (MPO) are abundantly expressed by activated neutrophils, and both of them have been implicated to induce high-fat diet-induced obesity, IR and atherosclerosis in mice. Recently, remarkable high expression of NE and MPO genes in peripheral blood leukocytes was found in morbidly obese African-American young adults compared to lean controls. We aimed to explore the expressions of NE and MPO genes in peripheral blood leukocytes in overweight and obese subjects, and to find out the association of expressions of those genes with BMI, IR and atherogenic index of plasma (AIP).

Materials and methods: Fifty apparently healthy subjects were grouped as control or lean (BMI, 18.5-22.9 kg/m2), overweight (23.0-27.4) and obese (≥27.5) following the WHO recommendation for Asian BMI. Subjects suffering from diabetes, hypertension, chronic liver and kidney disease, infection and inflammatory disease were excluded. Fasting glucose, insulin and lipid profile were measured. IR was calculated as [(Glucose x Insulin) / 22.5] and AIP was calculated as [log (TG/HDL-C)]. The NE and MPO mRNA levels were quantified relative to reference gene beta-actin by real-time qPCR. Gene expression data were analyzed by comparative Ct method.

Results: The lean (n=15), overweight (n=18) and obese (n=17) groups were similar in terms of age (mean±SD, 33±5 yrs), sex distribution and fasting glucose level (4.7±0.8 mmol/L). The BMI was different among the 3 groups (21.99±0.89, 25.59±1.43 and 34.64±5.20 kg/m2; $p<0.001$). Fasting insulin level and IR were found significantly elevated in obese group compared to overweight ($p<0.05$) and lean ($p<0.01$) groups. However, compared to lean group the AIP was found significantly elevated in both overweight ($p=0.007$) and obese ($p=0.002$) groups. The NE gene expression as percentage of beta-actin was found significantly elevated in overweight [median (IQR), 0.62% (0.50-1.79)] and obese [1.79 (0.34-2.93)] groups compared to lean group [0.19 (0.15-0.35); $p<0.001$ for both]. Similarly, MPO gene expression was also found significantly elevated in overweight [0.29 (0.16-0.46)] and obese [0.26 (0.05-0.90)] groups compared to lean group [0.06 (0.03-0.16); $p<0.02$ for both]. Group-wise comparison of NE and MPO gene expression showed 4.25-fold ($p=0.001$) and 4.84-fold ($p=0.002$) up-regulation, respectively, in overweight group over lean group, and 5.39-fold ($p<0.001$) and 4.90-fold ($p=0.007$) up-regulation in obese group over lean group. The NE gene expression showed significant correlation with BMI ($r=0.49$, $p=0.001$) and a positive trend with AIP ($r=0.30$, $p=0.06$) but no correlation with IR. The MPO gene expression also correlated with BMI ($r=0.32$, $p<0.05$) and AIP ($r=0.34$, $p<0.05$) but not with IR.

Conclusion: The NE and MPO genes are up-regulated in both overweight and obese subjects and are associated with BMI and AIP. These findings suggest that the expressions of NE and MPO genes may act as early marker of obesity and atherosclerosis.

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PS 048 Novel and updated biomarkers associated with obesity

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The decline in serum DPP-4 activity after weight loss is associated with the decrease in cholesterol levels but not with improved insulin resistance or inflammation

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Background and aims: Besides its prominent role in the degradation of the incretins glucagon-like peptide-1 and gastric inhibitory polypetide and thus contributing to decreased insulin sensitivity, rendering it an established anti-diabetic target, DPP-IV exerts systematic pro-inflammatory effects and has been identified as an adipose-derived cytokine. It is suggested to play a dual, synergistic, role in the pathogenesis and progression of diabetes mellitus as well as atherosclerotic vascular disease. The aim of this study was to evaluate whether serum DPP-IV activity is modified by weight loss induced by bariatric surgery, an established model for the investigation of cardiometabolic risk factors.
Materials and methods: We included 62 morbidly obese patients undergoing bariatric surgery. Exclusion criteria for this study included current anti-diabetic medication or HbA1C ≥ 7 rel. %. The patients were examined and samples obtained shortly before and 1-2 years after surgery. Serum DPP-IV enzymatic activity was measured by a commercially available kit using a fluorogenic substrate (H-Gly-Pro-AMC) from samples stored at -80°C. Additional laboratory parameters as well as an two-hour oral glucose tolerance test were measured at the institution's central laboratory. Data are displayed as median (25^{th}-75^{th} percentile) or count (percentage), as appropriate. Differences between paired and independent samples are estimated by Wilcoxon's signed-rank test or Mann-Whitney U test. Correlations between continuous parameters including the calculated differences between pre-surgery and post-surgery values are depicted by Spearman's rank-correlation coefficient. Al p-value ≤ 0.05 was deemed statistically significant.
Results: At baseline, the sample consisted of 48 women (77.4%) with a BMI of 45 (42.9-48.3) kg/m^2 and 39 (31-49) years of age. After surgery, the BMI dropped to 30.1 (25.7-34.5) kg/m^2 ($p<0.001$) while serum DPP-IV significantly decreased from 113.1 (97.5-132.6) to 95.9 (81.6-119.9) nmol/min/ml) ($p=0.008$). This change in DPP-IV activity positively correlated with the change in total cholesterol ($r=0.395$, $p=0.003$) as well as LDL-cholesterol ($r=0.373$, $p=0.005$). However, there were no notable associations between the change in DPP-IV activity and the changes in other parameters, such as HbA1C, fasting glucose, fasting insulin, HOMA-IR index, triglycerides, HDL-cholesterol, C-reactive protein (CRP), systolic blood pressure or the BMI. At follow-up, DPP-IV activity correlated positively with CRP levels (0.314, $p=0.018$).
Conclusion: This is the first study to demonstrate a reduction in the activity of circulating DPP-IV after massive weight loss due to bariatric surgery. Contrary to the expectations, this decline was solely associated with the decline in total cholesterol and LDL-cholesterol and not with markers of insulin resistance or inflammation. This finding might reflect the reduction of visceral fat and subsequent diminishing release of both circulating DPP-IV as well as free fatty acids, the latter leading to improved dyslipidemia including cholesterol levels.
Disclosure: **C.T. Herz:** None.

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The effect of bariatric surgery on fatty acid uptake and associated gene expression in abdominal subcutaneous adipose tissue of morbidly obese women

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Background and aims: Abdominal fat distribution often results in elevated plasma free fatty acid (FFA) levels, which can cause insulin resistance and associated metabolic complications. In the current study, we assessed the effect of bariatric surgery-induced weight loss on mRNA expression of genes regulating fatty acid metabolism in abdominal subcutaneous adipose tissue (SC) of obese women.
Materials and methods: A total of 23 obese women (BMI 42 kg/m^2) undergoing surgery and 15 age- and sex- matched controls (BMI 22 kg/m^2) participated in this study. Oral glucose tolerance test (OGTT), biochemical measurements and abdominal SC tissue biopsies were assessed prior and 6 months after surgery and once for control subjects.Abdominal SC distribution was assessed with magnetic resonance imaging and free fatty acid uptake (FAU) measured with positron emission tomography and 14(R,S)-[^{18}F] fluoro-6-thia-heptadecanoic acid tracer. Genes related to fatty acid metabolism were analyzed using illumina Truseq technology.
Results: Six months after surgery, subjects lost 23% ($p<0.001$) of their body weight. Compared to preoperative values, fasting and 2-hour plasma glucose decreased but plasma FFA levels were unchanged. There was no difference in baseline FAU (μmol/100g/min) in SC of obese compared to controls. Post-surgery, FAU per whole SC depot (μmol/min) decreased significantly ($p<0.001$) mainly due to decreased fat mass, as FAU per 100g tissue did not change (NS). Baseline expression of Elovl6 was higher in SC of controls compared to obese ($p<0.001$). Compared to before surgery values, Elovl6 was highly expressed in SC after surgery ($p=0.01$). The increase in expression of Elovl6 correlated positively with the decrease in FAU per whole SC depot ($r=0.53$, $p=0.01$) and with the decrease in glucose levels after 2-hour OGTT ($r=0.50$, $p=0.01$). There were reduced mRNA expressions of ACOX2, ADIPOR2, AGPAT1, AGPAT3, CPPED1 and Elovl1 (all $p\leq0.05$), and SCD expression increased ($p<0.01$) after surgery but these changes did not associate with FAU.
Conclusion: Elongation of Very Long Chain fatty acids 6 (Elovl6) gene is important for energy metabolism and insulin sensitivity. Here, we found that decrease depot fat volume may increase mRNA expression of Elovl6 which may be related to the increased glucose clearance observed in obese women after surgery.
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Disclosure: **P. Dadson:** None.

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The alternative pathway of complement activation is longitudinally associated with obesity: the CODAM study

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Background and aims: Animal studies suggest that silencing of genes of the alternative complement pathway may causally contribute to weight change. Also, human studies showed that systemic concentrations of

several complement factors were higher in obese and decreased after weight loss. In addition, in a longitudinal cohort study, the central complement component C3 was suggested to be a risk factor for future weight gain. Still, human data on the relationship of other components of the alternative pathway with obesity are scarce. Therefore, we investigated the associations of components of the alternative complement pathway, i.e. C3, factor H (FH), factor D (FD), properdin, and the activation products C3a and Bb, with obesity (measured as BMI) over a 7 year (yr) period.

Materials and methods: At baseline, the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study included 574 subjects (age: 60±7 yr, 61% men). After 7 yr, 495 subjects were re-investigated. Plasma concentrations of properdin, C3a, and Bb were measured at baseline, while C3, FH, FD and as well as BMI and other covariates were measured at baseline and follow-up. Standardized values were calculated for the various complement components to allow direct comparison of their effect sizes. Generalized estimating equations (GEE) were used to evaluate the longitudinal associations between complement factors (main independent variables) and BMI (outcome). Linear regression models were used to investigate the relationship between change in complement levels (C3, FH and FD) and change in BMI. All analyses were adjusted for age, sex, follow-up time, use of medication (glucose-, blood pressure- and/or lipid-lowering), smoking, physical activity, and energy intake.

Results: Over the 7 yr period, baseline concentrations of C3 were positively and significantly associated with BMI (β: 1.97 [95% CI: 1.60; 2.35]). Thus 1 SD higher baseline concentration of C3 was associated with 1.97 kg/m2 higher BMI, over the 7 yr period. Positive associations were also observed for FH (β: 1.31 [0.94; 1.68]), FD (β: 0.88 [0.42; 1.35]), properdin (β: 0.83 [0.46; 1.21]), and C3a (β: 0.78 [0.41; 1.16]). The association of Bb with BMI was not significant (β: -0.15 [-0.52; 0.23]). When baseline and follow-up concentrations for C3, FH and FD were included in the GEE analysis, again a positive association with BMI was observed, which was significant for C3 and FH, but not for FD (β C3: 1.04 [0.82; 1.27], β FH: 0.84 [0.60; 1.07], β FD: 0.36 [-0.24; 0.95]). Lastly, we evaluated associations, between complement and BMI, of within-subject changes over time. This was significant for C3 (β: 0.52 [0.33; 0.72]) but not for FH (β: 0.13 [-0.09; 0.35] or FD (β: 0.13 [-0.10; 0.35]).

Conclusion: In this middle-aged to elderly Caucasian cohort, higher concentrations of C3, its regulators, FH, FD, and properdin, and its downstream activation product C3a -but not Bb- were consistently associated with higher BMI, over a 7 yr period. In addition, for C3, greater changes during follow-up were associated with greater changes in BMI. These associations extend current knowledge on the intricate relation between the alternative pathway of complement activation and obesity. Whether activation of alternative pathway and/or its individual components can causally contribute to obesity remains to be investigated in humans.

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Disclosure: **Y. Xin:** None.

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Acute neuro-endocrine effects of deep Transcranial Magnetic Stimulation (TMS) in obesity

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Background and aims: TMS is a novel, non-invasive method inducing long lasting and reversible changes in neural excitability and dopamine release. When applied at a low frequency, TMS suppresses cortical excitability, whilst high frequency TMS enhances it. TMS has therapeutic benefits for several neuropsychiatric disorders, however the effects of TMS on neurophysiological parameters are not fully known. Aim of this study was to investigate the effects of a single deep TMS session on neuro-endocrine orexygenic pathways in obesity.

Materials and methods: We investigated the acute effects of a single session (30 min) of deep TMS on metabolic and neuroendocrine parameters in 15 obese patients (5 M, 10 F; age: 50.2±6.7; BMI: 34.7±3.8 kg/m2). Patients randomly received one session of high frequency (18 Hz), low frequency (1 Hz) or sham stimulation via an H-coil deep TMS. Prior to stimulation, the patients observed a series of palatable food images (cue). H-coil was targeted to Prefrontal Cortex and Insula, bilaterally. After an overnight fasting, blood was drawn before and after a single session of deep TMS.

Results: Blood pressure, heart rate, insulin, glucagon, cortisol, ghrelin, neuropeptide Y and GLP-1 did not show any changes during both 18 Hz and 1 Hz stimulations. After the 18 Hz TMS session, a significant increase in glucose levels (+8.2±3.6%, p=0.020) and a significant decrease in leptin levels (-15.6±3.9%, p=0.028) were found. A trend to rise in beta-endorphins levels (+26.0±20.7%, p=0.089) was also shown after 18 Hz TMS; this increase was significant when compared to sham (p=0.016). Following the 1 Hz TMS session, a reduction of epinephrine (-10.0 ±3.3%, p=0.037) was found. A significant reduction of prolactin levels was shown after both 18 Hz (-45.4±16.9%, p=0.036) and 1 Hz (-44.7 ±5.0%, p=0.017) TMS.

Conclusion: These findings suggest that deep TMS can acutely modulate orexygenic pathways, being a novel potential tool for the study of brain pathophysiology of obesity. The increase of beta-endorphins, induced by a single deep TMS session, could suggest an effect of TMS on the dopaminergic system activation. Therefore, a role of deep TMS, in acutely modulating food reward circuitry, should be hypothesized.

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Disclosure: **A. Ferrulli:** None.

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Up-regulation of proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) mRNAs by intermittent hypoxia in human neuronal cells

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Background and aims: Sleep apnea syndrome (SAS) is a highly prevalent sleep disorder characterized by repetitive episodes of oxygen desaturation during sleep, leading to exposure to alternation of low oxygen pressure and normal oxygen pressure, namely intermittent hypoxia (IH). Obesity is a predominant risk factor for SAS in addition to insulin resistance, hyperglycemia and type 2 diabetes. Accumulating evidence indicates that SAS, obesity and type 2 diabetes are strongly related with each other. The central nervous system (CNS), particularly the hypothalamic arcuate nucleus is known to control food intake and energy expenditure through close coordination between multiple neuronal populations to maintain body weight by the orexigenic and anorexigenic actions of the neuropeptides expressed in this area. In SAS patients, the direct effect of IH on the regulation of appetite and feeding behavior remains elusive. In the present study, using human neuronal cells and an in vitro IH system, we investigated the direct impact of IH, a hallmark of SAS, on the expression of major appetite regulatory neuropeptide and receptor genes.

Materials and methods: Human neuroblastoma NB-1, SH-SY5Y and SK-N-SH cells were exposed to IH (64 cycles of 5 min hypoxia (1% O_2) and 10 min normoxia (21% O_2)), mimicking neuronal cells of SAS patients, to normoxia, or to sustained hypoxia (SH) for 24 hours. After the treatment, the mRNA levels of appetite-related neuropeptide genes such as *proopiomelanocortin (POMC)*, *cocaine- and amphetamine-regulated transcript (CART)*, *galanin (GAL)*, *galanin-like peptide (GALP)*, *ghrelin (GHRL)*, *pyroglutamylated RFamide peptide (QRFP)*, *agouti-related peptide (AGRP)*, and *neuropeptide Y (NPY)* and a relevant receptor gene

melanocortin 4 receptor (MC4R) were measured by real-time RT-PCR. The reporter plasmids were constructed by inserting various lengths of *POMC* and *CART* promoter fragments upstream of a firefly luciferase reporter gene in pGL4.17 vector and were transfected into NB-1 cells for luciferase assay.

Results: IH significantly increased the mRNA levels of *POMC* (2.12 fold vs normoxia, P=0.0081; 1.62 fold vs SH, P=0.0287) and *CART* (4.78 fold vs normoxia, P<0.0001) in NB-1 cells, while the IH-specific increases were not observed in *GAL*, *GALP*, *GHRL*, *QRFP*, *AGRP*, *NPY* nor *MC4R*. Similar results that the mRNA levels of *POMC* and *CART* were increased by IH were observed in SH-SY5Y and SK-N-SH cells. Deletion analysis revealed that the -705 to -686 promoter region of the *POMC* gene and the -950 to -929 region of the *CART* gene are essential for the IH-induced *POMC* and *CART* promoter activity, and GATA factor binding consensus was located in each region.

Conclusion: These results indicate that IH-stress up-regulates the mRNA levels of *POMC* and *CART*, which are anorexigenic, in human neuronal cells via GATA binding sequence in the -705 to -686 region of *POMC* promoter and the -950 to -929 region of *CART* promoter. It is possible that the cyclic changes of hypoxia-reoxygenation in SAS patients act inhibitory for appetite and food intake at the level of transcription in the CNS.

Disclosure: **R. Shobatake:** None.

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Evaluation of hsCRP levels as a marker of cardiovascular risk in metabolically healthy obese individuals

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Background and aims: Metabolically Healthy Obese (MHO) characterises a subset of obese individuals who are free of any metabolic abnormalities. However, whether they will remain healthy in the long term or will develop metabolic syndrome in the future, is not clear yet. There is very limited data on cardiovascular (CV) risk markers in MHO individuals. We studied hsCRP levels as a marker for CV risk in MHO individuals in North Indian population. Our study aimed to: 1. Identify the metabolically healthy obese among the obese individuals coming to the hospital for preventive health check- up. 2. Evaluate CV risk in the metabolically healthy obese by measuring the hsCRP levels.

Materials and methods: All the individuals visiting our preventive health check-up facility were screened for overweight and obesity over 1 year period. Diagnosis of overweight and obesity was based on measure of Body Mass Index (BMI). BMI cut-off of less than 23kg/m2 was used to define normal weight, a value between 23-25kg/m2 indicated overweight, and more than 25kg/m2 defined obesity. In a case controlled study we included 120 metabolically healthy overweight and obese individuals(MHO) and 60 normal weight healthy controls from Preventive Health Check-up Department. Metabolic health was defined on the basis of normal values of fasting blood sugar, HbA1c, blood pressure, and lipid levels. All the subjects were evaluated for hsCRP levels.

Results: A total of 1624 patients visited the preventive health check-up department out of which 907 were overweight and obese (55.9%). Among the obese population, 192 were metabolically healthy comprising 11.8% of total population and 21.1% of obese population. A total of 120 MHO individulas and 60 normal weight healthy controls were taken up for study. All the subjects were 18-60 years of age. Control and study groups were well matched with slightly more males in both the groups. Of 120 subjects in the study group 94 had a BMI>25 and 26 had a BMI>23 but <25kg/m2. All subjects in the control group had a BMI<23kg/m2. Mean hsCRP in the study group was higher(2.77±1.94) as compared to control group(1.44±0.84) and was statistically significant(P value<0.0001). Within the study group, obese individuals had a significantly higher hsCRP levels(3.08±2.04) as compared to control group(1.44 ±0.84)(p90cms vs. 1.25±0.69 for males80cms vs. 1.65±0.95 for females<80cms, p=0.0001). There was no difference in hsCRP levels in males<90cms in study group vs control group(1.22±0.57 vs. 1.22±0.57, p=0.97) There was a strong correlation between hsCRP levels and BMI(r=0.46, p=0.0001) as well as hsCRP levels and waist circumference(r=0.28, p=0.002) in metabolically healthy obese individuals

Conclusion: Our study showed that hsCRP levels are raised in metabolically healthy obese individuals as compared to healthy normal weight individuals, indicating presence of vascular inflammation and that MHO may not be completely free of CV risk. Thus, our findings suggest that obesity per se in the absence of metabolic risk factors is not entirely benign and is in-fact associated with subclinical vascular inflammation and has increased cardiovascular risk.

Disclosure: **B.M. Makkar:** None.

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Clinical and anthropometric characteristics of sarcopenia in patients with type 2 diabetes and relevant risk factors

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Background and aims: Several studies have shown that type 2 diabetes is associated with increased risk of sarcopenia, which is considered as a major cause of frailty, falls and fractures in the elderly population. It may be essential to evaluate skeletal muscle mass and prevent sarcopenia in the management of elderly patients with type 2 diabetes. In this study we assessed the prevalence and characteristics of sarcopenia in patients with type 2 diabetes, and analyzed its underlying risk factors.

Materials and methods: The subjects consisted of 352 male and 245 female patients with type 2 diabetes, aged 62±11 years, with a BMI of 25.5±5.1 kg/m^2. Patients <40 years of age and those with malignant disease, massive proteinuria, severe liver disease and chronic pancreatitis were excluded. β-Cell function was assessed by C-peptide (CPR) responses to a standardized breakfast. Body composition was measured with a multi-frequency bioelectrical impedance analyzer (InBody^{TM}720). Sarcopenia was defined as a skeletal muscle index (total appendicular muscle mass/height^2) less than 7.0 kg/m^2 in men and 5.7 kg/m^2 in women according to the criteria by the Asian Working Group for Sarcopenia.

Results: The prevalence of sarcopenia was 32.6% in male and 30.2% in female patients. Sarcopenia increased with age in both sexes; 14.0%, 18.0%, 33.6%, 45.5%, and 66.7% in 40s, 50s, 60s, 70s, and 80s, respectively. Sarcopenic patients had lower BMI and lower body fat percentage than non-sarcopenic patients (21.4±2.8 vs. 27.4±4.9 kg/m^2 and 26.8±8.9 vs. 32.2±9.6%, respectively). Accordingly, they had higher HDL-cholesterol, lower triglyceride, lower IRI, and lower blood pressure. There was no significant difference in fasting plasma glucose or HbA1c. β-Cell function was significantly lower in patients with sarcopenia (p=0.008). Despite the fewer risk factors, the prevalence of vascular complications was comparable. Whereas no significant difference was found in daily physical activity between sarcopenic and non-sarcopenic subjects, sarcopenic patients had significantly lower serum levels of albumin and cholinesterase suggesting the presence of undernutrition. Furthermore, serum amylase levels were higher in sarcopenic subjects in both sexes. In males, the rate of habitual drinking tended to be higher in sarcopenic subjects (p=0.07), and those with habitual drinking had lower appendicular muscle mass (p=0.02). In females, sarcopenic subjects were more frequently positive for fecal fat than non-sarcopenic subjects (p=0.047), especially in patients <65 years of age (p=0.010).

Conclusion: Here we showed that although the prevalence of sarcopenia steeply increases with age, it is not a rare complication of type 2 diabetes

even in 40s. Our observations suggest that reduced β-cell function and undernutrition are major contributors to sarcopenia in patients with type 2 diabetes. Habitual drinking of alcoholic beverages may be a risk factor for sarcopenia in men, and pancreatic exocrine insufficiency in women. Physical inactivity was not associated with sarcopenia in this population, although it may create a vicious cycle and aggravate the loss of muscle. Nutritional management may be the cornerstone for the prevention of sarcopenia in diabetic subjects at high risk.

Disclosure: **H. Nakayama:** None.

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Real-world weight change among patients treated with GLP-1, SU, or DPP-4 therapy for type 2 diabetes and the influence of medication adherence

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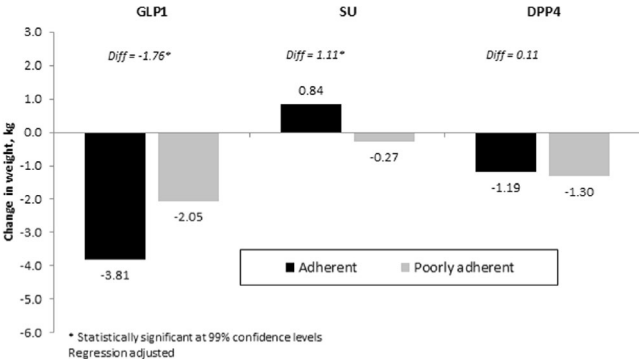
Background and aims: The study objective was to examine real-world weight change and the role of medication adherence in explaining variability in weight change among patients who initiated one of three drug classes: glucagon-like peptide 1 receptor agonist (GLP-1), dipeptidyl peptidase 4 inhibitor (DPP-4) and sulfonylureas (SU).

Materials and methods: This retrospective cohort study used a large US integrated EMR-administrative claims database to identify adult T2D patients who initiated GLP-1, DPP-4 or SU treatment. Adherence was defined as percent of days covered (PDC) ≥80% during the year following drug initiation. Patients who initiated treatment and continued it at some level as well as patients who may have discontinued the drug were included in the study. A sub-analysis compared very adherent (PDC≥90%) and very poorly adherent (PDC<50%) patients. Weight change was calculated from drug initiation (-180, + 30 days) to one year (±90 days) later; patients could contribute multiple observations. Multivariate regression controlled for baseline differences between adherent and poorly adherent patients (body mass index [BMI]), HBA1c, diabetes complications, T2D drug therapy), age and an indicator for additional therapy; standard errors were clustered to account for multiple observations per patient.

Results: The study included 833 GLP-1, 2,272 DPP-4 and 2,713 SU patients who contributed 2,279, 6,602 and 7,429 observations, respectively. Patients initiating a DPP-4 or SU were older (62 and 63 years), compared with GLP-1 (56 years, P<0.01). Baseline weight and BMI were highest among GLP-1 patients (110kg, 38kg/m2, P<0.01) compared with DPP-4 and SU (both 97 kg, 34kg/m2). Patients initiating a GLP-1 achieved the largest weight change (-2.46 kg GLP-1, -1.26 kg DPP-4, and 0.18 kg SU, P<0.01). After regression adjustment, the difference in weight change between adherent and poorly adherent GLP-1 patients remained significant (-3.81 vs -2.05 kg, P<0.01) (Figure). For DPP-4, weight loss was not significantly associated with adherence. SU patients achieved weight loss only if poorly adherent; adherent SU patients experienced weight gain. In the sub-analysis comparing very adherent and very poorly adherent patients, findings were accentuated, with larger weight changes associated with the level of adherence for GLP-1 and SU (both P<0.01) but non-significant differences in DPP-4 (P=0.36).

Conclusion: In the real-world setting, patients treated with GLP-1 experienced greater weight loss than those treated with SU or DPP-4. Greater adherence with GLP-1 treatment resulted in a greater level of weight loss. Adherence to DPP-4 did not have a significant association with weight change whereas adherence to SU was significantly associated with weight gain.



Disclosure: **G.S. Carls:** Employment/Consultancy; Intarcia Therapeutics (consultant).

PS 049 Fat distribution in humans

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Contribution of a first-degree family history of diabetes to increased serum adipocyte fatty acid binding protein levels independent of body fat content and distribution

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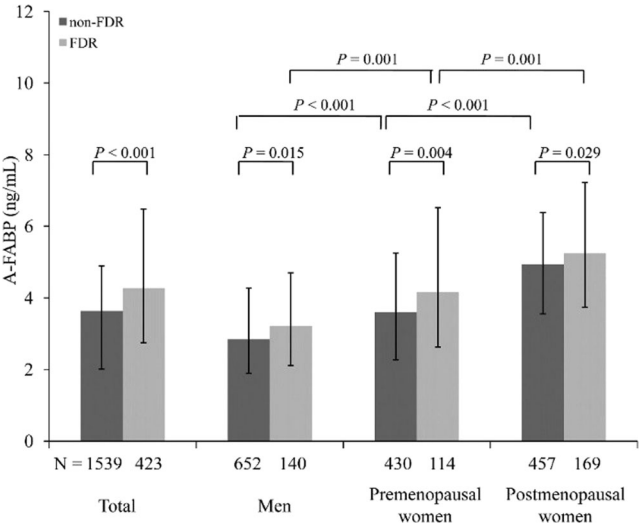
Background and aims: First-degree relatives of patients with type 2 diabetes mellitus (FDRs) bear an increased risk of diabetes, overweight/obesity, and cardiovascular disease. Accumulating evidence indicates that circulating concentrations of several adipokines are altered in FDRs, but the adipokine adipocyte fatty acid binding protein (A-FABP) has been rarely studied in FDRs. The present study explored the association between a first-degree family history of diabetes (FHD) and serum A-FABP levels.
Materials and methods: A total of 1962 normoglycemic participants were divided into subgroups of men, premenopausal women, and postmenopausal women. Serum A-FABP levels were measured using a sandwich enzyme-linked immunoabsorbent assay. Abdominal fat distribution, including visceral fat area and subcutaneous fat area, was assessed by magnetic resonance imaging.
Results: Totals of 792 men, 544 premenopausal women, and 626 postmenopausal women were enrolled. Serum A-FABP levels were much higher in FDRs than in those without a FHD in the entire study population and in all subgroups (all $P < 0.05$). Logistic regression analysis revealed an independent and positive relationship between a first-degree FHD and serum A-FABP levels in men ($P = 0.029$), premenopausal women ($P = 0.036$), and postmenopausal women ($P = 0.008$). Multiple stepwise regression analysis showed that a first-degree FHD was an independent factor positively associated with serum A-FABP levels in men (standardized $\beta = 0.068$, $P = 0.029$), premenopausal women (standardized $\beta = 0.090$, $P = 0.018$), and postmenopausal women (standardized $\beta = 0.102$, $P = 0.004$).
Conclusion: Serum A-FABP levels were increased significantly in normoglycemic FDRs. The contribution of the first-degree FHD to the elevated serum A-FABP levels was independent of total body fat content and abdominal fat distribution. Thus, use of serum A-FABP as a biomarker in FDRs may result in overestimation of the risk of obesity-induced metabolic disease and cardiovascular disease.



Supported by: 973 Program of China; Grant from Shanghai HFPC; Project of NSFC
Disclosure: **Y. Bao:** None.

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RSPO3 functions via LGR4 to regulate human body fat distribution by eliciting diverse biological responses in abdominal and gluteal progenitors

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Background and aims: Body fat distribution is a heritable trait that independently predicts type 2 diabetes and cardiovascular risk. Genome-wide association studies (GWAS) meta-analyses have identified sexually dimorphic associations, with greater effect in women, between loci within RSPO3 (e.g. rs9491696) and BMI-adjusted waist-to-hip ratio (WHR). RSPO3 is a LGR4 receptor ligand and a Wnt/β-catenin signalling agonist. Our aim was to translate genetic findings into functional insights.
Materials and methods: 1. We performed genotyping (n=3544) and gene expression studies of RSPO3 in paired subcutaneous (SC) abdominal and gluteal fat biopsies from 103 women and 97 men from the Oxford Biobank to determine if rs9491696 was associated with changes in dual-energy X-ray absorptiometry-determined adipose tissue (AT) distribution and RSPO3 expression. 2. We knocked-down (KD) and over-expressed RSPO3 and LGR4 in SC abdominal and gluteal adipose progenitors (APs) and determined their effects on proliferation and adipogenesis (lipid staining and adipogenic gene expression). 3. We studied an rspo3 loss-of-function zebrafish mutant to determine if RSPO3 modulates AT biology in vivo.
Results: Consistent with a role in modulating regional adiposity, RSPO3 expression was higher in abdominal vs gluteal AT (2.7-fold, $p<0.001$, paired t-test) and in males vs females (1.5-fold, $p<0.001$ for abdominal AT, 1.4-fold, $p=0.03$ for gluteal AT, t-test). The WHR-increasing allele (G) at rs9491696 was associated with ~2-fold higher RSPO3 expression in both abdominal ($p<0.001$, t-test) and gluteal ($p<0.02$, t-test) AT, despite being associated with increased android and reduced leg fat mass. Accordingly, RSPO3 had distinct effects on abdominal and gluteal AP biology due, in part, to differential modulation of Wnt/β-catenin signalling. Specifically, ectopic RSPO3 expression led to increased proliferation selectively in abdominal APs (doubling time, T_d, 32.3±1.6h vs 34.1±2.1h in control APs; $p=0.03$, paired t-test). Adipogenesis was impaired in both abdominal and gluteal RSPO3 over-expressing cells ($p<0.001$, t-test). Reciprocally, RSPO3 KD impaired proliferation only in abdominal APs (T_d 32.9±1.5h vs 26±1.1h in control APs; $p<0.001$, paired t-test) whilst promoting adipogenesis more robustly in gluteal cells (5- vs 1.7-fold in abdominal APs; $p<0.001$, t-test). LGR4 KD replicated the effects of RSPO3 KD on AP proliferation (T_d of 50.2±3.9h vs 34.7±0.9h in LGR4 KD and control abdominal APs, respectively; $p=0.004$, paired t-test) and adipogenesis (2.5- and 1.3-fold increase for gluteal and abdominal LGR4 KD cells, respectively; $p<0.001$, t-test). In keeping with GWAS and in vitro data, a single bp mutation in zebrafish rspo3, which resulted in ~70-97% reduction in AT rspo3 mRNA levels, led to increased generalised adiposity and enhanced 'peripheral' body fat distribution in female homozygous mutants vs wildtype siblings ($p<0.001$, t-test).
Conclusion: RSPO3 signals through LGR4 to differentially regulate abdominal and gluteal adipose progenitor cell proliferation and adipogenesis, thereby modulating body fat distribution.
Supported by: BHF, EFSD, NIH
Disclosure: **N.Y. Loh:** None.

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Phenotypical heterogeneity linked to adipose tissue dysfunction in patients with type 2 diabetes

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Background and aims: Adipose tissue (AT) inflammation, in condition of caloric excess, leads to increased free fatty acids efflux and ectopic fat deposition. Whether AT dysfunction drives to selective fat accumulation in specific sites remains unknown. Aim of this study was to investigate the association between AT dysfunction, hepatic/pancreatic fat accumulation and the associated metabolic phenotype in patients with type 2 diabetes (T2D).
Materials and methods: For this cross-sectional study we enrolled sixty-five consecutive overweight and obese patients affected by T2D. Study population underwent clinical examination and blood sampling for routine biochemistry and for estimating insulin secretion (HOMA-β%), whole-body and AT insulin-resistance (HOMA-IR, ADIPO-IR). Subcutaneous (SAT) and visceral (VAT) adipose tissue area, hepatic (HFF) and pancreatic (PFF) fat fraction were determined by magnetic resonance (MRI).
Results: 55.4% of T2D patients had MRI-diagnosed NAFLD; they were significantly younger and insulin-resistant than non-NAFLD patients. ADIPO-IR was the main determinant of HHF independent of age, sex, HOMA-IR, VAT and SAT and was highly predictive of severe NAFLD with AUROC=0.796 (C.I. 95%: 0.65-0.94, p= 0.001), for ADIPO-IR ≥6.9 mmol/L·μU/mL with sensitivity: 84.6% and specificity: 79.1%. The PFF was independently associated with increased total adiposity (r2= 0.32, p= 0.012), but did not correlate with AT dysfunction, insulin resistance and secretion or NAFLD in T2D patients.
Conclusion: The ADIPO-IR index was capable of predicting NAFLD independent of all confounders whereas it did not seem to be related to intrapancreatic fat deposition; unlike HFF, higher PFF was not associated with relevant alterations in the metabolic profile. In conclusion, variable degrees of AT dysfunction in T2D patients are associated with a heterogeneous distribution of ectopic fat and with a different metabolic profile. Therefore, evaluation of AT dysfunction may contribute to identify different risk profiles among T2D patients.
Supported by: SID Fellowship; Sapienza University Ateneo Scientific Research
Disclosure: **I. Barchetta:** None.

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Lipidomic profiling of human fat tissues in subjects with type 2 diabetes and normal glucose tolerance

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Background and aims: The accumulation of ectopic fat is related with the occurrence of type 2 diabetes mellitus (T2DM). However, underlying mechanisms are poorly understood and there is limited data of lipidomic signatures of human fat tissues.
Materials and methods: We investigated the differences of subcutaneous, visceral and pericardial lipidomes and compared their lipidomic signatures between subjects with normal glucose tolerance (NGT) and T2DM. Liquid chromatography/mass spectrometry was used for lipid profiling in different fat tissues from patients with NGT (n = 22) and T2DM (n = 27) who underwent coronary artery bypass graft. Principal component analysis was performed to compare lipidomic signatures between tissues and between patient groups.
Results: Total 26 lipid classes and more than 400 lipid species were identified. The diacylglycerol (DG), dihydroceramide, and sphinganine in the visceral fat tissue were significantly lower in the T2DM group than the NGT group. However, the DG level in pericardial fat tissue was higher in the T2DM group than the NGT group. At the lipid species level, lipidomic signatures of DG, and lysophosphatidylserine distinguished the presence of T2DM.
Conclusion: In this lipidomic analysis, the selective enrichment of lipidomic signatures was observed between T2DM and NGT. Further validation will provide a novel insight for characteristics of ectopic fat in subjects with T2DM.
Disclosure: **S. Choi:** None.

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Validation of circulating Wnt1 inducible signalling pathway protein 1 (WISP1) as a novel biomarker of visceral obesity and associated diseases

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Background and aims: The secreted extracellular matrix protein WISP1 (WNT1 inducible signaling pathway protein 1) is a novel adipokine that induces a pro-inflammatory response in macrophages. Our aims were 1) to assess the WISP1 reliability, as expressed by intraclass correlation coefficient (ICC), over a 4-month period of time in a population-based sample of healthy individuals (n=207) within the European Prospective Investigation into Cancer and Nutrition (EPIC) -Potsdam Cohort (2007-2008) and 2) to evaluate its suitability as a circulating marker for visceral adiposity and associated diseases in individuals with different stages of glucose tolerance (n=172).
Materials and methods: Circulating plasma and serum WISP1 concentrations were measured using commercially available assay kits (Human WISP-1/CCN4 DuoSet ELISA; R&D Systems, Wiesbaden, Germany).
Results: In the EPIC-Potsdam study among individuals without apparent metabolic diseases, plasma concentrations of WISP1 were detectable in 27 individuals (13% of the full sample). There was a pronounced difference in WISP1 concentrations according to sex with male individuals having higher concentrations compared to females (155.5±190.4 pg/ml vs. 55.0±58.9 pg/ml, respectively, NS). The ICC of plasma WISP1 concentrations over a 4-month period was 0.93 (95% CI: 0.84-0.96) in all individuals, 0.94 (95% CI: 0.78-0.98) in men and 0.91 (95% CI: 0.70-0.97) in women. In serum samples of the second study cohort among metabolically afflicted participants, 34% of subjects have values under detection limit of assay. Similarly to observations within EPIC, male individuals showed a trend to elevated WISP1 levelss compared to females (100.4±16.6 pg/ml vs. 86.4±11.9 pg/ml, respectively, NS). A linear regression model with backward elimination revealed as independent determinants of WISP1 glycated hemoglobin (ß=-0.335, p=0.004) and low-density cholesterol (ß=0.219, p=0.056). There were no apparent differences in WISP1 concentrations between individuals with normal glucose tolerance and type 2 diabetes.
Conclusion: Circulating plasma WISP1 showed high reliability in the EPIC-Potsdam study. Serum samples seem to be particularly promising for detection of WISP1. The high number of values below the limit of detection in healthy subjects suggests that WISP1 is possibly sex related "disease marker".
Supported by: German Center for Diabetes Research (DZD)
Disclosure: **C. Tacke:** Grants; German Center for Diabetes Research (DZD).

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Impact of IRS1 promoter methylation,mRNA expression and the SNP variant rs2943650 on fat distribution and metabolic traits in human subcutaneous and visceral fat depots

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Background and aims: Body fat composition is a much more sensitive measure in predicting obesity associated co-morbidities than BMI. Previously, a SNP variant near the *IRS1* gene locus was found to be associated with decreased body fat, *IRS1* gene expression levels and an adverse metabolic profile in humans. We hypothesized that these effects may be mediated by epigenetic alterations.
Materials and methods: We investigated DNA methylation levels of the *IRS1* promoter in paired samples of SAT and OVAT from 146 Caucasian individuals including gene expression data for a subgroup of 60 subjects. Genotyping of the body fat associated variant rs2943650 near *IRS1* was performed in all individuals (N=146). We tested for i) differential DNA methylation and mRNA expression in SAT vs. OVAT and for ii) correlation of DNA methylation with gene expression levels in the same tissues. Furthermore, we analyzed the association of the rs2943650 effect allele (T) with both methylation and expression. iii) Finally, we performed bivariate correlation analysis to assess potential relationships between DNA methylation, mRNA expression and rs2943650 genotypes and metabolic and anthropometric variables.
Results: In the total cohort, we observed a significantly higher *IRS1* promoter DNA methylation in VAT compared to SAT ($P<0.0001$), while expression levels show the opposite effect directions. VAT methylation levels are nominally significantly correlated to VAT expression. The rs2943650 T-allele was nominally significantly associated with increased DNA methylation in VAT and mRNA expression in SAT. Finally, we found nominally significant associations of DNA methylation, gene expression and rs2943650 genotypes with several clinical variables including waist, waist-to-hip ratio (WHR) and CT-ratio.
Conclusion: Our data suggest that body fat composition and related metabolic variables may be influenced, in addition to and in concert with genetic factors, also by epigenetic modifications such as DNA methylation. Functional data are warranted to further understand the interplay between genetic variants, DNA methylation and gene expression of *IRS1* and its influence on metabolic traits.
Supported by: DDG;DDS;IFBK6e-96;EFSD/NovoNordisk;BMBF; FKZ01EO1501;FKZ 01GI1128;SFB 1052/1
Disclosure: **K. Rohde:** None.

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Adipose tissue dysfunction and metabolic syndrome among childhood acute leukaemia survivors

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Background and aims: Long-term survivors of childhood acute leukemia are at very high risk for cardiovascular diseases (CVD). Metabolic syndrome (MS), a major risk factor for CVD, is a common complication in this population, especially for patients who received total body irradiation (TBI). Consequences of radiation exposure on adipose tissue (AT) in human in unknown.
Materials and methods: To assess impact of TBI on AT, we compared the morphological and functional characteristics of AT between long-term survivors of childhood acute leukemia (patients from LEA cohort) with MS who received TBI (n=12) or not (n=12). We analyzed AT repartition by DEXA and MRI, and TG content in the liver by proton magnetic resonance spectroscopy. We performed needle-aspirated of abdominal subcutaneous AT and studied characteristics of this tissue using RT-qPCR, flow cytometry and preadipocytes' cultures.
Results: Mean age was 32 years old. Patients who received TBI had more components of MS (and components were more pronounced) than those without TBI, despite a trend for a lower body mass index and waist circumference. Hepatic triglycerides content was two time higher in TBI group. No difference was found in body composition (DEXA) and visceral and subcutaneous fat surface (IRM) between the two groups. Subcutaneous AT gene expression of fibrosis and lipidic fat storage was significantly lower in AT of TBI group. Furthermore, AT gene expression of several genes involved in preadipocytes' differentiation were decreased in TBI group.
Conclusion: These functional abnormalities of subcutaneous AT observed in patients treated with TBI could be the cause of ectopic fat storage in the liver, increasing the risk of Insulin resistance and MS.
Supported by: AORC AP-HM
Disclosure: **S. Béliard:** None.

PS 050 Inflammation in metabolism

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An inducer of glyoxalase 1 down regulates inflammatory gene expression in overweight and obese non-diabetic subjects

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Background and aims: Glo1 deficiency was identified as a driver of cardiovascular disease in a large integrative genomics study. Induction of glyoxalase 1 (Glo1) expression is a novel strategy to prevent inflammatory signalling and decrease risk of cardiovascular disease in overweight and obese populations. Increasing Glo1 expression is unaddressed by current therapy. Glo1 is part of the glyoxalase metabolic pathway which catalyses the metabolism of the reactive metabolite and glycating agent, methylglyoxal (MG), and thereby prevents formation of advanced glycation endproducts (AGEs). We previously described a regulatory antioxidant response element in the GLO1 gene which, when bound by transcription factor Nrf2, increases basal and inducible expression of Glo1. We screened dietary bioactive compounds for Glo1 inducer activity, confirmed hits and improvement of cell function in human cell primary cultures. The aim of this study was to validate target pharmacology of an optimised Glo1 inducer formulation in Phase 1 clinical trial in overweight and obese subjects and assess inflammatory gene expression in peripheral blood mononuclear cells (PBMCs).
Materials and methods: The Glo1 inducer formulation was evaluated in a randomised, placebo-controlled, double-blinded crossover clinical trial in overweight and obese subjects. Treatment was with Glo1 inducer or placebo once daily for 8 weeks with 6 week washout period before crossover. Thirty-two subjects were recruited and 29 completed the study. Data are analysed per protocol. PBMCs were collected at baseline and post-supplementation, RNA extracted and relative mRNA copy number analysed by the NanoString nCounter method. Significance testing in paired data was assessed by Wilcoxon signed-ranks test.
Results: Subject characteristics at baseline were: age 45 ± 13 years, gender (M/F) 8/21, body mass index (BMI; kg/m2) 30.0 ± 3.8 (18 overweight and 11 obese subjects), A1C 36.2 ± 4.3 mmol/mol Hb (5.5 ± 0.7%) and prediabetes (Y/N) 9/20 and hypertension (Y/N) 11/18. In highly overweight subjects (BMI >27.5 kg/m2, n = 20), the Glo1 inducer formulation increased PBMC Glo1 activity +27%, P<0.05. In all subjects there was increased PBMC expression of GLO1 (+6%), decreased expression of the HIF1A (-6%) and inflammation linked genes, IL8 (-39%) and PTGS2 or COX2 (-30%); P<0.05. In highly overweight subjects (n = 20) there was also decreased expression of FTH1 (-19%), RAGE or AGER (-37%) and MCP-1 or CCL2 (-49%); and in the obese subgroup (N = 11) also decreased expression of KEAP1 (-18%) and TNFA (-12%); P<0.05. Vascular inflammatory proteins were also assessed. In all subjects, Glo1 inducer decreased vascular inflammation marker sICAM1 (-10%, P<0.01). There was no effect of the placebo.
Conclusion: The Glo1 inducer increased activity and expression of Glo1, It decreased gene expression linked to hypoxia and vascular and tissue inflammation - including marked down regulation of RAGE, MCP1 and COX2. The Glo1 inducer could be a suitable treatment for improved metabolic and vascular health in overweight and obese populations.
Clinical Trial Registration Number: NCT02095873
Supported by: Unilever and Innovate UK (Project no 101129).
Disclosure: **M. Xue:** Yes.

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Detection of diabetes specific autoimmunity in obese subjects regardless the presence of diabetes

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Background and aims: We have recently demonstrated that the frequency of autoantibodies (Abs) directed against the 256-760 domain of the tyrosine phosphatase 2 increases by increasing the degree of obesity in adult autoimmune diabetes (LADA), hypothesizing that humoral autoimmune response may be secondary to the low-grade inflammation that characterizes visceral obesity. The aim of the present study was to evaluate, in a population of adult obese individuals with or without type 2 diabetes (T2D), the frequency of humoral immunoreactivities characteristic of type 1 diabetes (T1D).
Materials and methods: N= 978 subjects: 444 obese (OB) subjects (173m/271f; median age 42 years), 322 obese patients with T2D (OB-T2D) (127m/195m; median age 52 years) and 212 controls (CTRL) (90m/122f; median age 46 years) were analysed. Glutamic acid decarboxylase (GAD), tyrosine phosphatase 2 (IA-2ic(605-979) and e IA-2256-760) and Zinc cation efflux transporter (ZnT8) autoantibodies (Abs), were detected by using a radioimmunoprecipitation assay.
Results: Overall 20/444 (4.5%) OB, 12/322 (3.7%) OB-T2D and 4/212 controls (1.8%) were positive for one or more of the Abs investigated. OB patients were positive for at least one among GAD (5/20, 25%), IA-2(256-760) (14/20, 70%) and ZnT8 Abs (1/20, 5%), whereas no positivity was found for IA-2ic(605-979) Abs (0/20); interestingly, the IA-2(256-760)Abs were significantly more frequent than GAD-, IA-2ic(605-979)- and ZnT8-Abs (p=0.01, p<0.0001 and p<0.0001, respectively). OB-T2D patients were positive for all four diabetes-specific Abs, the most frequent was GAD (8/12, 66.7%), IA-2(256-760) (4/12, 33.3%), IA-2ic (605-979) (4/12, 33.3%) and ZnT8 antibodies (2/12, 16.7%). In OB-T2D GAD were significantly more frequent than ZnT8 Abs (p=0.036), but not than IA-2(256-760) or IA-2ic(605-979) Abs. Comparing the two groups, GAD and IA-2ic(605-979) antibodies were more frequent in OB-T2D than in OB subjects ((p = 0.03 and p = 0.014, respectively).
Conclusion: Obesity does not seem to be a "passive bystander" in the development of autoimmunity, IA-2(256-760)Abs appear as the main marker of diabetes-specific autoimmunity in obese subjects without diabetes and its presence could underline a mechanism of eliciting a humoral immune response possibly secondary to chronic low-grade systemic inflammation associated with obesity.
Supported by: an unconditioned grant from Novo Nordisk
Disclosure: **C. Tiberti:** None.

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TLR4 deficiency ameliorates ageing-dependent, diet-induced inflammation, glucose intolerance and pancreatic beta cell failure

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Background and aims: Ageing is a major risk factor for the development of type 2 diabetes (T2D), and it has been shown that ageing is coupled with reduced insulin secretion and insulin action in both human and animal studies. Toll-like receptor-4 (TLR-4) signaling is one of the major pro-inflammatory pathway; its ligands as well as downstream products are increased systemically in patients with T2D as well as in at-risk individuals. TLR4 knockout mice are protected from the metabolic consequences of a high fat diet. In the present study we investigated the effect of high fat diet feeding and TLR4 deficiency on aged mice, with a special focus on beta-cell function and survival and tissue inflammation.

Materials and methods: C57BL/10ScCr (TLR4 knockout; TLR4-KO) and wild type (WT) male mice comprising of young (1-2 months old) and old (12-14 months old) mice were fed with normal chow diet (ND) or high fat/high sucrose diet (HFD) for a period of 8 weeks. Intraperitoneal glucose tolerance (ipGTT), glucose stimulated insulin secretion (GSIS) and insulin tolerance tests (ipITT) were performed to evaluate glycemia, insulin secretion and insulin sensitivity. mRNA from adipose tissue, liver and pancreatic islets was extracted and cDNA was analyzed by real-time PCR for pro- and anti-inflammatory cytokines and macrophage markers. beta-cell mass was measured by immunostaining for insulin.
Results: In vivo ipGTT, ipITT and GSIS showed impaired glucose and insulin tolerance by both ageing and HFD, with the most intolerance observed in old mice fed with a HFD, while TLR4-KO mice displayed improved insulin secretion and glucose and insulin tolerance. Young mice fed a HFD showed a compensation in beta-cell mass, which was lost in old HFD-fed mice, whereas TLR4 deficiency restored such compensation. This was paralleled with augmented cytokine and macrophage marker mRNA expression in the adipose tissue of TLR4-KO mice. HFD-fed young mice expressed IL-1beta and the general macrophage markers CD68 and Emr1, while in old mice, chemokine CCL2, pan-myeloid cell marker CD11b, and M1 macrophage marker CD11c were additionally induced and the anti-inflammatory cytokine IL-10 was reduced. TLR4-KO was able to inhibit IL-1beta in HFD-fed young and old mice. In addition, expression of IL-6, TNFalpha, CCL2, CD11c was inhibited in old TLR4-KO mice together with an increase in IL-10. In liver and pancreatic islets, the pattern of enhanced inflammatory cytokines in old HFD-fed mice was similar to that in fat. Noteworthy, TLR4-KO increased liver M2 macrophage marker CD206 and Arg1 expression in the old HFD, compared to the old HFD WT mice.
Conclusion: Our results show that HFD feeding could jeopardize glucose and insulin tolerance and beta-cell function, with a notably stronger impairment in old mice, while TLR4 deficiency was able to ameliorate such impairment. Under the HFD feeding, fat tissue of old mice was more inflamed compared to that of young mice, with characteristical higher pro-inflammatory cytokine expression and more pro-inflammatory macrophages. TLR4 deficiency inhibited inflammation and shifted macrophages to more anti-inflammatory, which suggests its pharmaceutical potential in treating T2D.
Disclosure: **W. He:** None.

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Identification and characterisation of miRNAs as potential modulators of TNF-induced insulin resistance in obesity

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Background and aims: Adipose tissue secretes multiple cytokines and adipokines which can cause the complications of obesity, especially insulin resistance (IR). Among others, TNF-α, has been identified as one of the key players of IR. Although several miRNAs are thought to be involved in the development of adipose tissue IR, the role of miRNAs in the association between inflammation and IR is poorly understood. MicroRNAs are post-transcriptional regulatory molecules which mediate diverse biological processes. Dysregulation of miRNAs may contribute to metabolic abnormalities, suggesting that miRNAs may potentially serve as therapeutic targets for ameliorating or preventing obesity related disorders. Our aim is to search for validated microRNAs directly implicated in obesity and TNFα induced IR and to elucidate the mechanisms of those selected miRNAs within the obesity and IR context.
Materials and methods: Biopsies from subcutaneous and visceral adipose tissue from Subjects: n=28 BMI<30 and n=30 BMI≥30 with different degree of glucose tolerance. For in vitro studies: human preadipocytes, SGBS adipocytic cell line and the THP-1 human macrophage cell line were used. Real time PCR from human biopsies for the selected validated miRNAS: miR-149-5p, miR-181a-5p, and miR-23a-3p were assessed. Human preadipocytes and SGBS cell line were differentiated to mature adipocytes in vitro and subjected to TNFα inflammatory stimuli and processed to perform by miRNA expression. Functional analyses were performed in vitro in the SGBS cells and in THP-1 M1 macrophage using miRNA mimics and not target control siRNA as negative control. The transfection reagent Lipofectamine 2000 was used to transfect mature adipocytes at day 10 of differentiation as well as M1-polarized THP-1 macrophages with the selected miRNA mimics. Western blot analysis was performed for key molecules of the insulin pathway in the SGBS protein extracts. p-IRS-1, pAKT, PTEN and pAS160 antibodies were used in the western blots. qRT-PCR technique was used for the detection of expression levels of TNFα, IL1-β, IL-8 as phenotype markers on the M1-polarized THP-1 cells.
Results: miR-149-5p, miR-181a-5p and miR-23a-5p expression in adipose tissue was found inversely correlated to adiposity and HOMA-IR, while only miR-23a-5p was negatively correlated with TNFα mRNA tissue expression levels. In vitro, TNFα stimulus over human adipocytes decreased miR-149-5p and miR-181a-5p expression. Functional analysis disclosed that miR-181a-5p over-expression significantly increased phosphorilation of IRS-1, AKT and AS160 in human SGBS mature adipocytes, whereas in M1 polarized THP1 macrophages, forced expression of miR-149-5p impaired the production of TNF-α.
Conclusion: Collectively, our data highlights a role of miR-149-5p in ameliorating inflammation and a role of miR-181a-5p in modulating insulin-resistance in the context of obesity.
Supported by: ISCIII, FEDER, IISPV, ICS, CIBERDEM
Disclosure: **J. Lozano Bartolomé:** None.

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Visceral adipose tissue NK cells contribute to obesity-associated insulin resistance through macrophage polarisation and low-grade inflammation

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Background and aims: Induction of insulin resistance is a key pathway through which obesity increases risk of type 2 diabetes. Although the effects of obesity on insulin sensitivity are incompletely understood, inflammation induced by an enhanced ratio of inflammatory "M1" macrophages to regulatory "M2" macrophages in visceral adipose tissue (vAT) is thought be crucial. Recently, it has been shown in mice that early NK cell accumulation in vAT is crucial for inflammatory macrophage accumulation and insulin resistance development. However, it is unknown whether NK cells play a role in the etiology of chronic vAT inflammation and insulin resistance in humans.
Materials and methods: In a first study, we investigated whether vAT NK cells are reflected by CD11b expression on blood NK cells and whether they associate with inflammatory macrophage polarisation in vAT, defined as CD11c+ M1 to CD11C- M2 macrophage ratio. Flow cytometry was performed on blood and vAT from 17 lean and 27 obese, age-matched men to quantify CD11b expression on circulating NK cells, measure NK cell numbers in vAT, and to determine vAT macrophage subsets. In a second study, we investigated whether CD11b expression on circulating NK cells, as a reflection of vAT NK cells, is associated with vAT volume on the one hand and with low-grade inflammation and insulin resistance on the other. In this study, we included 53 non-smoking, abdominally obese men (waist circumference 102-110 cm; aged 18-65;

no CVD or T2DM) and 25 lean men (waist circumference <94 cm). VAT volume was determined through T1-weighted TSE MRI, low-grade inflammation by TNF levels, and insulin sensitivity as whole body glucose disposal (WBGD) during a 1 mU/kg/min euglycaemic insulin clamp. Studies were reviewed by the Local Ethics Committee and were performed in accordance with the ethical standards laid down in the Helsinki Declaration.

Results: In the first study, we found that CD11b expression on NK cells associated with the number of vAT NK cells ($r = 0.598$, $p < 0.001$). CD11b expression on circulating cells ($r = 0.364$, $p < 0.05$) and NK cell numbers in vAT ($r = 0.395$, $p < 0.05$) were associated with M1/M2 ratio in vAT. The second study showed an association between vAT volume and CD11b expression on circulating NK cells ($r = 0.292$, $p < 0.05$). In addition, CD11b expression on circulating NK cells was associated with both plasma TNF levels ($r = 0.459$, $p < 0.001$) and WBGD ($r = -0.255$, $p < 0.05$). Moreover, mediation analysis showed that the association between vAT volume and whole body glucose disposal ($\beta = -1.575$, $p < 0.001$) is partially explained by the NK cell-TNF axis ($\beta = -0.063$; -0.072 to -0.008).

Conclusion: CD11b expression on circulating NK cells reflects vAT NK cells and associates with vAT volume, inflammatory macrophage polarisation, low-grade inflammation, and insulin resistance. Moreover, the NK cell-TNF axis mediates the association between vAT and insulin resistance, suggesting an important contribution of NK cell accumulation in vAT to obesity-associated insulin resistance through macrophage polarisation and subsequent low-grade inflammation.

Clinical Trial Registration Number: NCT01675401

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Disclosure: **K. Wouters:** Grants; NWO-Veni, Netherlands Heart Foundation, FP7, and research grant CH001 from the Top Institute Food and Nutrition.

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CD93: a novel causal factor in type 2 diabetes

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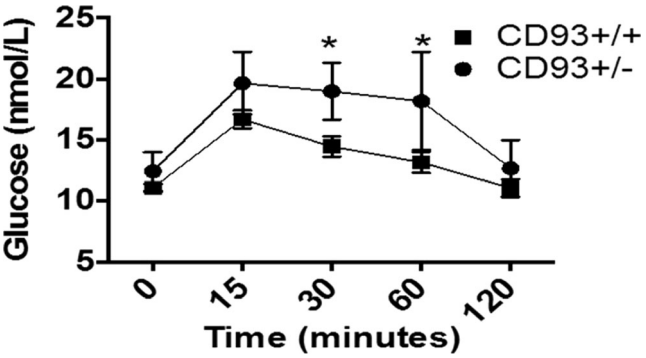
Background and aims: The exact mechanisms by which type 2 diabetes (T2D) leads to increased risk of cardiovascular disease (CVD) are poorly understood. Whilst genetic studies have identified many variants which influence risk of T2D or CVD, there is surprisingly little overlap. Both diseases involve a complex interaction between metabolic and immune functions, thus proteins involved in both processes are potential candidates for preventative therapies. One such molecule is CD93, first described as a component of the complement cascade. CD93 is also suggested as the candidate gene at a mouse diabetes locus and has been implicated in pathways common to T2D and CVD. In addition, levels of the soluble fragment of CD93 have been associated with myocardial infarction. The aim was to determine whether levels of soluble CD93 (sCD93) influence early atherosclerosis or glucometabolic processes.

Materials and methods: Epidemiological studies were conducted using the prospective IMPROVE study (for early atherosclerosis and T2D) and the Stockholm Diabetes Prevention Program (SDPP, for T2D). sCD93 levels were measured using the MesoScale platform. In parallel, a *cd93*-knockout mouse model was generated on a genetically pure background. For metabolic studies, the mice were fed a high fat diet. For atherosclerosis studies the mice were crossed wth *apoe*-knockout mice for 6 generations and fed a high fat diet. Standard statistical methods were applied and $p<0.05$ considered significant.

Results: In the IMPROVE study, there was no asssociation between levels of sCD93 and measures of vascular changes associated with atherosclerosis at baseline or 30 months followup, but a reduction in levels of sCD93 was observed in subjects with T2D (157±40ng/mL) compared to those without (164±45ng/mL, $p<0.0001$). The SDPP cohort confirmed that baseline levels of sCD93 in normal glucose tolerant subjects were reduced in subjects who went on to develop T2D (158±45ng/mL) compared to those who remained normal glucose tolerant (166±44ng/mL, $p=0.016$). The mouse studies were consistent with the human studies, in that cd93-deficient mice did not differ from the wildtype, with respect to atherosclerosis development, however they did demonstrate reduced glucose tolerance (Figure), despite no difference in weight.

Conclusion: CD93 is a novel component of glucometabolic regulation, which influences CVD endpoints (previously reported), but not early vascular changes



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The increase of klotho after weight loss is most pronounced in insulin sensitive patients and is associated with a decrease in inflammation

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Background and aims: Klotho, a gene originally identified in 1997 that encodes a novel protein, has been postulated as a regulator of the human aging process. Deletion of Klotho causes a phenotype similar to premature human aging, including endothelial dysfunction, progressive atherosclerosis, and even a shortened lifespan. Furthermore in vitro studies suggest that Klotho might have an important role in adipocyte turnover as well as in glucose metabolism. Therefore it was of interest to investigate Klotho levels in patients before and after weight loss induced by bariatric surgery (BS).

Materials and methods: We investigated 52 patients (median age: 39 (33-46) years; median BMI: 45(41-50) kg/m^2) with morbid obesity (MO) and 18 controls (CO; 30 (25-34) years; 23 (22-25) kg/m^2). Weight, cardiovascular risk factors, glucose tolerance tests, renal and inflammatory parameters, as well as Klotho levels were obtained.

Results: Morbidly obese patients exhibited significantly lower Klotho levels than healthy controls (565.4 [444.7 - 701.7] pg/ml vs. 817.41 [669.1 - 1228] pg/ml, $p=0.001$). When comparing pre-surgery with post-surgery values, Klotho significantly increased to 617.2 (502.1 - 777.6) pg/ml ($p=0.014$). In a correlation analysis, delta Klotho levels only correlated with delta HOMA-IR ($r=0.331$, $p=0.034$). We found that

patients with an HbA1c below the median of 5.7 rel. % at baseline showed a much steeper increase in Klotho levels after surgery compared to patients with an HbA1c ≥ 5.7 rel. % (p=0.005). In the first group Klotho levels increased from 564.9 (442 - 699.5) to 647 (503.4 to 890.5) pg/ml while in the latter, Klotho levels remained unaltered with 574.5 (434.2 - 713.8) pg/ml before and 583.4 (484.9 - 681.8) pg/ml after surgery. In those with HbA1c below 5.7% delta Klotho was significantly correlated with delta C-reactive protein (r=-0.445, p=0.049).

Conclusion: We hypothesize, that the decrease of inflammation is only associated with an increase in Klotho levels in patients with a normal glucose metabolism. In contrast, in highly insulin resistant prediabetic patients, the in comparison stronger reduction of insulin resistance seems to outweigh the reduction of inflammation. Our data are first to confirm a hypothesized association between Klotho and insulin resistance in humans.

Disclosure: **J.M. Brix:** None.

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SGLT2 inhibition by empagliflozin attenuates obesity-induced insulin resistance and inflammation by enhancing fat utilisation and alternative activation of macrophages

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Background and aims: Obesity activates the innate immune system, with subsequent recruitment of macrophages and T cells into white adipose tissue (WAT) and liver, contributing to the development of insulin resistance. In particular, macrophage recruitment and polarization are pivotal in obesity-induced inflammation and insulin resistance. Sodium glucose cotransporter-2 (SGLT2) inhibitors increase urinary glucose excretion (UGE), thereby leading to blood glucose reduction as well as weight loss, with apparent pleiotropic effects. However, the impact of SGLT2 inhibition on energy homeostasis and obesity-related inflammation remains largely unknown. Here, we show that empagliflozin, an SGLT2 inhibitor, enhances fat utilization and attenuates inflammation and insulin resistance in both WAT and liver of high-fat diet (HFD)-induced obese mice.

Materials and methods: Eight-week-old C57BL/6J mice were pair-fed a HFD or a HFD containing 0.01% empagliflozin (HFD + Empa) for 16 weeks. To assess the effect of empagliflozin on energy metabolism, oxygen consumption and energy expenditure were measured by indirect calorimetry. Immune cells in the stromal vascular fraction of epididymal WAT (eWAT), and in liver, were quantified by fluorescence-activated cell sorting (FACS).

Results: After 16 weeks, empagliflozin administration increased UGE ($P < 0.01$) in mice fed a HFD, and suppressed HFD-induced weight gain by 16% ($P < 0.01$). Weight reduction with empagliflozin was attributed to decreases in both visceral and subcutaneous fat depots. In contrast, femoral muscle weight was unaffected by empagliflozin. Moreover, empagliflozin shifted energy metabolism toward fat utilization, accompanied by elevated AMPKα and ACC phosphorylation and by increased expression of fatty-acid oxidation genes (Pparα/Cpt1a) in skeletal muscle. Importantly, HFD + Empa mice exhibited consistently higher oxygen consumption and exhaled more carbon dioxide than did HFD mice, leading to increased energy expenditures prior to measurable body mass differences. Empagliflozin improved HFD-induced glucose intolerance, hyperinsulinemia, and hepatic steatosis, and enhanced insulin signaling assessed by IRβ and Akt phosphorylation in eWAT, liver, and muscle. F4/80+ macrophage migration into eWAT and liver, as well as crown-like structure formation in eWAT, were reduced in HFD + Empa mice relative to HFD controls. These findings were associated with a reduction in markers of endoplasmic reticulum stress (GRP78/CHOP) and with attenuation of MAPK (JNK/p38MAPK) and NF-kB pathway activity in eWAT and liver. Furthermore, plasma levels of TNF-α were markedly lower in HFD + Empa mice than in HFD mice. FACS analysis revealed that empagliflozin decreased the number of adipose tissue macrophages (ATMs) and liver macrophages (LMs), identified as CD45+CD11b+F4/80+ cells, by 24% and 21%, respectively in mice fed a HFD. In addition, HFD + Empa mice had 49% and 29% fewer CD11c+CD206-(M1) ATMs and LMs, respectively, and 70% and 31% more CD11c-CD206+ (M2) ATMs and LMs, respectively, than did HFD mice, indicating a predominance of M2 over M1 ATM and LM populations.

Conclusion: Empagliflozin suppresses weight gain, adiposity, and hepatic steatosis by enhancing fat utilization, and attenuates obesity-induced inflammation and insulin resistance by alternative activation of macrophages in WAT and in liver.

Supported by: Japan Diabetes Association

Disclosure: **L. Xu:** None.

PS 051 Insulin and IGF-1: signalling and action

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Prep1 enhances neuronal activity by increasing insulin-like growth factor 1 pro-survival signalling

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Background and aims: Several studies show a strong association between alterations of insulin and/or insulin-like growth factor 1 (IGF1) signaling and the onset of neurodegenerative disorders, such as Parkinson disease or Alzheimer disease. Diabetic patients display an increased risk of developing neurological alterations. On the other hand, about 80% of subjects with neurodegenerative diseases show impaired glucose tolerance or diabetes mellitus. Indeed, insulin and IGF1 may regulate neuronal metabolism and survival in specific brain areas. Previous studies have identified the transcriptional factor Prep1 as a key regulator of insulin signaling. Prep1 hypomorphic mice (Prep1i/+), which express about 55-57% of protein, show a better insulin sensitivity and are protected from streptozotocin-induced diabetes. In addition, Prep1 has an essential role during embryonic development of the hindbrain. Morphological data, reported on web-based atlas, revealed that Prep1 expression is kept also in adult mouse brain and, in particular, in the olfactory bulbs (OBs). OBs represent the central nervous system area with the highest density of insulin and IGF1 receptors. Moreover, olfactory impairments have been associated to a wide range of neurological disorders, both in mouse and in human. Thus, in our study we investigated the role of Prep1 in insulin/IGF1 signaling in OBs and the possibility that Prep1 may be involved in neurodegenerative events linked to insulin/IGF1 pathways dysfunction.
Materials and methods: OBs of WT and Prep1i/+ mice have been analyzed by cytocrome C oxidase (COX) and Hemalum histological staining. Western blot experiments have been used to measure protein phosphorylation levels. Growth-curves and Sulphorodamine B assays have been performed on mouse neuroblastoma cell lines (N2A) to assess cell growth and viability.
Results: Histological analysis of OBs sections obtained from Prep1i/+ mice revealed a significant ($p<0.05$) 20% decrease of COX activity and cell density in glomerular and granular cell layers, compared to WT mice. Moreover, Western blot analysis showed 30% reduction of phosphorylation levels of the main kinases involved in cell proliferation and metabolism (Akt/PKB and ERK). Consistently, N2A stably transfected with Prep1 cDNA show a 35% increase of cell growth and viability, with 50% higher phosphorylation levels of ERK and Akt/PKB kinases, both in basal growth conditions and after IGF1 stimulation.
Conclusion: Our data suggest that Prep1 has a role in IGF1-mediated neuro-survival signalling pathway, and give a rationale to further investigate Prep1 as possible candidate in neurodegenerative disorders linked to insulin/IGF1-resistance.
Disclosure: **S. Ricci:** None.

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A new player on the insulin receptor internalisation mechanism: circular dorsal ruffles

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Background and aims: Whole-body glucose homeostasis is achieved through the regulated action of several cellular mechanisms that ultimately lead to glucose uptake from the bloodstream into insulin-sensitive organs and tissues, such as the liver and skeletal muscle. Insulin binds to the insulin receptor and the newly formed complex is rapidly internalized, via clathrin- or caveolae-mediated endocytosis. After internalization, the activated receptors concentrate in endosomes to further stimulate pathways that regulate metabolism and mitogenesis. The uncoupling of the insulin-IR complex leads to signal termination and receptors can then be degraded, recycled back to the surface or translocated to the perinuclear area. However, the number of receptors that undertake each pathway, how the cell decides the preferred route and how many times a receptor is recycled before degradation, are questions without an answer. IR trafficking is critical for normal development of an organism, maintenance of glucose homeostasis, and peripheral insulin levels mediated by both insulin secretion and insulin clearance processes, as well as to control magnitude and specificity of the cell's response. It is crucial then to determine the mechanisms involved in this process, both in physiology and pathophysiology. Our working hypothesis is that IR internalization in hepatocytes and skeletal muscle cells is mediated by actin-rich ring-shaped structures known as circular dorsal ruffles (CDRs).
Materials and methods: We used Hepa 1-6 mouse and HUH-7 human hepatocyte cell lines, as well as primary mouse hepatocytes and L6 muscle cell line, to characterize the trafficking of the IR, in physiological conditions. Cells were stimulated with various insulin concentrations (50, 75 and 100 nM), for different time points, and processed for immunofluorescence, using phalloidin to label the actin cytoskeleton as well as cortactin, an actin-binding protein, and antibodies against the receptor.
Results: Our results suggest that, upon insulin stimulation, both human and mouse hepatocytes form CDRs, which are dynamic and transient structures that form solely on the dorsal surface of the cell, upon growth factor stimulation. CDRs formed immediately after insulin stimulation (1 min), and we established that the IR localizes to these structures, suggesting that it is being internalized through this pathway. Moreover, CDRs are also formed in L6 rat muscle cells, upon insulin stimulation, suggesting its important role in insulin receptor internalization in insulin-sensitive tissues.
Conclusion: These membrane-derived structures, CDRs, were already known to rapidly internalize tyrosine kinase receptors, a characteristic of IR, through macropinosomes. Subsequently, receptors can be returned to the plasma membrane, following a recycling route, or be degraded in lysosomes. In this work, we showed for the first time CDRs formation in hepatocytes and skeletal muscle cells, upon insulin stimulation. This IR recycling route might be a major pathway not only in the receptor's availability to activate insulin signaling pathways, to promote glucose uptake in the skeletal muscle, but also in the internalization of insulin itself, towards being metabolized in the liver, a process known as insulin clearance.
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Disclosure: **M. Araújo-Correia:** None.

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Decrement in Rho-kinase 1 and adenylate energy charge contribute to the interactive regulation of insulin and fatty acid on AMPK/SREBP-1c pathway in skeletal muscle cells

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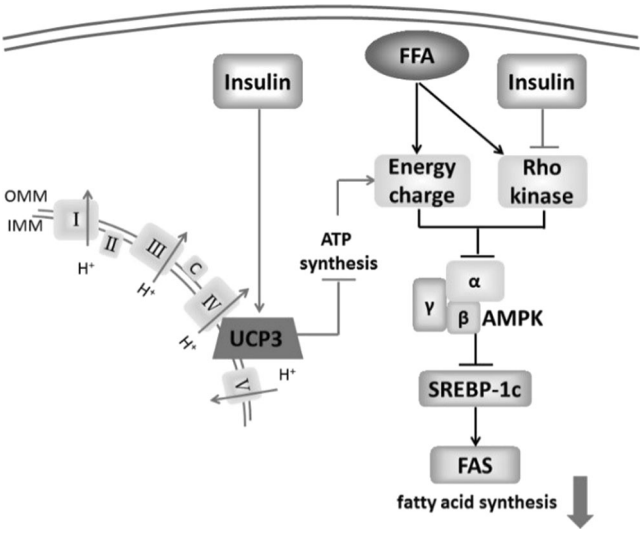
Background and aims: Clinical and experimental data suggest that early insulin therapy can reduce lipotoxicity in subjects and animal models with type 2 diabetes mellitus. However, the underling mechanisms need to be clarified. Sterol regulatory element-binding protein 1c (SREBP-1c), which is known negatively regulated by AMP-activated protein kinase (AMPK), is reported to play a critical role in lipotoxicity and insulin resistance of skeletal muscle cells by promoting intramuscular triglyceride (IMTG) accumulation and inflammatory stress. Here we investigated the effect and molecular mechanism of insulin intervention on AMPK activation and SREBP-1c-associated pathway in skeletal muscle cells with chronic exposure to palmitate.

Materials and methods: Male C57BL/6 mice were fed with a high-fat-diet for 12 weeks and were then treated with insulin, AMPK inhibitor, or metformin. L6 myotubes incubated with palmitic acid (PA) were treated with insulin or metformin. Dominant negative AMPKα2 (DN-AMPKα2) lentivirus, AMPKα2 siRNA, or Rho-kinase 1 (ROCK1) siRNA were transfected into PA-treated L6 myotubes. AMP/ADP/ATP levels were measured by HPLC to calculate cellular adenylate energy charge (EC).

Results: In normal control cells, insulin inhibited AMPK activity and increased SREBP-1c expression. However, we found that, in L6 cells with chronic PA treatment and high fat diet-induced obese C57BL/6 mice, insulin improved glucose metabolism, increased AMPKα2 phosphorylation, and reduced SREBP-1c protein expression. Interestingly, SREBP-1c expression decreased by insulin was blocked by AMPK inhibitor, but not PI3K inhibitor. Down-regulation of AMPK activity by DN-AMPKα2 lentivirus and AMPKα2 siRNA attenuated the impact of insulin on the inhibition of SREBP-1c in PA-treated L6 myotubes. Further, our study showed that insulin inhibited ROCK1 protein expression that was activated by PA, and knockdown of ROCK1 by siRNA blocked the down-regulation of AMPK phosphorylation under PA-treated L6 myotubes. In addition, PA treatment decreased the ratio of AMP/ATP which was reversed by insulin. Meanwhile, compared with metformin which down-regulated the adenylate energy charge (EC) via inhibition of respiratory complex I, insulin decreased EC in palmitate-pretreated L6 myotubes which representing energy surplus, via up-regulating UCP3 expression. (Figure)

Conclusion: Our studies for the first time demonstrated that under PA-induced skeletal cells with lipid energy surplus, the suppression of ROCK1 and the decrement of high EC are sufficient and necessary for insulin regulation of AMPK/SREBP-1c activity, revealed the interactive and synergic network of insulin and fatty acid on AMPK/SREBP-1c pathway and provided the important evidence of the timing and target of insulin therapy for type 2 diabetes.



Supported by: NNSF of China Grant Award
Disclosure: **S. Tang:** None.

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Insulin regulates SRBI and ABCG8 in a mTOR dependent pathway in CaCo-2 cells

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Background and aims: Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia and alterations in the metabolism of lipids and proteins. In the post-prandial phase most of the triglyceride rich lipoproteins have intestinal origin and diabetic patients have higher levels of triglyceride-rich lipoproteins either in fasting and postprandial state. The aim of this study was to determine whether excessive insulin changes the mRNA levels of critical mediators in a cellular model of intestinal epithelium.

Materials and methods: : CaCo-2 cells were grown until 80% confluence and subcultivated into plates with polycarbonate inserts Transwell to induce differentiation into a polarized epithelium. CaCo-2 cells were treated with insulin (100 nM), and inhibitors of signaling pathway PI3K/Akt/mTOR, wortmannin (100 nM), triciribine (1μM), rapamycin (100 nM). mRNA was isolated from cells and reverse transcription was performed using 200 ng of total RNA. qRT-PCR analysis was used to quantify mRNA levels by Taqman technology. mRNAs were normalized with a housekeeping gene (Cyclophilin) and expressed as fold change relative to vehicle treated cells by the ΔΔCt method. Biotin was added to both apical or basolateral epithelial surfaces. Cell lysates were digested and precipitated with streptavidin and analyzed by western blot in standardized conditions. Neutral lipids in the cells were stained with BODIPY and visualized by confocal fluorescence microscopy (493/503). Data are means±S.D. of 3 independent experiments. All samples were analyzed by ANOVA and posterior Tukey's multiple comparison test.

Results: Insulin (100 nM) significantly increased the mRNA levels of SRBI, ABCA1 and SREBP1c (fold changes relative to vehicle treated cells: 6.2±2.5, 2.2±1.0 and 1.4±0.6 respectively) in polarized CaCo-2 cells. Antagonists of PI3K, AKT and mTOR with Wortmannin, Triciribine and Rapamycin, respectively, prevented completely the actions of insulin on the mRNA levels of SREBP1c and ABCA1, but their effect on SRBI mRNA level was only partial (~70% of inhibition). At the protein level, insulin (100nM) increased the whole cell abundance of SRBI but decreased ABCG8 (fold change relative to vehicle treated cells: 1.5±0.8 and 0.25 ±0.001, respectively). Antagonists of PI3K or AKT did not affect the effects

of insulin on SRBI and ABCG8 proteins but antagonism of mTOR with Rapamycin prevent both the rise of SRBI and the decrease of ABCG8 induced by insulin. At the subcellular level, insulin preferentially increased SRBI in the apical plasma membrane. Confocal fluorescence microscopy revealed that insulin increased the abundance of lipid droplets in CaCo-2 cells, preferentially on the basolateral side of the cells.
Conclusion: Insulin increases SRBI protein levels in the apical surface of CaCo-2 cells. This regulation requires mTOR but not PI3K or AKT signaling and appears to be at the transcriptional level. Insulin also decreases ABCG8 protein levels in a mTOR dependent way. Insulin increases the neutral lipids content in CaCo-2 cells and this lipids are mostly stored at basolateral side of the cells.
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Disclosure: **M. Fuentes:** None.

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Central leptin and insulin signalling are required to maintain glucose homeostasis in healthy mice

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Background and aims: The synergistic actions of central leptin and insulin have been widely shown to regulate peripheral glucose metabolism using genetically engineered mice. However, the data surrounding the rapid action of central leptin and insulin is limited. Moreover, genetically engineered mice tend to display compensatory mechanisms which can be challenging to dissect.
Materials and methods: Here, we performed a single intracerebroventricular (ICV) injection of highly specific and competitive leptin (LAN-3) and insulin (S961) receptor antagonists in C57BL6 mice prior to conscious and unrestrained hyperinsulinemic-euglycemic clamp.
Results: We found that LAN-3 did not modify basal blood glucose levels. Conversely, S961 rendered lean mice hyperglycemic before at the start of hyperinsulinemic-euglycemic clamp. Interestingly, the inhibition of central leptin or insulin receptors alone is sufficient to induce insulin resistance in the periphery. We found that central administration of LAN-3 or S961 alone decreases glucose infusion rate (GIR) and whole body glucose turnover (GDR) in lean mice. In addition, hepatic glucose production (HGP) was markedly increased in both groups relative to control mice (aCSF group). This suggests that intact leptin and insulin signaling in the brain are not only required to regulate HGP but also GDR in peripheral tissues. In addition, we showed a synergistic reduction in insulin sensitivity by acutely antagonizing both leptin and insulin receptors in the brain. GlR was significantly decreased compared to leptin or insulin inhibition alone and we observed that the reduction in whole body insulin sensitivity was a consequence of a further change in HGP and not GDR.
Conclusion: In essence, this study has corroborated the important role of central leptin and insulin signaling in controlling peripheral glucose homeostasis especially HGP. More importantly, this work can explain the loss of peripheral insulin sensitivity by the rapid onset of hypothalamic leptin and insulin resistance in diet-induced obese mice, which may unravel the profound association between obesity and type 2 diabetes.
Disclosure: **W. Chen:** None.

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Duration of morning hyperinsulinaemia impacts hepatic glucose uptake and storage later in the same day

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Background and aims: We have previously shown in conscious dogs that 4 hours of hyperinsulinemic euglycemia in the morning significantly stimulates hepatic glucose uptake (HGU) and glycogen synthesis during a subsequent same-day hyperinsulinemic, hyperglycemic clamp with portal glucose infusion. To determine whether the pattern of morning insulin delivery affects the response, we compared 2 groups of dogs (n=6/group) that received the same amount of insulin delivered over either 2 or 4h in the morning.
Materials and methods: During the morning, somatostatin was infused i.v., glucagon was replaced via the portal vein at a basal rate, insulin was infused intraportally with the rates in the 2h group being double those in the 4h group (total dose in both groups=405 mU/kg), and blood glucose was clamped at basal (76±1 mg/dL) with i.v. glucose infusion. After the morning infusions were completed, all infusions were stopped and a primed, continuous i.v. infusion of 3H-glucose began. There was a 90 min period of equilibration and 30 min of BASAL sampling before the PM clamp. In the PM clamp, somatostatin and glucagon were infused in the same manner as in the morning, insulin was infused intraportally at 4xbasal, glucose was infused intraportally at 4 mg/kg/min to mimic meal glucose absorption, and variable-rate IV glucose infusion was used to clamp the arterial blood glucose at 2x basal. Data from the 2 groups are reported in relation to a group (SAL; n=6) that received a 4h infusion of saline, with no clamp, in the morning before undergoing the BASAL and PM procedures in an identical manner to the 2h and 4h groups.
Results: During the PM clamp, the arterial blood glucose (147±1 mg/dL) and plasma insulin (23±2 μU/mL) concentrations in the groups were indistinguishable. Glucagon concentrations remained at BASAL levels throughout the PM in all groups. The rates of net hepatic glucose output during the BASAL period were virtually identical among groups (1.4 ±0.1, 1.4±0.2 and 1.3±0.1 mg/kg/min in SAL, 4h and 2h, respectively). All groups rapidly shifted to NHGU with the start of the PM clamp. See Table 1 for clamp period data.
Conclusion: Morning hyperinsulinemia stimulated hepatic glucose uptake and storage during a hyperinsulinemic hyperglycemic clamp, mimicking a meal, later in the same day. Despite an identical mass of insulin delivered, prolonged hyperinsulinemia had a significantly greater effect than a shorter hyperinsulinemic period. Since adequate hepatic glycogen stores reduce the risk of hypoglycemia in diabetic individuals, these findings suggest that sustained hyperinsulinemia in the morning might improve glucoregulation later in the day.

Table 1. Results from PM clamp

Group	NHGU (AUC_{4h}; mg/kg)	HGU (AUC_{4h}; mg/kg)	Net hepatic glycogen synthesis (AUC_{4h}; mg/kg)	NEFA (Δ from basal; μM)
SAL	835±53	432±27	610±49	-890±67
4h	1412±162*	915±132*	1200±156*	-719±34
2h	1044±39*†	559±46*†	758±35*†	-902±105

*P<0.05 vs SAL; †P<0.05 vs 4h; NEFA, nonesterified fatty acids

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Disclosure: **M.C. Moore:** Grants; National Institutes of Health.

PS 052 Liver and lipids

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Polycomb controls metabolic homeostasis through a novel liver-to-adipose axis

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Background and aims: Diabetes and Obesity are complex diseases with multifactorial etiologies, involving both genetic and environmental components. While Genome Wide Association Studies (GWAS) have established a genetic framework for our current understanding of diabetes and obesity, little is known about the contributions of additional regulatory layers, especially epigenetics. Polycomb and Trithorax Group (PcG/TrxG) proteins were first identified in Drosophila for their roles in silencing homeotic genes and are now recognized as constituting a chromatin-based transcriptional regulatory system with key roles in multicellular development, stem cell biology and cancer. Perhaps the most striking feature of PcG proteins is their ability to effect cellular memory, that is, to transmit silenced gene expression states through mitosis. PcG target genes have been profiled in human embryonic fibroblasts and murine embryonic stem cells. Targets showed a very strong bias for genes controlling development and cell fate decisions, and interestingly, Gene Ontology analysis revealed Cellular Metabolism as one of the top GO terms for PcG protein-bound regions. Moreover, our own genome-wide in-vivo RNAi fly screen for obesity factors identified high enrichment for PcG/TrxG members. These findings strongly suggest a novel, prominent role for the PcG/TrxG system in metabolic control.
Materials and methods: To study the role of PcG in liver function and metabolic control, we crossed Eed (Embryonic Ectoderm Development) floxed C57/BL6J mice with Albumin-Cre-tg and generate liver specific Eed knockout (LEeKO). Mice were fed either normal chow or high fat diet (HFD - 60% Kcal from fat) and thoroughly metabolically phenotyped. Whole liver and adipose tissue, as well as, isolated hepatocytes and adipocytes were used for downstream molecular analyses. For the human studies, liver biopsies and plasma were obtained from subjects stratified per age and body mass index (BMI).
Results: Despite normal development and growth, LEeKO mice have better glucose tolerance on regular chow diet and are surprisingly protected from diet-induced obesity and metabolic syndrome on HFD. PcG inhibition in the liver derepresses the expression, and subsequentely, the secretion of Indian Hedgehog (Ihh). While not activating autocrine and paracrine signaling in the liver, Ihh mediates a functional browning of the subcutaneous white adipose tissue, as verified by LEeKO mice being resistant to temperature stress. Moreover, histological analysis of subcutaneous white adipose tissue showed increased number of multilocular adipocytes, consistent with induced browning; and validated by genome-wide profiling of isolated adipocytes. Conversely, genetic and pharmacological ablation of the hedgehog signaling pathway impairs brown adipose tissue function and metabolic homeostasis. Strikingly, two independent human cohorts stratified per age and BMI show strong negative correlation of Ihh expression and secretion with obesity.
Conclusion: Our identify Ihh as a novel hepatokine and suggest the Eed-Ihh axis as a novel liver-to-adipose axis controlling obesity and metabolic homeostasis in mice and humans.
Supported by: Marie-Curie IEF, ERC-StG Metabolic Polycombics
Disclosure: **R. Teperino:** None.

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T-cadherin gene (CDH13) polymorphisms, adiponectin levels and Fatty Liver Index (FLI) in D.E.S.I.R.

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) often occurs along with diabetes. The main diagnosis method remains the invasive hepatic biopsy. The Fatty Liver Index (FLI) is a non-invasive hepatic diagnosis score. FLI predicted the onset of type 2 diabetes at 9 years follow-up in the D.E.S.I.R. study. Adiponectin is an insulin sensitizing adipokine inversely associated with NAFLD. T-cadherin is a receptor for adiponectin. In GWAS, T-cadherin gene (CDH13) polymorphisms are associated with plasma adiponectin levels. In a previous case-control study, we found that CDH13 variants were associated with the risk of type 2 diabetes. The aim of the present study was to investigate the association between allelic variations of CDH13, circulating adiponectin levels and FLI.
Materials and methods: Three polymorphisms of CDH13 (rs11646213, rs3865188, rs4783244) were genotyped within a cohort from the general French population, D.E.S.I.R. (Data from an Epidemiological Study on the Insulin Resistance Syndrome) (N=5212). We selected people consuming less than 30 g/day of alcohol for men and 20 g/day for women (N=3650). Adiponectin was measured in people who became hyperglycemic at 3 years and their matched controls for sex, age and body mass index (BMI) (N=456). Plasma adiponectin concentrations were log-transformed to improve the approximation to normality. The FLI score was calculated with an algorithm including BMI, waist circumference, plasma triglycerides, and gamma-glutamyl-transferase. The FLI levels were dichotomized according to the type 2 diabetes onset threshold value in D.E.S.I.R. (<70 and ≥ 70). The associations between the polymorphisms and continuous quantitative variables were estimated by ANCOVA. Odds-Ratios (OR) were estimated for the associations between the polymorphisms and FLI were estimated by logistic regression. All analyses were adjusted for confounding factors such as age, sex, BMI, alcohol consumption and HbA1C, when appropriate.
Results: In D.E.S.I.R., the FLI was negatively associated with plasma adiponectin levels ($p<0.0001$). The three polymorphisms were associated with FLI and plasma adiponectin levels. The minor allele of the variant rs11646213 was associated with an increased risk of an FLI≥ 70 at the end of the study (baseline: p=0.35 OR=1.12 [95% IC 0.88-1.43]; end: p=0.05 OR=1.22 [1.00-1.49]) and lower plasma adiponectin levels (p=0.03). The minor alleles of rs3865188 (baseline: p=0.01 OR=0.72 [0.57-0.92]; end: p=0.005 OR=0.75 [0.61-0.92]) and rs4783244 (baseline: p=0.02 OR=0.74 [0.58-0.95]; end: p=0.01 OR=0.77 [0.62-0.94]) were associated with a lower risk of a FLI≥ 70 and an increase in plasma adiponectin levels (p=0.002 and 0.003, respectively). The associations with FLI remained significant after adjustment on HbA1c and BMI.
Conclusion: Three CDH13 variants, associated with type 2 diabetes in a case-control study, were also associated with adiponectin levels and FLI in the general French population. These associations are in favour of a protective role of circulating adiponectin towards NAFLD. Further investigations will determine whether these associations could be explained by changes in plasma adiponectin levels or by a direct T-cadherin effect on the liver.
Disclosure: **A. Nicolas:** None.

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Glycation impairs hepatic lipid metabolism and glucose tolerance in high-fat diet-induced obese rats

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is an obesity- and diabetes-related condition involving insulin resistance and lipotoxicity. However, the mechanisms involved in NAFLD progression in obesity are unknown. We intended to assess the role of glycation in the induction of glucose and lipid dysmetabolism and NAFLD progression in obesity.
Materials and methods: Wistar rats were fed a high-fat diet (HFD group) in order to induce obesity. Glycation was induced by MG supplementation, alone (MG group) or in combination with the high-fat diet (HFDMG group) and compared with standard diet controls (n=6/ group). Lipid species in liver were assessed in vivo by 1H nuclear magnetic resonance spectroscopy (MRS) and ex vivo by mass spectrometry (MS) and gas chromatography (GC), followed by western blotting and histologic analysis.
Results: Liver fat mass measured by MRS was increased in HFD and HFDMG groups. However, only HFDMG group had decreased unsaturated lipids and increased fraction of saturated lipids (measured by both MRS and MS), mainly due to reduced oleic acid levels (common in high-fat diets and increased in HFD rats). HFDMG group also showed lower lipid esterification, due to decreased percentage of esterified glycerol carbons and higher fatty acids/glycerol ratio observed in the MRS, as well as decreased triglycerides levels, measured by MS, suggesting an increased concentration of non-esterified FA levels and diacylglycerols in relation to triglycerides. MS and GC also revealed further decreased levels of plasmalogens (antioxidant phospholipids) and cardiolipins (mitochondrial phospholipids) in HFDMG group, showing higher oxidative damage in this group. Moreover, insulin signaling was decreased only in the HFDMG group, together with dysregulation of lipid synthesis pathways and portal inflammation, despite microvesicular steatosis was found in both high-fat diet-fed groups (HDF and HFDMG).
Conclusion: Our data suggest that glycation changes lipid metabolism in a context of hyperlipidemia/obesity, independently of the total amount of liver fat. This is likely to contribute to the hepatic lipotoxicity observed in the metabolic syndrome and (pre)diabetic patients and to the vicious circle between insulin resistance, glucose dysmetabolism and NAFLD.
Supported by: FCT, FEDER-PT2020, DoIT – Diamarker, RNEM
Disclosure: **C. Neves:** None.

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Liver-specific deletion of ROCK1 prevents hepatic steatosis in leptin-deficient ob/ob mice

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Background and aims: Obesity is a risk factor for non-alcoholic fatty liver disease (NAFLD) and is strongly associated with insulin resistance. The prevalence of NAFLD is 2-44% in the European population and 42.6-69.5% in people with type 2 diabetes. Furthermore, over 50% of adults in the European Union are overweight or obese. Our previous data showed that liver-specific deletion of Rho-kinase 1 (ROCK1) improved systemic insulin sensitivity and ameliorated hepatosteatosis in obese mice induced by a high-fat diet. The current study was designed to further determine whether inactivation of hepatic ROCK1 prevents the development of hepatic steatosis in leptin-deficient ob/ob mice.
Materials and methods: Leptin-deficient ob/ob mice lacking hepatic ROCK1 (L-ROCK1 -/-; ob/ob) were studied. ROCK1 protein expression and enzymatic activity was measured. Metabolic parameters, including body weight, blood glucose and serum insulin levels, were measured. Lipid profiles, including hepatic and serum content in triglycerides and cholesterol, were also determined. Hematoxylin and eosin stain (H&E stain) of liver sections from control and L-ROCK1 -/-; ob/ob mice was performed. Gene expression of key molecules involved in lipid metabolism was also determined.
Results: At 14 weeks of age, ROCK1 protein levels (1.00 ± 0.02 A.U. vs. 1.60 ± 0.06 A.U., N=4, $p < 0.001$) and enzymatic activity (1.00 ± 0.14 A.U. vs. 1.67 ± 0.07 A.U., N = 3-6, $p < 0.001$) were increased in ob/ob mice compared with control mice. In contrast, ROCK2 protein levels and its enzymatic activity are comparable between ob/ob mice and their control littermates. Hepatic deletion of ROCK in ob/ob mice resulted in a significant decrease in blood glucose (487.6 ± 42.27 mg/dL vs. 348.6 ± 47.18 mg/dL, N = 9-12, $p < 0.05$) and serum insulin (18.87 ± 3.29 ng/mL vs. 9.29 ± 2.10 ng/mL, N = 6-11, $p < 0.05$), independently of adiposity. Hepatic triglycerides (44.30 ± 4.00 mg/g vs. 32.09 ± 1.68 mg/g, N = 5-8, $p < 0.05$) and cholesterol (9.21 ± 0.58 mg/g vs. 7.44 ± 0.23 mg/g, N = 4-7, $p < 0.05$) were greatly decreased in L-ROCK1 -/-; ob/ob mice compared to ob/ob mice. These effects were accompanied by decreased gene expressions of key lipogenic enzymes, including FAS, SCD1 and DGAT2. However, loss of ROCK1 from liver had no effect on gene expression involved in fatty acid oxidation and fatty acid uptake. Hepatic H&E staining also confirmed reduced lipid accumulations in mice lacking ROCK1 in ob/ob mice.
Conclusion: Our data demonstrate that hepatic ROCK1 activation is required to protect from hyperglycemia, hyperinsulinemia and hepatic steatosis, suggesting an important role for ROCK1 in hepatic metabolism. Thus, targeting hepatic ROCK1 could be a therapeutic option for the treatment of obesity-related metabolic disorders such as NAFLD.
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Disclosure: **I.S. Lima:** None.

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Pyridoxamine prevents diet-induced insulin resistance through the regulation of ceramide/sphingosine/sphingosine-1-phosphate equilibrium in mice liver

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Background and aims: Dietary Advanced Glycation End-Products (AGEs) have been linked to the onset of obesity, adiposity, and insulin-resistance. Recently we documented that AGEs accumulate in liver, gastrocnemius muscle, and brain of diet-induced insulin-resistant mice, leading to dysregulation of lipid synthesis. Recent studies have revealed that the enhanced synthesis of the sfingolipids ceramide (Cer) and sphingosine-1-phosphate (S1P) contributes to insulin resistance development. Indeed, it has been evidenced that the maintenance of the Cer/sphingosine (Sph)/S1P equilibrium by the enzymes ceramidase, that degrades Cer to Sph, and S1P kinase, that phosphorylates Sph to S1P, has a role in the modulation of insulin signaling. Moreover, emerging evidence

has shown that AGEs can alter Cer/Sph/S1P equilibrium by affecting the neutral ceramidase and the S1P kinase activities. We, thus, investigated whether the administration of the anti-glycative compound pyridoxamine to high-fat fed mice could prevent insulin resistance onset by restoring the Cer/Sph/S1P equilibrium.

Materials and methods: C57Bl/6J mice were fed a standard diet (SD) or a 60% saturated fat diet (HFD) for 12 weeks. Two subgroups of SD and HFD mice received the anti-glycative compound pyridoxamine (150 mg/kg/day) in the drinking water. Glucose and lipid metabolism were analyzed. AGEs levels and the expression of the AGE-receptor RAGE were evaluated in the liver by liquid chromatography-mass spectrometry (LC-MS) and western blotting. The liver concentrations of Cer, Sph, and S1P were evaluated by using an ultra performance liquid chromatography-mass spectrometry (UPLC/TQMS) system. The expressions of neutral ceramidase, S1P kinase, and S1P receptors S1PR1-3 were assessed by western blotting.

Results: At the end of protocol HFD mice showed increased plasma glucose ($P<0.05$ vs SD) and cholesterol ($P<0.05$ vs SD), impaired OGTT curve, and hepatosteatosis. The pyridoxamine administration efficiently prevented glucose intolerance and ameliorated hypercholesterolemia ($P<0.01$ vs HFD) and hepatosteatosis. High levels of AGEs and RAGE were detected in the liver of HFD mice ($P<0.01$ vs SD) and pyridoxamine prevented their accumulation ($P<0.05$ vs HFD). HFD induced a marked reduction of the liver Sph levels ($P<0.01$), paralleled by the increase in Cer levels ($P<0.05$). Interestingly, pyridoxamine administration prevented the Cer increase by enhancing ceramidase expression and significantly inhibited S1P kinase expression. Sph depletion was thus completely reverted reaching levels over the SD values ($P<0.05$ vs SD; $P<0.01$ vs HFD).

Conclusion: The present data indicate for the first time that the anti-glycative compound pyridoxamine can move the equilibrium from Cer and S1P towards Sph by the modulation of the involved enzymes. The regulation of Sph metabolism could contribute to the prevention of insulin resistance observed in the pyridoxamine-treated HFD mice. Therefore, it could be of relevance to further investigate on the predictive value of Cer/Sph/S1P imbalance for insulin resistance onset and on pyridoxamine supplementation as preventive strategy.

Disclosure: **R. Mastrocola:** None.

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Towards a multi-organ-chip combining human liver, pancreatic islets, skeletal muscle and kidney equivalents to study metabolic diseases

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Background and aims: Currently in vivo experiments are crucial for the systemic study of pathogenesis and potential therapeutic intervention in metabolic diseases like type II diabetes. However due to phylogenetic differences between humans and animals, results are known to be limited in their transferability. Therefore an approach to systemically combine several human organ models to emulate their physiological cross-talk is highly desirable. We developed a platform bridging this gap between animal models and conventional monoculture of human cells.

Materials and methods: At the size of a microscopic glass slide, the multi-organ-chip consists of two independent microfluidic circuits, representing the blood circulation on the one site and the urinary tract on the other site. At their interconnection the circuits are separated by a PET membrane, allowing for the cultivation of kidney proximal tubule cells. The blood circuit connects the kidney cells with up to 3 additional organ equivalents, each in spatially separated cavities. An integrated on-chip micropump circulates a nutrient solution through the microfluidic channel system. This connection of all organ models enables their cross-talk. The cultivation cavities can be opened at any time during culture allowing samples to be taken for metabolite analysis. Here we analyzed glucose and insulin levels after a glucose load (11 mM) to investigate the cross-talk between liver spheroids and primary pancreatic islets. At the end of the cultivation, tissues are removed from the cultivation cavities and stained immunohistochemically.

Results: Co-cultures of human liver spheroids and human pancreatic islets in the multi-organ-chip are able to drop glucose levels from high glucose (11 mM) to physiological values (5.5 mM) within 24 h. Glucose homeostasis can be maintained for up to 48 h without adding fresh medium to the system. While the insulin level in islet single cultures steadily increases, it stagnates in co-culture with liver aggregates. In order to simulate the in vivo glucose-insulin balance more closely, tissue equivalents for skeletal muscle as well as the kidney are envisioned to be cultured in their intended cultivation cavity. Data on the cultivation of 3D skeletal muscle tissue made from iPS derived cells in a fibrin matrix and the polarization of the Na+/Ka+-ATPase in a proximal tubulus cell line cultured on the PET membrane in our microphysiological device will be presented. Finally, progress on the generation of insulin resistant liver cells will be shown by accumulation of Nile-Red stained vesicles in human liver cells treated with 0,6 mM palmitate.

Conclusion: Here, we present a multi-organ-chip platform for the cultivation of three-dimensional culture models of liver, pancreatic islets, skeletal muscle and kidney for the development of in vitro metabolic disease models.

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PS 053 Cholesterol and dyslipidaemia

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Chemokine receptor5 facilitates endothelial progenitor cells recruitment and ameliorates hypercholesterolaemia in ApoE-deficient mice

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Background and aims: The major event initiating atherosclerosis is dyslipidemia-induced disruption of vascular endothelium integrity. Chemokine receptor 5(CCR5) promotes the mobilization of endothelial progenitor cells (EPCs) and thereby mediates endothelial repair. The aim of this study was to investigate the therapeutic potential of CCR5 overexpressing EPCs on prevention of dyslipidemia in ApoE-/- mouse model.
Materials and methods: Splenectomized ApoE-/- C57BL/6J mice fed with high-fat diet for 24 weeks were intravenously injected with EPCs transfected with CCR5 overexpression lentivirus. The recruitment of EPCs was evaluated by immunofluorescence, and the endothelial cells content on hepatic venous sinus was assayed by specific immunostaining. The serum levels of fasting plasma lipids were measured by colorimetric analysis. The gene and protein levels of SREBP2, HMGCR, ABCA1 and ABCG1 were detected by real-time PCR and western blot analysis.
Results: Six weeks after treatment, CCR5 overexpression enhanced the homing of EPCs and increased endothelial cells content on hepatic venous sinus. CCR5 overexpressing EPCs treatment reduced circulating cholesterol and low-density lipoprotein level but not triglyceride levels in ApoE-/- mice. In addition, the mRNA and protein expressions of SREBP2 and HMGCR were reduced in mice with CCR5 overexpressing EPC treatment. The mRNA and protein expressions of ABCA1 and ABCG1 were increased in CCR5 overexpressing EPC treatment.
Conclusion: We provided evidence for ameliorate hyperlipidemia through treatment of CCR5 overexpressing EPCs. If CCR5 has a similar function in humans, stimulation of CCR5 expression in EPCs may promote EPC recruitment, restore endothelial cell function, decrease cholesterol synthesis, enhance the cholesterol efflux, and eventually reduce the risk of atherosclerotic disease.
Supported by: National Natural Science Foundation of China Grants
Disclosure: **Z. Zhang:** None.

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Endoplasmic reticulum stress might play a pivotal role in lipid metabolic disorders detected in a novel subclinical hypothyroidism mouse model

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Background and aims: Subclinical hypothyroidism (SCH) is becoming a global health problem for its potential deleterious effects. However, the molecular mechanism of lipid metabolic disorders in SCH has not been fully clarified. And, the progression of detecting the exact pathogenesis of SCH was hampered by lacking of optimized mice models. Therefore, establishing an appropriate SCH animal model will be conductive to investigate the pathological characteristics of SCH.
Materials and methods: A common antithyroid drug — methimazole (MMI, 0.08 mg/kg•d) was applied to C57BL/6 mice to construct a non-invasive SCH mice model. After MMI were respectively administered for 12 weeks, 16 weeks and 20 weeks, thyroid function, serum lipid spectrum and liver lipid deposition were detected. Hepatic triglyceride and cholesterol dyeing were determined by Oil red O staining and Filipin staining, respectively. The whole body metabolism was measured by metabolic chambers at the 16th week. The expression of endoplasmic reticulum stress (ERS) molecules in liver was detected by Western blotting. 4PBA was used in vivo to alleviate ERS to observe the role of ERS in lipid metabolic disorders in SCH.
Results: Compared with the control mice, the mice treated by MMI for 12 weeks showed the diagnostic variation of SCH: increased serum thyrotropin (TSH) level (1.2925±0.483 vs. 0.4607 ±0.209mIU/L, $p<0.05$) while thyroid hormone levels remained constant. Interestingly, the SCH status persisted for approximately 8 weeks. Meanwhile, the whole body metabolic situation detected by metabolic chambers showed no difference between SCH and control mice, which is corresponding to the clinical features of SCH. Drinking MMI for 16weeks, serum TC, LDL-C and TG in SCH mice were higher than controls ($p<0.05$ for all). Hepatic TG (158.765 ± 77.893 vs. 80.335 ± 22.339 μmol/g, $p<0.05$) and cholesterol (64.024 ± 7.282 vs. 46.159 ± 4.143 μmol/g, $p<0.05$) contents increased significantly in SCH mice compared with controls. The Oil red and Filipin staining also confirmed the above results. Compared to controls, the expression of Bip was up regulated in the liver of SCH mice. Corresponding to this result, the expressions of p-IRE1α and XBP-1s in SCH mice were higher than that of controls. But, the expression of ATF6α and p-eif2α did not show obvious differences in two groups. Then, 4PBA was used to block ERS in SCH mice. As expected, hepatic ERS were improved in 4PBA treated SCH mice compared with that in vehicle treated SCH mice, along with alleviating hepatic triglyceride deposition (99.901±32.641 vs. 165.587 ±54.026μmol/g, $p<0.05$) and cholesterol accumulation (60.035 ±4.565 vs. 75.693±9.658μmol/g, $p<0.05$). Meanwhile, serum TC, LDL-C and TG levels in 4PBA treated SCH mice restored to normal ranges.
Conclusion: Our findings suggested that an optimized SCH mice model could be established using MMI and hepatic ERS might play a pivotal role in lipid metabolic disorders in SCH.
Disclosure: **L. Zhou:** None.

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Liraglutide reduces postprandial hyperlipidaemia by increasing apoB48 catabolism and by reducing apoB48 production

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Background and aims: Postprandial hyperlipidemia is an important feature of diabetic dyslipidemia. Treatment with GLP1 agonist, liraglutide, has been shown to reduce postprandial triglycerides in patients with type 2 diabetes. However, the mechanisms responsible for this postprandial lipemia reduction remain unknown. This prompted us to study the effect of liraglutide on the metabolism of apoB48, the major apolipoprotein of postprandial lipoproteins (chylomicrons, chylomicron-remnants).
Materials and methods: We performed an in vivo kinetic study with stable isotopes in the fed state (food intake fractionated in small portions provided every 2 hours starting 6 hours prior to the tracer infusion up to the end of the study) in 10 patients with type 2 diabetes and the typical diabetic dyslipidemia (triglycerides ≥1.7 Mmol/L and/or HDL-C < 1.29 (F)/1.03 (M)), before then 6 months after the initiation of a treatment with liraglutide, at a dose of 1.2 mg/day. Triglyceride-rich lipoproteins were isolated by ultracentrifugation and apoB48 isolated by electrophoresis.Isotopic enrichlment was measured by mass spectrometry.
Results: Six months after the initiation of liraglutide treatment, significant reductions of HbA1c (7.13 ± 1.08 vs. 9.62 ± 2.65%, p=0.009), of body weight (100.5 ± 19.6 vs.104.9 ± 19.6 kg, p=0.021), of fasting triglycerides (2.01 ± 0.93 vs.2.78 ± 1.48 Mmol/L, p=0.005) and of postprandial triglycerides (2.04 ± 0.98 vs.2.90 ± 1.52 Mmol/L, p=0.005) were observed, as compared to baseline. ApoB48 pool was dramatically reduced (86 ± 47 vs. 231 ± 100 mg, p=0.018). Data from the kinetic study showed a significant increase in apoB48 Fractional Catabolic Rate (from 3.52 ± 0.84 at baseline to 4.87 ± 0.88 pool/d on liraglutide, p=0.005) and a significant

reduction in apoB48 Production Rate (from 8.05 ± 4.57 at baseline vs. 4.27 ± 2.42 mg.kg-1.d-1 on liraglutide, p=0.009).
Conclusion: Treatment with liraglutide significantly decreases postprandial hyperlipidemia in patients with type 2 diabetes by reducing apoB48 production and by increasing apoB48 catabolism. The effect of liraglutide on apoB48 kinetics leads to reduce postprandial hyperlipidemia and, thus, might be beneficial to reduce cardiovascular risk.
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Supported by: NovoNordisk Grant
Disclosure: **B. Vergès:** Grants; NovoNordisk Research Grant.

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Altered HDL lipidome in pre-diabetic and diabetic individuals with normal HDL-C levels studied by proton Nuclear Magnetic Resonance spectroscopy

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Background and aims: The low HDL cholesterol (HDL-C) levels that commonly characterizes patients with Type 2 Diabetes Mellitus has been partially attributed to compositional changes in HDL particles, such as depletion in cholesteryl esters and enrichment in triglycerides. However, data on the HDL particles composition in pre-diabetic and diabetic individuals with normal HDL-C levels are limited. Proton nuclear magnetic resonance (1H NMR) spectroscopy is one of the preferred tools for lipid analysis of biological matrixes. It is a rapid and reproducible technique, requires minimum sample pretreatment and provides an overall qualitative and quantitative lipid assay, including detailed information on molecular structure. In the present study we have used 1H NMR spectroscopy to characterize the HDL lipidome in pre-diabetic and diabetic individuals with normal HDL-C levels compared to non-diabetic individuals.
Materials and methods: Serum samples from 20 non-diabetic (serum glucose levels< 100 mg/dL and HbA1c< 5.7%), 12 pre-diabetic (glucose levels: 100-125 mg/dl and HbA1c: 5.7-6.4%) and 18 diabetic (glucose levels equal or greater than 126 mg/dl and HbA1c equal or greater than 6.5%) individuals were collected after an overnight fast. Patients, in the three groups studied, were selected as having no statistically different levels of total, HDL and LDL cholesterol and triglyceride in order to avoid a possible confounding effect of these parameters on the lipid composition of the HDL particles. HDL-C levels in non-diabetic, pre-diabetic and diabetic individuals were 44.7 ± 11.9, 48.9 ± 11.2 and 49.6 ± 11.6, respectively (no statistically different). HDL lipoprotein particles were isolated from the non-HDL lipoproteins by precipitation with Dextran Sulfate/ MgCl2 and the lipid content was extracted with methanol-chloroform. Multivariate pattern recognition analysis was applied on the 1H NMR HDL lipidomic data recorded on a Bruker DRX-600 Spectrometer.
Results: The NMR-based lipidomic analysis showed that pre-diabetic and diabetic individuals were progressively differentiated from the non-diabetic group. Pattern Recognition scores plots revealed a significantly different lipid profiling of serum HDL lipoproteins of both, pre-diabetic and diabetic individuals, compared to that recorded from non-diabetics. Individuals with diabetes were characterized by altered HDL fatty acid pattern (mainly higher saturated and lower unsaturated and omega-3 fatty acids), lower levels of surface phospholipids (phosphatidylcholine and sphingomyelin) and cholesterol and higher levels of core triglycerides compared to non-diabetic individuals. A similar atherogenic lipid profiling in HDL lipoproteins was also found to characterize the pre-diabetic stage.
Conclusion: Despite normal HDL-C levels, an altered composition in HDL lipoprotein particles has been detected in pre-diabetes and diabetes containing atherogenic features that possibly affect their functionality and metabolism. Proton NMR-based lipid approach is a promising technique in clinical medicine for the identification of lipid diagnostic biomarkers of a disease state and therapy monitoring.
Disclosure: **V. Tsimihodimos:** None.

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Dyslipidaemia in 68,482 adults with type 2 diabetes: the impact of age, gender and treatment based on a joint analysis of the German DIVE and DPV registries

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Background and aims: Dyslipidemia is a common cardiovascular risk factor in adults with type-2 diabetes. While most guidelines recommend narrow target values for lipids in this high-risk-group, real-life data indicate that these targets are frequently missed.
Materials and methods: DIVE and DPV, two large multicenter registries on real-life care for adult patients with diabetes (type 1, type 2 and others) continuously and longitudinally record data on clinical care and outcome in Germany. The DIVE-registry currently includes 89,871 patients with type-2-diabetes at 159 centers, while DPV includes 303,996 patients with type-2 diabetes at 442 centers. This analysis is based on 68,482 adult patients with type-2 diabetes treated in 2014 or 2015 and having a complete lipid status (Total Cholesterol [TC], HDL-C, LDL-C, Triglycerides) available. Non-HDL-C was calculated as TC minus HDL-C. Target values from the European Society of Cardiology (ESC / EAS) guidelines for the management of dyslipidemias were applied.
Results: 94% of patients with type-2 diabetes failed to achieve the ESC/EAS target for lipid values, with 84% of patients failing on two target values. LDL-cholesterol below 70 mg/dl, as recommended was achieved by 13% of patients only, followed by only 18% of subjects achieving a non-HDL-cholesterol < 100 mg/dl. Prevalence of dyslipidemia was higher in females compared to males, and in younger patients (80 years of age. Patients on statins displayed lower TC, LDL-C and non-HDL-C values and thus a lower rate of dyslipidemia. Remarkably, only 32% of patients were treated with statins. Treatment rate was higher in males (34%) compared to females (30%) and highest in the age-group 60-80 years (35%) as compared to younger (26%) and older (31%) patients.
Conclusion: These real-world data indicate a high rate of dyslipidemia, if current guideline recommendations are used to define target lipid values. These guidelines recommend a much higher rate of statin therapy in patients with type-2 diabetes as was observed. Patients treated with statins display better lipid profiles, however the majority still does not reach recommended target values.

Table 1: Dyslipidemia in patients with type-2 diabetes

	Total	Gender		Age groups			Treatment	
		Male	Female	Age < 60	Age 60-80	Age > 80	No statins	On statins
Number of pts	68,482	37,225	31,257	17,878	40,285	10,319	46,515	21967
Median lab values								
TC (mg/dl)	182	174	193	193	180	174	188	172
HDL-C (mg/dl)	46	43	50	43	46	47	46	45
LDL-C (mg/dl)	110	105	116	120	108	102	115	100
Non-HDL-C (mg/dl)	134	130	139	147	131	124	139	124
TG (mg/dl)	153	153	153	175	149	132	151	155
Lipid target achieved								
TC (mg/dl)	88.4 %	91.7 %	84.5 %	83.6 %	89.9 %	90.9 %	87.2 %	91.1 %
HDL-C (mg/dl)	70.7 %	75.2 %	65.4 %	65.9 %	72.9 %	70.4 %	70.8 %	70.5 %
LDL C (mg/dl)	12.6 %	14.0 %	9.6 %	8.3 %	13.2 %	17.6 %	10.4 %	17.0 %
Non-HDL C (mg/dl)	18.1 %	20.8 %	14.9 %	10.3 %	19.3 %	26.8 %	15.2 %	24.3 %
TG (mg/dl)	69.7 %	68.5 %	71.1 %	59.3 %	71.4 %	80.7 %	70.1 %	68.8 %
Dyslipidemia								
1 lipid abnormal	93.8 %	92.2 %	95.8 %	96.9 %	93.3 %	90.0 %	95.0 %	91.4 %
2 lipids abnormal	83.9 %	81.2 %	87.2 %	91.3 %	82.7 %	75.7 %	86.5 %	78.4 %

Values are median lipid values in mg/dl and percentage of patients within target range / patients with dyslipidemia.

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A common gene variant in glucokinase regulatory protein interacts with glucose metabolism on diabetic dyslipidaemia: the combined CODAM and Hoorn studies

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Background and aims: Small molecule disruptors of the glucokinase-glucokinase regulatory protein (GKRP) complex are potential new glucose-lowering targets. They stimulate hepatic glucose uptake by increasing glucokinase activity in the liver. It can, however, be anticipated that an increased hepatic glucose uptake will affect intrahepatic glucose metabolism, including stimulation of de novo lipogenesis. This might accelerate the development of nonalcoholic fatty liver disease and hypertriglyceridemia, in particular in patients with type 2 diabetes mellitus (T2DM). Of interest, rs1260326 is a common, functional variant in the GKRP gene (GCKR) that allows investigation of the effects of glucokinase-GKRP disruption at the population level. The minor T-allele encodes GKRP that binds glucokinase less effectively. In the present study, we examined whether the strength of association between rs1260326 and plasma lipids is affected by glucose metabolism.

Materials and methods: Genotyping of rs1260326 was done in subjects with normal glucose metabolism (NGM; n=497), impaired glucose metabolism (IGM; n=256) and patients with T2DM (n=351) who participated in the combined Hoorn and CODAM studies, two prospective cohorts designed to study determinants and (cardiovascular) complications of T2DM. Associations between the rs1260326 minor T-allele and plasma lipid levels were analyzed using linear regression, with adjustments for age, sex, and cohort, and stratified by glucose metabolism subgroups.

Results: The strength of association between the rs1260326 minor T-allele and plasma triglycerides increased from NGM to IGM to T2DM (β=0.006, 0.018 and 0.067, respectively; p for interaction=0.002). The inverse relation between the rs1260326 T-allele and HDL cholesterol was again most prominent in patients with T2DM (β=0.012, 0.020 and -0.096 for NGM, IGM and T2DM, respectively; p for interaction=0.004). Similar trends were observed when the Hoorn and CODAM cohorts were analyzed separately. In addition, comparable results were obtained when glucose metabolism strata were replaced by continuous indices of glucose metabolism, i.e. plasma HbA1c and fasting glucose levels.

Conclusion: These findings demonstrate that stimulation of hepatic glucose uptake will have downstream metabolic consequences. Moreover, our results suggest that small molecule disruptors of the glucokinase-GKRP complex aggravate dyslipidemia in particular when glucose control is suboptimal.

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Disclosure: **N. Simons:** Grants; The Netherlands Heart Foundation (NHS; grant #2015T042).

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Severe hypertriglyceridaemia in an adolescent with lipoprotein lipase deficiency: responding to triple drug combination

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Background and aims: Severe hypertriglyceredemia due to lipoprotein lipase (LPL) deficiency is a very rare disorder. Generally they respond to single or dual triglycerides(Tg) lowering therapy. We report a very rare case of LPL gene mutation where three drugs including saroglitazar were used to reduce serum Tg to a safer level. To the best of our knowledge this kind of drug combination in this condition has not been reported.

Materials and methods: An adolescent male was admitted in surgical ward for acute appendicitis. During the pre op work up his serum was found to be lipemic. Serum Tg were 6500 mg/dl. He never had any acute pancreatitis in the past. There was no family history of severe dyslipidemia or pancreatitis. Clinical examination was unremarkable. He was normotensive, his BMI was 21 and there were no xanthomas or xanthelesma. There was no hepatosplenomegaly and fundus examination was normal. Plasma glucose, renal and liver functions, blood counts, TSH and cortisol were estimated.Pre operatively he was treated with fenofibrate and he underwent an uneventful appendicectomy.Fasting serum lipid profile, HbA1c and glucose were measured periodically thereafter. Saroglitazar 4 mg and Gemfibrogil 300 mg tid were added subsequently. After about 2 years he had acute pancreatitis which was managed conservatively. Serum Tg was 2400 mg/dl during acute pancreatitis which was due to treatment non compliance.

Results: After surgery he was continued on 165 mg fenofibrate and 10 mg atorvastatin. His Tg fell to 2500 after 3 weeks .Capsules of omega 6 FA were added. He followed a rigorous diet pattern and physical activity suggested by the nutritionist during repeated counseling. Atorvastatin was withdrawn when serum cholesterol began to fall below 125mg/dl. When fenofibrate couldn't bring down tg further, 4 mg of saroglitazar, a PPAR alpha & gamma receptor agonist was added once daily. After 3 months of addition of saroglitazar the Tg levels varied between 1100 to 1800 mg/dl (table 1). When Tg was not falling below 1000 mg/dl(a number which is considered safe to prevent acute pancreatitis) gemfibrogil was increased to 600 mg bid. Tg got stabilised at around 900 mg/dl.He tolerated all the three drugs well. Fasting glucose readings had a linear relationship with serum Tg, ranging from 104 to 154. HbA1c was always in normal range. Glucose measured by fingerstick glucose meter was always in euglycemic range. The lab glucose was measured by hexokinase technique. This indicates a falsely elevated glucose due to interference in the glucose measurement by hexokinase method in the presence of severe hypertriglyceridemia. At present the patient is on fenofibrate 145 mg,saroglitazar 4 mg and gemfibrogil 600mg bid. He is asymptomatic. Exome sequencing revealed a known mutation in lipoprotein lipase gene. None of the parents and siblings have similar dyslipidemia.

Conclusion: Severe hypertriglyceredemia this case was due to a known LPL gene mutation which is a rare disease. Acute pancreatitis is a known complication which can be prevented by successful management with a tripple drug therapy.

Disclosure: **V. Lodha:** None.

PS 054 Ectopic lipids

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Brain leptin signalling reduces hepatic lipid content by increasing hepatic VLDL secretion and reducing de novo lipogenesis

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) is closely associated with obesity and diabetes. An imbalance between uptake and production versus export and utilization of triglycerides (TG) in the liver results in hepatic lipid accumulation, thus leading to hepatic steatosis. The adipokine leptin is implicated in the regulation of hepatic TG content. Leptin deficient lipodystrophic patients suffer from severe NAFLD, while leptin treatment dramatically ameliorates steatosis in these patients. The mechanism for this is unclear, however leptin may reduce steatosis by boosting hepatic TG export. Since leptin exerts its function mainly via signaling in the central nervous system (CNS), we hypothesized that brain leptin modulates hepatic TG secretion.
Materials and methods: To study the role of brain leptin in regulating hepatic TG flux we performed acute (4 hour) tyloxapol infusion studies in male Sprague Dawley rats in combination with isolated brain hyperleptinemia, which was achieved by infusing leptin or vehicle directly into the 3rd ventricle via stereotaxic intracerebroventricular (ICV) cannulae. To further test, whether acute changes in hepatic triglyceride secretion caused by brain leptin signaling also translate into measureable differences in hepatic lipid content, we complemented these studies by performing chronic ICV infusion studies, where we assessed hepatic lipid content non-invasively using 1H-magnetic resonance spectroscopy. Rats on either regular chow (RC) or high fat diet (HFD) were infused with leptin (RC 0.3 ug/d; HFD 0.9 ug/d), a leptin receptor antagonist (6ug/d) or vehicle over 4 weeks using osmotic mini-pumps attached to stereotaxic cannulae. Control animals were food restricted to account for body weight differences caused by the chronic leptin infusion.
Results: Acute ICV leptin infusion markedly increased hepatic TG export (1.9 ± 0.22 vs 2.9 ± 0.17 umol/kg/min; p=0.00013; n≥13 per group). In agreement with an increase in TG secretion, chronic leptin infusion reduced hepatic fat content compared to controls (0.61 ± 0.06 vs 0.42 ± 0.06% water signal; p=0.038; n=8 per group). These changes where mirrored by a reduction in multiple hepatic lipid species and a suppression in key de novo lipogenic proteins as assessed by western blot of liver tissue samples. Conversely, blocking endogenous CNS leptin signaling chronically by ICV infusion of a leptin receptor antagonist resulted in hepatic steatosis (+4.1 ± 7.0 vs +39.8 ± 10.4% change to baseline; p=0.021; n=5 per group). In HFD-challenged rats the effects of brain leptin on hepatic steatosis were absent, suggesting that obesity alters the ability of CNS leptin to promote TG secretion.
Conclusion: Here we show that brain leptin improves hepatic steatosis by increasing hepatic TG secretion and reducing de novo lipogenesis. Thus, these studies identify CNS leptin signaling as an important regulatory factor in determining hepatic lipid content. Restoration of brain leptin signaling could be a novel therapeutic strategy to ameliorate hepatic steatosis in obesity.
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Hepatic lipid content and composition in diet-induced metabolic dysregulation and recuperation

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Background and aims: The liver participates in the regulation of whole body energy balance by taking up and releasing both glucose and lipids, accordingly with the nutritional demands. We aimed at elucidating the temporal frame of hepatic lipid alterations in the ontogeny the diet-induced metabolic dysregulation and recuperation, notably in relation with the loss of glucose homeostasis, in mice fed a high fat diet (HFD).
Materials and methods: C57BL/6j mice (27.4 ± 0.4 g) were fed a HFD (60% kcal from fat, N=13) or a control diet (10% kcal from fat, CD N=9) for 26 weeks. At week 18, 4 mice in HFD were switched back to CD for the remainder 8 weeks. Glucose homeostasis was evaluated by fasting glucose and insulin levels and OGTTs (1.5 g/kg dose). HLC was measured by magnetic resonance spectroscopy (MRS) in vivo at 14.1 T and the contributions of saturated-, monounsaturated- and polyunsaturated fatty-acids (SFA, MUFA and PUFA) resolved using indices derived from water-suppressed spectra. MRS was performed in ad libitum fed mice with additional measurements after an overnight fast at weeks 18 and 26. Statistical significance was determined with unpaired Student's t test or Two-way ANOVA and One-way ANOVA with Newman-Keuls Multiple Comparison test when comparing more than two data sets.
Results: Mice in HFD showed higher body weight relative to CD from week 3 onwards, reaching a final weight of 51.5 ± 1.1 g at week 26 ($P<0.001$ vs CD, 30.8 ± 0.8 g), without differences in caloric intake. Reversal from HFD to CD reduced body weight to 35 ± 1.0 g, at the end of the study ($P<0.001$). Altered glucose tolerance was noticeable within a week in HFD, with modest increases in AUC and glycaemia at 2h ($P<0.05$), relative to CD. Fasting hyperinsulinemia was present in HFD from week 4 ($P<0.01$) and aggravated with time. Glucose tolerance was recovered within 2 weeks of dietary reversal while complete normalization of fasting insulin was achieved after 6 weeks. HLC at the beginning of the study was 1.5 ± 0.1% (pooled groups) and the fatty acid composition 0.4:0.7:0.4 (SFA:MUFA:PUFA). Alterations in hepatic lipids were not detected before 9 weeks in HFD, when HLC was 3.4 ± 0.5% ($P<0.01$ vs CD, 1.1 ± 0.1%) with SFA:MUFA:PUFA: of 0.8:0.7:1.9. HLC further increased in HFD until 18 weeks, not changing until the end of the study (18.6 ± 3.3% at 26 weeks with SFA:MUFA:PUFA: of 3.4:4.6:10.6). An overnight fast induced a 3-fold increase in HLC in CD mice but no changes in HFD mice. Reversal from HFD to CD progressively reduced HLC, which reached 2.4 ± 0.3% at the end of the study, still above that of CD mice (1.5 ± 0.2%, $P<0.01$). With dietary reversal, HLC doubled after an overnight fast, resembling the dynamics observed in CD mice.
Conclusion: In our paradigm, hepatic lipid alterations were a late-stage event in HFD-induced metabolic dysregulation. Ectopic fat accumulation in the liver was initiated and supported by PUFA and, to a less extent SFA, likely from unsuppressed adipose tissue lipolysis in an insulin resistance setting. HFD also hampered oscillations in hepatic lipids normally observed between fasted and fed states. Recuperation of metabolic control was achieved with dietary reversal to CD: glucose homeostasis was promptly and completely reasserted but hepatic lipids not fully normalized. Therefore, the hepatic lipid pool and its dynamic oscillations are relevant biomarkers of long term metabolic health status in the scope of nutritional challenges.
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Disclosure: **A.F. Soares:** None.

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Fasting plasma insulin concentrations are associated with changes in hepatic fatty acid synthesis and partitioning prior to changes in liver fat content in healthy adults

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Background and aims: Resistance to the action of insulin impacts on fatty acid delivery to the liver, fatty acid synthesis and oxidation within the liver and triglyceride export from the liver. To understand the metabolic consequences of hepatic fatty acid synthesis, partitioning, oxidation and net liver fat content in the fasted and postprandial states we studied healthy men and women with varying degrees of insulin resistance before and after consumption of a mixed meal using stable-isotope tracer methodologies.
Materials and methods: Thirty-seven healthy subjects were classified on their fasting plasma insulin concentration as normo-insulinemic (NI) (fasting plasma insulin less than 11.2 mU/L, n=18) or hyper-insulinemic (HI) (fasting plasma insulin greater than 11.2 mU/L, n=19). Postprandial hepatic de novo lipogenesis (DNL and fatty acid partitioning was investigated using metabolic substrates labelled with stable-isotope tracers (2H2O for DNL and oral U13Cpalmitate in a mixed meal for postprandial fatty acid metabolism). Blood and breath samples were taken in the fasted state and then regularly after consumption of a mixed test meal for 7 hours; with a second meal (75 g glucose drink) being consumed at 6 hours. Whole-body respiratory exchange ratio (RER) was measured in the fasting and postprandial state. Liver fat content was measured using MRS.
Results: There was no difference in liver fat content between HI (3.4 (1.4 - 27.6%) median (range)) and NI (3.4 (0.7 - 24.4%)) individuals, despite HI subjects having significantly (P<0.05) more visceral fat. Fasting plasma TG concentrations were significantly (P<0.05) higher in HI compared to NI subjects. Compared with NI subjects hepatic de novo lipogenesis (DNL) was higher (P<0.05) in HI subjects. Postprandial fatty acid oxidation, assessed by whole-body RER, and the incorporation of 13C from dietary fat into plasma 3-hydroxybutyrate (13C-3OHB) and expired breath was notably (P<0.05) lower in the HI compared to the NI group. We found a robust association between fasting plasma insulin concentration and fasting hepatic DNL in the HI (rs=0.53, P<0.05) but not NI (rs=0.20, P=NS) group. In contrast we observed a strong inverse association between postprandial DNL and 13C-3OHB in the NI (rs= -0.75, P<0.001) but not HI (rs= -0.14, P=NS) group.
Conclusion: These data suggest that metabolic pathways promoting fat accumulation are enhanced in HI but paradoxically without any significant impact on liver fat content when observed in healthy people. This is likely to be explained by increased triglyceride secretion as observed by hypertriglycerideaemia.
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Differential storage pattern of intramyocellular lipid in type 2 diabetes patients and trained athletes

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Background and aims: Intramyocellular lipid (IMCL) storage negatively associates with insulin resistance albeit not in endurance-trained athletes, in whom high IMCL levels coincide with high insulin sensitivity. Thus not the amount of IMCL per se, but other factors seem responsible for this relationship. With the aim to elucidate (one of) these factors we compared lipid droplet (LD) morphology and subcellular localization in muscle of type 2 diabetes patients (T2D) vs. trained athletes (TA).
Materials and methods: We performed morphometric analysis of LDs using quantitative immunofluorescent confocal imaging in sections of muscle biopsies obtained from T2D (n=8) and TA (n=8), similar in IMCL content (1.06±0.35% vs. 0.73±0.39%, p=0.087), but discrepant in insulin sensitivity (M-value=77.4±15.1 μmol/kg/min vs. 18.5±5.3 μmol/kg/min, p<0.001).
Results: In TA IMCL content was higher in type I compared to type II fibers (1.49±0.53% vs. 0.42±0.23%, p<0.001), whereas T2D had similar IMCL content in both fiber types (0.86±0.41% in type I vs. 0.65±0.40% in type II, p=0.316). In type II fibers, LDs were smaller in TA than T2D (0.24±0.06 μm^2 vs. 0.37±0.14 μm^2, p=0.022), whereas in type I fibers the number of LDs per fiber area was higher in TA compared to T2D (0.057 ±0.022 LDs/μm^2 vs. 0.031±0.013 LDs/μm^2, p=0.012). Interestingly, not only fiber type specific differences in size and number were observed; also subcellular distribution of LDs was different between TA and T2D. TA stored more IMCL in the intramyofibrillar area in type I fibers (1.45 ±0.40% for TA vs. 0.79±0.36% for T2D, p=0.003), whereas in T2D IMCL content was higher in the subsarcolemmal area of type II fibers (0.43±0.29% for TA vs. 1.93±1.21% for T2D, p=0.004).
Conclusion: Despite similar amounts of IMCL, TA and T2D show a distinct pattern of lipid storage in LDs in muscle. Whether this is (part of) the underlying mechanism for the difference in insulin sensitivity remains to be elucidated.
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Disclosure: **S. Daemen:** None.

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Challenging of AS160 alters lipid status in L6 myotubes

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Background and aims: The Akt substrate of 160 kDa (AS160) is a key regulator of GLUT4 translocation from intracellular depots to the plasma membrane of skeletal muscles. Similarly, long chain fatty acids (LCFAs) transport require relocation of protein transporters such as fatty acid translocase (FAT/CD36), fatty acid transport proteins (FATPs) and plasma membrane fatty acid-binding protein (FABPpm). The aim of our study was to determine the impact of AS160 silencing on intramyocellular lipid milieu in L6 myotubes. Moreover, in an attempt to better understand the role of AS160 in fatty acid induced insulin resistance, the present study compares two different settings, namely: 1) AS160 knockdown prior to palmitate stimulation, 2) palmitate stimulation with subsequent AS160 knockdown.
Materials and methods: In one of the groups, AS160 was knocked down (for 48 h) using gene silencing method before incubation with palmitate (0.75 mM) for next 16 h (designation of group: pre-silencing of AS160, AS160-/PA). In the second set of experiments, cells were incubated with palmitate (0.75 mM) for 16 h and then subjected to knockdown of AS160 (designation of group: post-silencing of AS160, PA/AS160-). The efficiency of AS160 silencing was checked at mRNA level with Real Time PCR. The expression of fatty acid transporters was determined via Western blot technique. Additionally, the effect(s) of AS160 challenging on the intracellular distribution of FA transport proteins was examined using immunofluorescence. Intracellular lipid content and FA

composition in selected fractions (FFA, DAG, TAG and PL) was estimated by GLC, whereas basal palmitic acid uptake was analyzed by means of scintigraphy.

Results: AS160 knockdown resulted in a decrease of its mRNA expression (-82%, $p < 0.05$) and protein content (-25%, $p < 0.05$), as confirmed by RT-PCR and Western blot, respectively. Both groups with silenced AS160 were characterized by a greater expression of the analyzed fatty acid transporters when compared with the cells incubated with palmitate alone (PA) [AS160-/PA and PA/AS160- vs. PA: FAT/CD36: +67%, +72%, FATP-1: +44%, +59%, and FATP-4: +64%, +59%, respectively, $p < 0.05$]. Moreover, the abovementioned had possibly contributed to an increased FA cellular influx (AS160-/PA and PA/AS160- vs. PA: +104% and +212%, respectively, $p < 0.05$). This enhanced FA uptake took place despite the decreased FABPpm expression (AS160/PA- and PA/AS160- vs. PA; -30% and -28%, respectively, $p < 0.05$). Accordingly, we observed that only AS160 post-silencing resulted in a marked decrement in DAG, TAG and PL content, but increased FFA content (-28%, -58%, -39% and +45%, respectively, $p < 0.05$). These changes were accompanied by a more pronounced decrease in the saturated fatty acids (SFAs) constituting TAG and PL. Interestingly, an opposite effect was observed for a group of pre-silencing of AS160, in which knockdown of AS160, in general, did not affect on lipid pool(s) as well as on fatty acids composition.

Conclusion: Our results indicate that under potentially damaging conditions (palmitate induced insulin resistance), post-silencing of AS160 significantly decreases the lipotoxic effect of PA followed by reduction of lipid fraction (mainly DAG and PL), the compounds with the potential to promote insulin resistance in L6 myotubes.

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The ratio of deep to whole subcutaneous adipose tissue thickness is higher in type 2 diabetes and relates to insulin resistance and liver fat content

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Background and aims: Deep subcutaneous adipose tissue (DSAT) could contribute to regulation of insulin sensitivity (IS). In glucose-tolerant individuals, the ratio of deep to whole abdominal subcutaneous adipose tissue thickness (DSAT/wSAT) correlates with insulin resistance (IR). Hepatocellular lipid content (HCL) relates to IR in both glucose-tolerant humans and type 2 diabetes patients (T2D). However, the relevance of DSAT/wSAT and fatty acid (FA) composition in DSAT in T2D is yet unknown. We hypothesized that (i) DSAT/wSAT is higher, (ii) FA unsaturation is increased and (iii) FA chain length in DSAT is reduced in T2D compared to glucose tolerant humans (CON).

Materials and methods: T2D and CON of similar age and body mass index (BMI) (T2D, n=22, age 54±2 yrs, BMI 32±1 kg/m^2 vs. CON, n=16, age 51±2 yrs, BMI 30±1 kg/m^2) underwent in vivo proton magnetic resonance (MR) spectroscopy to measure HCL as well as to assess FA double bond content from the unsaturation index (UI) and saturated chain length from the CH2/CH3 ratio, in DSAT. The UI was calculated as the ratio of the olefinic (5.3 ppm) to methylene (1.3 ppm) resonance intensities. The CH2/CH3 was calculated as the ratio of the methylene (1.3 ppm) to methyl (0.9 ppm) resonance intensities. In a subgroup of 10 T2D and 8 CON, thickness of DSAT and wSAT was measured with ultrasound imaging at the level of rectus abdominis muscle and validated by single-slice MR imaging. Biopsies of DSAT were taken under ultrasound guidance below the fascia scarpa, which separates superficial from deep subcutaneous adipose tissue. Mitochondrial respiratory capacity of octanoylcarnitine oxidation was measured ex vivo by high-resolution respirometry in biopsies of DSAT. The M-value was assessed using euglycemic-hyperinsulinemic clamp tests.

Results: The M-value was 33% lower and HCL was 68% higher in T2D ($p<0.001$ and $p<0.05$ vs. CON). T2D had higher DSAT/wSAT (0.71±0.01 vs. 0.58±0.02, $p<0.001$) and slightly lower CH2/CH3 (5.5±0.1 vs. 5.6 ±0.1, $p<0.05$) upon adjustment for age, sex and BMI, whereas UI did not differ between groups. DSAT/wSAT correlated negatively with M-value and positively with HCL ($r=-0.60$, $p<0.01$ and $r=0.66$, $p<0.005$), even upon adjustment for age and BMI. UI and CH2/CH3 did not correlate with M-value. Mitochondrial oxidative capacity in DSAT was not different between T2D and CON.

Conclusion: The relative increase of thickness and altered FA composition in deep subcutaneous adipose tissue suggest the presence of a novel phenotype in insulin resistance and T2D.

Disclosure: **K. Bódis:** None.

PS 055 Experimental therapies

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Preclinical, toxicological and safety assessments of MOD-6031, a novel long-acting GLP-1/Glucagon agonist, support Phase 1 study in healthy overweight and obese subjects

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Background and aims: MOD-6031 is a novel long-acting dual GLP-1/Glucagon agonist developed by OPKO Biologics for the indication of weight management and potentially type 2 diabetes. It is comprised of a 37 amino acid synthetic oxyntomodulin (OXM) peptide, identical in sequence to the endogenous human OXM, conjugated to polyethylene glycol (PEG) via a hydrolysable linker that spontaneously hydrolyzes under physiological conditions. This technology enables a slow and controllable release of the intact OXM and substantially prolongs its exposure. This consequently improves the potential of the released OXM to interact with its target receptors (GLP-1 and Glucagon) and effectively cross the blood-brain barrier. The study objectives were to characterize the in vitro properties of MOD-6031 and to provide pre-clinical as well as toxicological and safety assessment of MOD-6031 in order to support the initiation of a Phase 1 clinical study in overweight and obese healthy subjects.
Materials and methods: The in vitro properties of MOD-6031 were assessed by evaluating its ex vivo hydrolysis under different conditions, its ability to activate both GLP-1 and GCG receptors, by measurement of the DPPIV cleavage rate of MOD-6031, and by evaluating MOD-6031 role as activator of glucose-dependent insulin secretion. The prolonged pharmacological effects of MOD-6031, including weight loss and glycemic control, were evaluated in diet-induced obesity (DIO) and ob/ob mice models following once/twice a week subcutaneous (SC) injections for 30 days in comparison to twice daily administration of OXM and/or commercial GLP1R agonist. In addition, the anti-obesity and anti-diabetic effects of MOD-6031 were correlated to the prolonged pharmacokinetic profile.
Results: MOD-6031 hydrolysis was strongly effected by high pH level and high temperature, which cause the release of the native and active OXM peptide. The extended half-life of OXM (from ~10 min to ~9 h) and its pharmacological efficacy were confirmed in vitro and in diabetes/obesity models following SC administration of MOD-6031. Remarkable induced weight loss and food intake inhibition were observed, as well as significant improvement of glycemic and lipid profiles. A comprehensive battery of toxicological and safety pharmacological studies demonstrated an excellent safety profile without any unexpected adverse events at significant margins above the clinical doses. The changes noted were related to an exaggerated pharmacological response to the drug's weight loss effect at the high doses. No anti-MOD-6031 antibodies were detected.
Conclusion: The enhanced exposures and half-life of hydrolyzed OXM, together with the safety profile of MOD-6031, support the initiation of Phase 1a study aimed to assess the safety, tolerability and pharmacokinetics of MOD-6031 in healthy overweight or obese subjects receiving single escalating doses in a placebo-controlled design. Exploratory pharmacodynamic responses will be evaluated by monitoring glucose, insulin, FFA, triglycerides and adiponectin blood levels, and by monitoring glucose levels following a mixed-meal tolerance test.
Disclosure: **G. Hart:** None.

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Chronic treatment with cobalt protoporphyrin or hemin compromises adipogenesis and adiponectin production in a manner indenpendent of heme-oxygenase-1

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Background and aims: Adiponectin is a beneficial adipokine with insulin-sensitising, anti-inflammatory, cardioprotective properties. Type 2 diabetes is characterised by reduced circulating adiponectin levels. Hence therapeutic strategies to increase adiponectin levels are attractive. In preclinical models, induction of heme-oxygenase-1 (HO-1) by agents such as cobalt protoporphyrin (CoPP) or hemin has been reported to increase circulating adiponectin levels concomitant with decreased inflammatory cytokines prompting the proposal of a 'HO-1 - adiponectin axis'. We recently performed a series of experiments designed to provide direct evidence of this axis. Surprisingly, acute (24-48 h) treatment with CoPP or hemin had no effect on adiponectin expression or secretion in either healthy or TNFα-treated human adipocytes. Here, we have extended these studies to characterise the effects of chronic treatment during differentiation of human preadipocytes.
Materials and methods: Primary and SGBS human adipocytes were differentiated in the presence of increasing concentrations of CoPP or hemin. HO-1 activity or expression was blocked using an inhibitor (SnMP) or validated siRNA. Differentiation was determined morphologically and by determination of expression of genes including adipocyte markers (PPARγ, ADIPOQ, AdipoR2 & GLUT4), inflammatory markers (IL-6), markers of Nrf2 activation (NQO1 & GCLM) as well as HO-1 by qRT-PCR. Intracellular HO-1 and secreted adiponectin and IL-6 protein levels were determined by ELISA. Functional integrity of adipocytes was determined by measurement of basal and insulin-stimulated glucose uptake.
Results: Chronic treatment with CoPP or hemin throughout differentiation promoted a dose-dependent increase in HO-1 mRNA and protein (both ↑ 30-40 fold) concomitant with reduced adipogenesis (↓ upto 95%), decreased adiponectin production (↓ upto 95%), increased IL-6 production (↑ 10-20 fold) and basal glucose uptake (↑ upto 3 fold) and reduced insulin-stimulated glucose uptake (↓ upto 75%). Co-treatment with SnMP failed to reverse these effects. Co-treatment with HO-1 siRNA reduced the induction of HO-1 (by ≥ 60%) but also failed to ameliorate these effects. The expression of two genes NQO1 & GCLM which, like HO-1, are situated downstream of the Nrf2 transcription factor were increased by CoPP or hemin in a manner that was not prevented by co-treatment with SnMP or siRNA.
Conclusion: These results demonstrate that chronic treatment with CoPP or hemin interferes with differentiation of human adipocytes in a manner that appears incompatible with adipocyte function and increased adiponectin production. Intriguingly, these effects appear to be independent of induction of HO-1 activity or protein but may instead be linked to chronic activation of the Nrf2 transcription factor and downstream effectors implicated in oxidative stress.
Disclosure: **M. Yang:** None.

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Gut microbiota regulate pancreatic beta cell function in the host through epigenetic programming of gene expression

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Background and aims: Intrauterine undernutrition is a major causative factor for health outcomes in later life. We recently described a rat model

of multigenerational (50 generations) undernutrition, which closely mimics human populations in developing countries, to understand intergenerational transmission of environmental impact. Our current studies in these animals confirm that: i) the gut microbiota composition of lean rats differs from obese rats; and ii) that obese rats benefit metabolically after ingestion of lean rat faeces following coprophagic feeding behaviour. This corroborates with previous reports suggesting associations between the gut microbiome and metabolic syndrome (diabetes and obesity). Similar human studies confirm that metabolic benefits can be derived through faecal transplants; however, underlying mechanisms are not yet fully elucidated. The aim of this study was to identify the underlying mechanisms involved in association of gut microbial composition with obesity and Type 2 diabetes (T2D).

Materials and methods: Several aspects of metabolic syndrome were analysed using techniques including anthropometry, MRI imaging, DXA analysis, serum biochemistry, hyperinsulinemic-euglycemic clamp studies, gene expression analyses and chromatin (histone) methylation analysis. Susceptibility to diabetes was measured by assessing dose response to the diabetogenic agent; Streptozotocin (STZ). Short chain fatty acids (SCFAs) are administered to obese animals using osmotic pumps following a modified protocol that we described earlier. Bacterial DNA was isolated starting with 150mg of feces / gut contents (from ileum, caecum or colon) and gut microbiota was assessed using 16s pyrosequencing followed by validation of specific bacteria using real-time quantitative (q)PCR.

Results: Using various in vitro and molecular analyses, we demonstrate that i) diet and lifestyle over multiple generations selects for specific microbiota to populate the gut; ii) the biota of lean animals contains greater proportion of bacteria that produce specific short chain fatty acids (SCFAs), as compared to those of obese animals; iii) SCFAs directly regulate the expression of incretin genes through epigenetic programming of intestinal epithelial cells, thereby inducing them to produce higher levels of incretins; iv) increased incretin secretion programs better insulin release from the pancreas, which in turn v) improves the lifelong risk of Type 2 diabetes. We also demonstrate that localized delivery of SCFAs can change the gut biota of obese mice to mimic lean gut biota in just 4 days from transplantation.

Conclusion: Our data demonstrate the effect of microbial SCFAs on epigenetic programming of gut epithelial cells and ultimately insulin secretion. These studies identify metabolic pathways that may direct future pre- and pro-biotic or other therapies in early life to prevent adult obesity and T2D.

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Disclosure: **A.A. Hardikar:** None.

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Glucoraphanin-mediated Nrf2 activation reduces obesity and insulin resistance by increasing energy expenditure and limiting endotoxaemia-related chronic inflammation

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Background and aims: Increased oxidative stress in accumulated fat underlies obesity-associated insulin resistance. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a master regulator of the antioxidant response and plays a role in maintaining glucose homeostasis. However, the mechanisms by which activation of Nrf2 reduces obesity and insulin resistance remain largely unknown. In addition, although synthetic Nrf2 inducers alleviate obesity and insulin resistance in animal models, they are not clinically available due to safety concerns. Sulforaphane is a potent, naturally occurring Nrf2 inducer. Glucoraphanin is a stable glucosinolate precursor of sulforaphane that is hydrolyzed to yield bioactive sulforaphane prior to its intestinal absorption. Here, we examined the effect of glucoraphanin on high-fat diet (HFD)-induced obesity and insulin resistance in mice.

Materials and methods: Eight-week-old male C57BL/6J mice were fed either a HFD or a HFD containing glucoraphanin (300 mg/kg, HFD + GR) for 14 weeks. Oxidative stress markers and insulin sensitivity in the liver and epididymal white adipose tissue (eWAT) were examined. To assess the effect of glucoraphanin on energy metabolism, oxygen consumption and energy expenditure were assessed using indirect calorimetry. In addition, the immune cells present in the stromal vascular fraction of eWAT and in the liver were quantified using flow cytometry.

Results: Glucoraphanin suppressed HFD-induced weight gain by 15% ($p < 0.01$) without affecting food intake. HFD + GR mice exhibited consistently higher oxygen consumption and exhaled more carbon dioxide than did HFD mice, leading to a 12% increase in energy expenditure ($p < 0.01$) prior to measurable body mass differences. Glucoraphanin improved glucose tolerance, hyperinsulinemia, and fatty liver and enhanced insulin signaling, as assessed by phospho-Akt in liver, skeletal muscle, and eWAT. The hepatic levels of malondialdehyde, a marker of lipid peroxidation, were increased by HFD, while glucoraphanin treatment attenuated lipid peroxidation, accompanied by reduced gene expression of NADPH oxidase subunits gp91phox, p22phox, p47phox, and p67phox. A flow cytometry analysis revealed that glucoraphanin decreased the number of liver macrophages by 44% ($p < 0.05$) in HFD mice. In addition, HFD+GR mice had 67% fewer CD11c+CD206− (M1) macrophages but 20% more CD11c−CD206+ (M2) macrophages than HFD mice, resulting in a predominance of the hepatic M2 vs. M1 macrophage population. Glucoraphanin also caused an M2-dominant phenotypic shift of macrophages in eWAT. HFD-induced metabolic endotoxemia, driven by gut-microbiota-derived lipopolysaccharide (LPS), causes chronic inflammation. Although plasma LPS and LPS binding protein (LBP) levels were markedly higher in HFD mice than in mice fed normal chow, glucoraphanin markedly diminished the plasma levels of both markers. Moreover, the weight-reducing and insulin-sensitizing effects of glucoraphanin were abolished in HFD-fed Nrf2 knockout mice, indicating that the anti-obesity effect of glucoraphanin was Nrf2-dependnent.

Conclusion: Glucoraphanin-mediated activation of Nrf2 alleviated HFD-induced obesity and metabolic endotoxemia, thereby attenuating chronic inflammation in the liver and WAT and improving whole-body insulin resistance in mice.

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Disclosure: **T. Ota:** None.

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A limonene-derivative from Sudachi peel activates sirt1 and improves lipid and glucose metabolism in high fat diet-fed mice

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Background and aims: Sudachi (Citrus Sudachi) is a small sour citrus, and it is quite a typical seasoning for roast as well as raw fish in Japanese cuisine. Interestingly, it grows exclusively in Tokushima region of Japan, and Sudachi is a unique specialty of Tokushima prefecture. Recently our collaborators reported that administration of freeze-dried peel of Sudachi decreases serum triglyceride (TG) levels in obese human subjects. Thus we aimed to determine active components from Sudachi peel in the current study. Here we reports identification of the active ingredient to ameliorate lipid and glucose metabolism which are possibly dependent on sirt1.

Materials and methods: The effects of crude Sudachi peel were evaluated in Zucker diabetic fatty (ZDF) rats. Hexane-extract of Sudachi peel was fractionated by silica gel column and subjected to a screening using C2C12 myotubes by an index of intracellular TG content. The positive fractions were further purified by octadecylsilyl column. Activities of the compound were evaluated in high fat diet-fed male ddY mice as well as C2C12 cells.
Results: Serum TG level of ZDF rats was improved by daily administration of the freeze-dried Sudachi peel. It extended lifespan of ZDF rats without changing body weights. Stimulation by crude Sudachi peel reduced TG levels in cultured C2C12 myotubes as well. Thus we conducted the cell-based screening and found a limonene-derivative as an active compound affecting intracellular lipid content from Sudachi peel. The TG-lowering effects of the compound was sensitive to nicotinamide, a sirt1 inhibitor, and stimulation by this molecule increased sirt1 expression levels in a dose-responsive manner in the C2C12 cells. Phosphorylation and expression levels of AMPK alpha subunit were also increased by exposure to the limonene-derivative, which were inhibited by nicotinamide. In high fat diet-fed mice, repetitive administration of the compound for 10 days improved glucose tolerance, fatty liver, serum TG and cholesterol to the same levels as those of healthy mice with increase in sirt1 activities in the gastrocnemius muscle and liver.
Conclusion: We successfully identified at least one limonene-related compound, which can ameliorate lipid and glucose metabolism in vivo as well as in cultured cells. The metabolic effects of the compound should be mediated by activation of sirt1-AMPK pathway. The sirt1 activation is supposed to be principally due to upregulation of the expression levels. Repetitive administration of the compound might stretch the lifespan through sirt1 activation, though it remains to be determined. In conclusion, we identified a limonene-derivative which ameliorates metabolism in cultured cells and in vivo with increase in the activities and expression levels of sirt1 which should be involved in the metabolic action. It will be a novel lead compound for drug discovery which regulates metabolic homeostasis by modulating sirt1 expression levels.
Disclosure: **L. Miyamoto:** None.

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Beneficial effects of lobeglitazone on white adipose tissue inflammation and brown adipocyte differentiation in db/db mice

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Background and aims: Lobeglitazone, a novel thiazolidinedione (TZD)-based activator of peroxisome proliferator-activated receptor gamma (PPARr), has been recently approved for the treatment of type 2 diabetes mellitus (T2DM) in Korea.
Materials and methods: We investigated effect of lobeglitazone on adipose tissues (AT) in db/db mice. Seven-week-old male db/db mice were randomly assigned to two groups with (1)vehicle-treated (N=8), and (2)lobeglitazone-treated groups (N=8). Lobeglitazone (1mg/kg) was intraperitoneally injected daily for 20 weeks.
Results: Lobeglitazone treatment of 20 weeks increased the body weight by 15% than vehicle group (P=0.002). While the mean random glucose level at 20 weeks was 416.5 mg/dL in the vehicle group, lobeglitazone treatment significantly lowered it from 1 week after treatment and decreased it further by the mean value of 72.0 mg/dL at 20 weeks. Whereas epididymal AT mass was significantly decreased, subcutaneous AT mass was increased in lobeglitazone-treated group. We measured the adipocytes' size and found that lobeglitazone resulted in smaller adipocytes in both epididymal and subcutaneous AT (both Ps<0.001). Using flow cytometry, the CD11c-positive M1 macrophages and CD206-positive M2 macrophages in the epididymal fat tissue exhibited decreased M1-to-M2 ratio by lobeglitazone treatment. Furthermore, in lobeglitazone-treated group, interscapular brown AT mass was significantly increased (P<0.001), and it also clearly visualized by Positron Emission Tomography -Computed Tomography (PET-CT) compared to vehicle group. In immortalized brown preadipocytes, lobeglitazone treatment further promoted expression of the uncoupling protein 1 (UCP1) and significantly activated a central thermogenic factor interferon regulatory factor-4 (IRF-4) compared to treatment of pioglitazone or rosiglitazone, even in the absence of the normally required hormonal induction cocktail.
Conclusion: These findings point towards a beneficial role of lobeglitazone treatment in white AT inflammation and biology as well as glycemic control in db/db mice. To our knowledge, we for the first time observed the induction of brown adipocyte differentiation by lobeglitazone in db/db mice.
Disclosure: **B. Cha:** None.

PS 056 Adipose tissue crosstalk

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Obesity and type 2 diabetes alters the immune properties of human adipose derived stem cells

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Background and aims: Adipose tissue-derived stem cells (ASCs) are proposed as an alternative stem cell source to bone marrow-derived cells for immune cell therapy. However, microenvironmental factors may contribute to the functionality of the stem cell population in human adipose tissue (AT). Once viewed as an energy storage depot, AT is now considered as a bona fide immune organ, at the crossroads between metabolism and immunity. Besides adipocytes, AT contains various immune cells involved in adaptive (B and T lymphocytes) and innate (macrophages) immunity. Indeed, AT-resident lymphocytes and macrophages might play a key role in the etiopathogenesis of obesity. Accordingly, ASCs not only participate in the turnover of mature adipocytes in humans (ca. 10% per year), but they also possess immunoregulatory properties that might have an important yet unknown pathophysiological function. Analogous to other mesenchymal stem cells (MSCs), human ASCs might be recruited to sites of tissue damage where they could regenerate new tissues and repair the defects. Our previous study revealed that obesity determines the stem cell population within adipose tissue. We hypothesized that the fat depot in addition to the donor phenotype controls the immunomodulatory capacity of ASCs.
Materials and methods: Focusing on obesity and type 2 diabetes (T2D) as metabolic disorders which might affect the immune response of ASCs, we compared the inflammatory response of ASCs isolated from subcutaneous and visceral AT from age-matched donors (lean n=4, body mass index [BMI] 21.98±1.9; obese n=4 BMI 33.1±2.1 and T2D n=4 BMI 35.3 ±1.5).
Results: Obese but remarkably T2D-derived ASCs showed increased expression of inflammatory markers, activation of the NLRP3 inflammasome and higher migration, invasion and phagocytosis capacities than those derived from lean donors. Remarkably, ASCs derived from obese and T2D subjects exhibited a reduction in typical immunosuppressive activities attributed to stem cells. Accordingly, obese and T2D-ASCs were less effective in suppressing T cell and B cell proliferation, activating the M2 macrophage phenotype as well as in increasing TGF-B1 secretion than lean-derived ASCs.
Conclusion: Overall, these data indicate that the metabolic phenotype of the donor compromises the immunomodulatory properties of ASCs, particularly in cells derived from visceral AT. These results are relevant not only for understanding the physiology of ASCs in terms of cell-based therapies but also for their role as key regulators of the immune response.
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Disclosure: **C. Serena:** None.

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Essential role of fat tissue PIKfyve in systemic glucose homeostasis and insulin sensitivity revealed through pikfyve disruption by two adipocyte promoters driving Cre

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Background and aims: We have previously demonstrated that systemic deficiency of PIKfyve, the evolutionarily conserved phosphoinositide kinase synthesizing cellular PtdIns5P and PtdIns(3,5)P2 and implicated in insulin signaling, causes early embryonic death in mice. By contrast, mice with muscle-specific pikfyve disruption have normal lifespan but exhibit early-age whole-body glucose intolerance and muscle insulin resistance, thus, establishing the key role of muscle PIKfyve in glucose homeostasis. Fat and muscle tissues control postprandial glucose clearance through different mechanisms, raising questions as to whether adipose pikfyve disruption will also trigger whole-body metabolic abnormalities and, if so, what the mechanism might be.
Materials and methods: To answer these questions, we have generated two new transgenic mouse lines with adipose tissue disruption of pikfyve by crossing our PIKfyvefl/fl mice with mice expressing Cre recombinase under the adipose-specific fatty acid binding protein 4 (aP2) or adiponectin (Aq) promoter. Glucose and insulin tolerance tests (GTT and ITT) were performed after injecting glucose and insulin, respectively, and subsequent glucose measurements in mouse-tail blood at different time intervals. Adipose tissue akt phosphorylation was measured by immunoblotting subsequent to insulin or saline intraperitoneal injections in 14 h fasting Aq-Cre-PIKfyvefl/fl or control fl/fl mice. Lipolysis was measured in freshly isolated fat cells. Plasma concentration of free fatty acids and adiponectin were measured by a commercial kit and immunoblotting with anti-adiponectin antibodies, respectively. PIKfyve protein levels were assessed in different tissues by western blotting with anti-PIKfyve antibodies.
Results: Both PIKfyvefl/fl,aP2-Cre+ and PIKfyvefl/fl,Aq-Cre+ mice were born at the expected Mendelian ratio. They were ostensibly normal, healthy and fertile with growth curves for body weight and a life span similar to those of littermate PIKfyvefl/fl brothers and sisters up to >10 months of age. Western blotting revealed that PIKfyve protein levels were selectively decreased by ~50-80% in fat pads of both male and female PIKfyvefl/fl,aP2-Cre+ or PIKfyvefl/fl,Aq-Cre mice vs. corresponding PIKfyvefl/fl littermates. Both aP2-Cre-PIKfyvefl/fl and Aq-Cre-PIKfyvefl/fl mice exhibited severely dysregulated glucose homeostasis and systemic insulin sensitivity as judged by the area under the curve during GTT and ITT. These abnormalities stemmed in part from accelerated fat-cell lipolysis and elevated serum FFA, but not from altered adiponectin secretion that remained similar in mutant and control mice. Adipose tissue was severely insulin resistant as judged by the profound reduction of Akt activation in response to intraperitoneal injection of insulin in Aq-Cre-PIKfyvefl/fl mice, consistent with the requirement of adipose PIKfyve and its lipid products for efficient Akt phosphorylation by insulin. Reduced ability of insulin to restrain beta-adrenergic receptor-activated lipolysis in Aq-Cre-PIKfyvefl/fl fat cells was also evident.
Conclusion: Our data identify for the first time that adipose tissue pikfyve plays a key role in the mechanisms regulating whole-body glucose homeostasis and adipose tissue insulin sensitivity, and reveal an unexpected role of adipose pikfyve in the mechanisms regulating fat tissue triglyceride storage.
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Disclosure: **A. Shisheva:** None.

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Early perivascular adipose tissue dysfunction impairs insulin induced vasodilation via JNK2

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Background and aims: In obese humans insulin induced vasodilation and skeletal muscle perfusion are reduced due to changes in perivascular adipose tissue. We hypothesized that a short term western diet (WD) impairs insulin-induced vasodilatation via changes in PVAT, and that infiltration of circulating JNK2 expressing cells in PVAT is involved in this process.

Materials and methods: A two week WD or chow intervention was applied to male C57BL/6J mice after transplantation with c-Jun N-terminal kinase isoform 2 (JNK2) JNK2+/+ or JNK2-/- bone marrow. Resistance arteries were isolated for ex-vivo vasoreactivity assay. Insulin sensitivity was assessed using a euglycemic hyperinsulinemic clamp in combination with microvascular perfusion imaging. RNA was isolated from PVAT, epididymal and subcutaneous adipose tissue for qRT-PCR of target genes.
Results: Here, we show that two weeks of WD blunts the ability of PVAT to induce insulin-induced vasodilation in an ex vivo vasoreactivity assay. In contrast, exposure of resistance arteries to PVAT from WD JNK2-/- bone marrow transplanted C57BL6 mice restores vasodilation. Deletion of JNK2 in bone marrow salvages the vasodilator phenotype of PVAT during WD exposure. In vivo, insulin sensitivity was partially recovered in WD JNK2-/- bone marrow transplanted C57BL6 mice to chow JNK2+/+ levels. In addition, WD alone blunts insulin induced muscle perfusion compared to an increase in perfusion in chow fed mice. While the JNK2 deletion in PVAT prevents this perfusion problem in WD fed mice. Furthermore we assessed whether JNK2 in hematopoietic cells is directly involved in in obesity induced inflammation of different adipose tissue depots. We found that there was no difference in mRNA expression of inflammatory genes Tnfα, Il1β, Il6, or Pias1 in PVAT, epididymal or subcutaneous adipose tissue depots. However, pro- inflammatory monocyte gene F4/80 and adipokine Leptin were increased in PVAT by WD while increase was prevented by JNK2 deletion. Increase of fat mas and obesity associated protein (Fto) in WD-JNK2-/- mice suggests PVAT remodeling.
Conclusion: Impaired muscle perfusion in WD without signs of inflammation in PVAT is caused by PVAT dysfunction via infiltration of JNK2 positive cells from the bone marrow into PVAT. This results in a change of PVAT composition which is responsible for the changed vasoreactivity.
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Disclosure: **F.P.M. Hoevenaars:** None.

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The novel FAHFA lipids synthesised by human adipose tissue are related to adipose cell GLUT4, lipogenesis and improved adipogenesis

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Background and aims: Adipose tissue dysfunction, associated with hypertrophic obesity and low levels of the insulin-regulated glucose transporter GLUT4 in the adipose cells, is an important contributor to systemic insulin resistance and associated complications including Type 2 diabetes (T2D). Recently, a novel class of endogenous lipids, branched fatty-acid esters of hydroxy-fatty acids (FAHFAs), was discovered. A subfamily of FAHFAs, palmitic acid esters of hydroxystearic acids (PAHSAs), was shown to have anti-diabetic and anti-inflammatory effects in mouse models and to be reduced in insulin-resistant humans. Synthesis of these lipids is regulated by GLUT4-associated glucose uptake in the adipose tissue, activation of ChREBP and enhanced de novo lipogenesis as shown in mouse models. The aim of the present study was to investigate if markers of adipose tissue dysfunction, i.e., adipocyte hypertrophy, low adipose cell GLUT4 and markers of impaired adipogenesis, are related to PAHSA levels in human subjects. In addition, we examined if PAHSAs regulate adipocyte differentiation.
Materials and methods: Serum and adipose tissue biopsies from metabolically well-characterized human subjects were analysis for PAHSA and markers of adipose tissue dysfunction. 3T3-L1 pre-adipocytes were used for adipocyte differentiation studies. Statistical analyses were performed using Wilcoxon non-parametric test and Spearman correlation.
Results: We found that adipose cell GLUT4 protein expression is reduced in insulin-resistant subjects and correlates with systemic insulin sensitivity (R=0.40, p=0.009). We also found adipose cell GLUT4 levels to be positively correlated to adipogenic genes; PPARγ, C/EBPα and adiponectin. Thus, GLUT4 is a good marker of local and systemic insulin sensitivity and fully functional, non-dysregulated adipose cells. Furthermore, adipose cell GLUT4 expression is closely correlated with mRNA levels of two central enzymes for de novo lipogenesis ACACA (R=0.77, p<0.001) and FASN (R=0.73, p<0.001), both known to be regulated by ChREBP. These results confirm the close link between GLUT4 protein, glucose uptake and lipogenesis also in human adipose cells. Furthermore, we found adipose cell GLUT4 protein levels to be strongly and positively correlated with adipose tissue levels of 5-, 9-, 10-, 12/13- and total-PAHSA (R=0.74, p=0.006) indicating that high levels of GLUT4 and glucose uptake regulate PAHSA production, degradation or release from human adipose tissue. In addition, we found adipocyte cell size, a marker of hypertrophic obesity and dysregulated adipose tissue, to be inversely related to adipose tissue levels of all PAHSA isomers measured including total-PAHSAs (R=-0.84, p=0.001). We also showed that PAHSAs improve preadipocyte differentiation through a mechanism not involving direct activation of PPARγ.
Conclusion: The discovery of FAHFAs and our current results provide novel insights into positive effects of secreted adipokines and why a dysfunctional and hypertrophic adipose tissue is associated with insulin resistance and risk of T2D. We conclude that adipose tissue dysfunction is related to reduced levels of GLUT4 and reduced production and secretion of the novel PAHSAs. Further studies are under way to understand the synthetic and degradative pathways and biological functions of these novel lipids.
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Disclosure: **A. Hammarstedt:** None.

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Skeletal muscle-specific expression of DGKδ protects against diet-induced obesity through metabolic crosstalk with adipose tissue

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Background and aims: Diacylglycerol (DAG) is a precursor for the formation of triacylglycerides and phospholipids, and acts as a second messenger molecule. DAG can be phosphorylated to phosphatidic acid by diacylglycerol kinases (DGKs), a family of kinases with 10 different isoforms, all with heterogeneous tissue distribution. We have previously shown that DGKδ deficiency increased diacylglycerol content, reduced peripheral insulin sensitivity, and led to age-dependent obesity. Here we tested the hypothesis that increased skeletal muscle DGKδ abundance will protect against diet-induced obesity (DIO) and insulin resistance.
Materials and methods: Skeletal muscle-specific DGKδ transgenic (MLC-DGKδ Tg) and wild-type (WT) male mice were fed chow or high fat diet for 12 weeks. Body composition was assessed using magnetic resonance imaging. Intraperitoneal glucose tolerance tests were performed. Fatty acid oxidation and glucose uptake was determined in isolated extensor digitorum longus (EDL) muscle. Basal and noradrenaline-stimulated lipolysis was assessed in adipose tissue. Cell culture experiments were performed in 3T3-L1 adipocytes exposed to serum from either MLC-DGKδ Tg or WT mice. Statistical analysis was performed using either a two-way ANOVA with Bonferroni post-test, or Student's t-test where appropriate.
Results: Body weight and fat mass was reduced and glucose tolerance was enhanced in chow-fed MLC-DGKδ Tg mice. Importantly, MLC-DGKδ Tg mice were protected against the development of obesity and glucose intolerance when challenged with a high fat diet. Fatty acid

oxidation and glucose uptake was unaltered in isolated EDL muscle from MLC-DGKδ Tg mice. However, basal and noradrenaline-stimulated lipolysis was increased in gonadal fat from MLC-DGKδ Tg mice. Furthermore, mRNA expression of the adrenoceptor beta 1, 2 and 3 was increased in gonadal fat from DIO MLC-DGKδ Tg mice. These results suggest skeletal muscle overexpression of DGKδ promotes tissue cross-talk with adipose tissue presumably via a skeletal muscle derived factor that increases the sensitivity of adipose tissue to adrenaline stimulation. In support of such a mechanism, in vitro experiments revealed conditioned media containing serum from MLC-DGKδ Tg mice increased lipolysis in 3T3-L1 adipocytes.

Conclusion: Overexpression of DGKδ in skeletal muscle plays an important role in whole body energy homeostasis by preventing DIO and glucose intolerance. Moreover, skeletal muscle overexpression of DGKδ promotes tissue cross-talk with adipose tissue presumably via a skeletal muscle derived factor that increases the sensitivity of adipose tissue to catecholamine stimulation. Future research is now aimed at identifying the nature of this factor and mechanisms for skeletal muscle-adipose tissue cross-talk on glucose and lipid metabolism.

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Disclosure: **M.L. Borg:** None.

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Changes in the metabolic gene NRIP1 expression in response to weight loss and exercise

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Background and aims: Nuclear receptor interacting protein 1 (NRIP1) is an important regulator of energy expenditure. Here we investigated changes in NRIP1 gene expression in adipose tissue and skeletal muscles; and protein levels in serum in response to weight loss and exercise.

Materials and methods: NRIP1 expression in subcutaneous adipose tissue biopsies was measured in 14 obese adults subjected to a 3-month weight loss (WL) followed by 6-month weight maintenance (WM) intervention. NRIP1 protein level was measured in the serum of 113 patients. To investigate the changes in NRIP1 expression in response to exercise, we also analysed skeletal muscle transcriptome from database GSE59088.

Results: In obese subjects, we observed significantly increased NRIP1 mRNA levels after WL compared to baseline, which remained to be elevated after WM. Gas chromatography-mass spectrometry metabolomics analysis revealed positive association between NRIP1 expression and HDL cholesterol and glycine levels; and negative association with triglycerides, BMI, fat mass, and waist circumference. In the serum of non-obese subjects, males (n=42) have 3-fold higher NRIP1 than the females (n=71), which is negatively associated with BMI, waist-hip ratio, acute myocardial infarction, age, etc. In skeletal muscle, NRIP1 expression increased 80% in the sedentary rest group and ~25% in the strength training group compared to before sessions; while no changes in the endurance training group. Furthermore, NRIP1 expression became sensitive to insulin stimulation during a euglycemic clamp after sedentary living, whereas re-training led to decreased NRIP1 levels.

Conclusion: These observations imply that expression of NRIP1, a suppressor of mitochondrial function, is elevated in states characterized by low energy demand, either due to lack of surplus energy (after weight loss), or under non-oxidative conditions (resistance training). During endurance training where the major energy comes from oxidative metabolism of carbohydrate and lipid, NRIP1 expression is suppressed to ensure high energy output. NRIP1 may therefore serve as an important marker and target for various interventions.

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ASCs from obese environment are more resistant to apoptosis: a potential role of survivin

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Background and aims: Adult adipose tissue (AT) contains a pool of abundant multipotent stem cells, designated as adipose-derived stem cells (ASCs) that are able to replicate as undifferentiated cells, and to develop as mature adipocytes. Current available information indicates that ASCs are important players in the dynamics of AT remodeling, an ongoing process that is dysregulated in obesity. Thus, obesity appears to be linked to defective cellular turnover and remodeling of AT. The size of the progenitor´s cell compartment is governed by the balance between self-renewal (i.e. proliferation) and cell loss (apoptosis). We have recently shown that ASCs from obese patients have enhanced proliferation capacity; however, their sensitivity to apoptosis is unknown. The aim of this work is to study the sensitivity of ASCs to apoptosis and to gain insight into the molecular players involved.

Materials and methods: ASCs were isolated from subcutaneous adipose tissue of lean and obese subjects. Serum was also collected. Apoptosis was measured using annexin-propidium iodide staining by flow cytometry. Gene and protein expression were assessed by qPCR, WB and ELISA. Recombinant adenovirus was used to perform overexpression studies.

Results: The profiling of several anti- and pro-apoptotic proteins showed increased levels of survivin, a member of the IAP family of antiapoptotic proteins, in both AT and ASCs from obese individuals. Accordingly, serum survivin levels were enhanced in obese patients and survivin secretion was higher in conditioned media from obese ASCs compared to those isolated from lean subjects. Additionally, inflammatory stimuli such as conditioned media from M1 macrophages, enhanced survivin expression in ASCs from lean patients. Increased levels of survivin in obese context correlate with higher protein stability, lower levels of protein ubiquitinization and lesser mir-203 expression (a direct upstream negative regulator of survivin) in obese ASCs compared to their lean counterparts. Remarkably, survivin promoter was more methylated, turning into decreased mRNA levels as a possible counteracting mechanism to such large amount of protein and secretion. Apoptotic stimulus, such as leptin and hypoxia, induced apoptosis in ASCs from lean but not from obese donors. More interestingly, adenovirally overexpression of survivin in lean ASCs mimicked the apoptosis-resistant phenotype of obese ASCs.

Conclusion: ASCs from obese environment are more resistant to apoptosis and survivin appears to play a key role. These results uncover that an obese environment could promote the stem cell niche.

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Disclosure: **V. Ceperuelo-Mallafre:** None.

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Stem cells isolated from adipose tissue of obese subjects show different methylation patterns that compromise their biological functions

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Background and aims: Human adipose derived mesenchymal stem cells (hASCs) are progenitor cells that are part of a structural component of the stroma surrounding mature adipocytes and maintain tissue homeostasis. Available information indicates that hASCs are important players in the

metabolic dynamics of the adipose tissue (AT) participating in the development of obesity and related comorbidities. We have proved that AT from obese individuals contains a dysfunctional population of hASCs. In this sense, we hypothesize that the hostile environment associated with obesity (as inflammation and hypoxia) could be the underlying cause of the defective properties of AT-resident stem cells through epigenetic modifications

Materials and methods: We isolated hASCs from subcutaneous (SAT) adipose tissue of lean (BMI 20-24,9 Kg/m2; N=6) and obese (BMI 30-34,9 Kg/m2; N=6) subjects. gDNA was extracted from all hASCs and its mature adipocytes descendants (mAd-hASCs). An Infinium Human Methylation 450 Bead Chip was performed for epigenome-wide association studies (a total of 4 comparisons were completed (lean vs obese hASCs, lean hASCs vs lean mAd-hASCs, obese hASCs vs obese mAd-hASCs, lean vs obese mAd-hASCs). Gene expression of some affected genes were also validated by qPCR. Functional studies were also performed.

Results: Differentially DNA methylation profiles exist due to the obese environment in the hASCs niche (650 significant differentially methylated regions (DMR)) and these differences diminish in mature adipocytes (206 DMR). We showed that DNA methylation is quite static during the transition from stem to the fully mature adipocyte, and most of the differences observed are due to the obese phenotype in the hASCs niche. Interestingly, most of these changes are located in transcribed regions, which have also been actively correlated with gene expression. Gene Ontology analysis revealed adipogenesis, inflammation and migration as the biological functions most significantly represented among the DMR identified in the hASCs niche. Specifically, TBX15, a transcription factor of the T-box family of genes involved in adipogenic differentiation and mitochondrial metabolism, is differentially methylated in 13 DMRs in obese hASCs. We confirmed that TBX15 down-methylation found in hASCs isolated from obese donors compared to those isolated from lean subjects correlates with an increase in mRNA expression, which may contribute to the phenotype of obese hASCs.

Conclusion: Our study reveals that methylation status is significantly modified in an obese environment supporting hASCs dysfunction as a key regulatory event in obesity.

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Disclosure: **S. Fernandez-Veledo:** None.

PS 057 Fatty acids: long and short chain

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The free fatty acid receptor GPR84: A new player in glucose tolerance and mitochondrial function?

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Background and aims: Free fatty acid receptors, such as GPR40 and GPR120, have been recognized as important mediators of favourable metabolic effects on insulin secretion, inflammation and glucose uptake. Here we describe a potential new player involved in the regulation of glucose tolerance and mitochondrial function, the G-protein coupled receptor GPR84. GPR84 is a medium-chain fatty acid (MCFA; C8-C14) receptor that was originally thought to be expressed only in immune cells. However, our new data suggest that GPR84 is also abundantly expressed in skeletal muscle. We previously showed that mice and rats fed a MCFA diet (based on coconut oil) were partially protected from lipid-induced glucose intolerance and insulin resistance, when compared to animals fed a lard-based high-fat diet (enriched in long-chain fatty acids). This study aimed to determine if GPR84 was involved in potentiating those beneficial metabolic effects.

Materials and methods: Global GPR84 knockout (KO) and wild type (WT) male mice were fed either a low-fat control diet, a lard-based high-fat diet or a MCFA-enriched high-fat diet for 8 weeks. Following the feeding period, various metabolic parameters were investigated including obesity, glucose tolerance, oxidative stress and mitochondrial oxidative metabolism.

Results: The MCFA diet and lard diet led to increased fat deposition that was independent of genotype. WT mice fed the MCFA diet showed protection from lipid-induced glucose intolerance compared to the lard diet but this protection was completely lost in KO mice (iAUC *WT*: C 222.1±66.0, MCFA 345.3±36.6, lard 543.2±109.2; *KO*: C 266.3±27.7, MCFA 470.9±69.8, lard 406.7±86.2). In addition, GPR84 KO mice fed the MCFA diet exhibited a substantial impairment in mitochondrial function in skeletal muscle. Mitochondrial respiration was decreased while mitochondrial superoxide generation was increased in muscle of MCFA-fed KO mice (state 3 respiration with succinate: WT 127.4±33.7, KO 88.5±16.9 nmol/min/mg protein; superoxide generation: WT 460.1±71.1, KO 638.5±99.8 pmol/min/mg protein). Higher superoxide generation in MCFA-fed KO mice was accompanied by higher oxidative damage, suggesting an overall increase in oxidative stress in muscle of MCFA-fed GPR84 KO mice. Interestingly, decreased mitochondrial respiration was observed despite an increase in overall muscle mitochondrial content (determined as protein content of the mitochondrial membrane protein VDAC, mitochondrial/nuclear DNA copy number, and citrate synthase activity). The disconnect between an increase in mitochondrial content but a decrease in mitochondrial function is potentially due to defects in autophagic removal of mitochondria leading to accumulation of 'defective' mitochondria.

Conclusion: The results suggest that the MCFA receptor GPR84 plays an important role in glucose tolerance, potentially via the regulation of mitochondrial health and function in skeletal muscle.

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Disclosure: **M.K. Montgomery:** None.

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The nuclear receptor FXR decreases murine enteroendocrine L cell response to gut microbiota metabolites, the short chain fatty acids

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Background and aims: Diabetes mellitus involves many metabolic disorders including a decrease in incretin effect. One of the incretine hormones, Glucagon-Like Peptide-1 (GLP-1) is produced and secreted by enteroendocrine L cells which represent 1% of the intestinal epithelial cells. We have recently shown that nuclear receptor Farnesoid X Receptor (FXR) activation in enteroendocrine L cells decreases glucose-induced ChREBP-dependant proglucagon gene transcription. By inhibiting glycolysis pathway, FXR also decreases glucose-induced GLP-1 secretion. The aim of this study is to investigate the role of FXR in the L cell response to other GLP-1 secretagogues and especially to short chain fatty acids (SCFA) that are metabolites produced by the gut microbiota by fermentation of non digestible polysaccharides. Indeed, in addition to their contribution of 5 to 10% of the daily energetic resources, SCFA are also signalling molecules as they bind to the transmembrane receptor GPR43/FFAR2, thereby promoting GLP-1 secretion by L cells.
Materials and methods: FXR was activated in vitro in the murine cell line GLUTag and in vivo in C57Bl6/J mice by the synthetic agonist GW4064. GLP-1 secretion tests (ELISA) in response to Butyrate, one of the SCFA, were performed in vitro in GLUTag and ex vivo on murine colonic explants. GPR43 mRNA levels were evaluated by qPCR in colon from mice treated with GW4064, KO FXR mice and mice treated with colesevelam, a bile acid sequestrant, which display a drastic down regulation of FXR transcriptional activity.
Results: FXR activation decreases GLP-1 secretion in response to butyrate both in vitro in GLUTag and ex vivo in mouse colonic explants. In parallel, FXR activation decreases GPR43 mRNA levels. As a mirror effect, FXR KO mice and colesevelam treated mice exhibit an increased GPR43 mRNA level in colon.
Conclusion: FXR activation decreases GPR43 expression and L cell capacity to respond to SCFA in terms of GLP-1 secretion. Disregulation of FXR seems to be a good way to increase L cell capacity to respond to glucose and to gut microbiota metabolites.
Disclosure: **S. Ducastel:** None.

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Impaired adipose tissue lipid storage, but not altered lipolysis, contributes to elevated levels of free fatty acids in type 2 diabetes

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Background and aims: Elevated levels of circulating free fatty acids (FFA) mediate many adverse metabolic effects including insulin resistance and beta cell failure. We aimed to elucidate adipose tissue (AT) mechanisms related to FFA metabolism in type 2 diabetes (T2D).
Materials and methods: 20 control and 20 metformin-treated T2D (HbA1c 6.6 ± 0.3%, mean±SD) subjects were matched for gender (10M/10F), age (58 ± 11 vs 58 ± 9 y) and BMI (30.8 ± 4.6 vs 30.7 ± 4.9 kg/m2). In vivo lipolysis was assessed during a 3 h-OGTT with plasma glycerol and FFA levels. MRI was performed for body fat measurements. Subcutaneous AT samples were obtained by abdominal needle biopsies in fasting and 90 min post-OGTT to measure mRNA and metabolite levels of factors related with lipolysis and lipid storage. In vitro lipolysis was measured in fasting isolated adipocytes.
Results: Plasma FFA levels during the OGTT where higher (30% for AUC, p=0.005) in T2D than in control subjects, but plasma glycerol AUC did not differ. Adipocyte lipolysis in vitro (glycerol release) was similar, for basal, isoproterenol-stimulated ± insulin conditions. This suggests normal AT lipolysis regulation. In multivariate analyses, HbA1c, visceral AT volume and males were significantly associated with FFA AUC in T2D. Expression in AT of genes involved in lipid storage (DGAT1, DGAT2, FABP4, FASN, PC, PDHA1 and PPARG, P<0.05) were significantly reduced in T2D compared with control subjects, but no difference was seen for genes involved in lipolysis. T2D had significantly elevated markers of beta-oxidation, 2-hydroxybutyrate (1.4-fold, p<0.01) and 3-hydroxybutyrate (1.5-fold, p<0.05) in plasma, but not in AT. OGTT decreased 3-hydroxybutyrate (80-90%, p<0.01), a broad range of acyl carnitines (eg decanoylcarnitine, 40-60%, p<0.001) and 3-hydroxy fatty acids (eg 3-hydroxyoctanoate, 50-60%, p<0.001) in plasma of both controls and T2D subjects, which is consistent with decreased lipolysis and beta-oxidation. However, 3-hydroxybutyrate and 3-hydroxy fatty acids remained elevated (about 2-fold, p<001) in the obese T2D group compared with the obese control group in the post-OGTT period. The % of molecular species of unsaturated fatty acids including diacylglycerols (eg DG20:3n6, 1.9 fold, p<0.05), triacylglycerols (eg TG20:3n6, 2.1-fold, p<0.01), cholesterol esters (eg CE18:3n3, 2.1-fold, p<0.01), phosphatidylcholines (eg PC20:3n6, 1.6-fold, p<0.001) and sphingomyelins (eg SM22:1, 1.5-fold, p<0.01) were increased in the AT of obese T2D compared with the obese control following OGTT. Surprisingly, this may provide an anti-inflammatory component to the AT environment after nutritional challenge in obese T2D.
Conclusion: In conclusion, glycemic control, visceral adiposity and gender (male) contribute to elevated plasma FFA in T2D. Underlying mechanisms may include impaired FFA re-esterification or lipogenesis but not lipolysis regulation in AT.
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Fatty acid trafficking in familial partial lipodystrophy: discovery of a new pathological pathway for the generation of non-esterified fatty acids

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Background and aims: Familial partial lipodystrophy of Dunnigan-Kobberling type is invariably associated with extreme insulin resistance and often with early presentation of type 2 diabetes. Patients with this condition have high plasma concentrations of non-esterified fatty acids (NEFA). Plasma NEFA concentrations are normally determined by the release rate from adipose tissue and in light of the relative absence of major fat depots in this condition, the high NEFA concentrations are paradoxical. In this physiological study we are investigating the origins of NEFA in patients with the Dunnigan-Kobberling syndrome.
Materials and methods: Five patient with verified mutations in the LMNA gene and age, sex and abdominal fat mass-matched healthy controls were studied. In order to assess the regional adipose tissue NEFA release rate we established arterio-venous blood sampling across the subcutaneous abdominal and femoral tissue. We traced endogenous NEFA by a constant infusion of 2H2-palmitate and exogenous fatty acids by including U-13C-palmitate in a mixed meal. Blood samples were taken in the fasted state and after intake of the meal for 6 hours.
Results: Despite the absence of noticeable subcutaneous fat in the legs, the patients showed extraction of plasma triglycerides across the

subcutaneous tissue of the leg. However, there we clear signs of that the fatty acids were not appropriately stored - the spillover fraction of fatty acids from chylomicrons (13C) was drastically higher in patients than control. Also, the accumulation of 2H2-palmitate in the NEFA fraction increased with time (by 100%, $p<0.01$), indicating continuous spillover of fatty acids from VLDL.

Conclusion: This study demonstrates a novel and pathological pathway for NEFA release into the systemic circulation in patients with familial partial lipodystrophy, which could partly explain the hypertriglyceridaemia and severe insulin resistance seen in this condition. It also emphasises the important role of lower body fat in normal fatty acid trafficking.

Supported by: British Heart Foundation

Disclosure: **F. Karpe:** None.

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Targeted metabolomics study on bile acid profiles in db/db mice and their wild type littermates

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Background and aims: Bile acids are known to be associated with type 2 diabetes. Hence, the bile acid profiles in type 2 diabetes are valuable information for the understanding of pathogenesis and discovery of potential biomarkers.

Materials and methods: A LC-MS/MS based targeted metabolomics approach was applied to determine 25 bile acids in plasmic, urinary and fecal samples collected from db/db mice and their wild type (wt) littermates from age Week 6 to Week 19. OPLS-DA and Kruskal-Wallis tests were used to compare the differences between the two groups, and pairwise correlations among the bile acids were calculated to show their associations.

Results: (1) 13 out of the 25 bile acids were readily detected in plasma samples, while 7 out of the 25 bile acids were readily detected in urine and feces samples, respectively. (2) Bile acid concentrations varied notably interindividually and temporally. (3) Bile acid profiles in all sample types displayed discriminating trends between the db/db and wt groups. (4) Among the detected bile acids, the plasmic CDCA, DCA, HDCA, TCA and TCDCA concentrations of db/db mice were significantly higher than the wt mice; urinary CA/creatinine and CDCA/creatinine values were higher in db/db group, while DCA/creatinine and HDCA/creatinine values were lower in db/db group; and fecal CA, DCA, HDCA, UDCA concentrations were higher, whereas 12-oxoCDCA concentration was lower in db/db group compared with the controls. (5) In plasma, the concentrations of taurine-conjugated bile acids were more closely associated with the other taurine-conjugated bile acids, and vice versa for the unconjugated bile acids; the seven bile acids readily detected in urine and feces, respectively, displayed the same phenomenon. (6) Additional real-time RT-PCR tests revealed that the transcription of hepatic gene Cyp7b1 was downregulated and Hsd3b7 upregulated in db/db mice.

Conclusion: Bile acid profile is significantly altered in db/db mice, associated with modulated hepatic gene transcription. This suggests bile acids are promising targets for the discovery of type 2 diabetes biomarkers.

Supported by: China Postdoctoral Science Foundation

Disclosure: **C. Chen:** None.

PS 058 Circadian rhythm

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Insulin directly regulates clock gene expression in the insulin-dependent tissues in vivo and in vitro

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Background and aims: Action and secretion of insulin, a major hormonal regulator of metabolism in the body, are both on circadian control. Here we tested hypothesis that insulin may regulate circadian clocks directly or via metabolic-induced changes.

Materials and methods: We conducted a gene-metabolites network supported analyse of human adipose tissue biopsies from obese subjects during hyperinsulinemic-euglycemic clamp (EC) experiments and control saline infusion (CS). Using bioluminescence assays of cell/tissue cultures received from PER2::LUC knockin mice and the culture of primary human adipocytes transfected with PER2 Lenti-reporter-Luciferase vector we tested the effect of insulin on clock genes in vitro.

Results: Expression levels of five of eighteen clock genes were changed in EC and closely connected to insulin in the EC network. Period circadian protein homolog 2 (PER2) gene was included in the CS network but it was disintegrated from the molecular information exchange in the EC network, suggesting that insulin regulates its expression. In the next step, we observed the up-regulation of PER2 gene expression by insulin in the adipocytes derived from human mesenchymal stem cells in vitro. Using bioluminescence assays of cell/tissue cultures received from PER2::LUC knockin mice we confirmed insulin effects on clock gene expression in adipose tissue and hepatocytes but not in macrophages and lungs. In the culture of transfected primary adipocytes the increase of PER2 oscillations amplitude was observed after treatment with 100nM insulin and 150 μl of human serum from euglacemic clamp experiments.

Conclusion: Our results demonstrate that insulin directly regulates clock gene expression in the insulin-dependent tissues in vivo and in vitro. This data contribute to the further understanding the complexity of the interplay between circadian clock and metabolism.

Clinical Trial Registration Number: NCT00774488
Supported by: DFG and DAAD

Disclosure: **N.N. Rudovich:** Grants; German Science Foundation (DFG Grant Pf164/021002I and German Academic Exchange Service.

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Demonstration of a day-night rhythm in skeletal muscle oxidative capacity

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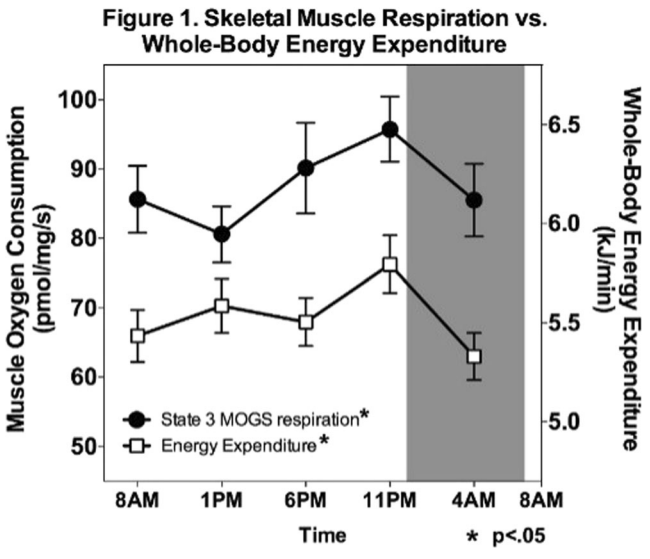
Background and aims: A disturbed day-night rhythm is associated with obesity and type 2 diabetes mellitus (T2DM). Experimental

mouse and cell studies revealed that the molecular clock is causally linked to mitochondrial function. In skeletal muscle, mitochondrial dysfunction is associated with the development of T2DM. Thus, mitochondrial dysfunction may be the link between disturbances in day-night rhythm and insulin resistance. Therefore, the aim of this study was to examine the existence of a day-night rhythm in skeletal muscle oxidative capacity in humans. In addition, we are the first to show the presence of a functioning core molecular clock in human skeletal muscle.

Materials and methods: Twelve healthy young male subjects (22.2 ± 2.3 years) with a normal day-night rhythm were subjected to standardized meals (based on energy requirements) and physical activity. Five biopsies were taken sequentially from the quadriceps muscle, interspaced by 5 hours and at least 4h post-prandially. We determined oxidative capacity (using high-resolution respirometry), mitochondrial copy number and protein abundance along with mRNA expression of core molecular clock genes. In parallel, we measured whole-body energy expenditure by indirect calorimetry and core-body temperature by an ingested telemetric pill. Variation over time was analyzed by repeated measures ANOVA.

Results: Core body temperature was lower during the early night, confirming a normal day-night rhythm. Skeletal muscle oxidative capacity demonstrated a robust day-night rhythm, with a significant time effect in ADP-stimulated respiration (state 3 MO, state 3 MOG and state 3 MOGS, $p<0.05$). Respiration was lowest at 1PM and highest at 11PM (state 3 MOGS: 80.6 ± 4.0 vs. 95.8 ± 4.7 pmol/mg/s, figure 1). Interestingly, the fluctuation in mitochondrial function was also observed in whole-body energy expenditure, with peak energy expenditure at 11PM and lowest energy expenditure at 4AM ($p<0.001$, figure 1). In skeletal muscle, BMAL1 exhibited a robust sinusoidal rhythm with highest mRNA expression at 1PM while PER2 demonstrated peak expression at 8AM. Mitochondrial copy number and protein appeared stable throughout the 24-hour period.

Conclusion: We demonstrate for the first time the presence of a molecular clock rhythm in human skeletal muscle. Moreover, we demonstrate the presence of a profound day-night rhythm in human skeletal muscle oxidative capacity. Peak oxidative capacity and highest resting energy expenditure coincide at the end of the active phase.



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Supported by: NWO TOP Grant
Disclosure: **D. van Moorsel:** None.

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Fatty acid-induced cross-talk between internal clock genes and insulin sensitivity in skeletal muscle cells

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Background and aims: Circadian rhythms occur in all species and modulate fundamental physiological processes. Day-night cycles align the phase of circadian rhythms to earth rotation, but most cells of the body follow an endogenous circadian clock independent of light exposure. Disruption of circadian cycles is associated with metabolic imbalance, and leads to increased risk of type 2 diabetes in individuals working night shifts. This suggests that alterations in circadian rhythms could contribute to the worldwide epidemic of metabolic syndrome, but the pathophysiological mechanisms are poorly understood. Skeletal muscle is engaged in locomotor activity, and glucose uptake and metabolism in this tissue are exquisitely regulated by insulin-independent contractile activity. Skeletal muscle is indeed the major determinant of post-prandial glycaemia and whole-body insulin sensitivity. But surprisingly little is known about the cross-talk between glucose/lipid metabolism and the internal clock in skeletal muscle and subsequent impact on whole body insulin sensitivity. The aim of this study is to determine if and how metabolic challenges impair the skeletal muscle internal clock leading to disturbances in glucose and fatty acid metabolism.

Materials and methods: Primary skeletal muscle cells were isolated from human biopsies. Satellite cells were grown and differentiated into myotubes by serum depletion. Muscle cells were synchronized using dexamethasone shock (200 nM, 1h) and exposed to 0.2 mM of palmitate (PA), oleate (OL) or a combination of both for up to 64h following synchronization. Samples were collected every 4-8h for RNA or protein extraction followed by qPCR and Western blot for the analysis of gene expression and signalling pathways.

Results: Primary skeletal muscle cells exhibit a circadian rhythm (period~24h) on the expression of several genes of the clock machinery (REVERB, CLOCK, PER1/2), as well as genes involved in metabolism and inflammation-related processes (PGC1a, IL6). Palmitate, oleate or a combination of both reduced the amplitude of PER2 cycling while increasing the amplitude of CLOCK, suggesting that fatty acids modulate the core clock. In parallel, palmitate, but not oleate impaired insulin-induced phosphorylation of Akt and glycogen synthesis in skeletal muscle cells, which occurred only after 24h exposure to fatty acids and concomitant with the induction of clock gene disturbances.

Conclusion: Fatty acids discretely affect elements of the core clock in skeletal muscle. Future experiments will analyse the interaction between insulin resistance and clock impairments to decipher the cause-consequence relationship between these two events. Identification of pathways linking metabolic and clock disturbances could lead to new pharmacological targets to target fatty acid-induced insulin resistance.

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Disclosure: **N.J. Pillon:** None.

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Congenital suprachiasmatic nucleus denervation irreversibly disrupts central but not peripheral clocks regulating time-restricted feeding-dependent energy metabolism

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Background and aims: The hypothalamic suprachiasmatic nucleus (SCN) is a master regulator of circadian rhythmicity by synchronizing

metabolic activity with daily light-dark cycles. The synchronizing function of the central molecular clock, consisting of self-regulated genes encoding transcriptional activators and repressors, is critically dependent on light-dependent stimuli reaching the SCN directly from the retina. In turn, the SCN translates this information to entrain molecular clocks in peripheral organs, which are essential to adapt metabolic activity to circadian variation. Here, we investigated the relative importance of the input pathway relying light information to the SCN for the function of central and peripheral clocks and for the circadian regulation of energy metabolism.

Materials and methods: We used Pitx3-deficient Aphakia (Pitx3Ak) mice, characterized by a congenital defect that severely disrupts eye development, as well as control C57BL/6. Mice were maintained under 12 hour light-dark cycles (lights on from 8 am to 8 pm). Electroretinogram recordings were used to test eye function. Expression of clock genes was determined by quantitative RT-PCR, and energy metabolism was assessed by indirect calorimetry.

Results: Electroretinogram recordings showed that Pitx3Ak mice were completely unresponsive to light. Neural tracing experiments indicated the SCN of these mice do not receive inrvation from retinohypothalamic axons, which do not project beyond the optic chiasm. Indirect calorimetry revealed that, contrary to control mice, Pitx3Ak lacked cyclic day-night oscillations in oxygen consumption, energy expenditure, CO2 production and respiratory exchange ratio. Expression levels of clock genes in the SCN and the liver of control mice, including Per1, Per2, Bmal1 and Clock showed night-day oscillations, but in Pitx3Ak mice they were reduced and showed no significant day-night variation. Time-restricted-feeding (TRF) allowing Pitx3Ak mice access to food only during the night resulted in the partial restoration of circadian rhythmicity in energy metabolism and expression of liver clock genes, but no change was observed in the expression of SCN clock genes.

Conclusion: Our data show that congenital eye defects preventing the relay of light-dependent information to the SCN result in permanent defects in the expression of clock genes in the SCN. In addition, our results indicate that clock genes in peripheral tissues retain an autonomous capacity to be entrained by external stimuli in the absence of SCN-dependent activity.

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Disclosure: **A. Fernández-Pérez:** None.

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Effects of ghrelin on the expression of clock genes in gastric tissue: a possible mechanism of altered food: anticipatory activity

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Background and aims: Altered food anticipatory response has been implicated as a mechanism underlying eating disorders and obesity. The activity is driven by meal-entrained clock genes that are independent of the suprachiasmatic nuclei. It was reported that gastric oxyntic cells coexpress ghrelin and the circadian clock proteins Per1 and Per2, and act as meal-entrainable circadian clocks. Thus there may be crosstalk between ghrelin production and clock gene expression in the stomach. Although diurnal rhythm of plasma ghrelin is regulated by the internal clocks, the role of ghrelin in the regulation of clock gene expression remains to be determined. In this study we assessed the effect of ghrelin on the expression of clock genes in gastric tissue using ghrelin knockout (GKO) mice.

Materials and methods: All mice were maintained under specific-pathogen-free conditions with a regular light cycle of 12 h light/12 h darkness. Locomotor activity (ACT) and food intake (FI) were measured in real time by feeding behavior analyzing system for mice (MFD-RQ, Shinfactory Co.). Plasma active ghrelin concentration was assayed by RIA. Clock gene expression in the stomach was measured by quantitative RT-PCR.

Results: Plasma levels of ghrelin showed a peak at the end of the light phase (ZT12) in 16-week-old C57BL/6 mice fed a standard chow (CD). However, in mice fed high-fat diet (HFD; 60 kcal% fat) for 12 weeks, the peak was shifted to ZT8 and rather showed nadir at ZT12.The diurnal pattern of ACT and FI were disrupted in HFD mice which consumed 31% of the food in light phase while CD mice consumed only 6% in light phase. To test the hypothesis that altered diurnal pattern of plasma ghrelin may affect the rhythm of the internal clock, next we assessed the effect of ghrelin per se on the expression of clock genes. Per1, Per2, Cry1 and Cry2 genes showed obvious circadian rhythmicity in gastric tissue of wild type mice. In gastric tissue of GKO mice, the rhythm of the clock genes was delayed by 1.9, 0.8, 0.9, and 2.4 h, respectively. An intraperitoneal injection of GHRP6, a ghrelin agonist, at a dose of 2 mg/kg at ZT10 resulted in the up-regulation of the clock genes at ZT12.

Conclusion: It has been reported that the diurnal variation of ghrelin was regulated not only by food ingestion but also by the internal clock in the stomach. In this study we found that ghrelin conversely modulates the expression of clock genes in gastric tissue. The elevation of plasma ghrelin at the end of light phase was likely associated with the increase in Per1/2 and Cry1/2 expressions in the beginning of dark phase. Interestingly, ghrelin upregulated the expression of the clock genes when injected at the end of light phase. These observations suggest that ghrelin per se plays a role in the entrainment of the gastric clock genes to meal time. Thus decreased plasma ghrelin levels at the beginning of active phase in obese individuals may be associated with the alteration of eating behaviour. Furthermore, high fat diet and night eating often observed in obese subjects may disrupt the gastric clock through the impairment of ghrelin rhythm, leading to persistent eating disorder and the exacerbation of obesity.

Disclosure: **K. Hara:** None.

PS 059 Other hormones

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The role of 17-beta-estradiol and estrogen receptors on glucose homeostasis in malnourishment mice

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Background and aims: Malnourishment mice show low insulin plasma level and a reduced insulin secretion. To maintain the normal glucose homeostasis, these animals increased insulin sensitivity on peripheral tissues. Estrogen receptors (ERs) are implicated in insulin secretion and biosynthesis, as well as, insulin sensitivity. The aim of this study was to verify the role of ER and estradiol upon glycemic control of male malnourished mice.
Materials and methods: Post-weaned Swiss male mice were fed with normoprotein diet (14%, NP) or low protein diet (6%, LP) protein diet during 8 weeks. We characterized the malnourishment phenotype through analyses of the body weight, insulin, protein and albumin plasma levels. We analyzed the gene expression and protein content of estrogen receptor (ER) in the liver, adipose tissue, skeletal muscle and pancreatic islets. We measured the effect of 10μg/Kg of E2 on the insulin sensitivity with a hyperinsulinemic-euglycemic clamp assay, and also, the effect of 1nM estradiol (E2) on *in*-vitro glucose stimulated insulin secretion (GSIS). Data are expressed as mean ± SEM and statistical significance was determined using two-tailed T-test.
Results: Low protein diet (6%) induced the malnourishment phenotype, indicated by a lower body weight (control: 40.43±3.9g; LP: 35.83±1.99g; p-value:0.0001), lower plasma levels of protein (control: 5.61±0.23g/dL; LP: 4.86±0. 19g/dL; p-value:0.02), albumin (control: 2.16±0.07g/dL; LP: 1.92±0.07g/dL; p-value:0.02) and insulin (control: 2.94±0.51ng/mL; LP:1.20±0.18ng/mL; p-value:0.002) compared with the control group. The analysis of estrogen receptor gene expression showed, in liver, a down regulation of ERα and an up regulation of ERβ in LP animals; a decreased expression in both isoforms in adipose tissue; and a higher expression of both receptors in muscle. In pancreatic islets, ERα was increased in LP mice but ERβ did not change. The protein content of estrogen receptors did not show changes in any tissues in LP animals compared with control. The stimulation with 10μg/Kg of E2 in a hyperinsulinemic-euglycemic clamp showed a statistically significant increase in glucose infusion rate in LP group compared with control (basal phase: control 0.57±0.08mL/min; LP 0.89±0.1mL/min; p-value:0.005).This date indicates increased insulin sensitivity in LP mice. GSIS was increased when islets from LP animals were stimulated with 10nM E2 in low and intermedia glucose concentration compared with control group (2.8mM glucose: control: 0.54ng/mL·ng protein^{-1}; LP: 1.17ng/mL·ng protein^{-1}; 8.3mM glucose: control:1.45ng/mL·ng protein^{-1}; LP: 2.88ng/mL·ng protein^{-1}; 16.6mM glucose: control: 3.36ng/mL·ng protein^{-1}; LP: 4.01ng/mL·ng protein^{-1}); these changes were statistically significant (p-value:0.04).
Conclusion: We conclude that the effects of steroid hormone augmented insulin secretion and action in malnourishment mice may be through mechanisms independent of ER protein express.
Supported by: FAPESP CEPID-OCRC
Disclosure: **M. García-Arévalo:** None.

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Amelioration of oral glucose tolerance by neuromedin U is actually due to a blockade of gastric emptying

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Background and aims: The gut and brain peptide neuromedin U (NmU) is reported to decrease food intake and body weight, and to improve oral glucose tolerance. These anorexigenic and incretin properties make NmU a promising candidate for the treatment of obesity and diabetes. However and in contradiction with previous observations, NmU was recently presented as a "decretin" hormone able to decrease insulin secretion during a long-lasting period of starvation in mice. Thus, the underlying mechanisms of NmU contribution to glucose homeostasis still need to be clarified.
Materials and methods: We used C57Bl6 mice fed a standard chow diet (CD) or a high fat diet (HFD) for 12 weeks to investigate the evolution of NmU sensitivity and intestinal secretion of NmU during the setting of obesity. Oral (OGTT) and intraperitoneal (IPGTT) glucose tolerance tests, insulin secretion in response to oral glucose and insulin sensitivity were measured after an intraperitoneal injection of NmU or PBS. [14]-C-glucose uptake in isolated intestinal loops in presence or absence of NmU was assessed. Gastric retention of a phenol red gavage and total intestinal transit time were also evaluated in presence or not of NmU. Results are expressed as mean ± SEM and were analyzed with non-parametric statistical tests.
Results: In CD mice, OGTT-induced rise of glycemia was prevented by a single intraperitoneal injection of NmU. Unexpectedly, in these conditions glucose-induced insulin secretion was also prevented by NmU injection. On the contrary, IPGTT was not modified and insulin sensitivity was lightly ameliorated by intraperitoneal injection of NmU (AUC 0-150min - 4% vs PBS treated mice; $P<0.05$). In addition, intestinal glucose uptake was barely reduced by NmU (-17% $P<0.05$ vs PBS). Actually, intraperitoneal injection of NmU blocked gastric emptying (19% vs 79% in PBS treated mice $P< 0.0001$) and increased intestinal transit time (+150% vs PBS $P< 0.05$). HFD fed mice displayed a 2-fold decrease of intestinal NmU mRNA ($P<0.05$ vs CD mice). In addition, despite oral glucose intolerance, NmU still prevented OGTT-induced rise of glycemia in diet-induced obese mice.
Conclusion: These data demonstrate that a single intraperitoneal injection of NmU strongly delays gastric emptying, reduces intestinal absorption of nutrients and indirectly improves oral glucose tolerance. This effect could also contribute to the anorexigenic effect of Nmu. In obese mice, accelerated gastric emptying is known to contribute to satiety defect and we show that NmU production is decreased suggesting an implication of NmU in the setting of obesity.
Disclosure: **A. Jarry:** None.

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Deficiency in catechol-o-methyltransferase is linked to a disruption of glucose homeostasis in mice

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Background and aims: 2-methoxyestradiol (2-ME), an estrogen metabolite generated via catechol-o-methyltransferase (COMT), exerts diverse biological functions. Lower enzymatic human SNPs of COMT have been associated with hypertension, preeclampsia and obesity in diabetic individuals. Here we found that COMT deficiency is associated with glucose tolerance defects in high fat diet (HFD) fed or in pregnant mice, and 2-ME replacement could be the potential strategy to combat such COMT deficiency-associated metabolic defects.
Materials and methods: Eight-week-old male C57/B6 mice were obtained from CLEA Japan, Inc. (Tokyo, Japan). The mice were fed with a control diet or HFD starting at 8 weeks of age. The metabolic status and histological/biochemical evaluations were performed at 2 or 10 weeks after the initiation of indicated diet. For the 2-week protocol, metformin (250 mg/kgBW/day: intraperitoneal), Ro41-0960 (25 mg/kgBW/day: subcutaneous) or 2-ME (10 ng/day: subcutaneous) were injected into the mice after the initiation of indicated diet. The interval of the drug or molecules was 1 week. For the 10-week protocol, 2-ME was injected at week 6 following the initiation of the indicated diet. 2-ME intervention

was performed during the last 4 weeks. For in vitro experiment, we utilized MIN-6 beta cell line.
Results: COMT protein suppression in the liver of HFD mice was associated with glucose tolerance defects, whereas a COMT-inhibitor or COMT siRNA-mediated COMT suppression in HFD fed mice exacerbated such glucose intolerance. 2-ME intervention displayed a remarkable amelioration of these defects. 2-ME also ameliorated glucose tolerance defects in pregnant mice with COMT inhibitor treatment. Such beneficial effects of 2-ME on glucose tolerance were associated with an induction of AMPK phosphorylation in both the liver and also in islet cells. Metformin restored COMT protein levels in the liver, and metformin-induced AMPK phosphorylation in the liver was abolished by COMT inhibitor treatment. Interestingly, the amelioration in glucose tolerance by 2-ME was associated with biphasic insulin secretion in a disease condition-dependent manner, [the amelioration of insulin resistance (long term HFD and COMT inhibitor-treated pregnant mice), or the induction of insulin secretion]. 2-ME-induced insulin secretion was associated with the activation of AMPK, phosphorylation of PDX-1, and the suppression of MST-1 in MIN-6 cells.
Conclusion: These results suggest that COMT is essential enzyme to keep homeostasis of plasma glucose level in both HFD fed and pregnant mice. 2-ME is a potential endogenous anti-diabetic candidate molecule via diverse mechanisms.
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Disclosure: **K. Kanasaki:** Employment/Consultancy; KK is under the consultancy agreement with Boehringer Ingelheim. Grants; Japan Society for the Promotion of Science, Daiichi-Sankyo Foundation of Life Science, Ono Medical Research Foundation, Takeda Science Foundation, Novo Nordisk Insulin Research Foundation, Banyu Life Science Foundation International, Boehringer Ingelheim, Tanabe-Mitsubishi, Ono Pharmasuetical. Honorarium; Boehringer Ingelheim,, Eli Lilly, Sanofi, Dainippon-Sumitomo, MSD, Asteras, Tanabe-Mitsubishi, AstraZeneca.

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Low carbohydrate diet impairs the glucose restoring effect of glucagon in patients with type 1 diabetes

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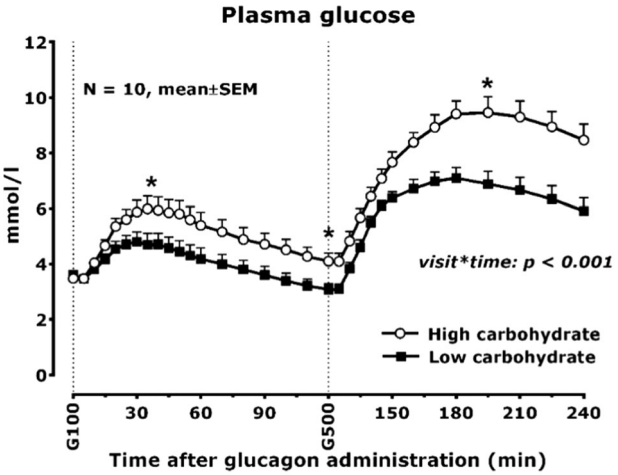
Background and aims: Dual-hormone closed-loop treatment with insulin and glucagon reduces the risk of mild hypoglycaemia. However, the glycaemic responses of exogenous glucagon when liver glycogen stores are partly depleted are unknown.
This two-way crossover randomized clinical study investigated the ability of low-dose glucagon to restore plasma glucose (PG) from mild hypoglycaemia in patients on high (HCD) (≥250 g daily) vs low (LCD) carbohydrate diets (≤50 g daily).
Materials and methods: Ten patients with insulin pump treated type 1 diabetes (median±SD age 48±10 years, HbA1c 6.9±2.7%, diabetes duration 22.5±7 years, and BMI 24.5±1.8 kg/m2) completed one week of HCD and one week of LCD in random order. Each week ended with a study visit, where fasting patients received a subcutaneous insulin bolus estimated to induce a mild hypoglycaemia. Once PG reached ≤ 3.9 mmol/l, 100 μg glucagon was given subcutaneously, followed by a repeated glucagon injection of 500 μg subcutaneously two hours later. Patients were observed for four hours after the first glucagon injection and were measured for concentrations of glucose, glucagon and insulin. Data presented as mean±SEM.
Results: Fasting PG (9.3±0.8 vs 8.3±0.6 mmol/l), the insulin dose (2.6 ±0.3 vs 2.3±0.4 U), and the time (155±20 vs 126±14 min) to achieve hypoglycaemia (3.5±0.1 vs 3.6±0.1 mmol/l) did not differ between HCD and LCD (all p>0.05). The PG time course after the first glucagon bolus differed between HCD vs LCD (repeated measures ANOVA: time x visit, p0.05) and second glucagon bolus (48.1±14 vs 44±14 min, p>0.05).
Conclusion: We conclude that LCD may impair the treatment effect of glucagon on mild hypoglycaemia. Thus, carbohydrate intake should be assessed when low doses of glucagon are used in open-loop and maybe also in closed-loop settings.



*Plasma concentration of glucose on study visits after the subcutaneous injection of 100 μg glucagon (G100) followed by 500 μg glucagon (G500) in ten patients with type 1 diabetes, respectively after one week of high (empty circles) versus one week of low carbohydrate diet (filled squares). Data expressed as mean±standard error of the mean (SEM). Repeated measures ANOVA was performed (visit: p<0.001; time: p<0.001; interaction: visit*time:p<0.001).*
** designates p<0.05.*

Clinical Trial Registration Number: NCT02578498
Supported by: The Danish Diabetes Academy supported by Novo Nordisk Foundation
Disclosure: **A. Ranjan:** Grants; The Danish Diabetes Academy supported by Novo Nordisk Foundation.

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The upregulation of non-selective cation channels in isolated NPY neurons contributes to ghrelin action

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Background and aims: Define the ionic effectors of ghrelin action in ventromedial hypothalamic NPY neurons.
Materials and methods: Ca2+ imaging of hypothalamic neurons isolated from NPY-GFP mice was used to assess activation following ghrelin application.
Results: Acylated ghrelin produced a sustained elevation of [Ca2+]c in isolated NPY-GFP neurons with an EC50 of ~110 pM. Pharmacologic studies confirm reports on the importance of the adenylate cyclase/protein kinase A and phospholipase C/IP3 signaling pathways as activators of AMPK following the interaction of ghrelin with the growth hormone secretory receptor, GHS-R. Ghrelin action is unaffected by tetrodotoxin, an antagonist of voltage-gated Na+ channels, but reducing [Na+]o suppresses activation implying a potential role for non-selective cation

channels. Although SUR1/Kir6.2-type KATP channels are described in NPY neurons in situ, diazoxide, a KATP channel agonist, elevates [Ca2+]c mimicking the action of ghrelin, while glibenclamide, a channel antagonist, partially suppresses ghrelin action. The results are consistent with their effects on SUR1/TRPM4 channels, with the identification of TRPM4 in isolated NPY-GFP, but not NPY neurons in situ, and with the inhibition of ghrelin action by 9-phenanthrol and flufenamic acid, selective antagonists of TRPM4 channels. Ghrelin activation is unaffected by known inhibitors of L- and N-type Ca2+ channels, nifedipine and ω-conotoxin, respectively. Ni2+, mibefradil, and TTA-P2 completely or partially inhibit ghrelin action implicating T-type Ca2+ channels. Additionally, ghrelin activation is sensitive to SNX-482, a spider toxin selective for R-type Ca2+ channels. Nanomolar concentrations of GABA markedly inhibit isolated NPY-GFP neurons consistent with a potential chronic suppression of ghrelin action in vivo.

Conclusion: The results are consistent with previous studies where ghrelin activates isolated hypothalamic NPY neurons via multiple signaling pathways. T- and SNX482-sensitive R-type voltage dependent Ca2+ channels are important for the response of isolated NPY-GFP neurons to ghrelin. In view of previous reports of KATP channels in NPY neurons, the paradoxical response to SUR1 agonists and antagonists is consistent with the hypothesis that isolation, like other 'traumatic' or inflammatory stimuli, upregulates TRPM4 which couples with SUR1 to produce non-selective cation channels that are activated by diazoxide and inhibited by glibenclamide. The potential for upregulation of SUR1/TRPM4 channels in NPY neurons under pathologic, inflammatory conditions is not known.

Clinical Trial Registration Number: N/A
Supported by: NIH grant DK084242 (JB)
Disclosure: **H. Hashiguchi:** Grants; NIH grant DK084242 (JB).

PS 060 GLP-1 RA and obesity

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Liraglutide 3.0 mg reduces body weight and improves cardiometabolic risk factors: SCALE obesity and prediabetes randomised, double-blind, placebo-controlled 3-year trial

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Background and aims: Obesity and prediabetes are risk factors for developing T2DM. A 5-10% weight loss may reduce the risk of developing T2DM. This phase 3 trial investigated effects of liraglutide 3.0 mg, as an adjunct to diet and exercise, in delaying the onset of T2DM (primary endpoint) over 3 years, as well as in improving body weight and cardiometabolic risk factors.

Materials and methods: Individuals with overweight or obesity (BMI ≥30 kg/m^2 or ≥27 kg/m^2 with dyslipidaemia and/or hypertension) were advised on a 500 kcal/day deficit diet and 150 minutes/week physical activity programme and randomised 2:1 to once-daily s.c. liraglutide 3.0 mg (n=1505) or placebo (n=749). Efficacy data are observed means, with last observation carried forward.

Results: Baseline characteristics of the 2254 randomised individuals (mean ±SD) were: age 47.5±11.7 years, 76.0% female, body weight 107.6±21.6 kg, BMI 38.8±6.4 kg/m^2. While on treatment, the time to onset of T2DM over 3 years was 2.7 times longer with liraglutide 3.0 mg than with placebo [95%CI 1.9;3.9], corresponding to a hazard ratio of 0.2 (p<0.0001). More individuals regressed from prediabetes (ADA 2010 criteria) to normoglycaemia while on treatment with liraglutide 3.0 mg vs placebo by week 160 (66% vs 36%; odds ratio 3.6 [3.0;4.4], p<0.0001), corresponding to a number needed to treat of ~3. At week 160, individuals treated with liraglutide 3.0 mg had lost more weight (6.1%) than those treated with placebo (1.9%) (with an estimated treatment difference [ETD] of -4.3% [95%CI 4.9; 3.7], p<0.0001), and had a greater reduction in mean waist circumference (ETD -3.5 cm [-4.2;-2.8], p<0.0001). Weight loss was accompanied by reductions in mean systolic BP (ETD -2.8 mmHg [-3.8;-1.8], p<0.0001), total cholesterol (ETD -2% [-3;0], p=0.03), triglycerides (ETD -6%[-9;-3], p=0.0003) and high-sensitivity C-reactive protein (ETD 29% [-34;-23], p<0.0001), though mean pulse rate was increased (ETD 2.0 beats/min [1.2;2.7], p<0.0001). Adverse events were reported by 94.7% of individuals in the liraglutide 3.0 mg group vs 89.4% of those in the placebo group, serious events by 15.1% vs 12.9%, respectively. Gallbladder-related events (2.9 vs 1.2 events/100 patient years of observation [PYO]) and confirmed pancreatitis (0.29 vs 0.13 events/100 PYO) were low, but more frequent with liraglutide 3.0 mg vs placebo. Two deaths occurred in each treatment group, one of which, in the liraglutide 3.0 mg group, was externally adjudicated as cardiovascular-related (namely, cardiac arrest). Adjudicated major adverse cardiovascular events (non-fatal myocardial infarction or stroke, cardiovascular death) were low overall (0.19 vs 0.20 events/100 PYO).

Conclusion: With continued treatment over 3 years in individuals with overweight or obesity and prediabetes, liraglutide 3.0 mg vs placebo, as an adjunct to diet and exercise, was associated with lower risk of T2DM, weight loss and improvements in cardiometabolic risk factors, which if sustained in the long term may be associated with reduced cardiovascular risk. No new safety issues were identified as compared with the initial 56-week period of the trial.

Clinical Trial Registration Number: NCT01272219
Supported by: Novo Nordisk A/S
Disclosure: **C.W. le Roux:** Honorarium; Novo Nordisk, GI Dymanics, Fractyl, Herbalife.

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Greater weight loss and regression to normoglycaemia, and reduced risk of T2D, at 3 years in early weight loss responders to liraglutide 3.0 mg vs early non-responders

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Background and aims: The SCALE Obesity and Prediabetes trial randomised adults with prediabetes and obesity (BMI ≥30 kg/m^2) or overweight with comorbidities (≥27 kg/m^2; dyslipidaemia / hypertension) to liraglutide 3.0 mg (N=1505) or placebo (N=749) as adjunct to diet and exercise for 3 years. This *post hoc* analysis compared liraglutide 3.0 mg early responders (ERs: ≥5% weight loss at week 16) and early non-responders (ENRs: <5% weight loss at week 16), in keeping with EMA stopping-rule criteria.
Materials and methods: Efficacy outcomes are estimated means in ERs (n=580) and ENRs (n=210) who completed 160 weeks of treatment. Those developing T2D or regressing to normoglycaemia were analysed on the full analysis set with LOCF. Safety was based on the safety analysis set (n=886 ERs, n=416 ENRs). Placebo data are shown only for proportion of ERs/ENRs.
Results: Of individuals with week 16 data, for liraglutide 3.0 mg (n=1302) 68.0% were ERs and 32.0% ENRs; for placebo (n=640), 22.3% were ERs and 77.7% ENRs. At week 160, greater mean and categorical weight loss and greater improvements in cardiometabolic risk factors and patient-reported outcomes were observed in ERs to liraglutide 3.0 mg vs ENRs (Table). At week 160 0.5% of ERs and 3.2% of ENRs had been diagnosed with T2D; 69.8% of ERs and 55.4% of ENRs had regressed to normoglycaemia while on treatment. Adverse events (AEs) were reported in 97.1% and 95.0% of ERs and ENRs, respectively; serious AEs in 17.7% and 12.7%; gastrointestinal AEs in 75.3% and 71.6%; and gallbladder disorders in 6.3% and 2.2%.
Conclusion: Among individuals treated with liraglutide 3.0 mg, over 160 weeks a reduction in diagnoses of T2D and greater regression to normoglycaemia were observed in ERs vs. ENRs while on treatment. Among those completing 160 weeks of treatment, greater weight loss and improvements in cardiometabolic risk factors and patient-reported outcomes were observed in ERs vs. ENRs. Apart from gallbladder related events, which may relate to greater weight loss in ERs, AE rates were similar between ERs and ENRs.

Table. Outcomes in ERs and ENRs who completed 160 weeks of treatment.

Week 0–160	Early responders to liraglutide 3.0 mg n=580	Early non-responders to liraglutide 3.0 mg n=210
Change in body weight (%)	−8.6	−2.9
Change in body weight (kg)	−9.1	−3.1
Proportion achieving ≥5% weight loss (%)	65.4	33.3
Proportion achieving >10% weight loss (%)	36.7	14.1
Proportion achieving >15% weight loss (%)	19.0	5.5
Change in FPG (mmol/L)	−0.43	−0.32
Change in HbA_{1c} (%)	−0.44	−0.33
Change in SBP (mmHg)	−3.74	−3.26
Change in SF-36 physical component summary score[a]	+3.68	+1.81
Change in IWQoL-Lite total score[a]	+13.40	+9.53

[a]Increase in score=improvement

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Supported by: Novo Nordisk
Disclosure: **J.P.H. Wilding:** Grants; AstraZeneca, Bristol-Myers Squibb, Novo Nordisk. Honorarium; AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Janssen, Merck Sharp & Dohme, Novo Nordisk, Sanofi. Lecture/other fees; AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Janssen, Eli Lilly & Co, Novo Nordisk, Pfizer.

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The effect of liraglutide 3.0 mg for weight management on HRQoL, as measured by SF-36: 3-year data

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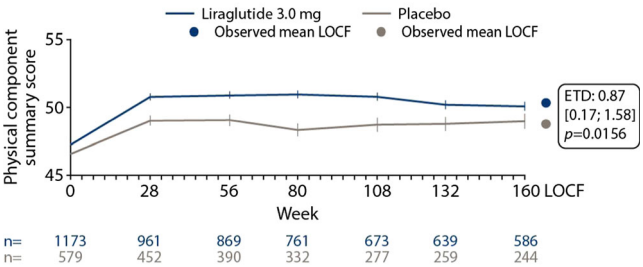
Background and aims: Obesity has negative impacts on health-related quality of life (HRQoL), the degree of which is dependent upon the severity of obesity. This analysis investigated the effect of liraglutide 3.0 mg at 160 weeks, as an adjunct to diet and exercise, on HRQoL in participants from the 3-year part of the SCALE Obesity and Prediabetes trial with prediabetes and obesity (BMI ≥30 kg/m^2) or overweight (BMI ≥27 kg/m^2) with hypertension and/or dyslipidaemia.
Materials and methods: Individuals were advised on a 500 kcal/day deficit diet and a minimum 150 min/week physical activity, and randomised 2:1 to once-daily s.c. liraglutide 3.0 mg (n=1505) or placebo (n=749). Physical and mental HRQoL outcomes, measured by the Short-Form 36 v2 (SF-36) questionnaire, were assessed in participants from countries with validated translations (73.6% of all randomised) over a period of 160 weeks. Data are reported as observed means and estimated treatment differences (ETD) [95% CI], derived using ANCOVA with LOCF.
Results: Mean baseline characteristics: 77.6% female, age 47.9 years, weight 108.2 kg, BMI 39.1 kg/m^2. Compared with placebo, individuals on liraglutide 3.0 mg had statistically significantly greater improvements from baseline on four SF-36 subscales: physical functioning (3.50 vs 2.48; ETD 1.47 [0.78;2.16]; $p<0.0001$), general health (2.25 vs 1.39; ETD 1.31 [0.54;2.08]; $p<0.001$), vitality (1.84 vs 1.07; ETD 0.94 [0.06;1.82]; $p=0.037$), and mental health (0.19 vs -0.81; ETD 1.27 [0.39;2.16]; $p=0.005$). No significant differences were found for four SF-36 subscales: role physical, bodily pain, social function, and role emotional. A statistically significant difference in favour of liraglutide 3.0 mg compared with placebo was also observed for the change in the physical component summary score (3.10 vs 2.61; ETD 0.87 [0.17;1.58]; $p=0.016$) (Figure) but not for the change in the mental component summary score (-0.46 vs -1.40; ETD 0.77 [-0.09;1.63]; $p=0.078$). Liraglutide 3.0 mg was generally well tolerated.
Conclusion: Compared with placebo at 160 weeks, treatment with liraglutide 3.0 mg in addition to diet and exercise in individuals with prediabetes who were overweight or had obesity was associated with significant improvements in HRQoL, with the largest treatment effect observed on the physical functioning subscale. These results demonstrate the benefit of combining pharmacological treatments, such as liraglutide 3.0 mg, with lifestyle intervention on HRQoL.

Figure: Physical component summary score over time



FAS. Line graphs are observed means (±SE). Circles are observed means LOCF.
FAS, full analysis set; LOCF, last observation carried forward; SE, standard error.

Clinical Trial Registration Number: NCT01272219
Supported by: Novo Nordisk
Disclosure: **J.B. Bjørner:** Employment/Consultancy; OptumInsight, Novo Nordisk.

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Improvements in 'Impact of Weight on Quality of Life-Lite' score with liraglutide 3.0 mg vs placebo for weight management: 3-year data

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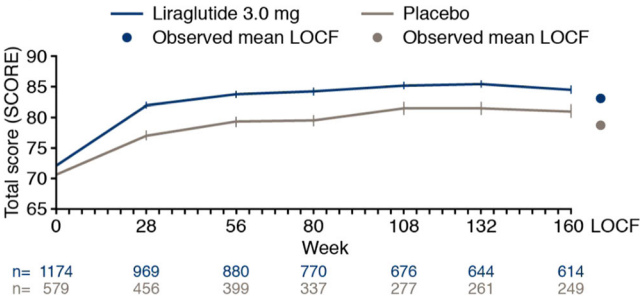
Background and aims: Obesity has negative impacts on health-related quality of life (HRQoL). This analysis investigated the effect of liraglutide 3.0 mg at 160 weeks, as an adjunct to diet and exercise, on HRQoL in patients from the SCALE Obesity and Prediabetes 3-year clinical trial with prediabetes and obesity (BMI ≥30 kg/m^2) or overweight (BMI ≥27 kg/m^2) with hypertension and/or dyslipidaemia.
Materials and methods: Individuals with prediabetes and BMI ≥30 kg/m^2 or ≥27 kg/m^2 with hypertension and/or dyslipidaemia were advised on a 500 kcal/day deficit diet and 150 min/week exercise programme, and randomised 2:1 to once-daily liraglutide 3.0 mg (n=1505) or placebo (n=749) for 160 weeks. HRQoL was assessed in trial participants from countries with linguistically validated translations using the Impact of Weight on Quality of Life-Lite (IWQOL-Lite) instrument. Missing post-baseline values were imputed with LOCF.
Results: Mean baseline characteristics: 78% female, age 47.9 years, weight 108.2 kg, BMI 39.1 kg/m^2, IWQOL-Lite total score 71.7. The liraglutide 3.0 mg group experienced greater improvements in IWQOL-Lite total score vs placebo (at week 160: 10.96 vs 8.11; ETD 3.35 [95% CI: 2.04; 4.66]; p<0.0001) (Figure shows mean total scores over time). All subscales of the IWQOL-Lite were also significantly improved with liraglutide 3.0 mg vs placebo: physical function (13.19 vs 9.59; ETD 4.48 [95% CI 2.87; 6.10]; p<0.0001), self-esteem (14.78 vs 11.82; ETD 3.53 [95% CI 1.64; 5.41]; p=0.0002), sexual life (9.29 vs 6.16; ETD 3.35 [95% CI 1.46; 5.25]; p=0.0005), public distress (5.99 vs 4.34; ETD 2.16 [95% CI 0.76; 3.56]; p=0.0026), work (5.87 vs 4.42; ETD 1.64 [95% CI 0.27; 3.02]; p=0.0192). Liraglutide 3.0 mg had an acceptable safety profile and was generally well tolerated.
Conclusion: Compared with placebo at 160 weeks, treatment with liraglutide 3.0 mg in addition to diet and exercise in individuals with prediabetes who were overweight or had obesity was associated with significant improvements in HRQoL. These results demonstrate the benefit of combining pharmacological treatments, such as liraglutide 3.0 mg, with diet and exercise on HRQoL.

Figure. IWQOL-Lite total score over time (0–160 weeks). Line graphs are observed means (±SE). Full analysis set, IWQOL-Lite, Impact of Weight on Quality of Life-Lite; LOCF, last observation carried forward; SE, standard error.



Clinical Trial Registration Number: 01272219
Supported by: Novo Nordisk
Disclosure: **R. Kolotkin:** Employment/Consultancy; Orexigen, Novo Nordisk, Eisai. Honorarium; Novo Nordisk, Janssen. Other; Duke University: royalty fees for IWQOL-Lite.

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GLP-1 receptor agonist therapy induces a greater reduction in glycaemic variability compared to gastric bypass in type 2 diabetic patients

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Background and aims: A relationship between the glycemic variability (GV) and the micro and macrovascular damage of diabetes has been established. This could explain in part why patients with similar levels of HbA1c have different prevalence or severity of complications. Few studies have shown the effect of GLP-1 receptor agonist (GLP-1RA) on GV, and no one has compared these drugs with gastric bypass (GBP). Our aim was to evaluated the impact of GLP-1 RA (Liraglutide or Lixisenatide) compared with Gastric Bypass (GBP) on GV in patients with type 2 diabetes (T2D) and mild obesity.
Materials and methods: PRODIGIES is a prospective randomized controlled trial of medical versus surgical treatment of diabetes in patients with BMI < 35kg/m2 and nephropathy. We reported results in the first 9 subjects after GBP and 9 after medical treatment with GLP-1RA (MED). A continuous subcutaneous glucose monitoring (CSGM) during 6 days was performed before and 6 months after the interventions. The parameters of GV analyzed were standard deviation (SD) and mean amplitude of glycemic excursions (MAGE).
Results: At baseline there were not differences in age, BMI, HbA1c, diabetes duration, insulin use, SD and MAGE. In MED 6 patients were on liraglutide 1.2 mg QD and 3 on lixisenatide 20 mg QD. After 6 months the percentage of weight loss was greater in GBP compared to MED (11.6 ± 4.1 vs.2.6 ± 2.5; p= 0.0001). There were not differences in Hba1c and fasting glycaemia reduction between GBP and MED (1.5 ± 1.1 vs. 1.7 ± 1.6%; 55.7 ± 51.1 vs. 33.4 ± 47.8 mg/dl, respectively). After 6 months, SD and MAGE had a significant reduction in GLP-1RA group, (46.2 ± 20.8 to 32.6 ± 9.2 mg/dl and 84.7 ± 29.5 to 64.5 ± 20.8 mg/dl, respectively, p < 0.05). There were no significant changes in GBP group. At 6 months SD and MAGE were significant lowers in GLP-1RA group than GBP (32,6 ± 9,2 vs. 45,7 ± 11,4 mg/dl and 64,5 ± 20,8 vs 93,9 ± 29,2 mg/dl, respectively, p < 0.05)
Conclusion: In diabetic patients with mild obesity, GBP does not induce improvement in GV in spite of significant reductions in HbA1c, fasting glycemia and weight. In this group of patients, GLP-1RA therapy is able to produce significant reduction in GV, a similar improvement of HbA1c compared to GBP, in spite of a lower weight reduction. This suggests that the magnitude of caloric intake does not explain our finding. The opposite actions on gastric emptying may explain it, because the GLP-1RA delays the gastric emptying while GBP accelerates it. The impact at long-term of a high GV in diabetic patients with GBP on micro and macro vascular complications it should be determined.
Clinical Trial Registration Number: NCT01974544
Supported by: FONDECYT Project N° 1120877
Disclosure: **J.P. Valderas:** None.

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Variations in the states of prediabetes in adolescents after a school programme for nutritional and behavioural intervention

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Background and aims: Prediabetes states represents a frequent complication associated with obesity in adolescent population. To study the

prevalence of prediabetes, diabetes and metabolic syndrome (MS) in a representative sample of adolescents with obesity before and after developing a school Program for Nutritional and Behavioral intervention (PNBI).
Materials and methods: 263 high school students (127 males) aged 12-16 yrs from Granada (Spain) were randomly selected to participate in PNBI during the school year. At the beginning and end of the school year their body mass index (BMI) and waist perimeter were determined. In addition, we determined their body composition by impedance measurement, their blood pressure and serum determinations of fasting plasma glucose (FPG), cholesterol (LDL and HDL) and triglycerides. They were also surveyed to determine their dietary habits by using a frequency test for the food consumption and exercise habits. Overweight and obesity were defined according to the criteria of Cole et al, and MS according to IDF criteria for the 10-16 yr age group. If glucose levels exceeded 100 mg/dl in the second determination, a standard oral glucose tolerance test (OGTT) was performed. The PNBI comprised fortnightly classes of 45 minutes duration on nutritional and lifestyle recommendations for adolescents, in addition, each day, they had a Mediterranean breakfast at school (275-350Kcal).
Results: After intervention, the prevalence of overweight decreased among male (31.5% vs 21.3%; p< 0.001) and female (21.7% vs 14%; p<0.001) students. The prevalence of obesity decreased in both males (7.9 vs 5.5%; p<0.001) and females (4.7% vs 3.9%; p<0.001). The PNBI reduced the prevalence of MS from 32% to 19.7% (p<0.001). Pre-intervention, 10.5% of adolescents had impaired glucose levels, 6.3% had basal glucose intolerance and 3.83% carbohydrate intolerance. No diabetes mellitus was diagnosed in any case. Post-intervention states of prediabetes decreased to 1.42%.
Conclusion: The PNBI decreases significantly the prevalence of overweight and obesity in adolescents of both sexes, and decreasing the prevalence of MS and prediabetes.
Disclosure: **M. Serrano:** None.

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Effect of exenatide on post-prandial glucose disposal, beta cell function and incretin hormones in non-diabetic obese subjects

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Background and aims: In diabetic patients, exenatide lowers glucose by potentiating glucose-induced insulin release, delaying gastric emptying and improving beta cell function. The effect of exenatide on glucose disposal and beta cell function in non-diabetic subject is not clear. We investigated the effect of exenatide on post-prandial glucose appearance and lipolysis, and its effect on beta cell function and glucagon concentrations in non-diabetic, morbidly obese subjects.
Materials and methods: Thirty morbidly obese non-diabetic patients (BMI 45.2±1.1 kg/m2) were randomised to exenatide 10 μg bpd (EX, n=15) or control (CT, n=15) for 3 months. At baseline and study end, patients received a meal test/double tracer study (MTT) with 2H5-glycerol (to estimate lipolysis).
Results: At 3 months, EX showed a significant reduction in body weight (121±6 to 114±6 kg, p<0.001, vs 121±3 to 118±5 of CT). During the MTT, mean glucose (5.7±0.2 vs 6.1±0.1 mmol/L, p<0.01) and insulin levels (295 ±37 vs 392±53 pmol/L, p=0.03) decreased significantly, while beta cell glucose sensitivity was unchanged. In EX compared to CT, the time-course of plasma glucose, insulin, and insulin secretion showed a lower peak at 60 min, with a rapid drop thereafter until 240 min. This pattern was due to a reduction in the rate of oral glucose appearance in the 2nd-3rd hr postmeal (AUC60-180min =1091 [1149] μmol/kgffm vs 2115 [777] of CT, median [IQR], p<0.01), followed by an increase during the subsequent 3 hrs (AUC60-180min =1843 [1161] μmol/kgffm vs 1045 [873] of CT, p=0.05), without any change in the total amount (AUC0-360min) recovered in the systemic circulation. Likewise, endogenous glucose production (EGP) resumed at significantly higher rates through the 2nd and 3rd hour postmeal with EX (AUC60-180min from 321 [164] to 588 [392] μmol/kgffm, p=0.03), the total amount of glucose release being similar over the whole 6 hours. In EX, post-prandial glycerol Ra was slightly reduced during the first two hours followed by an increase, but total Ra was similar between the two groups. In EX, post-prandial incremental GIP was reduced (AUC = 75±11 to 46±12 ng/ml, p=0.04), whilst the plasma glucagon response was flat (incremental AUC0-180min from 774±270 to -546±161 pmol/L, p=002). Insulin sensitivity was improved in EX (413 ± 14 vs 346 ± 10 ml.min-1.m-2, p=0.003) but not in CT (347±9 vs 350±10, p=ns).
Conclusion: In obese non-diabetic subjects exenatide causes weight loss, improvement of insulin sensitivity, decreased postprandial glycaemia and glucagon release without changes in β-cell function. These effects are in part consequent upon delayed oral glucose appearance in the systemic circulation.
Supported by: In part by a research grant from Lilly
Disclosure: **S. Camastra:** None.

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One year of treatment with dapagliflozin QD + exenatide QW in obese adults without diabetes: results of an open-label extension study

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Background and aims: GLP-1 receptor agonists and SGLT-2 inhibitors have complementary mechanisms of action, on energy intake and disposal, that may deliver sustained weight loss. In the initial 24-wk phase of a randomized, blinded, Phase 2 study, obese adults received dapagliflozin (DAPA) QD + exenatide (EXE) QW or placebo (PBO), with significant weight reduction, 4 kg, for DAPA+EXE vs PBO (ADA June 2016). Weight reduction is difficult to maintain over time. The goal of this extension was to address efficacy, including durability, and tolerability of DAPA+EXE in subjects treated continuously for 1 yr or switching from PBO to DAPA+EXE.
Materials and methods: This study was a single center, 52-wk, open-label extension of a 24-wk randomized double-blind study. In first 24 wks, 50 obese nondiabetic adults (age 18-70 yr; BMI 30-45 kg/m2) were randomized 1:1 to oral DAPA 10 mg QD + sc EXE 2 mg QW (n=25) or PBO pills + injections (n=25). At completion of 24-wk study, subjects were invited to enter 28-wk open label extension in which all subjects received DAPA+EXE. All subjects, switching from PBO (PBO→ DAPA+EXE) or continuous DAPA+EXE, were assessed with anthropometry and fasting blood chemistry. Oral glucose tolerance test (OGTT), including urinary glucose, performed only for DAPA+EXE.
Results: A total of 38 subjects (17 initially PBO; 21 DAPA+EXE) entered the extension. Baseline (wk 24) values for PBO→DAPA+EXE / DAPA+EXE were mean (SD) body weight 102.2 (14.5) / 102.2 (17.7) kg; HbA1c, 35.6 (2.7) / 33.4 (4.2) mmol/mol; and fasting plasma glucose (FPG), 5.94 (0.47) / 5.47 (0.54) mmol/L. PBO→DAPA+EXE subjects lost 4.7 kg in wks 24–52; weight was stable for DAPA+EXE (Table). Over the whole 1-yr period, weight loss was 5.0 and 5.9 kg, respectively. HbA1c and FPG declined from wk 24–52 in the PBO→ DAPA+EXE group only, and over 1 yr in both groups. From 24–52 wks, the proportion with impaired fasting glucose decreased in PBO→DAPA+EXE group (from 14 to 7 of 15 completers, P=0.058). In the DAPA+EXE group, urinary glucose excretion was maintained over 52 wks (Table). Systolic blood pressure (SBP), but not diastolic, was reduced in the active treatment periods; at 52 wks by 16 mmHg for DAPA+EXE. Overall, adverse events (AEs) in wks 24–52 were similar to the original study, but 1 case of angioedema occurred (serious AE). During wks 24–52, 7 subjects in DAPA+EXE and 2 in PBO→DAPA+EXE group discontinued or did not complete per protocol, mainly due to AEs or noncompliance.

Conclusion: In this 1-yr Phase 2 study (24 wks blinded + 28 wks open label) in obese adults without diabetes, DAPA+EXE treatment reduced and then maintained body weight (5–6 kg loss) and also levels of HbA1c, FPG, 2h-plasma glucose and SBP. Treatment effect in the PBO→DAPA+EXE group was similar to DAPA+EXE in the original study, supporting robust efficacy. This combination treatment may be efficacious for weight loss and maintenance and potentially for preventing type 2 diabetes.

Parameter	Wk 0 Mean±SD	Δ Wk 0–24	Δ Wk 24–52	Δ Wk 0–52
DAPA+EXE	**n=21**	**n=21**	**n=14**	**n=14**
Body weight (kg)	106.4±15.7	−4.24**	−1.08	−5.91*
Body weight change (%)	NA	−4.24**	−1.40	−6.03*
HbA1c (mmol/mol)	37.2±3.8	−3.9***	0.5	−3.1**
FPG (mmol/L)	5.91±0.64	−0.45***	0.19	−0.36*
2h-PG (OGTT; mmol/L)	7.82±2.19	−1.88**	−0.34	−2.45**
U-glucose (g per 3h)	NA	9.41***	−1.18	6.53**
SBP (mmHg)	132.9±12.6	−8.0**	−6.1	−16.3**
DBP (mmHg)	74.5±10.4	2.9	−0.6	3.2
PBO→ DAPA+EXE	**n=17**	**n=17**	**n=15**	**n=15**
Body weight (kg)	102.9±14.7	−0.72	−4.73**	−5.04**
Body weight change (%)	NA	−0.67	−4.60**	−4.93**
HbA1c (mmol/mol)	37.2±2.6	−1.6**	−1.7**	−3.3**
FPG (mmol/L)	5.78±0.42	0.16	−0.39**	−0.19
2h-PG (OGTT; mmol/L)	7.17±1.23	0.035	NA	NA
U-glucose (g per 3 h)	NA	0.0	NA	NA
SBP (mmHg)	137.4±13.8	−3.7	−7.2*	−11.7**
DBP (mmHg)	79.9±12.0	0.7	−0.8	−1.2

DBP, diastolic blood pressure; NA, not applicable; FPG; fasting plasma glucose; OGTT, oral glucose tolerance test; PG, plasma glucose; SBP, systolic blood pressure; U-glucose, urinary glucose.
Data are unadjusted means (±SD). *P<0.05; **P<0.01; ***P< 0.001 for change during the indicated study period.
Full analysis set of extension study used for 0- and 24-wk data.
Per protocol analysis set used for 52-wk data.

Clinical Trial Registration Number: NCT02313220
Supported by: AZ
Disclosure: **J.W. Eriksson:** Grants; AstraZeneca AB. Lecture/other fees; AstraZeneca AB.

PS 061 Nutrition and diet

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A survey of UK dietitians on current advice to people with type 1 diabetes using insulin pump therapy

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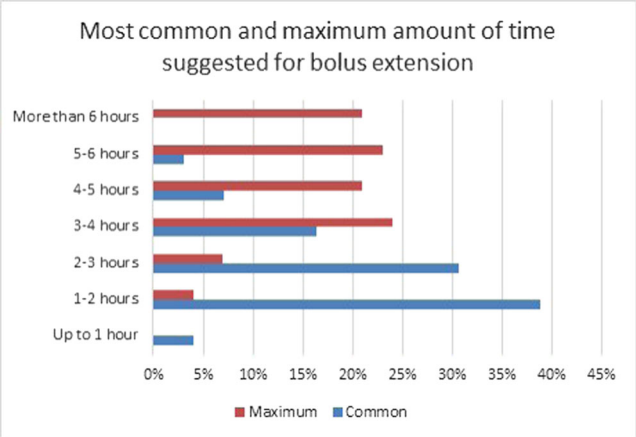
Background and aims: Continuous Subcutaneous Insulin Infusion (CSII) enables greater food freedom than multiple daily dose injection regimens and enables extended insulin delivery with slowly digested food. Quality evidence to support advanced insulin bolus options with CSII is lacking or conflicting, and clinical guidelines do not exist. The aim of this national survey was to establish current practice among type 1 diabetes specialist dietitians.
Materials and methods: A 10 question survey was sent out electronically to the British Dietetic Association's Diabetes Specialist Group, which consists of approximately 400 Dietitians working in, or with a special interest in Diabetes.
Results: 106 people responded to the questionnaire. 42% of dietitians advised their patients to bolus for a minimum of 1-5g carbohydrate, 35% advised 6-10g, 21% advised 11-15g and only 2% advised 16-20g carbohydrate. The majority (91%) did not advise their patients to count protein or fat. 7% advised to count both protein and fat, 1% advised to count fat, and 1% advised to count protein. 100% advise extended boluses. The most popular was a 50:50 dual wave bolus, with 89% advising its use. A square wave was next most popular (recommended by 80% of dietitians), then 30:70 dual wave (75%), 70:30 dual wave (66%), then 60:40 and 40:60 both with 54% recommending their use. Only 36% of dietitians recommended using split boluses. A range of bolus durations were recommended as shown in the graph. Extending over 1-2 hours was most commonly recommended (39%) followed by 2-3 hours (31%) then 3-4 hours (16%). The maximum duration advised was 3-4 hours (24%), 4-5 hours (21%), 5-6 hours (23%) or greater than 6 hours (21%). 84% said they would not give different advice depending on whether patients are eating freshly cooked or reheated starches. 52% provide one-to-one education with a Diabetes educator before commencing pump therapy. 48% provide structured education for Type 1 diabetes, 10% provide structured education specifically for patients considering pump therapy. 7% of respondents report that patients are not required to attend any of these services. 25% of respondents recommend super bolusing, either often (6%) or occasionally (19%) and 33% were unfamiliar with super bolusing.
Conclusion: There is varied dietetic practice across the UK. Bolusing for 1-10g instead of the traditional 15-20g carbohydrate may reflect ease of bolusing on pumps, and the ability of a pump to track active insulin. Relatively small numbers advise protein and fat counting. The range of bolus types recommended by dietitians was very varied reflecting the conflicting evidence. Structured education is central to diabetes care but 7% of centres do not have an educational programme supporting CSII initiation. Robust data and evidence-based guidelines are required to support advanced feature use for people with type 1 diabetes using CSII while the absence of structured education in some centres is concerning.



Disclosure: **S. Rilstone:** None.

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Nutritional impact of discrete strategies to reformulate or reduce discretionary food choices in diet of the Australian population

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Background and aims: In Australia, discretionary foods contribute over a third of energy intake and displace core foods such as fruit, vegetables, dairy, lean meats, and whole grains. Reformulating discretionary foods to reduce energy density or improve their nutritional profile, or simply reducing their intake has been suggested to reduce the population level of nutrient deficiencies, obesity and associated chronic disease. The aim of this study was to evaluate the potential impact of key discrete strategies to reformulate or reduce discretionary foods on the diet of the Australian population.
Materials and methods: Food and nutrient intake data from 12153 people who provided information to the 2011-13 Australian Health Survey were population weighted and aggregated at the food identifier level. Data modelling was conducted to simulate a range of scenarios about the potential impact of key discrete strategies to reduce discretionary foods in the Australian population (identified from a previously conducted scoping review of the literature). The modelled scenarios included: reducing the quantity (portion size) of discretionary foods; substituting non-caloric beverages for water based sugar sweetened beverages (SSBs); decreasing the sugar content of discretionary foods; and, decreasing the sodium content of discretionary foods. Modelling was conducted for the entire Australian population and a subset of people (4.7%) that self-reported having diabetes.
Results: For the overall Australian population, a 25% reduction in the portion size of all discretionary foods reduced average per person daily energy intake by 766kJ (9.0%) compared to the original diet. An increase in non-discretionary foods of 14.0% would be required to counter the energy deficit of a 25% reduction in discretionary foods, which would result in 6g per person (7.0%) greater protein intake and 6g (5.7%) less sugar compared to the original diet. Reducing sugar by 25% in discretionary foods within the food groups 'biscuits' (sweet and savoury) and 'cakes/ muffins/ scones/ cake type desserts' reduced total energy by just 36kJ (0.4%). Substituting water/non-caloric beverages to take the place of all SSBs reduced energy by 251kJ (2.9%) and sugar by 15g (14.3%). Reformulation of grain based discretionary foods to reduce sodium by 25% resulted in a 69mg (2.9%) lower sodium intake. The sub-population of people with diabetes reported a lower intake of discretionary foods compared to the overall population (2515kJ [32.4% total energy] vs. 3061kJ [35.8% energy]). This subgroup had a similar magnitude of response to the overall population for the modelled scenarios.
Conclusion: Key discrete strategies, identified from the literature, to reformulate or reduce discretionary foods would in theory have small to moderate impacts on the diet quality of the overall Australian population and a subset of those who self-report having diabetes. The impact of these strategies in combination, or for sub-populations with proportionally higher discretionary food intake may be more substantial.
Supported by: Funded from NHMRC Grants (631947 and 1053359)
Disclosure: **T. Wycherley:** None.

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Mediterranean diet increases circulating levels of endothelial progenitor cells in type 2 diabetes

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Background and aims: Mediterranean diet reduces the risk of coronary heart disease. The effect of Mediterranean diet on the regenerative capacity of the endothelium has never been investigated in type 2 diabetes. This study sought to evaluate whether Mediterranean diet affected circulating levels of endothelial progenitor cells (EPCs) in patients with type 2 diabetes.
Materials and methods: In a two-arm, single-center trial, 215 men and women with newly diagnosed type 2 diabetes were randomized to a Mediterranean diet (n = 108) or a low-fat diet (n = 107). At baseline visit and at 1 year seven EPCs subpopulations, resulting from the combinations of the three surface markers CD34, CD133, and KDR, were evaluated on peripheral mononuclear blood cells.
Results: At 1 year, both the CD34+KDR+ and CD34+KDR+CD133+ counts showed a significant increment with the Mediterranean diet compared with the low-fat diet ($P < 0.01$ for both). The diabetic patients with the highest scores (6-9) of adherence to the Mediterranean diet had higher circulating CD34+KDR+ level than the diabetic patients who scored <3 points on the scale ($P = 0.001$). The increase in circulating level of CD34+ KDR+ cells count was positively associated with changes in total and monounsaturated fat ($r = 0.21$ and 0.28, respectively, $P < 0.001$ for both), and negatively associated with change in carbohydrates ($r = -0.19$, $P = 0.01$).
Conclusion: This study is the first dietary intervention trial demonstrating a beneficial effect of the Mediterranean diet on the regenerative capacity of endothelium in newly-diagnosed type 2 diabetes.
Clinical Trial Registration Number: NCT00725257
Disclosure: **M.I. Maiorino:** None.

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Treatment of reactive hypoglycaemia with macrobiotic Ma-Pi 2 diet for prevention of type 2 diabetes: the MAHYP randomised crossover trial

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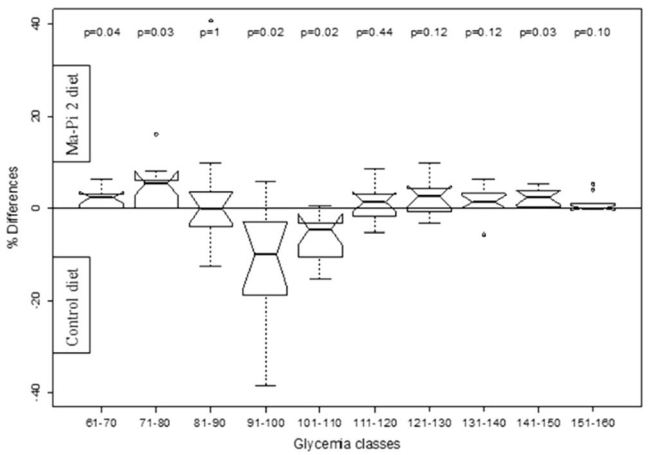
Background and aims: Postprandial endogenous hyperinsulinemic hypoglycemia, also called reactive hypoglycemia (RH), is one of the most common endocrine disorders. RH is featured by an altered synchrony between insulin secretion and glucose absorption along the

gastrointestinal tract and such condition leads to impaired fasting glucose (IFG) and ultimately to type 2 diabetes (T2D). Diet therapy represents the cornerstone of RH treatment. Based on the beneficial effects observed with the macrobiotic Ma-Pi 2 diet in the dietary treatment of T2D (MADIAB trial), we investigated the effect of the Ma-Pi 2 diet compared to a standard recommended diet for treatment of RH.

Materials and methods: The study was designed as a randomized cross-over trial and a residential setting in a farm outside Rome, Italy, was chosen to achieve proper control of the subjects by a team of doctors and nutritionists. Enrolled subjects affected by RH (n = 12) have been individually randomized (1:1) in a crossover design among two diet sequences to one of the two groups. One group has been administered the control diet for 3 days followed by the Ma-Pi 2 diet for a further 3 days; the other group was administered the Ma-Pi 2 diet first and then the control diet. Continuous glucose monitoring system (CGM) was adopted to assess blood glucose variations 24-hours a day, during both intervention periods. The primary endpoint of this crossover study was the number of RH events (blood glucose levels < 70 mg/dL) during 24 hours with and without snacks.

Results: The number of hypoglycemic events was 4.17 ± 3.95 during control diet period, whereas no episodes of hypoglycemia were reported during Ma-Pi 2 diet ($p < 0.03$). Percentage of glucose readings between 71 and 80 mg/dL was significantly higher in the control diet compared with Ma-Pi 2 diet ($p < 0.03$); percentage of glucose readings between 91 and 100 mg/dL was significantly higher in Ma-Pi 2 diet ($p < 0.03$) (Figure).

Conclusion: This trial demonstrated that the macrobiotic Ma-Pi 2 diet allows for good blood glucose homeostasis in subjects affected by RH as it managed to diminish blood glucose excursions during day and night hours. Ma-Pi 2 diet represents an effective nutritional tool for the management of RH and, based on these results and those of the MADIAB trial, we suggest that Ma-Pi 2 diet can be an ideal diet to be tested in trials for prevention of T2D.



Clinical Trial Registration Number: ISRCTN84079151
Supported by: Un Punto Macrobiotico Association
Disclosure: **Y. Khazrai:** None.

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Diet-induced acid load is associated with increased risks of cardiovascular disease independent of diabetes

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Background and aims: A western diet is associated with the increased prevalence of cardiometabolic diseases. Dietary patterns determine acid-base status, which recently has been reported to increase hypertension and diabetes incidence. However, the association between dietary acid load and cardiovascular disease (CVD) risk in general population has not been fully investigated.

Materials and methods: This was a population-based, cross-sectional study using 11,601 subjects from the Korea National Health and Nutrition Examination Survey 2008-2011. Individual's CVD risk was evaluated using atherosclerotic cardiovascular disease (ASCVD) risk equations. Acid-base status was assessed with both the potential renal acid load (PRAL) and the dietary acid load (DAL) score derived from nutrient intakes.

Results: Individuals with the highest PRAL tertile had a significant increase in ASCVD risks (10.1 vs. 8.3, $P<0.01$) and tended to belong to the high risk group compared to those with the lowest PRAL tertile (OR=1.43, 95% CI=1.30-1.58). Higher PRAL was linked to greater hypertension prevalence (OR=1.16, 95% CI=1.05-1.29). After stratification, non-obese subjects with higher PRAL had similar ASCVD risks in that of obese group with the lowest PRAL tertile. In addition, the lowest PRAL individuals who did not exercise showed comparable ASCVD risks with higher PRAL group who regularly exercised. The association between PRAL and ASCVD risk was stronger among non-sarcopenic subjects (OR=1.17, 95% CI=1.06-1.30). This association was still significant in multiple logistic regression models (OR=1.37, 95% CI=1.08-1.74). Similar trends were observed with the DAL scores.

Conclusion: Diet-induced acid load was associated with increased risks of CVD independent of hypertension or diabetes. In addition, the effect of dietary pattern on CVD risk was maintained after stratification with the status of obesity and exercise, but not sarcopenia.

Supported by: Korea Healthcare Technology R&D Project and NRF Basic Science Research Prog
Disclosure: **E. Han:** None.

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Efficacy and mechanism of chronic zinc supplementation in insulin resistant ob/ob mice

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Background and aims: High-dose chronic zinc supplementation is a pivotal component in traditional Indian and Chinese medicine to control Type-2 diabetes (T2DM). In clinical studies, chronic zinc supplementation improved metabolic control and dyslipidemia in T2DM via yet unknown mechanisms. In the current pre-clinical study, we investigated the metabolic effects of chronic zinc supplementation in insulin resistant ob/ob mice. Furthermore, we explored if the expression of selected genes which are relevant in the context of T2DM and for intestinal zinc uptake are affected by chronic zinc supplementation.

Materials and methods: Adult, female insulin resistant ob/ob and lean ob/- mice were chronically supplemented with zinc chloride ($ZnCl_2$, 20mM in drinking water) or placebo (n=8 / group). In vivo body composition measurement by NMR and blood sampling for measurement of circulating zinc concentrations and were performed after 5 weeks supplementation. After 7 and 8 weeks, metabolic status was assessed by insulin tolerance (ITT) and oral glucose tolerance tests (OGTT) following standard procedures. RNA-Expression of selected genes was quantified in liver, kidney, duodenum and jejunum by parallel microfluidic card real-time polymerase chain reactions. Results were statistically analyzed by ANOVA and regarded significant with $p<0.05$.

Results: Supplementation with zinc chloride resulted after 5 weeks in a significant increase in serum zinc concentration from 10.1 ± 0.5 µmol/L (mean ± SEM) to 35.9 ± 6.2 µmol/L for lean ob/- mice and from 17.8 ± 0.6 µmol/L to 23.8 ± 0.9 µmol/in obese ob/ob animals. After 8 weeks treatment, fasting blood glucose and HbA1c were significantly lower in

zinc supplemented ob/ob mice compared to controls (Glucose: 10.1 ± 0.5 mmol/L and 14.5 ± 1.2 mmol/L; HbA1c: 27.3 ± 0.9 mmol/mol and 34.0 ± 2.9 mmol/mol). Zinc supplementation did not significantly affect body weight, body composition and food intake. However, 20mM of ZnCl2 significantly improved insulin sensitivity and oral glucose tolerance in ob/ob mice vs. placebo. No significant metabolic effects of oral ZnCl2 supplementation were observed in lean ob/- mice. Major changes in expression of several genes could be observed most notably in the duodenum and only to a minor degree in jejunum, liver and kidney. In duodenum, zinc supplementation did not change expression of selected genes relevant in T2DM, but enhanced the duodenal expression of zinc transporters by a factor of 1.3 fold to 1.8 fold for different transporter families. The strongest induction by zinc could be observed for three isoforms of metallothioneins, Mt1 (10.2 fold), Mt2 (14.8 fold), and Mt4 (142-fold).
Conclusion: We confirmed that high-dose chronic zinc supplementation significantly improved glucose metabolism in ob/ob mice. Although ZnCl2 supplementation did not significantly affect the expression of selected genes which are directly involved in the physiology of glucose metabolism, a strong up-regulation of metallothioneins was detected in the duodenum. This may represent a key step to explain the beneficial effects of zinc supplementation because metallothioneins are known to trigger anti-oxidant systems and to reduce oxidative cell stress. The role of intestinal metallothionein expression in the context of T2DM may need to be investigated further.
Disclosure: **C. Gerbeth:** None.

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Chemerin concentrations are heritable and increase in response to a high-fat diet in healthy, lean subjects

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Background and aims: Chemerin is a secreted protein with adipose tissue and liver being major sites of synthesis. Besides its established role in inflammation, chemerin was shown to regulate adipocyte development and function and to be involved in glucose and lipid metabolism. Furthermore, serum chemerin levels are elevated in obese patients and are positively correlated with markers of the metabolic syndrome. In a human intervention study with mono- and dizygotic twins, we aimed to investigate the effects of a high-fat diet (HF) on chemerin and its receptor (CMKLR1) considering possible genetic determinants.
Materials and methods: In the NUGAT (NUtriGenomic Analysis in Twins) study, 46 healthy twin pairs received a carbohydrate-rich, low-fat diet for 6 weeks (LF) followed by an isocaloric HF diet for 1 (HF1) and additional 5 weeks (HF6). At each clinical investigation day (LF, HF1, HF6) subcutaneous adipose tissue biopsies were taken. Gene expression analyses were performed on Agilent 8x60K microarrays. For selected genes results were validated by quantitative real-time PCR. Serum parameters were measured using conventional ELISA. Genomic DNA extracted from whole blood was genotyped using Illumina HumanOmniExpressExome BeadChips. For heritability estimates the 'ACE' model was applied.
Results: The heritability estimates for circulating chemerin were > 55%, confirming a strong influence of genetic factors. After 6 weeks on HF serum chemerin, normalized to LF, increased by 5% (LF: 134.07±22.65 ng/ml vs. HF6: 139.31±28.35 ng/ml; $p<0.05$). In accordance with this, adipose tissue expression of RARRES2, the gene encoding chemerin, was also increased in response to HF (HF1: 1.20-fold, HF6: 1.23-fold; $p<0.01$). CMKLR1 mRNA was acutely downregulated by 17% after 1-week HF ($p<0.05$), whereas at HF6 CMKLR1 expression returned to basal levels (HF1 vs. HF6: $p<0.01$, LF vs. HF6: n.s.). Interestingly, the rs3735167 polymorphism, a tagging SNP located in the 5′ flanking region of RARRES2, was significantly associated with serum chemerin levels, RARRES2 expression in fat, and visceral fat mass.
Conclusion: The present study revealed that more than 55% of the variation in serum chemerin levels is determined by additive genetic factors. Furthermore, we could show in humans that a high-fat diet induces increased chemerin concentrations and gene expression. In the short term this seems to be compensated by an acute downregulation of CMKLR1. Genetic polymorphisms are, at least in part, contributing to the high inter-individual differences in serum chemerinlevels.
Clinical Trial Registration Number: NCT01631123
Supported by: BMBF
Disclosure: **N. Küffel:** None.

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Distance between dietary intake and self-rated dietary behavior in patients with diabetes: analysis from National Health and Nutrition survey in Japan

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Background and aims: Although knowledge of patients' dietary intake and dietary behavior is essential for medical nutritional therapy for diabetes, evidence is sparse from the concurrent examination of these two factors in persons with and without diabetes. We aimed to investigate whether patients with diabetes have different dietary intakes and self-rated dietary behavior than persons without diabetes from the National Health and Nutrition Survey in Japan. Studied were 3,271 participants (age: 56.2 years, men: 41%, patients with diabetes: 14%, duration of diabetes: 7.9 years) from a total of 18,513 participants.
Materials and methods: They completed a 1-day weighed household food record, questionnaire on dietary behavior and clinical laboratory tests. Results were examined by gender using multivariate analysis of covariance and logistic regression analysis adjusted for age, gender, body mass index, and other confounders.
Results: In men, total energy intake was not significantly different (2265 vs.2204 kcal, $p=0.32$) but energy intake from grain was lower in patients with diabetes than in persons without diabetes (910 vs. 994 kcal, $p=0.020$). No significant difference was observed for macro- and micronutrient intakes. Conversely, in women, total energy intake and energy intake from grain were higher in patients with diabetes than in persons without diabetes (2152 vs.1933 kcal, $p=0.029$; 881 vs.783 kcal, $p=0.043$), and tended toward higher consumption of carbohydrate, fat, vitamins and minerals. For self-rated dietary behavior, men with diabetes had a significantly higher tendency to avoid consuming too much energy such as in sweets, alcohol and fatty foods than persons without diabetes (OR= 1.78, 2.15, 1.44, p= 0.001, 0.001, 0.016, respectively). However, in women with diabetes the only significant difference from persons without diabetes was to avoid consuming too much sweets (OR= 1.72, p= 0.001).
Conclusion: We clarified characteristics of dietary intakes and self-rated dietary behavior in diabetes including sex differences. Translating self-rated dietary behavior into actual action could be important for effective medical nutritional therapy for diabetes.
Disclosure: **C. Horikawa:** None.

PS 062 SGLT-2 inhibitors: efficacy and safety

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Efficacy of canagliflozin versus dipeptidyl peptidase-4 inhibitors in patients with type 2 diabetes: results from randomised controlled trials and a real-world study

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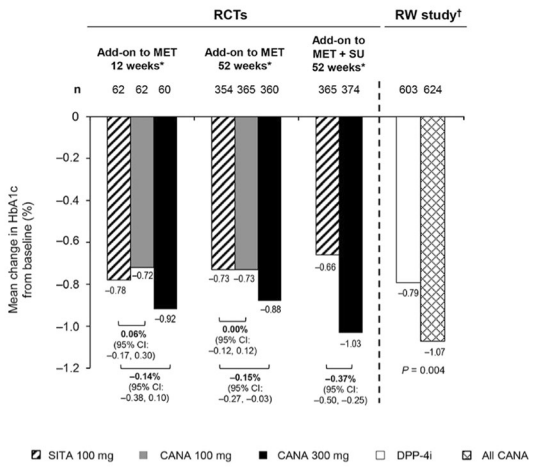
Background and aims: In randomised controlled trials (RCTs), canagliflozin (CANA) was shown to be more effective than the dipeptidyl peptidase-4 inhibitor (DPP-4i) sitagliptin (SITA) in lowering glucose. RCT and real-world (RW) results tend to differ as RW studies may include a broader set of patients with more advanced conditions; thus it is important to assess the effects of agents in clinical practice. The objective of this analysis was to compare the glycaemic efficacy of CANA versus DPP-4i using RCT and RW data.
Materials and methods: We compared the HbA1c-lowering efficacy of CANA 100 and 300 mg versus SITA 100 mg in 3 RCTs of patients with T2DM, and the effectiveness of CANA (pooled data for all doses) in a retrospective RW matched control-cohort study using US integrated claims and laboratory data from a large population of insured patients with T2DM (65% and 34% of patients received CANA 100 or 300 mg, respectively [1% other]). Patients in the CANA cohort were matched 1:1 to patients in the DPP-4i cohort using propensity score matching that incorporated demographics and baseline characteristics.
Results: In RCTs with baseline HbA1c ~8.0%, CANA 100 mg provided similar and CANA 300 mg provided greater HbA1c reductions versus SITA 100 mg (Figure). In the RW study with baseline HbA1c ~9.0%, greater HbA1c reductions were seen with CANA (-1.07%) versus DPP-4i (-0.79%).
Conclusion: The relative magnitude of HbA1c reduction with CANA and SITA was similar in the RCT and RW studies; CANA consistently lowered HbA1c versus DPP-4i in patients with T2DM.

Figure. Change from baseline in HbA1c: RCTs versus RW study.



MET, metformin; SU, sulphonylurea; CI, confidence interval.
*Data are least squares mean.
†Mean (median) follow-up of 182.3 (191.0) days for DPP-4i and 184.2 (197.0) days for CANA.

Clinical Trial Registration Number: NCT00642278, NCT01106677, NCT01137812
Supported by: Janssen Scientific Affairs, LLC
Disclosure: **W. Canovatchel:** Employment/Consultancy; Janssen Research & Development, LLC.

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Use of SGLT-2 inhibitors as replacement or intensification therapy in type 2 diabetes patients who use metformin: a Danish cohort study

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Background and aims: Limited real-life data are available on patient characteristics associated with use of SGLT-2 inhibitors when metformin-based therapy is changed.
Materials and methods: We used the nationwide Danish Centre for Strategical Research in type 2 Diabetes Project (DD2) cohort to identify 4,143 patients with recently diagnosed type 2 diabetes who was enrolled in DD2 during 2010-2014. Patients were eligible if they used metformin-based therapy with or without other glucose-lowering agents at enrollment, but had never used SGLT-2 inhibitors. We used binomial regression to examine age- and gender-adjusted associations between patient characteristics at baseline and later initiation of SGLT-2 inhibitors.
Results: Among 4,143 type 2 diabetes patients on metformin-based therapy (median age 61 years, 42% female), 127 initiated SGLT-2 treatment. Of these, 82 (2.0%) added an SGLT-2 inhibitor and another 45 (1.1%) replaced metformin with an SGLT-2 inhibitor. Patients who initiated an SGLT-2 inhibitor were substantially younger than SGLT-2 non-initiators (66% vs. 43% > 60 years). Patients who started an SGLT-2 inhibitor were more likely to be obese at baseline (73% vs. 54% with BMI > 30; adjusted relative risk (aRR)=1.25, 95% CI: 1.10-1.40), had more central obesity (83% vs. 68% with waist-hip ratio >1.0 (men)/>0.85 (women); aRR=1.19, 95% confidence interval (CI): 1.10-1.28), and reported more weight gain since age 20 (68% vs. 48% gained more than 30 kg; aRR=1.36, 96% CI: 1.19-1.55). Patients who started an SGLT-2 inhibitor were more likely to be enrolled at a hospital outpatient clinic than at a primary care setting, compared to SGLT-2 non-initiators (72% vs. 49%; aRR=1.34, 95% CI: 1.19-1.50). They also had poorer glycaemic control at baseline (HbA1c >7.5%, 71% vs. 41%; aRR=1.61, 95% CI: 1.41-1.83), higher C-peptide levels (indicating high insulin resistance) (50% vs. 36% C-peptide >800; aRR=1.34, 95% CI: 0.83-2.18), more microvascular complications (23% vs. 15%; aRR=1.86, 95% CI: 1.35-2.62). Prevalence of cardiovascular disease was similar at baseline (19% vs. 23%; aRR=1.10, 95% CI: 0.76-1.59). A tendency towards less physical activity was also reported among those initiating SGLT-2 inhibitors compared to non-initiators (34% vs. 39%; aRR=1.10, 95% CI: 0.96-1.24).
Conclusion: In this routine clinical care study of type 2 diabetes patients on metformin-based therapy, predictors of later initiation of SGLT-2 inhibitors were young age, obesity, high insulin resistance and poor baseline glycaemic control, presence of microvascular complications, and receipt of care at a hospital clinic.
Supported by: Danish Agency for Science (09-067009) and Novo Nordisk
Disclosure: **J.S. Nielsen:** None.

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Changes in HbA_{1c}, body weight, and systolic blood pressure in type 2 diabetes patients initiating dapagliflozin therapy: a German primary care database study

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Background and aims: Clinical trials have demonstrated that dapagliflozin has antihyperglycaemic efficacy as well as beneficial effects

on body weight and blood pressure. The aim of this study was to investigate changes in HbA1c, body weight (BW), and systolic blood pressure (SBP) in primary care type 2 diabetes (T2D) patients initiating dapagliflozin treatment.

Materials and methods: T2D patients who started dapagliflozin in 985 general and 32 diabetologist practices (Disease Analyser, Germany: 12/2012-10/2014) were analyzed (3 and 6 months follow-up). Multivariable linear regression analyses were applied to identify clinical characteristics and comorbidity associated with changes in HbA1c, BW, and SBP.

Results: 1,169 T2D patients (age: 62.5 years; men: 59%; diabetologist care: 23%) with new dapagliflozin therapy were included. At 3 months, dapagliflozin significantly reduced HbA1c (-0.8 ± 1.4%) from baseline (8.5 ± 1.5%) ($p<0.001$). Changes were maintained after 6 months (-0.8 ± 1.5%) (p9%) showed greater reductions in HbA1c than the whole sample (3 months -1.8%, 6 months -1.8%; both $p<0.05$). BW and SBP showed also statistically significant reductions with dapagliflozin over 3 and 6 months (-2.2 kg, $p<0.001$; -2.2 mmHg, $p=0.003$ and -2.5 kg, $p<0.001$; -2.3 mmHg, $p=0.011$), respectively. After 3 months, 53% achieved a reduction of both HbA1c and BW, and 45% at 6 months). Similar results were observed both in general and diabetologist practices. In multivariable analyses, baseline HbA1c (parameter estimate: -0.6479) and diabetologist care (-0.2553) were independent predictors of HbA1c change (6 months) (all $p<0.05$).

Conclusion: In German T2D patients treated with dapagliflozin therapy, there were statistically significant reductions in HbA1c, body weight, and systolic blood pressure in a real-world setting of primary and diabetologist care. The changes were comparable to results of the dapagliflozin clinical trial programme.

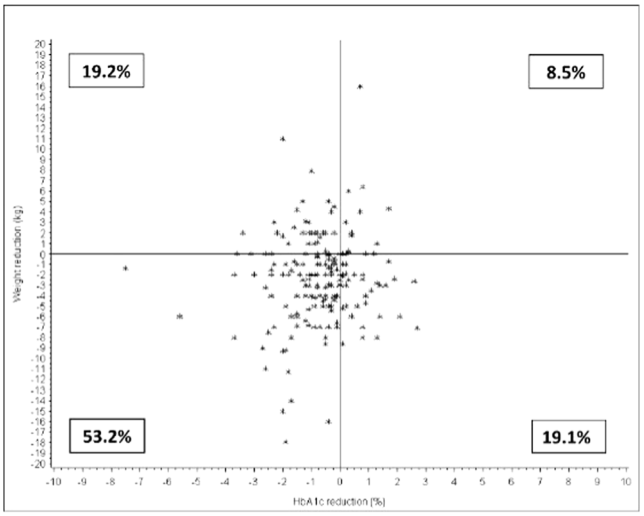

Figure Scatter plot representing the relationship between the change of HbA1c (%) and change of body weight (kg) after 3 months in type 2 diabetes patients initiating dapagliflozin in primary care

Supported by: AstraZeneca GmbH

Disclosure: **M.F. Scheerer:** Employment/Consultancy; Employee of AstraZeneca GmbH.

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Effect of dapagliflozin on HbA_{1c} and weight after its addition to various combinations of other diabetes medications: ABCD nationwide dapagliflozin audit

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Background and aims: To evaluate the effect on HbA1c and weight after adding dapagliflozin to various combinations of other diabetes medications, in patients with type 2 diabetes in the UK.

Materials and methods: We analysed data from the ABCD nationwide audit of dapagliflozin in real clinical use in the UK (October 2014-March 2016). In total, 156 contributors from 59 centres submitted data on 1753 dapagliflozin treated patients. Those with baseline and follow-up HbA1c within a median (interquartile range) of 6.2(4.1-9.4) months, after commencing dapagliflozin, were included. Patients were categorised into 6 groups according to their other diabetes therapies, dapagliflozin was added to: group 1 (metformin, n=148), group 2 (metformin and sulphonylurea, n=113), group 3 (metformin and dipeptidyl peptidase-4 inhibitor (DPP4i), n=68), group 4 (pioglitazone with or without any other diabetes medications, n=46), group 5 (glucagon-like peptide-1 receptor agonist with or without any other diabetes medications, n=248), group 6 (insulin with or without any other diabetes medications, n=542).

Results: Mean(±SE) HbA_1c fell by (-9.8(±1.2) mmol/mol from 76.7(±1.3) to 66.8(±1.4) mmol/mol, $p<0.001$) in group 1, (-12.0 (±1.2)mmol/mol from 80.8(±1.4) to 68.8 (±1.3)mmol/mol, $p<0.001$) in group 2, (-13.3(±1.9) mmol/mol from 79.7(±1.9) to 66.3(±2.1)mmol/mol, $p<0.001$) in group 3, (-10.2(±2.3) mmol/mol from 82.0(±2.1) to 71.7(±2.8) mmol/mol, $p<0.001$) in group 4, (-9.7 (±1.0) mmol/mol from 81.5(±0.9) to 71.8(±1.07) mmol/mol, $p<0.001$) in group 5 and (-9.2(±0.6)mmol/mol from 81.8(±0.7) to 72.6(±0.6)mmol/mol, $p<0.001$) in group 6. Weight fell by (-2.8(±0.5)kg from 101.1(±2.1)kg to 98.3 (±2.0)kg, $p<0.001$) in group 1, (-3.1(±0.4)kg from 99.6(±2.2)kg to 96.5(±2.2)kg, $p<0.001$) in group 2,(-3.8 (±0.6)kg from 94.8 (±2.2)kg to 90.9 (±2.1)kg, $p<0.001$) in group 3, (-2.9(±0.6)kg from 111.5(±3.9)kg to 108.6(±0.6)kg, $p<0.001$) in group 4, (-3.3 (±1.1)kg from 108.8(±1.4)kg to 105.5(±1.4)kg, $p<0.001$) in group 5, (-2.0 (±0.2) kg from 104.7(±0.9)kg to 102.6(±0.9)kg, $p<0.001$) in group 6(figure 1).

Conclusion: The biggest combined impact on HbA_1c and weight was when dapagliflozin was added to metformin and DPP4i and the least when added to insulin. Nevertheless, dapagliflozin reduced both HbA_1c and weight by clinically and statistically significant amounts in a wide range of real-world UK patients with type 2 diabetes on a variety of diabetes medications.

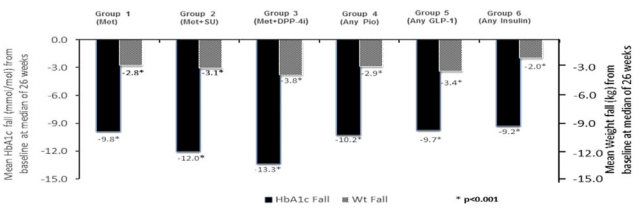

Figure1 shows the consistent reductions(*p<0.001) in both HbA_1c(mmol/mol) and weight(kg) when dapagliflozin is added to: **Group1**- metformin only, **Group2**- metformin and sulphonylurea, **Group3**- metformin and dipeptidyl peptidase-4 inhibitor, **Group4**– pioglitazone with or without any other diabetic medications, **Group5**- glucagon-like peptide-1 receptor agonist with or without any other medications, **Group6**- insulin with or without any other diabetic medications.

Supported by: ABCD

Disclosure: **M. Yadagiri:** None.

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Effects of dapagliflozin (DAPA), a sodium glucose cotransporter 2 inhibitor, on 24-hour glycaemic control in patients with type 2 diabetes

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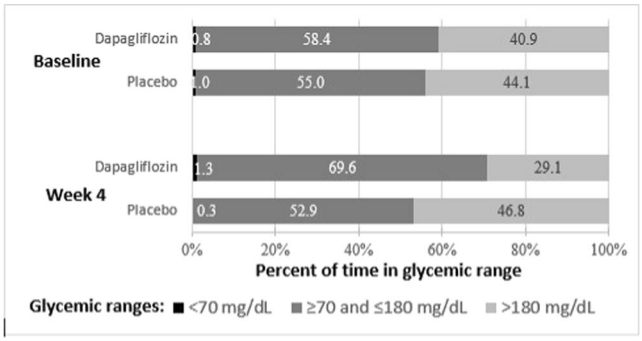
Background and aims: Daily glucose fluctuations may play an important role in diabetic complications, and drugs that deliver sustained efficacy over the course of the day may provide an advantage over the long term.
Materials and methods: This 4-wk randomized study compared the effects of DAPA 10 mg/d (N=50) vs placebo (PBO; N=50) on 24-h glycemic control in patients with T2D uncontrolled on stable doses of metformin alone (MET; ≥1500 mg/d) or insulin (INS; ≥30 U/d) ± ≤2 oral antidiabetes drugs. INS dose was adjusted at the investigator's discretion. Glucose was measured over 7 days at lead-in and wk 4 using continuous glucose monitoring (CGM). The primary outcome was change from baseline (BL) to wk 4 in 24-h mean weighted glucose (MWG). Glucose fluctuation was quantified using CGM data by change from BL in the 24-h mean amplitude of glucose excursion (MAGE). Fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) were evaluated from blood samples. Analyses were done for the overall population and by MET or INS strata and were based on the intent-to-treat population using an analysis of covariance model (ANCOVA).
Results: Treatment with DAPA significantly reduced 24-h MWG, 24-h MAGE, FPG and 2-h PPG vs PBO at wk 4. The 24-hour MWG decreased from 178 mg/dL to 161 mg/dL for DAPA and increased from 183 mg/dL to 188 mg/dL for PBO, for an LS mean difference of -24 mg/dL ($p<0.001$). For MAGE, the LS mean difference between the DAPA and PBO groups was -15 mg/dL ($p=0.010$). FPG decreased with DAPA and increased with PBO, for an LS mean difference of –30 mg/dL ($p<0.001$). The placebo-adjusted LS mean weighted decrease in 2-h PPG for DAPA vs PBO was –36 mg/dL ($p\leq0.001$). The proportion of time in euglycemia (blood glucose [BG]≥70 and ≤180 mg/dL) was increased and time with BG ≥180 mg/dL was decreased with DAPA vs PBO (Figure). There was a small increase in time with BG <70 mg/dL with DAPA vs PBO, driven by the DAPA + INS group, but no adverse events (AEs) of hypoglycemia were reported. The most common AE was urinary tract infections (6% in both arms).
Conclusion: These data show that DAPA is effective in reducing both overall glycemia and 24-h glucose fluctuations in patients with T2D uncontrolled on MET or INS.



Clinical Trial Registration Number: NCT#02429258
Supported by: AZ
Disclosure: **T. Mansfield:** Employment/Consultancy; AstraZeneca. Stock/Shareholding; AstraZeneca, Bristol-Myers Squibb.

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Achievement of glycaemic goals without hypoglycaemia with canagliflozin versus glimepiride in patients with type 2 diabetes

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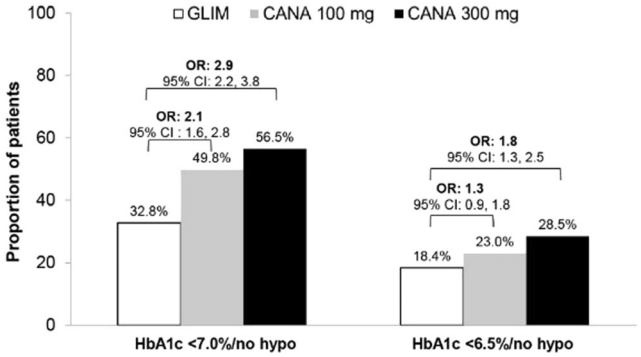
Background and aims: Guidelines for the management of type 2 diabetes mellitus (T2DM) recommend lowering HbA1c levels to <6.5% or <7.0% for most patients as long as they can be achieved safely, while avoiding hypoglycaemia. In a 52-week, Phase 3 study of patients with T2DM on background metformin (MET), canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor, improved glycaemic control and reduced body weight with fewer episodes of hypoglycaemia versus glimepiride (GLIM). This post hoc analysis evaluated the proportion of patients achieving HbA1c <7.0% and <6.5% without hypoglycaemia with CANA versus GLIM.
Materials and methods: Patients with T2DM (N = 1,450; mean age, 56.2 y; HbA1c, 7.8%; body mass index [BMI], 31.0 kg/m^2) were randomised to receive CANA 100 or 300 mg or GLIM (up- or down-titrated to maximum tolerated dose; mean maximal dose, 5.6 mg/day) as add-on to MET for 52 weeks. The proportion of patients achieving HbA1c <7.0% and <6.5% without documented hypoglycaemia (≤70 mg/dL [3.9 mmol/L]) was assessed.
Results: At Week 52, CANA 100 mg was non-inferior, and CANA 300 mg was superior to GLIM in HbA1c-lowering (LS mean changes of -0.82%, -0.93%, and -0.81%, respectively). The proportion of patients who achieved HbA1c <7.0% at Week 52 was similar across treatment groups (53.6%, 60.1%, and 55.8% with CANA 100 and 300 mg and GLIM, respectively); 25.5%, 30.6%, and 30.7% achieved HbA1c <6.5%, respectively. The incidence of documented hypoglycaemia was significantly lower with CANA 100 and 300 mg versus GLIM (5.6%, 4.9%, and 34.2%, respectively; P <0.001). A greater proportion of patients achieved HbA1c <7.0% without documented hypoglycaemia with CANA 100 and 300 mg versus GLIM (49.8%, 56.5%, and 32.8%, respectively; equating to odds ratios [ORs; 95% confidence interval (CI)] vs GLIM of 2.1 [1.6, 2.8] and 2.9 [2.2, 3.8], respectively [Figure]). Similarly, a greater proportion of patients treated with CANA 100 and 300 mg versus GLIM also achieved HbA1c <6.5% without documented hypoglycaemia (23.0%, 28.5%, and 18.4%, respectively; with relative ORs [95% CI] vs GLIM of 1.3 [0.9, 1.8] and 1.8 [1.3, 2.5], respectively). The overall incidence of adverse events (AEs) was similar with CANA 100 and 300 mg and GLIM (64.4%, 68.5%, and 68.5%, respectively). Incidences of male and female genital mycotic infections and osmotic diuresis-related AEs were higher with CANA 100 and 300 mg versus GLIM, consistent with other Phase 3 studies of CANA.
Conclusion: More patients with T2DM achieved HbA1c <7.0% and <6.5% without hypoglycaemia with CANA versus GLIM over 52 weeks as add-on to MET.

Figure. Proportion of patients achieving glycaemic goals without hypoglycaemia.



Clinical Trial Registration Number: NCT00968812
Supported by: Janssen Scientific Affairs, LLC
Disclosure: **F. Vercruysse:** Employment/Consultancy; Janssen Research & Development.

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The synergistic effect of the combination of liraglutide and dapagliflozin on glycaemic control and cardiometabolic risk factors in type 2 diabetes patients

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Background and aims: Sodium glucose cotransporter-2 inhibitors (SGLT2) represent a novel therapeutic strategy for the treatment of type 2 diabetes. One of the most exciting aspects of SGLT2 inhibition is that its metabolic effects do not depend on an insulin-dependent mechanism. Pharmacologically, the combination of an incretin-mimetic and an SGLT2-receptor blocker should result in a more significant weight loss and a greater reduction in postprandial glucose and HbA1c. The aim of this study is to examine the effect of liraglutide (a glucagon-like peptide-1 agonist) and dapagliflozin (a SGLT-2 inhibitor) combination on glycemic control and the change in weight, blood pressure and lipid profile either as an initial combination or as an add-on treatment in type 2 diabetes mellitus (T2DM) patients treated with liraglutide without any further improvement.
Materials and methods: 54 T2DM patients aged 55.11±9.64 years, with diabetes duration of 7.50±4.04 years, were enrolled in the study. 33 (61.1%) patients received both liraglutide and dapagliflozin (Group A) along with their existing treatment (with a modification according to previous treatment) and 21 patients (38.9%) received dapagliflozin in a treatment including liragloutide (Group B). Follow-up was 6 months and changes in HbA1c, weight, systolic arterial pressure (SAP), diastolic arterial pressure (DAP), total cholesterol (TC), LDL-c, HDL-c and triglycerides (TG) were recorded.
Results: There was no difference in age between the two groups' patients (54.72±9.14 vs 55.71±10.58 years, p=0.603), while there was a difference ενώ in diabetes duration, with a smaller duration for Group A (6.04±3.15 vs 9.85 ± 4.22 years, p=0.001). HbA1c at the starting point was 8.58±1.41% with Group A showing a higher initial HbA1c compared to Group B (8.68±1.39 vs 8.15±1.31, p=0.027). 77.8% of the patients were treated with metformin and 50% were under insulin treatment before the study while 33% remained on insulin after the end of the follow up period. A significant improvement of HbA1c was observed in both groups with the decrease in Group A showing higher (1.96±0.82 vs 1.40±0.68, p=0.033). Decrease of weight was also higher for Group A (6.68 ±2.59 vs 4.55±2.06, p=0.046). There was a significant decrease of SAP (10.36±4.81 vs 7.62±3.32 mmHg, p=0.211), as well as DAP (5.57±2.11 vs 4.02±1.96, p=0.287) for both groups. Both groups showed an increase of TC with Group A showing the lowest (10.66 ±2.32 vs 16.12±3.61, p=0.033). LDL-c showed a decrease in GroupA and an increase in Group B (-10.8±3.98 vs 8.56±4.12, p=0.003) while there was a higher increase of HDL-c in Group A (4.96±0.82 vs 2.44 ±1.07, p=0.188) at the end of the follow up period.
Conclusion: The combination of liraglutide and dapagliflozine leads to a significant decrease of HbA1c, body weight and blood arterial pressure. There is a positive effect of this combination on HDL-c and a negative effect on total cholesterol while there is a significant decrease of LDL-c with the silmutanous initiation of liraglutide and dapagliflozine and an increase of LDL-c with their sequential initiation. The effect on glycemic control and cardiovascular risk factors is higher with the simultaneous initiation of liraglutide and dapagliflozine which seem to synergize exceptionally.
Disclosure: **S. Pappas:** None.

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Canagliflozin provides greater improvement in risk factors of metabolic syndrome (MetS) versus glimepiride in patients with type 2 diabetes and MetS on background metformin

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Background and aims: MetS refers to a collection of risk factors associated with the development of cardiovascular disease and type 2 diabetes mellitus (T2DM). Canagliflozin (CANA), an SGLT2 inhibitor, increases urinary glucose excretion, leading to decreased plasma glucose levels and a net caloric loss. In patients with T2DM on background metformin (MET), CANA improved glycaemic control and reduced body weight and blood pressure (BP) compared with glimepiride (GLIM) over 52 weeks; this post hoc analysis assessed the effects of CANA versus GLIM on the components of MetS in patients with T2DM and MetS.
Materials and methods: In this randomised, double-blind study, patients with T2DM (N = 1,450; mean age, 56.2 y; HbA1c, 7.8%; body mass index [BMI], 31.0 kg/m^2) received CANA 100 or 300 mg or GLIM as add-on to MET over 52 weeks. In addition to T2DM, MetS was diagnosed if patients met ≥2 of the following criteria: triglycerides ≥1.7 mmol/L; high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/L (men), <1.3 mmol/L (women); waist circumference ≥102 cm (non-Asian men), ≥88 cm (non-Asian women), >90 cm (Asian men), >80 cm (Asian women); diagnosis of hypertension or BP-related criteria (systolic BP [SBP] ≥130 mmHg or diastolic BP [DBP] ≥85 mmHg). Changes from baseline in HbA1c, fasting plasma glucose (FPG), BP, waist circumference, body weight, BMI, and lipids were evaluated at Week 52.
Results: At baseline, 80.6% of patients with T2DM (n = 1,169) met the criteria for MetS; proportions were similar across treatment groups. Among patients with data available to assess all MetS criteria (n = 1,160), 39.7%, 33.7%, and 17.2% of patients met 3, 4, or 5 MetS criteria, respectively. Of those with MetS at baseline, 1,132 patients had data available to assess MetS criteria at Week 52; the proportion

of patients with MetS was lower in the CANA 100 and 300 mg groups (86.7% and 85.8%) compared with GLIM (92.7%). CANA reduced HbA1c more (300 mg) and similarly (100 mg) compared with GLIM (Table). Relative to GLIM, CANA 100 and 300 mg provided reductions in FPG, SBP, DBP, waist circumference, body weight, and BMI. CANA showed increases in low-density lipoprotein cholesterol (LDL-C, 300 mg only) and HDL-C (both doses) versus GLIM. Relative to GLIM, reductions in triglycerides were greater with CANA 100 mg and similar with CANA 300 mg. CANA was generally well tolerated.

Conclusion: CANA improved all components of MetS compared with GLIM over 52 weeks in patients with T2DM and MetS on background MET.

Table. Changes From Baseline in MetS Risk Factors at Week 52 (mITT, LOCF)*

Parameter	CANA 100 mg (n = 394)	CANA 300 mg (n = 396)	GLIM (n = 379)	Parameter	CANA 100 mg (n = 394)	CANA 300 mg (n = 396)	GLIM (n = 379)
HbA1c change, %	−0.84 (0.04)	−0.92 (0.04)	−0.80 (0.04)	Waist circumference change, cm	−3.2 (0.3)	−3.5 (0.3)	−0.2 (0.3)
Difference vs GLIM	−0.04 (−0.15, 0.07)	−0.13 (−0.24, −0.02)		*Difference vs GLIM*	−3.0 (−3.7, −2.2)	−3.3 (−4.0, −2.6)	
FPG change, mmol/L	−1.4 (0.1)	−1.6 (0.1)	−1.0 (0.1)	BMI change, kg/m²	−1.4 (0.1)	−1.5 (0.1)	0.3 (0.1)
Difference vs GLIM	−0.4 (−0.7, −0.1)	−0.5 (−0.8, −0.3)		*Difference vs GLIM*	−1.6 (−1.8, −1.4)	−1.8 (−1.9, −1.6)	
SBP change, mmHg	−3.9 (0.7)	−5.4 (0.6)	−0.5 (0.7)	LDL-C change, mmol/L	0.10 (0.04)	0.25 (0.04)	0.03 (0.04)
Difference vs GLIM	−3.4 (−5.0, −1.8)	−4.8 (−6.4, −3.2)		*Difference vs GLIM*	0.07 (−0.03, 0.17)	0.22 (0.12, 0.32)	
DBP change, mmHg	−1.8 (0.4)	−2.6 (0.4)	−0.2 (0.4)	HDL-C change, mmol/L	0.08 (0.01)	0.10 (0.01)	0.00 (0.01)
Difference vs GLIM	−1.7 (−2.7, −0.6)	−2.4 (−3.5, −1.4)		*Difference vs GLIM*	0.08 (0.05, 0.11)	0.10 (0.08, 0.13)	
Body weight change, kg	−3.8 (0.2)	−4.1 (0.2)	0.7 (0.2)	Triglycerides change, mmol/L	−0.26 (0.06)	−0.16 (0.06)	−0.04 (0.06)
Difference vs GLIM	−4.5 (−5.0, −4.0)	−4.8 (−5.4, −4.3)		*Difference vs GLIM*	−0.22 (−0.38, −0.06)	−0.12 (−0.28, 0.04)	

mITT, modified intent-to-treat; LOCF, last observation carried forward; LS, least squares; SE, standard error; CI, confidence interval.
*Data are LS mean (SE) change from baseline and GLIM-subtracted LS mean difference (95% CI).

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Disclosure: **M. Desai:** Employment/Consultancy; Janssen Research & Development, LLC.

PS 063 Novel agents

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Imeglimin improves vascular dysfunction in type 2 diabetes animal models

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Background and aims: Cardiovascular diseases are major complications of Type 2 Diabetes and new strategies to prevent and treat them are needed. Endothelial dysfunction contributes to the pathogenesis and clinical expression of atherosclerosis in Type 2 diabetic patients.

Materials and methods: 2 days post-streptozotocin (STZ) injection (200 mg/kg ip), diabetic rats were treated with Imeglimin (75, 150, 300mg/kg bid orally) during 1 week. Pressure-induced vasodilatation (PIV) was assessed by the measure of skin blood flow in response to local pressure using Laser Doppler Flowmetry (LDF). Endothelium-dependent response was assessed by acethylcholine (Ach) iontophoretic delivery. Aortic rings were isolated from diabetic db/db mice in order to assess Imeglimin effect on vascular reactivity using phenylephrine (PE)-induced contraction. Imeglimin (250µM and 1mM) and vehicle were added to the organ bath for 30 minutes and then incremental concentration from 1nM to 10µM of PE were added to induce contraction.

Results: Endothelium-dependent vasodilatation is reduced in diabetic control STZ rats compared to non-diabetic control rats (% Ach/baseline: 25±4% vs 61±15%, $p<0.05$). Imeglimin 150 mg/kg bid normalizes Ach-dependent vasodilatation, reaching almost the level of non-diabetic animals and was significantly higher than in the diabetic group (50±7% imeglimin 150mg/kg vs 25±4% control STZ; $p<0.05$). Same effects were measured in rats treated with 300mg/kg bid, whereas no effect was measured at 75mg/kg bid. The PIV response was reduced in diabetic rats compared to non-diabetic animals (% PIV/baseline: -8±5% vs 42±8% respectively, $p<0.01$). Imeglimin 150mg/kg (% PIV/baseline: 60±9%) and 300mg/kg (% PIV/baseline: 35±7%) restored the PIV response to the level of control non-diabetic rats. In aortic rings isolated from diabetic db/db mice cumulative concentrations of PE caused a concentration-dependent contraction. This vasoconstrictor response was significantly potentiated in diabetic mice compared to control mice with a 1.4 fold increase in maximal effect and an exacerbated sensitivity.. Imeglimin reduced significantly aortic rings sensitivity to PE in a dose-dependent manner. More remarkably, 1mM Imeglimin reduced the enhanced vascular reactivity to PE in diabetic mice to the level obtained in non-diabetic mice.

Conclusion: These data show that Imeglimin prevents cutaneous microcirculation alterations in diabetic mice partially induced by a restoration of the endothelium-dependent vasodilatation. Moreover, Imeglimin reduced PE-induced contraction and PE effectiveness which are highly increased in diabetic mice. We conclude that Imeglimin -through these improvements of vascular reactivity in both small and large arteries- could provide a protective effect on micro and macrovascular defects induced by diabetes.

Disclosure: **P. Fouqueray:** Employment/Consultancy; employment.

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Abscisic acid improves glucose tolerance in rodents and in humans by increasing muscle glucose uptake

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Background and aims: Abscisic acid (ABA) is a plant hormone also present in animals. ABA contributes to the regulation of glycemia in mammals: i) nanomolar ABA stimulates GLUT4-mediated glucose uptake by myoblasts and adipocytes in vitro; ii) plasma ABA increases in normal human subjects, but not in diabetic patients, after an oral glucose load (OGTT); iii) oral ABA at 1 μg/Kg body weight (BW) significantly lowers glycemia and insulinemia in rats undergoing an OGTT. We tested the effect of a nutraceutical containing ABA on glycemia and insulinemia in healthy volunteers consuming a standard meal. We also investigated the effect of chronic ABA treatment on blood glucose, lipids and body weight in mice fed a high-glucose (HG) or a high-fat (HF) diet. Finally, we measured the effect of a single oral ABA dose on muscle glucose uptake in rats by micro Positron Emission Tomography (μPET) analysis.
Materials and methods: After overnight fasting, ten healthy volunteers consumed a standard meal. Each subject volunteered in two experiments, one without (control), the other with a nutraceutical containing ABA at 1 μg/Kg BW, taken immediately before the meal (time zero). At time zero and after 30, 60 and 120 min, blood samples were taken for measurement of plasma glucose and insulin. Male CD1 or C57BL/6 mice (8 per group) were fed a HG or a HF diet, respectively, for 16 weeks, without (control) or with 1 μg/Kg BW of ABA, administered in the drinking water. At the end of treatment, blood glucose, triglycerides, glycated hemoglobin (HbA1c) and body weight were measured and an OGTT was performed. Male Wistar rats (8 per group), fasted for 14 h, underwent an OGTT without (control) or with ABA at 1 μg/Kg BW. Immediately after gavage, rats were anesthetized, injected i.v. with 37 MBq [18F]-deoxyglucose (FDG) and placed onto the bed of a μPET scanner.
Results: 1. In healthy human volunteers, intake of a nutraceutical containing ABA at a dose of 1 μg/Kg BW before a standard meal significantly reduced both the glycemia and insulinemia profiles. 2. In both HG- and HF- fed mice, ABA treatment significantly reduced HbA1c, fasting glycemia and lipidemia, the glycemia profile during an OGTT and BW gain compared to untreated controls. 3. Oral ABA at 1 μg/Kg BW reduced the glycemia and insulinemia profiles during the OGTT and significantly increased FDG blood clearance and muscle and brown fat uptake.
Conclusion: A single oral dose of ABA at 1 μg/Kg BW improves glucose tolerance in healthy humans fed a standard meal. Chronic oral ABA administration reduces glycemia, lipidemia and BW gain in mice fed a HG or a HF diet. μPET imaging reveals a significant increase of FDG blood clearance and muscle and brown fat FDG uptake in rats: stimulation of tissue glucose uptake is likely the reason for the reduced insulinemia observed in ABA-treated rats and humans. ABA administration could be a promising new intervention to improve glucose tolerance sparing insulin by stimulating tissue glucose uptake, which is usually compromised in pre-diabetic or diabetic subjects.
Supported by: Nutravis S.r.l.
Disclosure: **M. Magnone:** None.

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Esculentin-2CHa-GA30 and its substituted analogues: mechanisms of stimulatory action on pancreatic beta cells and acute anti-hyperglycaemic activity in vivo

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Background and aims: We recently reported antidiabetic potential of esculentin-2CHa (GFSSIFRGVAKFASKGLGKDLAKLGVDLVAC KISKQC) a natural peptide first isolated from the norepinephrine-stimulated skin secretions of the Chiricahua leopard frog Lithobates chiricahuensis (Ranidae). This peptide has relatively complex structure/chemistry and we now report on the insulin-releasing effects, cellular mechanisms of action and antihyperglycaemic activity of a series of 10 bioactive novel analogues lacking the cyclic C-terminal domain (CKISKQC), called esculentin-2CHa-GA30.
Materials and methods: Analogues of esculentin-2CHa-GA30 were designed for ease of synthesis, plasma enzyme resistance and increased biological activity. Effects on insulin release were assessed using clonal insulin-releasing BRIN BD11 cells, human 1.1B4 cells and isolated mouse islets. Effects on membrane potential, intracellular Ca2+ and cAMP levels were determined using commercially available kits. K-ATP currents were assessed using whole-cell mode of the patch clamp technique. Acute effects on glucose tolerance (18 mmol/kg) were investigated using fasted NIH Swiss mice.
Results: D-amino acid substitutions at positions 7 (Arg), 15 (Lys) and 23 (Lys) and fatty acid (L-octanoate) attachment to Lys at position 15 of esculentin-2Cha-GA30 conveyed resistance to plasma enzyme degradation whilst preserving 1.4-3.6 fold in vitro insulin-releasing activity at concentrations of 1 x 10-8 to 10-6M ($P<0.001$). Analogues [D-Arg7, D-Lys15,D-Lys23]-esculentin-2CHa-(GA30) and Lys15-octanoate-esculentin-2CHa-(GA30) (Peptides 7 and 10 respectively) which exhibited the most promising biological profiles were selected for further analysis. These peptides stimulated glucose-dependent insulin release from mouse islets ($P<0.01$) and stimulated insulin secretion by 1.5-3.5 fold ($P<0.001$) from human 1.1B4 cells at concentrations as low as 1 x 10-11M . Using chemical inhibitors of adenylate cyclase (30 uM NKY80), protein kinase C (10 nM PMA) or phospholipase C (5 uM U73122X) pathways, involvement of PLC/PKC mediated insulin secretion was confirmed in BRIN BD11 cells with similar action to that of CCK-8. Diazoxide (0.3mM) and verapamil (50uM) exerted small inhibitory effect on insulin secretion provoked by esculentin-2Cha-GA30 analogues ($P<0.05$) suggesting some action also on K+ and Ca2+ channels. Consistent with this, the analogues prompted weak plasma membrane depolarisation ($P<0.05$) and small increase of intracellular Ca2+ ($P<0.01$). Patch clamp experiments indicated lack of appreciable effect on K-ATP channels. Evaluation of cellular location with fluorescent-tagged esculentin-2Cha-GA30 indicated membrane action suggesting that the weak depolarisation action may be due to cationic nature of the peptides tested. Acute administration of either analogue to normal mice improved glucose tolerance ($P< 0.01$) and enhanced insulin release ($P< 0.05$) similar to that observed with equal dose of GLP-1 (75 nmol/kg).
Conclusion: These data suggest that multi-acting analogues of esculentin-2CHa-GA30 may prove useful for promotion of glycaemic control in obesity-diabetes.
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Disclosure: **C.R. Moffett:** None.

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Effect of oxymatrine on insulin resistance and lipid metabolism in HepG2 cells exposed to palmitic acid

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Background and aims: Insulin resistance(IR) is a common pathophysiological state associated with obesity, type 2 diabetes mellitus(T2DM), and non-alcoholic fatty liver disease(NFALD). Oxymatrine, a monomer component extracted from Sophora flavescens Ait, is reported to have many pharmacological effects. Recent studies have reported that Oxymatrine combined with Metformin could dramatically attenuated hepatic lipid accumulation and enhance insulin sensitivity in patients with NAFLD. However, it remains unclear about the underlying mechanism of these effects. The aim of this study was to explore the effect of Oxymatrine on IR and fat accumulation in HepG2 cells exposed to Palmitic acid (Pa).
Materials and methods: HepG2 cells were cultured in medium containing 0.25 mmol/L PA to induce insulin resistance. HepG2 cells were randomLy divided into two groups, normal-medium group and Pa-medium group, respectively cultured for 48 hours. After successful modeling, the PA-medium group were randomly subdivided into 3 groups: Pa-medium group (Pa) (0.25 mmol/L), Metformin-medium group (Met) (0.2 mg/mL), and Oxymatrine-medium group (Omt) (0.08 mg/mL). After 48 hours of drug intervention, the cells were collected respectively. Triglyceride content and the related markers of oxygen stress were measured by kits. Reactive oxygen radicals (ROS) were detected by 2, 7-dichlorofuorescin diacetate (DCFH-DA) staining. proteins and mRNA had been isolated to evaluate the expression of proteins and genes involved in insulin signal transduction and lipid metabolism, respectively by Western blot and real time PCR.
Results: The in vitro model of IR in HepG2 cells induced by Pa was successfully established, measured by the increased level of glucose concentration and decreased insulin sensitivity compared with N group($P<0.05$). Gene expression analysis revealed a significantly decreased of key markers in insulin signal pathway (INSR, IRS-1, AKT and GLUT4), while the intervention of Oxymatrine and Metformin increased the mRNA expression of these key markers($P<0.05$). Meanwhile, the ratio of p-Akt/t-Akt and GLUT4/β-actin in Pa group showed a significant decrease compared with N group($P<0.05$), while the ratio of p-IRS-1/IRS-1 increased. However, the administration of Oxymatrine and Metformin respectively increased the ratio of p-Akt/t-Akt and GLUT4/β-actin, and decreased the ratio of p-IRS-1/IRS-1($P<0.05$). On the other hand, Pa-induced accumulation of lipid in HepG2 cells was significantly alleviated in drug intervention groups. Compared with Pa group, the mRNA and protein expression of CD36 decreased significantly after Metformin and Oxymatrine administration, while the mRNA level of CPT-1 increased significantly($P<0.05$). Furthermore, there was an obvious increase in the generation of ROS and MDA in HepG2 cells exposed to Pa, as well as an decrease in the activities of antioxidative enzymes such as GSH-Px and T-SOD($P<0.05$). After drugs intervention, the generation of ROS and MDA decreased dramatically compared with Pa group, while the activities of GSH-Px and T-SOD increased($P<0.05$).
Conclusion: Oxymatrine may increase insulin sensitivity and lipid metabolism through improving oxygen stress state in HepG2 cells exposed to palmitic acid. Meanwhile it may ameliorate the lipid accumulation directly through inhibiting fatty acid uptake and increasing β-oxydation.
Disclosure: **W.J. Fei:** None.

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Emodin, a compound with acclaimed antidiabetic potential, deteriorates glucose tolerance in obese mice

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Background and aims: Emodin is found in herbal remedies used in Traditional Chinese Medicine. Alongside its undisputed laxative action, emodin has been reported to lower blood glucose in hyperglycaemic rodents. Although purely experimental, these results are heralded in non-scientific (online-) sources to promote sales of emodin-containing herbal products as a natural cure for diabetes. While emodin-induced glucose lowering has been attributed to a surprising multitude of molecular mechanisms (activation of PPARγ, inhibition of 11β-HSD, activation of AMPK, etc.), little effort has been made to sort out, whether emodin merely acts via loss of appetite and body weight.
Materials and methods: Mice were rendered obese and glucose intolerant by high fat diet and treated with emodin, which was given for 7 weeks as a food admixture of 2 or 5 g/kg (daily uptake was as in preceding studies that had reported anti-hyperglycaemic action). Comparison was made to control mice fed ad libitum as well as to mice weight-matched to the emodin-treated by restricted feeding. In addition, acute effects of single emodin doses were studied in mice and rats.
Results: The effects of emodin were dose-dependent, results given below refer only to the higher dose. Emodin blunted food intake (-20%) and reduced body weight (-14%). Since the same decrease in food intake without concomitant emodin treatement caused comparable weight reduction, emodin-induced weight loss could be explained entirely by reduced calorie consumption without changes in energy expenditure or food efficiency (weight change, g: fed ad libitum, +5.5±0.5; emodin, -2.0±0.6; weight-matched, -2.2±0.5 g; $p<0.001$ each vs. ad libitum). As described by others, emodin ameliorated hyperglycaemia versus control mice fed ad libitum, but comparison to weight-matched controls unmasked deterioration rather than improvement of basal blood glucose (mmol/l: fed ad libitum, 9.5±0.4; emodin, 7.2±0.4; weight-matched, 6.1±0.3; $p=0.03$ for emodin vs. weight-matched) and glucose tolerance (AUC, mol/l*min: fed ad libitum, 2.01±0.08; emodin, 1.89±0.07; weight-matched, 1.65 ±0.05; $p=0.01$ for emodin vs. weight-matched). Impaired glucose tolerance was associated with blunted insulin sensitivity (insulin tolerance test, decrease in blood glucose after 15 min, mmol/l: fed ad libitum, -2.37±0.33; emodin, -2.64±0.43; weight-matched, -3.88 ±0.34; $p<0.05$ each vs. weight-matched). A single oral dose of emodin did not affect glucose tolerance in mice, whereas i.v. injection in rats acutely blunted the rise of plasma insulin and deteriorated glucose tolerance.
Conclusion: We conclude that emodin´s anti-hyperglycaemic activity is merely the consequence of weight loss caused by a spoilt appetite, as it is known to accompany almost any type of sickness and discomfort in laboratory rodents. Our finding that emodin even impairs glucose homeostasis under weight-neutral conditions seriously compromises claims that have been raised about an antidiabetic potential of emodin and about molecular mechanisms mediating such action. The outcome illustrates the importance of an appropriate dose of professional scepticism in order to protect patients from potentially harmful misuse of medicinal products.
Disclosure: **S. Abu Eid:** None.

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Apabetalone (RVX-208) acts on epigenetics to lower Major Adverse Cardiovascular Events (MACE) in diabetes patients with atherosclerosis via microbiome activity

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Background and aims: RVX-208 selectively inhibits the second ligand domain in bromodomain extra-terminal (BET) proteins that are epigenetic readers of acetylated lysine marks on histone tails. In the SUSTAIN and ASSURE (n=499) trials, patients (n=192) with diabetes mellitus (DM) and cardiovascular disease (CVD) given 200 mg/d of RVX-208 had a 77% relative risk reduction (RRR) in MACE. This marked RRR likely arises from RVX-208's additive effects that include attenuation of known contributors to CVD such as the complement, inflammatory and coagulation pathways plus raising high density lipoproteins (HDL). In studies below, we tested a hypothesis that trimethylamine oxide (TMAO) a circulating metabolite connected to the microbiome may heighten complement activity. Next we asked how RVX-208 may beneficially affect the complement cascade (CC) in possibly underpinning the lower MACE observed in recent trials.
Materials and methods: Plasma from above human trials were analyzed. Gene expression data from RVX-208 exposed Huh-7 hepatoma cells and primary human hepatocytes.
Results: Heightened CC activity in CVD may perhaps be related to, a metabolite from dietary meat consumption, TMAO because of its ability to predict CVD risks. Human Huh-7 hepatoma cells (HC) grown in high glucose (25 mM) exposed to varying doses (0.1-100 uM) of TMAO revealed a dose and time dependent rise in mRNA encoding components of the CC; mannose-binding lectin (MBL) and C3 ranging from 1.2 to 1.5-fold. In high glucose and TMAO treated HC, adding 25 uM RVX-208 reduced levels of these mRNAs by >80%. Conversely, initial findings in HC grown exposed to euglycemia (5.6 mM glucose) TMAO failed to induce MBL or C3, but adding RVX-208 still suppressed both mRNAs by >80%. C5 another CC member was unlike MBL and C3 because TMAO did not induce C5 mRNA in HC exposed to hyperglycemia but RVX-208 also suppressed its expression. These findings point to the idea that TMAO enhances CC activity in hyperglycemia by inducing selected components of the CC but not all. In humans, plasma TMAO comes from flavin mono-oxygenase 3 (FMO3) activity on dietary components. To further explore the TMAO and CC link we had to use primary human hepatocytes (PHH) because FMO3 is not expressed in HC. Exposure of PHH to RVX-208 lowered FMO3 mRNA levels by 60%. Next we wondered whether these findings in vitro extended into humans by measuring CC proteins in plasma samples from our human trials. Consistent, in part with in vitro, MBL and C5 were significantly reduced by 15 and 12% respectively in plasma from patients given RVX-208 vs baseline in the ASSURE trial but C3 in the same samples did not change in a similar fashion.
Conclusion: HC grown in high glucose exposed to TMAO induces mRNAs encoding MBL and C3, two central components of the CC. Not only is TMAO induction of the mRNAs abrogated by apabetalone, it also suppresses by >80% levels of mRNAs encoding these CC. A separate effect of RVX-208 appears upstream of TMAO by suppressing FMO3 the enzyme that makes TMAO. Our findings suggest that RVX-208 has multiple ways of calming the CC at several points in liver cells exposed to hyperglycemia. Together our findings suggest that actions of RVX-208 may beneficially impact innate immunity by affecting CC in ways that could lead to lower MACE in patients with DM and CVD.
Clinical Trial Registration Number: NCT01058018
Disclosure: **N.C. Wong:** Employment/Consultancy; Employee of Resverlogix Corp. Stock/Shareholding; Holder of shares and options in Resverlogix.

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L-Carnitine attenuates fibrosis and inflammation in C57BL/6 mice with dietary-induced steatohepatitis

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Background and aims: Nonalcoholic steatohepatitis (NASH) represents an advanced stage of fatty liver disease developed in the absence of alcohol abuse and its prevalence is increasing in western countries in parallel with Type 2 Diabetes and metabolic syndrome incidence. NASH is a strong marker of cardiovascular risk and in the last few decades it has become evident that there is a mutual interaction between heart and liver, influencing individual functions. In effect NASH, characterized by excess of intracellular fatty acids, severe inflammatory and fibrotic state, plays a critical role in two principal tissues: liver and heart. Several studies have examined the effectiveness of L-Carnitine (LC) in liver function and have recognized LC as a nutritional supplementation in cardiovascular disease. The present study was designed to investigate the effects of LC administration on liver and heart function and morphology in mice models of steatohepatitis, induced by a methionine-choline deficient diet (MCD).
Materials and methods: C57BL/6 mice male (n=18, age:12 weeks) were divided in 3 different groups: control mice (CONTR) were fed for 6 weeks with a normal diet while both MCD and LC groups received MCD diet. From the 4th week LC group received MCD diet enriched with 200mg/kg/die LC (drinking water). IPGTT tests were performed. After sacrifice, livers and hearts were excised, subjected to histopathology, gene and protein expression analysis.
Results: The results showed that there are no significant differences in body weight between MCD and LC mice groups while, as expected, a significant weight loss occurred in MCD and LC groups in respect with CONTR. Furthermore, insulin sensitivity (IPGTT test) and GLUT4 protein content were unchanged among groups. Tissues fat deposition, inflammatory infiltration and fibrosis were, then, investigated. Different histopathology staining methods showed that LC significantly reduces fat accumulation. Immunofluorescence assay revealed an important downregulation of the two markers of tissue fibrosis: alpha smooth muscle actin protein level and Kruppel-like factor (KLF15). To clarify LC cellular mechanisms in counteracting inflammatory liver state, we evaluated NFkB pathway and PPARγ: LC markedly attenuated MCD-induced NFkB increase and restored PPARγ protein levels. In addition, LC significantly stimulated Ca2+/calmodulin-dependent kinases II activity, suggesting that LC could ameliorate mitochondrial function. Noteworthy, we observed LC actived ERKs pathway, which is correlated with a reduction of oxidative stress and hepatotoxicity. In parallel, to investigate LC anti-inflammatory and fibrotic role in heart, we studied STAT-3 activation: LC significantly decreased STAT-3 activation observed in MCD hearts. This data is in accordance with the reduction of Calcium signaling in LC hearts compared to MCD.
Conclusion: In conclusion, LC appears to exert different beneficial actions on the liver-heart axis counteracting steatohepatitis. LC supplementation may be used in order to prevent disease progression in these analyzed tissues, inhibiting the inflammation and fibrotic pathways.
Disclosure: **I. Terruzzi:** None.

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PXL770, a novel direct AMPK activator, inhibits hepatic de novo lipogenesis, for the treatment of metabolic disorders

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Background and aims: PXL770 directly activates adenosine monophosphate-activated protein kinase (AMPK), an enzyme that regulates cellular energy metabolism by affecting energy-consuming pathways such as De Novo Lipogenesis (DNL) including fatty acid and cholesterol synthesis as well as energy-producing pathways such as lipid oxidation and glucose uptake. Accordingly, PXL770 could play an important role in the management of diabetes and hyperlipidemia.
Materials and methods: The inhibitory potency of PXL770 on DNL was first evaluated in primary mouse and human hepatocytes. DNL was monitored by [1-14C] acetate incorporation into lipids to probe fatty acid synthesis. The AMPK dependency of this effect was then assessed in hepatocytes from liver AMPKα1α2-deficient mice. Finally, the effect of PXL770 on DNL was evaluated in vivo in nine week-old C57BL/6J male mice. Hepatic DNL was stimulated by fasting (24h) followed by high-carbohydrate diet refeeding (12h) manipulation. Mice were treated orally with vehicle, 35mg/kg or 75 mg/kg PXL770 3 times during the fasting/refeeding manipulation. The hepatic rate of lipid synthesis was assessed by the rate of in vivo incorporation of 3H2O into lipids in liver.
Results: PXL770 dose-dependently decreases liver DNL in human and mouse primary hepatocytes with similar potency, showing IC50 of respectively 3 and 2.8 µM. PXL770 inhibitory effect was totally blunted in AMPKα1α2-null hepatocytes, demonstrating an AMPK-dependent action of PXL770 on lipogenesis. Comparison between vehicle-treated 24h-fasted and vehicle-treated 12h-refed control groups showed a 15-fold induction of 3H2O incorporation in liver of refed mice indicating a strong stimulation of hepatic DNL by the fasting/refeeding manipulation. Importantly, a dose-dependent reduction of incorporation of 3H2O into lipids in liver of refed mice treated orally with PXL770 was observed, in comparison to vehicle-treated refed mice (-45%, P=0.0022 and -71%, P=0.000079 after PXL770 35mg/kg and 75mg/kg respectively).
Conclusion: These results show that PXL770 is a potent inhibitor of hepatic DNL as demonstrated by the strong reduction of 3H2O incorporation in lipids in the liver. The results are consistent with the decrease in fatty acid synthesis in response to PXL770 measured previously in vitro in primary hepatocytes and confirm the role of AMPK in this metabolic pathway. These data provide evidence for the therapeutic potential of PXL770 in hepatic lipid metabolism disorders. Treatment with PXL770 may contribute to improve the liver lipid content of patients suffering from hepatic steatosis and may potentially manage dyslipidemia and improve insulin sensitivity in patients with metabolic syndrome.
Disclosure: **S. Bozec:** Employment/Consultancy; Employment.

PS 064 SGLT-2 inhibitors: clinical trials

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Achieving composite endpoint of HbA_{1c}, body weight, and systolic blood pressure reduction with canagliflozin in patients with type 2 diabetes

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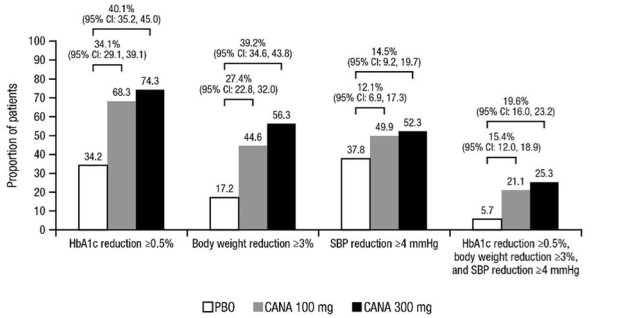
Background and aims: In addition to achieving glycaemic control, weight loss and blood pressure (BP) reduction are important components of type 2 diabetes mellitus (T2DM) management as many patients with T2DM are overweight/obese and/or have hypertension. Canagliflozin (CANA), an SGLT2 inhibitor, has demonstrated improvements in HbA1c, body weight, and systolic BP (SBP) across a broad range of patients with T2DM. The aim of this analysis was to evaluate the proportion of patients with T2DM achieving the composite endpoint of HbA1c reduction ≥0.5%, body weight reduction ≥3%, and SBP reduction ≥4 mmHg with CANA versus placebo (PBO).
Materials and methods: This post hoc analysis used pooled data from four randomised, double-blind, PBO-controlled, Phase 3 studies. Patients (N = 2,313; mean age, 56 y; HbA1c, 8.0%; body weight, 89 kg; SBP, 128 mmHg) received CANA 100 or 300 mg or PBO for 26 weeks.
Results: At baseline, similar proportions of patients met treatment goals across groups. At Week 26, a greater proportion of patients achieved individual HbA1c, body weight, and SBP targets with CANA versus PBO (Figure). A higher proportion of patients treated with CANA 100 or 300 mg versus PBO (21.1%, 25.3%, and 5.7%, respectively) achieved the composite endpoint of HbA1c reduction ≥0.5%, body weight reduction ≥3%, and SBP reduction ≥4 mmHg at Week 26. CANA was generally well tolerated, with a safety profile similar to that seen in other Phase 3 CANA studies.
Conclusion: Patients with T2DM were more likely to achieve clinically important reductions in HbA1c, body weight, and SBP with CANA versus PBO.

Figure. Proportion of patients achieving composite endpoint of HbA1c, body weight, and SBP reduction at Week 26 (LOCF).



LOCF, last observation carried forward; CI, confidence interval.

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Supported by: Janssen Scientific Affairs, LLC
Disclosure: **K. Merton:** Employment/Consultancy; Janssen Scientific Affairs, LLC.

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Effects of dapagliflozin on weight loss and metabolic changes in type 2 diabetes patients fasting in the month of Ramadan

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Background and aims: Fasting in the month of Ramadan can result in changes in body weight and metabolic profiles. These can be attributed to dietary factors, physical inactivity, medications and sleeping pattern. Dapagliflozin promotes excretion of excess glucose in the urine, therefore altering the body composition through caloric loss. The aim of this study was to assess the weight loss and metabolic changes associated with dapagliflozin in comparison to sulphonylurea in T2DM patients who fast during Ramadan.
Materials and methods: In this 12-week, randomised, open-label, two-arm parallel group study, 110 patients who were receiving sulphonylurea (SU) and metformin were randomised to either receive Dapagliflozin 10mg daily (n=58) or to continue receiving sulphonylurea (n=52). The primary outcome was to assess the mean change in body weight and mean change in metabolic parameters at week 6 (Ramadan fasting month) and week 12. Continuous variable were analysed using repeated measures ANOVA in general linear model (RM GLM). Data were analysed using IBM SPSS Statistics Version 22.
Results: Both groups showed a reduction in mean change in body weight at week 6, with greater reduction seen in dapagliflozin group compared to sulphonylurea (-3.9 ± 1.65 kg vs -1.8 ± 2.20 kg; p=0.004). There was a mild increase in mean change in weight at week 12 (0.43 vs 1.38 kg) in dapagliflozin and sulphonylurea group respectively. At the end of the study period, dapagliflozin continued to maintain a greater weight reduction of -3.47 ± 2.23 kg when compared to sulphonylurea (p<0.05). Adjustment of mean change in body weight to HbA1c, fructosamine, fasting plasma glucose and gender did not show any significant correlation. There was a significant reduction in mean change in systolic blood pressure (-8.7 ± 20.29mmhg vs -8.6 ± 16.91 mmhg) and diastolic blood pressure (-3.9 ± 11.84 mmhg vs -6.4 ± 8.25 mmhg) during the 12-week study period but it did not differ statistically dapagliflozin and sulphonylurea group. There were no significant differences in HbA1c, fasting plasma glucose, and fructosamine levels. A higher mean change in high density lipoprotein-cholesterol (HDL-C) seen in dapagliflozin compared to sulphonylurea group (0.05 ± 0.236 mmol/L vs -0.10 ± 0.158mmol/L; p=0.003). Although there were changes seen in the low density lipoprotein-cholesterol (LDL-C), total cholesterol, uric acid level, serum sodium and serum creatinine at week 6 and week 12, it did not differ between groups.
Conclusion: This study showed that patients on dapagliflozin attained a significant weight reduction of 3.47kg at week 12, an effect which was only seen at week 24 in previous dapagliflozin study. The initial weight loss at week 6 may have resulted from fluid loss associated with osmotic diuresis and accentuated by 13 hours of fasting and fluid restrictions, however subsequent weight loss is likely due to caloric loss from glycosuria. Whether fasting in Ramadan can be a good stepping stone for a boost to weight loss program needs to be further evaluated. In summary, the use of dapagliflozin resulted in a greater weight reduction when compared to sulphonylurea in T2DM patients who fast during Ramadan.
Supported by: Astra Zeneca
Disclosure: **W. Wan Seman:** None.

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Ertugliflozin effectively improves glycaemic control as monotherapy in patients with type 2 diabetes: the VERTIS MONO trial

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Background and aims: Ertugliflozin (ERTU) is an oral SGLT2 inhibitor in development for treatment of patients with T2DM. This presents results from the efficacy endpoints at Week 26 in this ongoing Phase 3, 52-week, randomized, double-blind, placebo-controlled trial, assessing the efficacy and safety of ERTU compared with placebo (PBO) in patients with T2DM and inadequate glycemic control (A1C 7.0-10.5%) on diet and exercise.
Materials and methods: Adult patients (age 56.4 ± 11.0 yrs; baseline A1C 8.21 ± 0.98%, and duration of T2DM 5.0 ± 5.1 years, mean ± SD) were randomized to receive PBO, ERTU 5 mg or ERTU 15 mg once daily in a 1:1:1 ratio (N=461).
Results: At Week 26, patients randomized to ERTU 5 mg and ERTU 15 mg had significantly greater reductions in A1C, FPG, body weight, and 2-hour post-prandial glucose (PPG) and were significantly more likely to have an A1C <7.0% when compared to PBO (Table 1). The placebo-adjusted least-squares mean reduction in A1C was 0.99% and 1.16% for ERTU 5 mg and ERTU 15 mg, respectively. Compared with PBO, ERTU treatment was associated with a trend toward lower blood pressure. Overall AE rates were comparable between ERTU and PBO and the rate of serious AEs was low across groups. A higher incidence of genital mycotic infections in females was observed in patients taking ERTU 15 mg (22.6%) and ERTU 5 mg (16.4%) compared to PBO (7.0%); there was no increase in the incidence of urinary tract infections with either dose of ERTU relative to placebo.
Conclusion: ERTU improved glycemic control and was generally well tolerated as monotherapy in patients with T2DM.

Table 1

Endpoint	Analysis Metric[†]	Ertugliflozin 5 mg vs. Placebo		Ertugliflozin 15 mg vs. Placebo	
		Estimate (95% CI)	p-value	Estimate (95% CI)	p-value
A1C (%)	Diff	-0.99 (-1.22, -0.76)	<0.001	-1.16 (-1.39, -0.93)	<0.001
FPG (mmol/L)	Diff	-1.92 (-2.37, -1.46)	<0.001	-2.44 (-2.90, -1.98)	<0.001
Body weight (kg)	Diff	-1.76 (-2.57, -0.95)	<0.001	-2.16 (-2.98, -1.34)	<0.001
Proportion of patients with A1C<7%	OR	3.59 (1.85, 6.95)	<0.001	6.77 (3.46, 13.24)	<0.001
2-hour PPG (mmol/L)	Diff	-3.83 (-4.62, -3.04)	<0.001	-3.74 (-4.54, -2.94)	<0.001
SBP (mmHg)	Diff	-3.31 (-5.98, -0.65)	0.015*	-1.71 (-4.40, 0.98)	0.213
DBP (mmHg)	Diff	-1.80 (-3.51, -0.09)	0.039*	-0.37 (-2.09, 1.35)	0.669*

[†]Diff = Difference in least squares means based on a constrained longitudinal data analysis model; OR = adjusted odds ratio.
*Nominal p-values
All efficacy analyses are based on the full analysis set, excluding data after initiation of glycemic rescue therapy.

Clinical Trial Registration Number: NCT01958671
Supported by: Pfizer and Merck and Co.
Disclosure: **S. Terra:** Employment/Consultancy; Pfizer. Stock/Shareholding; Pfizer.

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Safety and efficacy of ertugliflozin plus sitagliptin versus either treatment alone in subjects with type 2 diabetes inadequately controlled with metformin: the VERTIS FACTORIAL trial

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Background and aims: Ertugliflozin (ERTU) is an oral sodium/glucose cotransporter 2 (SGLT2) inhibitor in development for treatment of T2DM. This study compared the efficacy and safety of co-administration of ERTU 5 mg or 15 mg plus sitagliptin (SITA) 100 mg compared with either treatment alone.
Materials and methods: In a double-blind Phase 3 trial, subjects (n=1233) with HbA1c 7.5-11.0% on stable metformin ≥1500 mg/day were randomised to 1 of 5 treatment groups (Table). ERTU+SITA combinations were compared with corresponding ERTU doses alone (5 or 15 mg) and SITA alone.
Results: Baseline characteristics were comparable among groups (overall mean age 55.1 years, HbA1c 8.6%). After 26 weeks, co-administration of ERTU+SITA was significantly more effective than ERTU or SITA alone in reducing HbA1c, fasting plasma glucose (FPG), and achieving HbA1c <7.0%, and significantly more effective in reducing body weight and systolic blood pressure (BP) vs SITA (Table). Incidence of adverse events (AEs) was similar across groups, except for higher rates of genital mycotic infections in groups receiving ERTU vs SITA alone (females, 4.9-7.6% vs 1.1%; males, 2.4-4.7% vs 0%, respectively). Urinary tract infection rates were higher with ERTU alone (but not ERTU+SITA) vs SITA alone (range: 3.2% [SITA] to 5.6% [ERTU 15 mg]). Symptomatic hypoglycaemia rates ranged from 2.4% (ERTU 5 mg) to 4.9% (ERTU 15 mg+SITA). Hypovolaemia AE rates were 1.6 and 0.8% in ERTU 5 and 15 mg groups, respectively, and 0% in all other groups.
Conclusion: Co-administration of ERTU+SITA provided more effective glycaemic control vs ERTU or SITA alone and was generally well-tolerated.

Table. Summary of key efficacy endpoints at Week 26

		ERTU 5 mg (n=250)	ERTU 15 mg (n=248)	SITA 100 mg (n=247)	ERTU 5 mg + SITA 100 mg (n=243)	ERTU 15 mg + SITA 100 mg (n=244)
Change from baseline, LS mean (95% CI)	HbA1c (%)	−1.0 (−1.1, −0.9)	−1.1 (−1.2, −1.0)	−1.1 (−1.2, −0.9)	−1.5 (−1.6, −1.4)†	−1.5 (−1.6, −1.4)†
	FPG (mg/dL)	−35.7 (−40.0, 31.4)	−36.9 (−41.2, −32.6)	−25.6 (−29.9, −21.2)	−44.0 (−48.3, −39.6)†	−48.7 (−53.0 −44.4)†
	FPG (mmol/L)	−2.0 (−2.2, −1.7)	−2.1 (−2.3, −1.8)	−1.4 (−1.7, −1.2)	−2.4 (−2.7, −2.2) †	−2.7 (−2.9, −2.5) †
	Body weight (kg)	−2.7 (−3.1, −2.3)	−3.7 (−4.2, −3.3)	−0.7 (−1.1, −0.2)	−2.5 (−3.0, −2.1)‡	−2.9 (−3.4, −2.5)‡
	Systolic BP (mmHg)	−3.9 (−5.3, −2.5)	−3.7 (−5.1, −2.3)	−0.7 (−2.1, 0.8)	−3.4 (−4.8, −2.0)‡	−3.7 (−5.1, −2.3)‡
HbA1c <7.0% at Week 26, n (%)		66 (26.4)	79 (31.9)	81 (32.8)	127 (52.3)§	120 (49.2)§

†P≤0.004 vs individual treatments; ‡P≤0.005 vs SITA;
§P<0.001 based on model-estimated odds ratios comparing ERTU+SITA vs individual treatments
A constrained longitudinal model was used for continuous endpoints and logistic regression for binary endpoints.

Clinical Trial Registration Number: NCT02099110
Supported by: Merck & Co, Inc. in collaboration with Pfizer
Disclosure: **R. Pratley:** Employment/Consultancy; AstraZeneca, Boehringer-Ingelheim, GlaxoSmithKline, Hanmi Pharmaceutical Co., Inc., Janssen Pharmaceuticals, Inc., Ligand Pharmaceuticals, Inc., Lilly, Merck & Co., Inc., Novo-Nordisk, Takeda. Grants; Lilly, Merck & Co., Inc., Sanofi-Aventis US, LLC, Novo-Nordisk, Takeda. Honorarium; Novo-Nordisk. Lecture/other fees; AstraZeneca, Novo-Nordisk.

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Defining the potential "real-world" impact of the EMPA-REG OUTCOME trial on improving cardiovascular outcomes: observations from the Diabetes Collaborative Registry (DCR)

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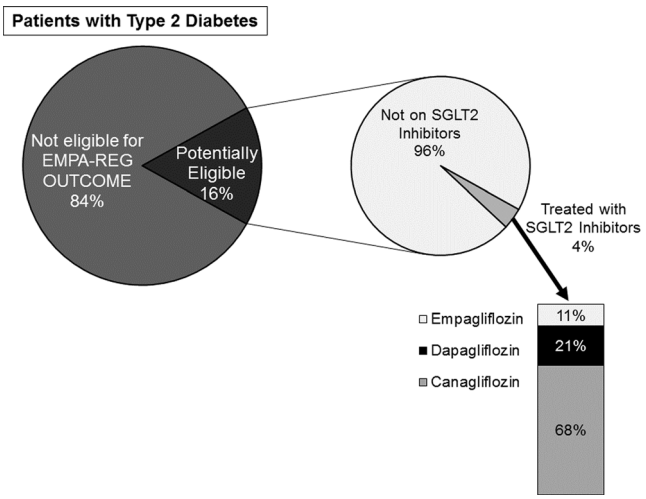
Background and aims: The EMPA-REG OUTCOME trial demonstrated beneficial cardiovascular (CV) effects of empagliflozin, a sodium-glucose co-transporter 2 inhibitor (SGLT2i) in patients with type 2 diabetes (T2D) and atherosclerotic CV disease (CVD). We estimated the current use and potential impact of empagliflozin, as well as any SGLT2i, in patients enrolled in the DCR.
Materials and methods: DCR is the first large-scale national outpatient registry of T2D patients recruited from cardiology, endocrinology, and primary care practices in the US and currently encompasses 299 practices and 4191 providers. Patients ≥18 years old with T2D, HbA1c 7-10%, and atherosclerotic CVD were considered potentially eligible for EMPA-REG OUTCOME.
Results: Among 979,175 patients in DCR, 284,632 had T2D and an HbA1c value. Of these, 45,948 (16.1%) had HbA1c 7-10% and atherosclerotic CVD. Mean age was 70 y, 62% were men, and mean HbA1c was 8.1%. Only 1788 of these patients (3.9%) were treated with an SGLT2i: canagliflozin 68%, dapagliflozin 21%, and empagliflozin 11%. (Figure). Patients treated vs. not treated with a SGLT2i were more likely to be younger (64 vs. 70 y), male (68 vs. 62%), have lower creatinine (1.1 vs. 1.3 mg/dl), and have less heart failure (22 vs. 35%; all p<0.001). Assuming similar risk reductions in this "real-world" patient population as observed in EMPA-REG OUTCOME, if all potentially eligible patients in DCR were treated with empagliflozin for 3 years, this may have prevented 1149 deaths, 1011 CV deaths, and 643 hospitalizations for heart failure.
Conclusion: In a large US-based outpatient registry of DM patients across the spectrum of primary and specialty care, we found that ~1 in 6 outpatients with T2D met the main eligibility criteria for EMPA-REG OUTCOME. In such patients, SGLT2i therapy is rarely used and tends to be prescribed in lower risk patients (younger, better kidney function, less heart failure). Expanded and better targeted use of empagliflozin (and, if they are shown to be equally effective, other SGLT2i's) in eligible patients, particularly those at highest risk for adverse CV outcomes, could significantly reduce CV morbidity/mortality. Longitudinal follow-up of the DCR cohort will provide the opportunity to assess adoption of SGLT2i's over time and potentially their real-world effectiveness in a large diabetes cohort.



Supported by: AstraZeneca and BI fund the DCR, but neither had any role in this analysis
Disclosure: **S.V. Arnold:** None.

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Effect of empagliflozin on anthropometry and indices of visceral and total adiposity in patients with type 2 diabetes and high cardiovascular risk: EMPA-REG OUTCOME

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Background and aims: Empagliflozin (EMPA) treatment significantly reduced cardiovascular (CV) events including CV death, hospitalisation for heart failure and all-cause mortality vs. placebo (PBO) in the EMPA-REG OUTCOME trial. Whether improvement in CV risk is related to treatment effects on total body and visceral adiposity (VA) is unknown. Therefore, we explored the effect of EMPA vs.placebo on anthropometry and validated surrogate indices of VA and total fat mass in EMPA-REG OUTCOME.
Materials and methods: Patients with type 2 diabetes and high CV risk were randomised to receive PBO, EMPA 10 mg, EMPA 25 mg in addition to standard of care. Changes from baseline to week 164 for body weight and validated indices of VA (waist circumference (WC), index of central obesity (ICO; ratio WC/height), and changes in estimated total body fat (eTBF) using the YMCA-formula ($eTBF_{men}$: 100*(-98.42 + [4.15*WC] -[0.082*weight])/weight; $eTBF_{women}$:100*(-76.76 + [4.15*WC] -[0.082*weight])/weight), were assessed between treatment and PBO groups using a mixed model repeated measures analysis.
Results: In total, 2333, 2345 and 2342 patients received PBO, EMPA 10 mg and EMPA 25 mg, respectively. Mean (SE) baseline weight for each group was 86.7 (0.4), 86.0 (0.4), 86.5 (0.4) kg. There were significantly greater reductions in body weight with EMPA treatment as compared with PBO: placebo-adjusted mean (95% CI) difference for EMPA 10 mg: -1.60 (-1.97,-1.23) and EMPA 25 mg: -1.98 (-2.34, -1.61), nominal p<0.0001 for both. Indices of VA and eTBF were also significantly reduced in both EMPA groups compared with placebo (Table).
Conclusion: These findings suggest that EMPA directly reduces total body and visceral adiposity in patients with type 2 diabetes and high CV risk. Further analyses are needed to determine the potential contribution of these changes to risk reduction in CV outcomes and all-cause mortality with EMPA.

Adiposity Parameter		Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
Waist circumference (cm)	N analysed at baseline/week 164	2259/1220	2272/1285	2273/1329
	Mean (SE) WC at baseline	105.0 (0.3)	104.8 (0.3)	104.8 (0.3)
	Placebo-adjusted mean (95% CI) difference from baseline at week 164		-1.5 (-1.9, -1.0)***	-1.6 (-2.0, -1.1)***
Index of central obesity	N analysed at baseline/week 164	2259/1220	2272/1285	2273/1329
	Mean (SE) ICO at baseline	0.627 (0.002)	0.627 (0.002)	0.625 (0.002)
	Placebo-adjusted mean (95% CI) difference from baseline at week 164		-0.008 (-0.011, -0.006)***	-0.009 (-0.012, -0.007)***
Estimated total body fat (%)	N analysed at baseline/week 164	2259/1217	2272/1282	2273/1327
	Mean (SE) eTBF at baseline	33.4 (0.2)	33.7 (0.2)	33.2 (0.2)
	Placebo-adjusted mean (95% CI) difference from baseline at week 164		-0.45 (-0.83, -0.07)*	-0.49 (-0.87, -0.12)*
Changes in markers of visceral and total adiposity with empagliflozin therapy in EMPA-REG OUTCOME. Nominal p-values for adjusted means based on mixed model repeated measures analysis in patients who received ≥1 dose of study drug. *p<0.05; **p<0.01; ***p<0.001 (all vs placebo).				

Clinical Trial Registration Number: NCT01131676
Supported by: BI/Eli Lilly
Disclosure: **I.J. Neeland:** None.

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Effect of baseline HbA_{1c}, renal function, age and glucose-lowering medication on hypoglycaemia in the EMPA-REG OUTCOME trial

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Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin (EMPA) given in addition to standard of care reduced the risk of cardiovascular (CV) death by 38%, hospitalisation for heart failure by 35% and all-cause mortality by 32% vs placebo (PBO) in patients with T2DM and high CV risk. Confirmed hypoglycaemic adverse events (AEs; plasma glucose ≤70 mg/dl and/or requiring assistance) were reported in similar proportions of patients on PBO, EMPA 10 mg and EMPA 25 mg (27.9%, 28.0% and 27.6%, respectively). We investigated hypoglycaemia in subgroups by baseline characteristics.
Materials and methods: Patients were randomised to receive PBO, EMPA 10 mg, or EMPA 25 mg. Background glucose-lowering therapy, including insulin dose, was to remain unchanged for 12 weeks then be adjusted according to local guidelines. Confirmed hypoglycaemic AEs that occurred during treatment or ≤7 days after last dose of study drug were analysed in subgroups by baseline HbA_{1c} (<7.5, 7.5 to ≤8.5, ≥8.5%), estimated glomerular filtration rate (eGFR [MDRD]; <45, 45 to <60, 60 to <90, ≥90 ml/min/$1.73m^2$), age (<50, 50 to <60, 65 to <75, ≥75 years) and background glucose-lowering medication (insulin, metformin, sulphonylurea [SU]).
Results: 2333, 2345 and 2342 patients were treated with PBO, EMPA 10 mg and EMPA 25 mg, respectively. The proportions of patients with confirmed hypoglycaemic AEs were similar between the PBO and EMPA groups in subgroups by baseline HbA_{1c}, eGFR and age, and increased with decreasing eGFR in all treatment groups (Table). Confirmed hypoglycaemic AEs were reported, respectively, in the PBO, EMPA 10 mg and EMPA 25 mg groups, in 42.6%, 43.6% and 41.4% of patients taking insulin at baseline vs 13.9%, 13.4% and 15.0% of patients not taking insulin; in 26.1%, 26.5% and 27.3% of patients taking metformin at baseline vs 32.9%, 32.1% and 28.4% of patients not taking metformin; and in 23.4%, 24.5% and 25.0% of patients taking SU at baseline vs 31.2%, 30.5% and 29.7% of patients not taking SU.
Conclusion: In the EMPA-REG OUTCOME trial in patients with T2DM and high CV risk, the proportions of patients with confirmed hypoglycaemic AEs were similar between the PBO and EMPA groups irrespective of baseline HbA_{1c}, eGFR, age, or the use of insulin, metformin, or SU at baseline.

n/N (%) with ≥1 confirmed hypoglycaemic AE		Placebo	Empagliflozin 10 mg	Empagliflozin 25 mg
Baseline HbA_{1c}	<7.5%	154/596 (25.8)	162/618 (26.2)	155/635 (24.4)
	7.5 to ≤8.5%	314/1090 (28.8)	313/1066 (29.4)	293/1051 (27.9)
	≥8.5%	182/647 (28.1)	181/660 (27.4)	199/655 (30.4)
Baseline age	<50 years	33/142 (23.2)	38/154 (24.7)	37/143 (25.9)
	50 to <65 years	309/1155 (26.8)	316/1146 (27.6)	315/1153 (27.3)
	65 to <75 years	246/808 (30.4)	239/834 (28.7)	241/833 (28.9)
	≥75 years	62/228 (27.2)	63/211 (29.9)	54/213 (25.4)
Baseline eGFR (MDRD)	≥90 ml/min/$1.73m^2$	102/488 (20.9)	116/519 (22.4)	135/531 (25.4)
	60 to <90 ml/min/$1.73m^2$	315/1238 (25.4)	344/1221 (28.2)	317/1202 (26.4)
	45 to <60 ml/min/$1.73m^2$	163/418 (39.0)	131/420 (31.2)	122/411 (29.7)
	<45 ml/min/$1.73m^2$	70/189 (37.0)	65/185 (35.1)	73/196 (37.2)

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **J. Duarte:** Honorarium; Boehringer Ingelheim. Lecture/other fees; Boehringer Ingelheim, Eli Lilly.

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Effect of empagliflozin when added to insulin in patients with type 2 diabetes and high cardiovascular (CV) risk: results from EMPA-REG OUTCOME

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Background and aims: In EMPA-REG OUTCOME, empagliflozin (EMPA) given in addition to standard of care significantly reduced 3-point major adverse CV events (MACE), CV death, hospitalisation for heart failure (HHF) and all-cause mortality vs placebo (PBO). As patients with T2DM using insulin represent a vulnerable population, we investigated changes in HbA_{1c} and weight, hypoglycaemia and CV outcomes in patients taking insulin at baseline.

Materials and methods: Patients were randomised to receive EMPA 10 mg, EMPA 25 mg, or PBO. Background glucose-lowering therapy, including insulin dose, was to remain unchanged for 12 weeks then be adjusted according to local guidelines. Confirmed hypoglycaemic adverse events (AEs) (plasma glucose ≤70 mg/dL and/or requiring assistance) that occurred during treatment or ≤7 days after last dose of study drug were analysed. HbA_{1c} and weight were assessed using a mixed model repeated measures analysis considering measurements until study end. CV outcomes were assessed using Cox regression analyses.

Results: In total, 2333, 2345 and 2342 patients received PBO, EMPA 10 mg and EMPA 25 mg, of whom 1135 (48.6%), 1132 (48.3%) and 1120 (47.8%), respectively, were taking insulin at baseline. At baseline, mean (SD) age of patients taking insulin was 63.4 (8.3) years, estimated glomerular filtration rate (MDRD) was 71.4 (21.5) ml/min/1.73m^2, body mass index was 31.6 (5.2) kg/m^2, 69.8% were male and 74.0% had been diagnosed with T2DM for >10 years. Mean insulin dose at baseline was similar between the EMPA and PBO groups, and decreased with EMPA and increased with PBO at study end. Similar proportions of patients had confirmed hypoglycaemic AEs in all treatment groups. Compared with PBO, EMPA reduced HbA_{1c} and weight at week 164 (patient numbers declined due to study design). In patients taking insulin at baseline, risk reductions with EMPA pooled vs PBO in 3-point MACE (hazard ratio [HR] 0.93; 95% CI 0.75, 1.13), CV death (HR 0.63; 95% CI 0.46, 0.85), HHF (HR 0.68; 95% CI 0.49, 0.94) and all-cause mortality (HR 0.72; 95% CI 0.56, 0.93) were consistent with patients not taking insulin at baseline (HR 0.79 [95% CI 0.64, 0.97]; 0.61 [95% CI 0.44, 0.85]; 0.61 [95% CI 0.39, 0.96]; 0.64 [95% CI 0.48, 0.84], respectively).

Conclusion: In patients with T2DM and high CV risk receiving insulin at baseline, use of EMPA led to reductions in HbA_{1c} and weight, without increased hypoglycaemia, vs PBO. Risk reductions in CV outcomes and all-cause mortality with EMPA in this subgroup were consistent with the overall population.

Parameter		Placebo (n=1135)	Empagliflozin 10 mg (n=1132)	Empagliflozin 25 mg (n=1120)
Insulin dose (U)	Mean (SD) insulin dose at baseline	65.0 (50.6)	65.2 (47.9)	65.5 (48.9)
	Mean (SD) insulin dose at study end	69.1 (50.9)	60.7 (45.5)	59.4 (44.5)
HbA_{1c} (%)	N analysed at baseline/week 164	1121/422	1110/449	1091/455
	Mean (SE) HbA_{1c} (%) at baseline	8.17 (0.03)	8.19 (0.02)	8.20 (0.02)
	Adjusted mean (SE) change from baseline in HbA_{1c} at week 164	0.08 (0.05)	-0.16 (0.05)	-0.27 (0.05)
	Difference vs placebo (95% CI)		-0.24 (-0.37, -0.11)	-0.35 (-0.48, -0.22)
Hypoglycaemia	n (%) with ≥1 confirmed hypoglycaemic adverse events	483 (42.6%)	494 (43.6%)	464 (41.4%)
Weight (kg)	N analysed at baseline/week 164	1116/573	1108/596	1086/616
	Mean (SE) weight at baseline	89.9 (0.6)	88.7 (0.6)	89.9 (0.6)
	Adjusted mean (SE) change from baseline in weight at week 164	-0.3 (0.2)	-2.2 (0.2)	-2.4 (0.2)
	Difference vs placebo (95% CI)		-1.9 (-2.5, -1.4)	-2.2 (-2.7, -1.6)

Clinical Trial Registration Number: NCT01131676
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Disclosure: **D. Jurišić-Eržen:** Employment/Consultancy; University Hospital Centre Rijeka. Lecture/other fees; Boehringer Ingelheim, Novo Nordisk,Takeda, MSD.

PS 065 SGLT-2 inhibitors: pathophysiology

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Empagliflozin improves beta cell function measured with the hyperglycaemic clamp in type 2 diabetes patients

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Background and aims: Chronic increase in plasma glucose concentration exerts a deleterious action on beta cell function, i.e. glucotoxicity. The aim of the present study was to examine whether lowering the plasma glucose concentration for 2 weeks with empagliflozin (SGLT2 inhibitor) improves beta cell function in T2DM.
Materials and methods: 15 T2DM patients (age=55±2; BMI= 31.2 ±1.1; FPG=195±9 mg/dl; HbA1c=7.8±0.2%; eGFR=107±7) received empagliflozin (25 mg/day) for 2 weeks, and beta cell function was measured with a 9-step hyperglycemic clamp (each step = +40 mg/dl) before and at 1 and 14 days after the start of empagliflozin.
Results: Empagliflozin caused 48±12% and 70±10% increase in the incremental area under the plasma C-Peptide concentration curve during the stepped hyperglycemic clamp on days 1 and 14 (from 23±5 to 34±7 and to 37±8 ng/ml.h, respectively, both $p<0.01$). Empagliflozin also caused an increase in the glucose infusion rate during the hyperglycemic clamp at days 1 and 14 compared to baseline by 15% and 16% ($p<0.05$), respectively. Beta cell function, measured as the insulin secretion/insulin resistance (IS/IR) index, increased by 76±21% and 100±20% ($p<0.05$ vs baseline) at days 1 and 14 versus baseline. Empagliflozin also caused a significant increase in beta cell glucose sensitivity during the hyperglycemic clamp (measured as the slope of the line relating the mean plasma C-peptide and plasma glucose conc during each hyperglycemic clamp step) by 42% and 54% at days 1 and 14 compared to baseline.
Conclusion: Lowering the plasma glucose conc with empagliflozin in T2DM patients: (1) enhances tissue glucose uptake during combined hyperinsulinemic/hyperglycemic conditions, (2) augments beta cell glucose sensitivity; (3) improves beta cell function (IS/IR index).
Supported by: Boehringer Ingelheim
Disclosure: **H. Al Jobori:** None.

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Safety and efficacy of dapagliflozin in combination with potassium-sparing agents

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Background and aims: Potassium-sparing agents are commonly used in patients with heart failure and hypertension. Sodium-glucose cotransporter 2 (SGLT2) inhibitors have recently been shown to reduce cardiovascular mortality and heart failure events in patients with type 2 diabetes and established cardiovascular disease. It is therefore likely that SGLT2 inhibitors and potassium-sparing agents will be co-administered, including in patients with hypertension and heart failure. While there are theoretical benefits to co-administration of potassium-sparing agents and SGLT2 inhibitors (including sodium loss, reduced blood pressure without increases in heart rate and complimentary effects on neurohormonal axis), it is unclear if such a combination increases hyperkalaemia risk.
Materials and methods: We examined the effects of dapagliflozin 10 mg versus placebo in patients treated with potassium-sparing agents, using pooled data from 14 phase 2b/3 trials over 24 weeks (dapagliflozin N=108; placebo N=119).
Results: Demographics and baseline characteristics were balanced between the groups (mean age 62 years, body mass index 35 kg/m^2, estimated glomerular filtration rate ~69 mL/min/1.73m^2, prior history of congestive heart failure ~25%, in both groups). Dapagliflozin lowered HbA1c, body weight and systolic blood pressure versus placebo (Table); the rate of serious adverse events was similar in both groups. No increase in serum potassium was seen with dapagliflozin; the proportion of patients with potassium ≥6 mmol/L during follow up was lower with dapagliflozin versus placebo.
Conclusion: When co-administered with potassium-sparing agents, dapagliflozin resulted in significantly lower HbA1c, weight and systolic blood pressure, with no evidence of an increase in serum potassium, and lower rates of significant hyperkalaemia compared with placebo.

Table. Effects of dapagliflozin on efficacy and safety in patients receiving potassium-sparing agents over 24 weeks.

	DAPA 10 mg-induced placebo-adjusted ΔBL at Week 24 (95% CI) (N = 119 [Placebo], 108 [DAPA])		Placebo (N=119) n (%)	DAPA 10 mg (N=108) n (%)
HbA1c (mmol/mol)	−4.3 (−6.6, −1.9)	AEs of renal impairment/ failure*	8 (6.7)	4 (3.7)
Body weight (kg)	−2.2 (−3. 0, −1.4)	AEs of hypotension/ dehydration/ hypovolaemia*	2 (1.7)	3 (2.8)
Systolic blood pressure (mmHg)	−5.2 (−8.8, −1.6)	Potassium ≥6 mmol/L	9 (7.6)	2 (1.9)
eGFR (mL/min/1.73m^2)	−3.2 (−6.7, 0.4)	Sodium <130 mmol/L	3 (2.5)	0 (0)
Serum sodium (mmol/L)	−0.1 (−0.7, 1.0)	*None were classified as serious AEs. AE, adverse event; ΔBL, adjusted mean change from baseline; DAPA, dapagliflozin; eGFR, estimated glomerular filtration rate.		
Serum potassium (mmol/L)	−0.1 (−0.3, 0.0)			

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Supported by: AstraZeneca
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Effects of canagliflozin on serum magnesium in patients with type 2 diabetes

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Background and aims: Hypomagnesaemia is an established risk factor for ventricular arrhythmia that is also associated with type 2 diabetes mellitus (T2DM) and its cardiometabolic complications. Canagliflozin (CANA), a sodium glucose co-transporter 2 inhibitor, has been shown to increase serum magnesium (Mg) without detectable changes in fractional excretion in patients with T2DM. The aim of this analysis was to

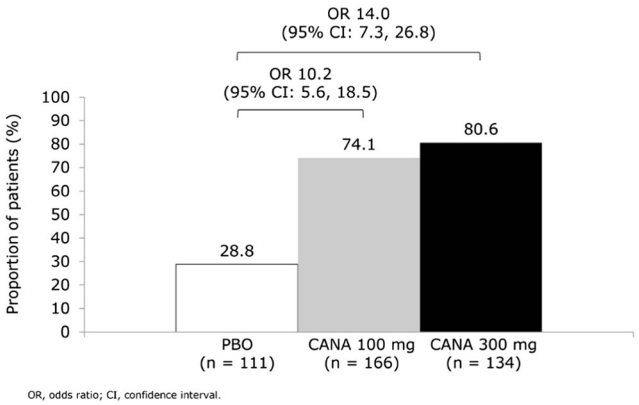
evaluate the effects of CANA treatment in patients with low serum Mg (<0.7 mmol/L).

Materials and methods: This post hoc analysis was based on pooled data from 4 placebo (PBO)-controlled studies of CANA in 2,313 patients with T2DM (mean serum Mg = 0.8 mmol/L; range = 0.4-1.2 mmol/L). The proportion of patients with serum Mg <0.7 mmol/L was evaluated at baseline and Week 26.

Results: At baseline, 18.3% of patients had serum Mg <0.7 mmol/L (hypomagnesaemia), 80.7% had Mg 0.7 to 0.9 mmol/L (normal Mg), and 1.0% had Mg >0.9 mmol/L (hypermagnesaemia). Patients with serum Mg <0.7 mmol/L were more likely to be female, white, have longer T2DM duration and have microvascular disease than those with serum Mg ≥0.7 mmol/L. At Week 26, increases in serum Mg were seen with CANA 100 and 300 mg versus PBO in patients with baseline serum Mg <0.7 mmol/L (17.0% and 19.0% vs 3.9%) and Mg ≥0.7 mmol/L (4.9% and 7.0% vs -1.4%). A greater proportion of patients with baseline serum Mg <0.7 mmol/L had Mg ≥0.7 mmol/L at Week 26 with CANA 100 and 300 mg versus PBO (74.1% and 80.6% vs 28.8%; Figure). Hypomagnesaemic patients treated with CANA versus PBO were 10 to 14 times more likely to achieve serum Mg ≥0.7 mmol/L.

Conclusion: CANA treatment was associated with normalisation of serum Mg in hypomagnesaemic patients with T2DM, potentially leading to improved cardiometabolic outcomes and reduced risk of arrhythmia.

Figure. Proportion of patients with baseline serum Mg <0.7 mmol/L who achieved serum Mg ≥0.7 mmol/L at Week 26.



OR, odds ratio; CI, confidence interval.

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SGLT-2 and SGLT-1 expression in renal tissue of patients with type 2 diabetes

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Background and aims: It is generally accepted that in patients with type 2 diabetes (T2DM) the expression of SGLT-2 in the proximal renal tubule is increased. This has been convincingly shown to be the case in mice, but in humans this notion mainly relies on a single study carried out in tubular cells harvested from the urine and examined in vitro. We sought to confirm this result by assessing the presence of both SGLT-2 and SGLT-1 in human renal tissues.

Materials and methods: Unaffected kidney samples were collected from 15 non-insulin-treated T2DM patients (age: 54±9 years, fasting plasma glucose 147±26 mg/dL, HbA1c 7.4±1.1%) and 15 age-matched non-diabetic subjects (CTL) undergoing unilateral nephrectomy for renal cell carcinoma. SGLT-1 and SGLT-2 gene and protein expression were quantified by real-time and digital PCR, western blot and immunofluorescence.

Results: Renal expression - reported as target/reference (β-actin) ratio - of both SGLT-1 and SGLT-2 was higher in CTL than in T2DM (SGLT-1: 1.04±0.20 vs 0.60±0.08, $p < 0.05$; SGLT-2: 1.61±0.02 vs 0.76±0.08, $p = 0.001$). These results were confirmed by western blot and immunofluorescence analyses. In the whole study group, a significant linear relationship was observed between SGLT-1 and SGLT-2 expression ($r = 0.57$, $p = 0.003$). Preliminary measurements performed by digital PCR showed that SGLT-1 is relatively more expressed than SGLT-2 in T2DM (CTL: 33.4±5.9 copies/μl for SGLT-2 vs 18.5±1.8 copies/μl for SGLT-1, $p = 0.02$; T2DM: 24.4±5.3 copies/μl for SGLT-2 vs 21.6±2.8 copies/μl for SGLT-1, p = ns). GAPDH was similar in the two groups (1523±217 in CTL vs 1622±291 copies/μl in T2DM, p = ns).

Conclusion: We do not confirm that SGLT-2 expression is increased in whole renal tissue of T2DM patients as compared to non-diabetic subjects. As a ratio to SGLT-2, renal tissue of T2DM patients appears to overexpress SGLT-1 compared to non-diabetic subjects.

Disclosure: **A. Solini:** None.

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Comparison in tissue distribution and selectivity among the 3 sodium-glucose co-transporter inhibitors empagliflozin, dapagliflozin and canagliflozin

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Background and aims: Three Sodium-Glucose co-Transporter 2 inhibitors empagliflozin; dapagliflozin and canagliflozin emerged recently as novel compounds for treating Type 2 diabetes. While no head to head trials have been conducted so far to compare these different drugs, it is interesting to note that empagliflozin has the highest renal excretion of parent drug. In regard to their different chemical structure and profile of selectivity toward SGLT1, we investigate the physicochemical property and tissue distribution of these 3 different compounds.

Materials and methods: The octanol/water partition-coefficient logP, was measured by HPLC method according to Donovan and Pescatore. The tissue distribution of these 3 drugs has been investigated by injecting intravenously the same amount of radiolabelled molecule as tracer added to the same dose of 10mg/kg SGLT2 inhibitor in Sprague Dawley rats. Animals were sacrificed at different time points (45min, 2h and 7h) and the amount of radioactivity was determined in blood and in 24 different tissues. The ratios (tissue/blood) were calculated for each tissue.

Results: The ratio of selectivity for human transporters (IC50 hSGLT1/IC50hSGLT2) is 2677, 1166 and 263 respectively for empagliflozin, dapagliflozin and canagliflozin. Therefore empagliflozin is about 2 and 10 fold more selective than dapagliflozin and canagliflozin versus hSGLT1. The measured logP (HPLC) of these 3 different drugs shows a clear difference in their lipophilicity, with lipophilicity increasing from empagliflozin (logP(HPLC) = 1.7) < dapagliflozin (logP(HPLC) =2.3) < canagliflozin (logP(HPLC) =3.5). Regarding tissue distribution, the radiolabelled compounds were found predominantly in the target organ (kidney) but also in the organs of absorption and elimination (gut, liver). The distribution into the other tissues was lowest but consistent with the rank order of lipophilicity of these 3 molecules. The tissue/

blood ratios were much higher for canagliflozin in comparison to dapagliflozin and empagliflozin, with empagliflozin being distributed into organs other than the kidney far less than the other two compounds. Assuming similar tissue/blood ratios between rat and man, we calculated the theoretical tissue distribution of these 3 molecules in man taking into account the respective Cmax or AUC values reported at clinically used doses of these compounds. Due to the highest dosage and plasma exposure of canagliflozin, the difference in the profile of calculated tissue distribution is exacerbated between the 3 compounds. Empagliflozin exhibited the lowest tissue distribution while being predominantly located in the organ of metabolism (liver) and the target organ (kidney).

Conclusion: In conclusion, in addition to the highest selectivity for SGLT2 transporters, the lowest tissue distribution of empagliflozin into other organs than the target organ (kidney) is consistent with its low lipophilicity properties in comparison to the other two molecules. While it is not known if these different physicochemical and selectivity properties may translate into a different drug profile, the more specific distribution of empagliflozin into the kidney may help to explain the positive results in regard to the reduction of CV death and hospitalisation for heart failure by 38% & 35%, respectively, as reported in the EMPA-REG OUTCOME study.

Disclosure: **S. Liebig:** None.

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Comparison of serum miRNAs expression of diabetic patients with healthy volunteers after type 2 diabetes drugs treatment

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Background and aims: MicroRNAs (miRNAs) are endogenous small noncoding highly conserved RNAs of 21-25 nucleotides that could bind to 3' untranslated region of the mRNAs of protein-coding genes to regulation of cellular function through effects on mRNA destabilisation and repression of transcriptional activity . Their expression profiles and functions have been extensively studied. Through modifying mRNA availability and protein synthesis, miRNAs regulate many cellular processes such as cell growth, proliferation, differentiation, and apoptosis. Recent studies suggested an association of endothelial microRNA-126 (miR-126) and miR-142 with type 2 diabetes mellitus (T2DM). In the current study, we examined how circulating miR-126 and miR-142 quantity are changed in serum of patients treated with common T2DM drugs in selected cohort of our patients

Materials and methods: The circulating miRNA profile was assessed in a pilot study of 18 men: 6 healthy volunteers and 12 T2D patients. Plasma was obtained by standard venipuncture and centrifugation in EDTA-coated vacutainer tubes. Total RNA was isolated with TRIzol reagent according to the protocol. For relative quantification miRNA expression we used the method of quantitative real-time PCR (qPCR). Total RNAs were reverse transcribed using miRNA-specific stem-looped primers, 1 mM dNTP-mix, 20 U ribolock RNAse inhibitor , 200 U Revers transcriptase and 1X Buffer (50 mM Tris-HCl (pH 8.3), 50 mM KCl, 4 mM MgCl2, 10 mM DTT. RT-qPCR measurments were perfomed on Bio-Rad CFX96 Touch™ Real-Time PCR Detection System in 96-well PCR-plates with 10 mkl final volume including 1,5 mkl RNA specific cDNA, 1X Sybr Green/ Rox mastermix and 1,5 pM miRNA specific primers. Samples were measured in triplicate. GenEx software were used for analized and normalized the qPCR data. Ct value of target miRNAs were normalized to each reference gene and the relative expression was calculated. The fold changes of differential miRNAs expression in plasma samples of diabet patients was calculated as normalization to expression of the same miRNAs in plasma sampes of health donors. Comparisons between cases and controls were performed using Student's t-test. Statistical significance was set at $p < 0.05$.

Results: A marked increase of miR-142 and decrease of miR-126 were observed in serum of T2D patients. We have found that by dapagliflozin monotherapy and dapagliflozin in combination with insulin and metformin treatment increases the amount of miR-126 to the level of healthy volunteers. In the treatment of metformin, sulfonylureas (gliclazide) alone or in combination with metformin or with dapagliflozin level of miR-126 becomes higher than the control serum. In the case of the miR-142 the level of this miRNA decreased below control in all treatment choices.

Conclusion: Thus, miRNA levels could serve as one of potential indicators of T2D drugs effects.

Disclosure: **V.M. Pushkarev:** None.

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SGLT2 inhibitors, via amplifying the effect of fasting moderately increases LDL-cholesterol and ketone levels

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Background and aims: SGLT2 inhibitors (SGLT2i) represent a novel class of drugs for treating Type 2 Diabetes with a unique mode of action. By triggering urinary glucose excretion, SGLT2i decrease blood glucose independently of the insulin pathway. SGLT2i are also reported to increase glucagon secretion and hepatic glucose production, to induce a switch towards lipid utilization and to moderately increase ketone bodies (ketones) and LDL-cholesterol (LDL-c) plasma levels. However, the mechanism of these effects is not yet fully understood. This study aims to investigate the pathways by which SGLT2i triggers a mild increase in ketones and LDL-c.

Materials and methods: For this purpose we use two preclinical species, Sprague Dawley rat and dyslipidemic hamster, the latter exhibiting a human-like cholesterol metabolism. In overnight fasted and non-fasted Sprague-Dawley (SD) rats, blood ketones levels were followed over 5h after a single dose of 3 and 10 mg/kg empagliflozin (SGLT2i). In hamsters the different parameters were measured after two weeks of empagliflozin (30mg/kg) treatment in overnight fasting versus non-fasting animals.

Results: In Sprague Dawley rats, ketones and blood glucose levels at baseline (t=0 and $AUC_{0\text{-}5h}$) were higher in fasted versus non-fasted animals. Hepatic glycogen of fasted rats, at t=0, was fully depleted (0.25 μmol/g) while it remained high (195 μmol/g) in non-fasted rats. Only in fasted rats, in which hepatic glycogen was depleted, SGLT2i increased the plasma ketone $AUC_{0\text{-}5h}$ (+17 & +32% (3 and 10 mg/kg respectively)). In non-fasted animals, empagliflozin did not trigger an increase in ketones, but diminished hepatic glycogen content versus vehicle (-10 and -28%, 3 and 10 mg/kg respectively)) during the 5h experimental period. A similar pattern was observed in fasting versus non-fasting hamsters determined after 2 weeks of treatment with empagliflozin (30mg/kg). In overnight fasted hamsters, empagliflozin significantly increased plasma ketone bodies by 60% associated with a drop in hepatic glycogen content. In parallel the level of LDL-c significantly increased from 120mg/dL, to 150mg/dL. Interestingly, hepatic HMGCoA activity was increased and hepatic aspartate levels (a marker of oxaloacetate pool) were significantly decreased in empagliflozin treated hamsters fasted overnight vs. vehicle.

Conclusion: Taken together, our studies in two different animal models allowed us to propose a scheme explaining the common pathway between ketone production and LDL-c homeostasis via a branching metabolic point. These pathways that are activated during fasting after glycogen depletion and gluconeogenesis activation are amplified by empagliflozin.

Disclosure: **E. Mayoux:** None.

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Dapagliflozin attenuates human vascular endothelial cell activation and induces vasorelaxation: a potential mechanism for inhibition of atherogenesis

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Background and aims: The sodium glucose transporter type 2 (SGLT-2) inhibitors represent a novel class of anti-diabetic agents used in the treatment of type 2 diabetes. Pre-clinical, clinical and cardiovascular safety studies suggest patients treated with SGLT-2 inhibitors may be afforded cardiovascular benefits via either direct SGLT-2 inhibition or novel molecular mechanisms. We aimed to investigate the effects and associated mechanisms of action of Dapagliflozin (DG) in pre-clinical models of cardiovascular disease with specific reference to endothelial cell activation and vascular reactivity in order to evaluate potential anti-atherogenic and vasculoprotective effects of DG.
Materials and methods: In-vitro studies utilised TNFα (10ng/ml) and hyperglycemia (10-30mM) stimulated human umbilical vein endothelial cells (HUVECs). Soluble intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) protein levels were determined from conditioned medium of TNFα or hyperglycemia stimulated, DG treated (24h, 1.0nM-5.0nM) HUVECs using ELISA assay. Real time qPCR was performed on mRNA extracted from TNFα stimulated, DG treated (24h, 1.0nM-5.0nM) HUVECs to evaluate NFkB expression levels. Abdominal aortic rings from 16 week-old C57Bl/6J mice maintained on a normal chow diet were utilised to evaluate direct vasorelaxation responses evoked by acute DG administration (1.0nM-10μM). Ex vivo endothelial-dependant vascular reactivity in response to acetylcholine (ACh) was examined using abdominal aortic rings from 7 month-old male apoE-/- mice on a high fat diet for 16 weeks that were treated with DG (1.0 mg/kg/day; drinking water) for the final 4 weeks.
Results: DG treatment of TNFα or hyperglycemia stimulated HUVEC resulted in a significant attenuation of TNFα and hyperglycemia-mediated induction of ICAM-1 and VCAM-1 protein expression at 24h, n=3-6, $p<0.05$. No significant effect of DG treatment on ICAM-1 or VCAM-1 protein expression was observed in unstimulated HUVEC. DG treatment of TNFα stimulated HUVEC also resulted in attenuation of induced NFkB expression, n=3-4, $p<0.05$. Acute DG treatment caused a marked concentration-dependent, and endothelium independent, relaxation of pre-contracted mouse aortae compared to time controls (maximum relaxation 60.45 +/- 5.04%) n=5-9, $p<0.0001$. In addition, there was a trend toward enhanced endothelial-dependent vasorelaxation evoked by ACh in the setting of chronic DG administration, n=3.
Conclusion: DG inhibits ICAM-1 and VCAM-1 expression in a human in-vitro model of endothelial cell activation, an effect associated with attenuation of induced NFkB expression. DG potently induces acute, direct vasorelaxation in mouse aortae whilst chronic in vivo administration of DG may afford improvements in endothelial dysfunction. Given the central role of vascular peptide expression and vasoreactivity in the atherogenic process formal in vivo characterisation of the effects of this agent on prevention of atherogenesis and plaque stabilisation are warranted.
Supported by: AstraZeneca Research Grant
Disclosure: **A.E. Dear:** Grants; Astra Zeneca Research Grant.

PS 066 Metformin and other oral agents

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WITHDRAWN

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Personalising therapy in type 2 diabetes: the effect of BMI and sex on glycaemic response and side effects to sulphonylureas and thiazolidinediones

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Background and aims: Sulphonylureas (SU) and thiazolidinediones (TZD) are second-line therapy options in type 2 diabetes, but there is no guidance as to which drug is best for which patients. We studied whether clinical characteristics could predict glycaemic response and side effects.
Materials and methods: Associations between clinical features and 1 year baseline-adjusted HbA1c fall were assessed in patients treated with SU (n=8748) and TZD (n=8876) in UK primary care data (CPRD). In the ADOPT trial (TZD n=1393; SU n=1337), mean HbA1c change/year and risk of 5% weight gain, oedema, fracture and hypoglycaemia over 5 years were compared in predefined subgroups showing the greatest differential response in CPRD.
Results: In CPRD, obese (BMI>30) female patients had 4.4mmol/mol better 1 year glycaemic response to TZD than SU ($p<0.001$), whilst non-obese males had a 3.3mmol/mol better response to SU than TZD ($p<0.001$). These findings were replicated in ADOPT: obese females mean HbA1c 4.8mmol/mol lower per year on TZD; non-obese males 2mmol/mol lower per year on SU. Obese males and non-obese females both had better glycaemic response with TZD (mean HbA1c 2.4 & 2.6mmol/mol lower per year). Risk of 5% weight gain on TZD compared with SU was greater in obese females (Hazard ratio(HR) 1.9, 95%CI (1.5-2.2) but similar in other subgroups (HR 0.98, (0.9-1.1)). Risks of oedema and fracture were greater on TZD compared with SU in females (oedema HR 1.5, (1.1-2.1); fracture HR 2.5, (1.4-4.2)) but not males (oedema HR 1.4, (0.98-2.1); fracture HR 1.1, (0.65-1.85)). Hypoglycaemia risk was lower with TZD and similar in all subgroups (HR range 0.18-0.23, all $p<0.001$).
Conclusion: BMI and sex should be considered when choosing between a TZD or SU. Non-obese males have the best glycaemic response to SU and are not at increased risk of side effects. Females have the best glycaemic response to TZD, but are at higher risk of side effects. Obese males have the best glycaemic response on TZD and may not have an increased side effect risk.
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Relationship of insulin sensitivity, beta cell function and glycaemic control with intensification of glucose-lowering therapy in newly diagnosed type 2 diabetes

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Background and aims: A second or third line glucose-lowering drug is often prescribed if target HbA1c levels are not reached on metformin

monotherapy in newly diagnosed type 2 diabetes. The aim of this study was to investigate whether insulin sensitivity, beta-cell function or glycemic control at diagnosis predict future therapy intensification.

Materials and methods: Patients with recently diagnosed type 2 diabetes and metformin monotherapy (n=138; mean age: 53 ± 10 years; 67% males; BMI: 32.2 ± 6.2 kg/m2) underwent detailed metabolic characterization within the first year after diagnosis. Associations of markers of glycemic control (HbA1c), insulin sensitivity (hyperinsulinemic-euglycemic clamp: M-value, mg·kg-1·min-1), and insulin secretion (i.v. glucose tolerance test: incremental C-peptide area under the curve0-60 min) with time to second-line therapy were assessed using parametric survival analysis accounting for interval-censoring, adjusting for age, sex, and baseline BMI.

Results: Thirty-eight (28%) patients initiated second-line therapy, mostly DPP-4 inhibitors or GLP-1 receptor agonists (71%), within a median follow-up of 3.3 years. Patients with second-line treatment had lower baseline M-values than patients who continued metformin monotherapy (median [Q1, Q3]: 4.4 [3.7, 6.8] vs. 5.7 [4.4, 6.7]) as well as higher baseline HbA1c (7.0 [6.1, 7.7]% vs. 6.2 [5.7, 6.6]%) (both $p<0.05$; Wilcoxon tests). No difference was observed for baseline incremental C-peptide (1.9 [1.4, 2.7] vs. 2.3 [1.6, 3.1]) ($p=0.11$). M-value (ln) (hazard ratio [95% confidence interval]: 0.34 [0.12-0.76]) and baseline HbA1c (HR: 1.72 [1.22-2.42]) were independent predictors of intensification of metformin monotherapy.

Conclusion: Insulin resistance, rather than deterioration of beta cell function, is an important predictor for intensification of glucose-lowering therapy within the first years after diagnosis of type 2 diabetes.

Disclosure: **W. Rathmann:** None.

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Metformin in type 1 diabetes: methods and baseline characteristics of the REMOVAL trial

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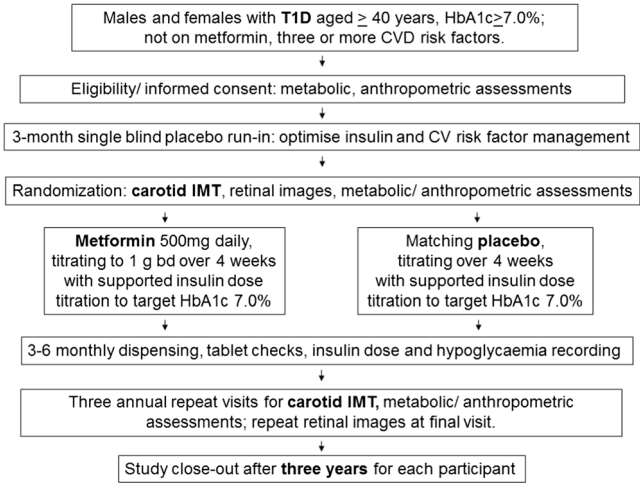
Background and aims: Cardiovascular disease (CVD) is a major cause of shortened life expectancy in type 1 diabetes (T1D). Long term glycaemic control prevents CVD but is difficult to achieve as intensive insulin therapy causes hypoglycaemia and carries a risk of weight gain with an associated rise in LDL cholesterol. Adjunct therapy with metformin reduces insulin dose requirement, promotes weight stabilization and lowers LDL cholesterol in T1D. We hypothesised that it would also reduce progression of atherosclerosis.

Materials and methods: REMOVAL is a double-blind randomized placebo-controlled trial of three years metformin therapy (1000 mg twice daily) added to insulin (target HbA1c 7.0%) in adults (40 years and over) with T1D. It involves a large team of international investigators (Australia, Canada, Denmark, the Netherlands, UK and USA) and is coordinated by the University of Glasgow, UK. The primary endpoint is progression of averaged mean far wall common carotid artery intima-media thickness (cIMT). Secondary and tertiary endpoints include HbA1c, LDL cholesterol, weight, insulin requirement, progression of retinopathy, endothelial function (reactive hyperaemia index by ENDOPAT) and frequency of hypoglycaemia.

Results: 429 participants have been randomised. Baseline characteristics: age 56±8.6 years; 41% female; diabetes duration 34±10.8 years; BMI 28 ±4.3 kg/m2; HbA1c 8.1±0.83%; BP 129±14.7/ 72±10.1 mmHg; total cholesterol 4.2±0.92 mmol/L; LDL cholesterol 2.2±0.74 mmol/L; cIMT 0.72±0.15 mm. Final results will be available in 2017.

Conclusion: REMOVAL is expected to provide clinically-meaningful information on the efficacy and safety of metformin as adjunct therapy in adults with T1D.



Clinical Trial Registration Number: NCT01483560
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Disclosure: **J.R. Petrie:** Grants; JDRF. Non-financial support; Merck KGaA, Itamar Medical.

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Latest metformin use patterns among type 2 diabetes patients in the UK

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Background and aims: Not all type 2 diabetes mellitus (T2DM) patients are appropriate for metformin, due to contra-indication and intolerance. There are limited publications on this topic. The objective of this database analysis was to assess the latest metformin use patterns and unmet needs in T2DM patients in the UK.

Materials and methods: A retrospective database analysis was conducted with the latest available UK Clinical Practice Research Datalink (CPRD). Eligible patients were T2DM patients 18 years and older with at least one metformin prescription in 2013. The date of first metformin prescription in 2013 was used as the index date. Continuous enrollments of 12 months for medical and pharmacy benefits were required both before and after the index date. Study endpoints included metformin discontinuation (a usage gap of 90 days or more), metformin non-adherence (proportion of days covered-PDC <80% among non-discontinuers), and suboptimal metformin dose (<1500 mg/day among in patients with ≥ 3 metformin prescriptions) in the 12-months following the index date. Multivariate regressions were performed to assess factors associated with metformin discontinuation, non-adherence and suboptimal dose.

Results: A total of 111,187 patients were included, with mean age of 66.4 years old and 58.6% of males. Overall, metformin was discontinued in 18% of patients (n=19,997). Among patients who continued metformin, 24% (n=21,505) were non-adherent. Among 105,449 patients with ≥ 3 metformin prescriptions, 24% (n=25,609) patients were on suboptimal (did not achieve optimal dose per highest dose of any individual metformin prescription) dose. Amongst the 11,227 patients who initiated

metformin in 2013, 75% of patients discontinued metformin. Among new metformin users, 36% of the metformin continuers were non-adherent, and 45% of patients with ≥ 3 metformin prescriptions (n=8,070) did not achieve optimal dose. Cox model results indicated that older age, female, higher HbA1c, lower BMI, hypoglycemia history, and higher Charlson comorbidity index were significantly associated with metformin discontinuation. Logistic regressions showed that younger patients, male, higher HbA1c, higher BMI, and without anti-diabetic drug use, microvascular complications and macrovascular diseases were associated with both non-adherence and suboptimal dose.

Conclusion: In our analysis of the latest UK CPRD data, metformin discontinuation and non-adherence were modest overall but very common among new metformin users. In addition, a quarter (of patient overall) or more (new metformin users) didn't achieve optimal dose in 12 months. Further study is needed to understand common clinical reasons for metformin discontinuation, suboptimal dose, and barriers to metformin adherence.

Disclosure: **J. Liu:** Employment/Consultancy; Merck & Co. Inc.

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Dual therapy with sulphonylurea and insulin is associated with increased mortality as compared with metformin and insulin combined treatment

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Background and aims: There is an intense debate regarding the effect of various diabetes medications on mortality risk, with most reports suggesting a beneficial effect of metformin (MET) and detrimental effect of sulphonylureas (SU). The aim of the current study was to investigate potential mortality differences associated with SU versus MET when used as combined dual treatment with insulin in diabetes patients.

Materials and methods: All patients aged ≥ 40 years, were included at their first prescription of insulin + MET or insulin + SU (dual therapy), during 2001-2008. They were considered at risk until outcome occurrence (death) or end of follow-up on December 31st, 2011. Time spent and deaths occurring on any other therapy besides insulin + SU/MET were excluded from the analysis. All mortality rates were calculated per 1000 person-years. Crude and adjusted (age and gender) rate ratios (RR) and 95%CI were calculated using time dependent analysis with insulin + MET as reference. Sensitivity analysis was done using four individual SU classes: glimepiride, gliclazide, glibenclamide and other SU.

Results: There were 7122 patients (60.8% women) included in the analysis, with a mean age at baseline 62.0±9.9 years (62.7±9.8 years women versus 60.9±10.1 years men, p<0.001). During the 11 years of study, patients on insulin + MET contributed 13620 person-years and 330 deaths (mortality rate 24.2, CI95% 21.8-27.0), while those on insulin + SU contributed 8720 person-years and 393 deaths (mortality rate 45.1, CI95% 40.8-49.8). Crude all-cause mortality RR (insulin + MET as reference) was 1.86 (CI95% 1.61-2.15, p<0.001), and decreased to 1.44 (CI95% 1.24-1.68, p<0.001) in adjusted analysis (for age and gender). Similar results were found for cardiovascular mortality. Individual SU adjusted RR for all-cause / cardiovascular mortality were: glimepiride 1.29 (CI95% 1.02-1.64, p=0.03) / 1.24 (CI95% 0.92-1.68, p=0.163), gliclazide 1.68 (CI95% 1.31-2.16, p<0.001) / 1.72 (CI95% 1.25-2.36, p<0.001), glibenclamide 2.05 (CI95% 1.47-2.85, p<0.001) / 1.63 (CI95% 1.03-2.58, p=0.034), other SU 1.36 (CI95% 1.13-1.65, p=0.001) / 1.23 (CI95% 0.96-1.57, p=0.105). Adjusted all-cause mortality RR using insulin + glimepiride as reference was 1.27 (CI95% 0.95-1.71, p=0.11) for gliclazide, 1.53 (CI95% 1.06-2.21, p=0.022) for glibenclamide, and 1.04 (CI95% 0.81-1.34, p=0.743) for other SU.

Conclusion: When combined with insulin as dual therapy, sulphonylureas (particularly glibenclamide) were associated with increased mortality as compared with insulin + metformin.

Disclosure: **M. Purcaru:** None.

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Monotherapy with sulphonylurea is associated with increased mortality as compared with metformin in type 2 diabetes

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Background and aims: To investigate possible mortality differences associated with sulphonylurea (SU) versus metformin (MET) when used as monotherapy in type 2 diabetes patients.

Materials and methods: All patients aged ≥ 40 years, were included at their first prescription of MET or SU as monotherapy, during 2001-2008. They were considered at risk until outcome occurrence (death) or end of follow-up on December 31st, 2011. Time spent and deaths occurring on any other therapy besides SU/MET monotherapy were excluded from the analysis. All cause and cardiovascular (CV) mortality rates were calculated per 1000 person-years. Crude and adjusted (age and gender) rate ratios (RR) and 95%CI were calculated using time dependent analysis with MET as reference. Sensitivity analysis was done using five individual SU compounds: glimepiride, gliclazide, glibenclamide, glipizide and gliquidonum.

Results: There were 47489 patients (52.8% women) included in the analysis, with a mean age at baseline 62.9±10.3 years (63.8±10.1 years women versus 61.9±10.4 years men, p<0.001). During the 11 years of study, patients on MET contributed 63795 person-years and 1169 deaths (mortality rate 18.3, CI95% 17.3-19.4), while those on SU contributed 129641 person-years and 5992 deaths (mortality rate 46.2, CI95% 45.1-47.4). Crude all-cause mortality RR (MET as reference) was 2.52 (CI95% 2.37-2.69, p<0.001), and decreased to 1.95 (CI95% 1.83-2.08, p<0.001) in adjusted analysis (for age and gender). Similar results were found for CV mortality. Individual SU adjusted RR for all-cause / CV mortality were: glimepiride 1.49 (CI95% 1.37-1.63, p<0.001) / 1.30 (CI95% 1.17-1.46, p<0.001), gliclazide 1.88 (CI95% 1.75-2.02, p<0.001) / 1.61 (CI95% 1.47-1.76, p<0.001), glibenclamide 1.97 (CI95% 1.80-2.15, p<0.001) / 1.74 (CI95% 1.55-1.94, p<0.001), glipizide 1.75 (CI95% 1.60-1.92, p<0.001) / 1.48 (CI95% 1.32-1.66, p<0.001), and gliquidonum 2.35 (CI95% 2.18-2.54, p<0.001) / 2.18 (CI95% 1.98-2.40, p<0.001). Adjusted all-cause mortality RR using glimepiride as reference was 1.23 (CI95% 1.11-1.36, p<0.001) for gliclazide, 1.34 (CI95% 1.19-1.51, p<0.001) for glibenclamide, 1.15 (CI95% 1.01-1.30, p=0.035) for glipizide, and 1.68 (CI95% 1.51-1.87, p<0.001) for gliquidonum.

Conclusion: When used as monotherapy, sulphonylureas were associated with increased mortality as compared with metformin. Similar results were found for cardiovascular mortality. There was a significant variation in individual SUs effect on mortality, with glimepiride having the lowest risk, but all had a detrimental effect when compared to metformin.

Disclosure: **L. Mintici:** None.

PS 067 New indications for GLP-1 RA

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The effect of liraglutide on cerebral electrical activity and cognitive performance in patients with type 1 diabetes

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Background and aims: While strong evidence from animal studies supports a potential role for glucagon-like peptide-1 in neuroprotection, memory and learning, the effects on cerebral electrical activity (EEG) and cognitive performance in patients with type 1 diabetes (T1D) are largely unknown. In a randomised, double-blinded placebo-controlled study we investigated the effect of 12-week treatment with liraglutide as an add-on to insulin in T1D during both normoglycaemia and hypoglycaemia.

Materials and methods: 20 patients with long-standing T1D were randomised to liraglutide 1.2 mg once daily (6 men/4 women, age 43.5 [23-66] years (median [range]), HbA1c 70 [61-83] mmol/mol) and placebo (8 men/2 women, age 30 [22-44] years (median [range]), HbA1c 73 [61-93] mmol/mol). The patients were studied before (day 1) and at end of treatment (day 2) during both eu- and hypoglycaemia induced by a hyperinsulinaemic glucose clamp. During each one-hour phase (eu- and hypoglycaemia) EEG, cognitive performance (evaluated with CalCAP, Trail Making Test, and Stroop test) and symptoms of hypoglycaemia were recorded, and blood samples were collected for analysis of glucagon, adrenaline, noradrenaline, cortisol, growth hormone, pancreatic polypeptide and GLP-1.

Results: Plasma glucose during hypoglycaemia was 2.3±0.2 and 2.4±0.2 mM with liraglutide and 2.5±0.2 and 2.4±0.3 mM with placebo on day 1 and 2, respectively. In the EEG, the peak amplitude of the theta and alpha and combined alpha-theta band significantly increased during hypoglycaemia compared to euglycaemia in both groups ($p<0.05$), but did not differ between day 1 and 2 within or between groups. The cognitive performance was compromised during hypoglycaemia compared to euglycaemia in both groups, but no treatment-related differences were found within or between groups at any time point. In both groups autonomic symptoms increased during hypoglycaemia compared to euglycaemia ($p<0.05$), but with no differences between day 1 and 2. Counterregulatory hormones increased during hypoglycaemia on both days with no difference within or between groups.

Conclusion: Liraglutide as an add-on to insulin treatment does not affect the cerebral electrical activity (EEG), cognitive performance, hypoglycaemia symptom scores or counterregulatory hormone responses during either normo- or hypoglycemia in patients T1D. These findings are in contrast to the improvement in cognitive performance and EEG changes observed in animal models.

Clinical Trial Registration Number: NCT02092896
Supported by: Unrestricted grant from Novo Nordisk A/S
Disclosure: **B. Thorsteinsson:** None.

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Cardiovascular effects of liraglutide in patients with type 1 diabetes: a randomised, double-blinded placebo-controlled trial (Lira-1)

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Background and aims: The add-on of a glucagon-like peptide-1 receptor agonist to insulin therapy in type 1 diabetes has proven beneficial with regard to insulin requirements, frequency of hypoglycaemia and loss of body weight. However, the effects on cardiovascular risk factors like 24-h ambulatory BP and heart rate (HR), carotid intima-media thickness (IMT) and aortic stiffness remain unknown.

Materials and methods: In this 24-week double-blinded, placebo-controlled trial, 100 overweight (BMI > 25 kg/m2) patients with type 1 diabetes and insufficient glycaemic control (HbA1c > 8%) were randomised to liraglutide 1.8 mg once daily or placebo as an add-on to intensive insulin therapy. At randomisation and at end of treatment (week 24) 24-h ambulatory BP and HR, carotid IMT and aortic pulse wave velocity (PWV) were measured.

Results: Baseline characteristics did not differ between groups: (liraglutide vs placebo, mean±SD) age 47±13 vs 49±12 years, HbA1c 8.7±0.7 vs 8.7±0.7%, BMI 30.3±3.5 vs 29.8±3.1 kg/m2, diurnal systolic BP (SBP) 137±11 vs 135±12 mmHg, nocturnal SBP 121±13 vs 116±12 mmHg, diurnal HR 79±9 vs 80±9 beats per min, nocturnal HR 67±9 vs 70 ±6 beats per min, carotid IMT 0.69±0.13 vs 0.71±0.14 mm, PWV 8.10 ±2.46 vs 8.85±2.86 m/s) except for diabetes duration 20±12 vs 25±12 years. After 24 weeks of treatment, diurnal and nocturnal HR and nocturnal diastolic BP (DBP) significantly increased by 3.7 beats per min [95% CI: 0.5; 7.0; p=0.024], 7.5 beats per min [95% CI: 3.7; 11.3; p<0.001] and 4.0 mmHg [95% CI: 0.2; 7.7; p=0.039], respectively, in liraglutide-treated patients compared with placebo-treated patients. Changes in diurnal and nocturnal SBP and diurnal DBP did not differ between groups (Table 1). In a sub-group analysis of patients with diurnal SBP above the median (136 mmHg), diurnal SBP decreased by 6.0 mmHg [95% CI: -11.5; -0.5] and 8.4 mmHg [95% CI: -17.6; 0.8] in the liraglutide and placebo group, respectively (p=0.490), and nocturnal SBP decreased by 4.7 mmHg [95% CI: -11.6; 2.2] and 5.1 mmHg [-15.6; 5.4] in the liraglutide and placebo group, respectively (p=0.241). Pulse pressure, carotid IMT and PWV did not differ within or between groups at end of treatment (Table 1).

Conclusion: In overweight patients with type 1 diabetes and insufficient glycaemic control, diurnal and nocturnal HR and nocturnal DBP increased when liraglutide was added to insulin therapy. No effect on diurnal and nocturnal SBP or diurnal DBP was seen. Additionally, no changes in pulse pressure, carotid IMT and PWV were found after 24 weeks of treatment.

Key results: Liraglutide 1.8 mg vs. placebo as an add-on to insulin in change from baseline to week 24

	Liraglutide, n=46 [95% CI]	Placebo, n=44 [95% CI]	Liraglutide vs. placebo* [95% CI]	p-value
Diurnal systolic BP (mmHg)	1.4 [-3.1; 5.9]	-1.2 [-7.9; 5.5]	3.4 [-2.8; 9.6]	0.281
Nocturnal systolic BP (mmHg)	-0.03 [-5.7; 5.6]	-0.7 [-7.2; 5.8]	4.2 [-2.3; 10.7]	0.206
Diurnal pulse pressure (mmHg)	-0.2 [-3.0; 2.5]	-0.7 [-4.5; 3.1]	1.1 [-2.6; 4.7]	0.572
Nocturnal pulse pressure (mmHg)	-2.8 [-6.4; 0.8]	0.1 [-3.7; 3.9]	-0.7 [-4.7; 3.3]	0.725
Diurnal heart rate (BPM)	4.1 [2.1; 6.1]	0.0 [-3.1; 3.1]	3.7 [0.5; 7.0]	0.024
Nocturnal heart rate (BPM)	6.5 [3.2; 9.7]	-1.7 [-3.8; 0.3]	7.5 [3.7; 11.3]	<0.001
Carotid IMT (mm)	0.01 [-0.01; 0.03]	0.00 [-0.02; 0.01]	0.01 [-0.01; 0.03]	0.361
PWV (m/s)**	0.24 [-0.34; 0.82]	-0.41 [-1.18; 0.37]	0.04 [-0.75; 0.83]	0.918

*All analysis adjusted for baseline value.
**Adjusted for blood pressure and heart rate at time of measurement.
BPM, beats per minute. IMT, intima-media thickness. PWV, pulse wave velocity.
Diurnal BP was measured between 08.00 a.m. and 09.00 p.m.
Nocturnal BP was measured between 00.00 p.m. and 06.00 a.m.

Clinical Trial Registration Number: NCT01612468

Supported by: Novo Nordisk
Disclosure: **T.F. Dejgaard:** Grants; Novo Nordisk A/S. Lecture/other fees; Novo Nordisk A/S.

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Efficacy and safety of liraglutide added to capped insulin treatment in type 1 diabetes, the ADJUNCT TWO™ randomised trial

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Background and aims: To investigate if treatment with liraglutide, a glucagon-like peptide-1 analogue, added to individually capped insulin treatment, improves glycaemic control and reduces weight and insulin dose in a broad population of adults with type 1 diabetes (T1D).
Materials and methods: The 26-week, double-blinded, multinational trial enrolled adults with T1D on basal-bolus or Continuous Subcutaneous Insulin Infusion (CSII) therapy with HbA1c 7-10%. Subjects with a history of severe hypoglycaemia, hypoglycaemia unawareness, diabetic ketoacidosis or renal impairment (with estimated GFR >30 mL/min/1.73 m^2) were not excluded. Subjects were randomised 3:1 to receive once-daily subcutaneous injections of liraglutide (1.8 mg, 1.2 mg, 0.6 mg) or placebo. Each subject titrated insulin with total daily dose capped at their own average dose from the week prior to randomisation.
Results: At baseline, n = 835, mean age = 43 years, T1D duration = 21 years, HbA1c = 8.1%, BMI = 29 kg/m^2 (60%, >27 kg/m^2) and body weight = 83.9 kg; 54% of subjects were women, 26% used CSII, 7% had severe hypoglycaemia in the last year, 6% were hypoglycaemia-unaware and 15% had baseline fasting C-peptide >0.03 nmol/L. Liraglutide treatment at all doses reduced HbA1c, body weight and insulin dose, both absolutely and relative to placebo, after 26 weeks. For liraglutide 1.8 mg, 1.2 mg and 0.6 mg, respectively: HbA1c estimated treatment differences (ETDs) [95% CIs] to placebo were -0.35% [-0.50;-0.20], -0.23% [-0.38;-0.08] and -0.24% [-0.39;-0.10]; body weight ETDs were -4.8 kg [-5.5;-4.1], -3.7 kg [-4.4;-3.0] and -2.2 kg [-2.9;-1.5]; insulin dose estimated treatment ratios to placebo were 0.90 [0.86;0.93], 0.93 [0.90;0.96], and 0.95 [0.92;0.99]. The rate of symptomatic hypoglycaemic episodes (severe by American Diabetes Association classification or <56 mg/dL with symptoms) was increased relative to placebo with liraglutide 1.2 mg (estimated rate ratio, ERR=1.31 [1.03;1.68]). Rates of severe hypoglycaemic episodes were similar across groups. The percentage of subjects achieving HbA1c reduction >1.0% with no severe hypoglycaemia was greater at all liraglutide doses (all p≤0.0078) compared with placebo (1.8 mg, 15.3%; 1.2 mg, 11.3%; 0.6 mg, 11.1%; placebo, 3.7%). The most frequent adverse events with liraglutide were dose-dependent nausea and vomiting. The rate of hyperglycaemic episodes accompanied by ketosis (>1.5 mmol/L) was increased with liraglutide 1.8 mg (ERR=3.96 [1.49;10.55]; liraglutide n=42; placebo n=10).
Conclusion: Liraglutide (1.8 mg, 1.2 mg and 0.6 mg) added to capped insulin treatment led to greater reductions in HbA1c, body weight and insulin dose compared with placebo, but a higher rate of symptomatic hypoglycaemia (lira 1.2 mg) may limit the clinical utility of liraglutide for a broad T1D population as studied in this trial.
Clinical Trial Registration Number: NCT01836523
Supported by: Novo Nordisk
Disclosure: **I.B. Hirsch:** Employment/Consultancy; Abbott Diabetes Care, Roche, Becton Dickinson. Grants; Novo Nordisk.

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Efficacy and safety of gemigliptin as add-on therapy in patients with type 2 diabetes inadequately controlled on metformin and glimepiride

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Background and aims: Gemigliptin is a potent, selective, competitive, and long-acting dipeptidyl peptidase (DPP) -4 inhibitor**.** This study evaluated the efficacy and safety of gemigliptin as add-on therapy to metformin and glimepiride for 24 weeks compared with placebo in patients with type 2 diabetes mellitus (T2DM) inadequate glycemic control.
Materials and methods: In this multicenter, randomized, double-blind, Phase III study, eligible patients with inadequate glycemic control (7% ≤ HbA1c ≤ 11%) were randomized to gemigliptin 50 mg q.d (n=109) or placebo (n=110). The primary endpoint was change from baseline in HbA1c after 24 weeks.
Results: Baseline demographics were similar between groups (age 60.9 years; BMI 24.9 kg/m^2, duration of T2DM 12.9 years), with mean ± SD baseline HbA1c of 8.12 ± 0.82% in the gemigliptin group and 8.15 ± 0.89% in the placebo group. At Week 24, adjusted mean ± SE change HbA1c with gemigliptin was -0.88 ± 0.17% (change with placebo -0.01 ± 0.18%; difference -0.87 ± 0.12%, 95% CI -1.09 to -0.64; p<0.0001). The differences in proportions achieving an HbA1c <7 or <6.5% were also statistically significant (p<0.0001) between groups. Gemigliptin was generally well tolerated, although there was a higher incidence of overall adverse events (AEs) in the gemigliptin group than in the placebo group (56.1% and 36.0%, respectively). Drug-related AEs were reported for 3.7% and 2.7% of gemigliptin and placebo, respectively. Hypoglycemia occurred in 9.4 and 2.7% of the gemigliptin and placebo groups, respectively.
Conclusion: In conclusion, triple therapy with gemigliptin 50 mg q.d in patients with T2DM inadequately controlled on metformin and glimepiride improved glycemic control and was generally well tolerated over 24weeks.
Clinical Trial Registration Number: NCT01990469
Supported by: LG Life Sciences
Disclosure: **S. Kim:** None.

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Efficacy and safety of evoglipitin monotherapy in patients with type 2 diabetes

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Background and aims: To evaluate the efficacy and safety of the newly developed dipeptidyl peptidase-4 (DPP-4) inhibitor evogliptin in drug-naïve patients with inadequately controlled type 2 diabetes.

Materials and methods: In randomized, double-blind, placebo-controlled, parallel-group, multicenter, phase III study, 160 patients with type 2 diabetes were randomized to evogliptin 5mg or placebo for 24 weeks. The primary efficacy outcome measure was mean changes from baseline to endpoint in hemoglobin A1c (HbA1c).
Results: The mean baseline HbA1c levels of the evoglipitin group and the placebo group were 7.20±0.56% and 7.20±0.63%, respectively ($p > 0.05$). Although the baseline HbA1c was very lower when compared with other phase III clinical trials using DPP-4 inhibitors, evogliptin provided significant placebo-corrected reductions in HbA1c from baseline of -0.23% ($p < 0.0001$) at 24 weeks. Also, the response rate achieving HbA1c <6.5% was significantly higher in the evoglipitin group than placebo group ($p = 0.008$). Overall, incidences of adverse event and hypoglycemia were similar between the two groups.
Conclusion: In this 24-weeks study, once-daily evoglipitin monotherapy significantly improved glycemic control and was well tolerated in patients with type 2 diabetes.
Disclosure: **D. Kim:** None.

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Evaluation of safety and efficacy of teneligliptin in newly diagnosed Indian type 2 diabetes patients

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Background and aims: India harbours the largest number of T2DM patients in the world and requires availability of more cost-effective and safer drugs for its management. This study was conducted to evaluate the safety and efficacy of Teneligliptin in Indian patients with T2DM inadequately controlled with diet and exercise alone.
Materials and methods: In this randomized, double blind, comparative, prospective, placebo-controlled, parallel-group, multicentre phase III study, drug naive Indian subjects with T2DM were randomized to either 20 mg Teneligliptin or to placebo. Efficacy and safety assessments were performed at 16 weeks and were compared with those at baseline. The primary efficacy endpoint which was the change from baseline in HbA1c was analysed using an analysis of covariance (ANCOVA) model with baseline HbA1c as a covariate. The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP) after the approval of protocol by regulatory authorities in India and the trial was registered at Clinical Trial Registry of India (CTRI).
Results: A total of 237 patients (158 in Teneligliptin group and 79 in Placebo group) were randomized in a 2:1 ratio to Teneligliptin 20 mg Tablets or Placebo Tablets. The least square mean change (LMS)in HbA1c levels from baseline to end of treatment were -0.304 and 0.251 in the Teneligliptin and Placebo groups, respectively and there was a statistically significant difference in change in HbA1c levels from baseline to end of treatment among patients receiving treatment with Teneligliptin, relative to Placebo (difference, 0.555; 95% CI, 0.176 to 0.934; $p=0.0043$) in the intent-to-treat (ITT) analysis. The proportion of patients who achieved HbA1c of ≤7% after 16 weeks of treatment was 43.4% in the Teneligliptin group and 27.3% in the Placebo group which was statistically significant for the Teneligliptin group compared to the Placebo group ($p=0.026$). Treatment emergent adverse events (TEAEs) were reported for 30/158 (19%) patients in the Teneligliptin group and 14/79 (17.7%) patients in the Placebo group. The majority of TEAEs were mild in severity.
Conclusion: The results of the study demonstrates that Teneligliptin was very well tolerated and superior on efficacy compared to Placebo in Indian patients with Type 2 diabetes mellitus inadequately controlled with diet and exercise alone. Teneligliptin can be considered as a promising alternative to other gliptins available in India.

Mean Change in HbA1c Levels (%) (ITT Population)

Visit	Statistics	Teneligliptin (N=145)	Placebo (N=77)
Mean Change from Screening to Week 16	LSM (SE)	-0.304 (0.1118)	0.251 (0.1565)
	Difference: LSM		0.555
	95% CI		(0.176, 0.934)
	p-value		0.0043

LSM: least square mean; SE: standard error; ITT: Intent-To-Treat; Difference: LSM (SE) between groups is calculated for Teneligliptin vs Placebo. The 95% confidence interval (CI) for the LSM mean difference in HbA1c between groups is calculated for Teneligliptin vs Placebo. p-value is calculated for the comparison of treatment groups using ANCOVA with treatment as main effect and by considering the baseline HbA1c value as covariate.

Clinical Trial Registration Number: CTRI/2014/01/004315
Supported by: Educational grant received from Glenmark TCRP
Disclosure: **S.Y. Suryawanshi:** Employment/Consultancy; Medical adviser to Glenmark Pharmaceuticals which sponsored the trial.

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The effects of once-weekly semaglutide on beta cell function in subjects with type 2 diabetes

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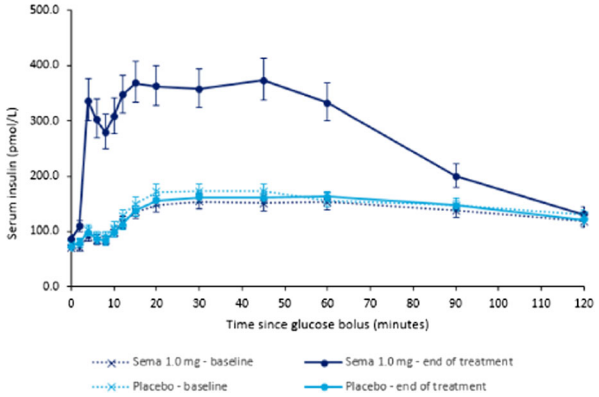
Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue with an extended half-life in phase 3 development for type 2 diabetes (T2D). This study evaluated the effect of once-weekly subcutaneous (s.c.) semaglutide in steady state versus placebo on beta-cell responsiveness in subjects with T2D.
Materials and methods: In this double-blind, parallel-group trial, 75 adults with T2D (mean HbA_{1c} 7.3%, duration of T2D 8.5 years, BMI 29.6 kg/m^2, age 56 years, 68% male) were randomised 1:1 to once-weekly once-weekly s.c. semaglutide (escalated to 1.0 mg) or placebo for 12 weeks. Intravenous glucose tolerance tests (IVGTT; 25 g glucose bolus), arginine stimulation tests (5 g arginine two hours after a glucose infusion) and graded glucose infusion (GGI) tests (target glucose levels of 5, 6, 7, 8, 9, 10, 11 and 12 mmol/L over 180 min) were performed at baseline and after 12 weeks of treatment. A control group of 12 untreated healthy subjects (mean BMI 26.8 kg/m^2, age 43 years, 67% male) was also subjected to a GGI test. Insulin response was measured as the semaglutide:placebo ratio for changes from baseline to end-of-treatment in the Area Under the Curve. The primary endpoint was first- (0-10 min) and second- (10-120 min) phase insulin secretion in the IVGTT.
Results: Following IVGTT, change from baseline for both first- and second-phase insulin responses in subjects receiving semaglutide was significantly greater than in those receiving placebo (estimated treatment ratio [ETR] 3.02 [95% CI: 2.53-3.60] and 2.10 [95% CI: 1.86-2.37], respectively; Fig 1). In the arginine stimulation test, increases from baseline for both insulin concentrations and insulin secretion rate in subjects receiving semaglutide were significantly greater than for those receiving placebo during 0-10 min (ETR 2.82 [95% CI: 2.39; 3.32] and 1.69 [95% CI: 1.49; 1.92], respectively) and 0-30 min (ETR 4.42 [95% CI: 3.74; 5.22] and 2.69 [95% CI: 2.38; 3.05], respectively). In the GGI test, both the insulin secretion rate and slope at end of treatment were significantly greater for semaglutide than placebo. In this group of subjects with T2D, insulin secretion and slope at end of treatment for subjects receiving semaglutide were comparable to those of untreated healthy subjects. No new safety or tolerability issues were identified for semaglutide.
Conclusion: Semaglutide (1.0 mg s.c. once weekly) signicantly improved first- and second-phase insulin secretion compared with placebo after 12 weeks of treatment. Beta-cell responsiveness at the end of the study in the semaglutide group was comparable to that of untreated healthy individuals. Semaglutide was well tolerated and had a safety profile similar to that of other GLP-1 receptor agonists.

Figure 1. Insulin response to the intravenous glucose tolerance test in subjects with T2D receiving semaglutide or placebo.



Clinical Trial Registration Number: NCT02212067
Supported by: Novo Nordisk A/S
Disclosure: **C. Kapitza:** Employment/Consultancy; Sanofi. Grants; Adocia, Astra Zeneca, Biocon, Boehringer Ingelheim, Dance Pharmaceuticals, Gulf Pharmaceutical Industries, Johnson & Johnson, Eli Lilly, Marvel LifeSciences Ltd., Medtronic, Medimmune, Novo Nordisk, Novartis, Roche Diagnostics, Sanofi, Senseonics, Zealand Pharma. Honorarium; Sanofi. Other; Additional grants: Novartis, Roche Diagnostics, Sanofi, Senseonics, Zealand Pharma, Advisory, speaker and travel grants: Sanofi.

PS 068 GLP-1 RA: renal aspects

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Effects of mild to moderate renal impairment on albiglutide (ALBI) in type 2 diabetes

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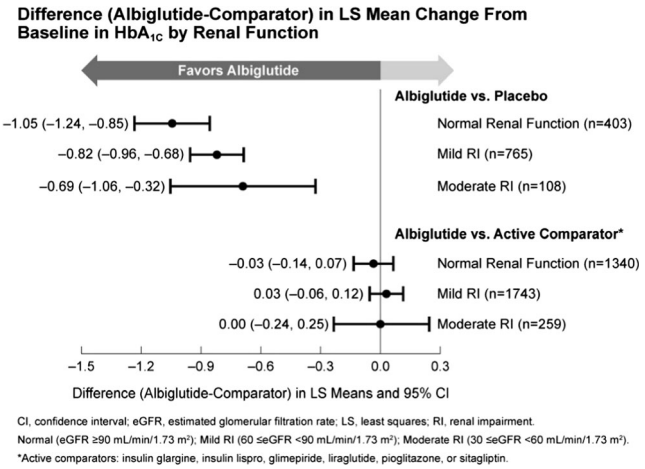
Background and aims: Renal impairment is a frequent comorbidity in type 2 diabetes mellitus (T2DM). In the HARMONY Phase 3 trials of patients with T2DM, treatment with the glucagon-like peptide-1 receptor agonist albiglutide (ALBI) resulted in HbA_{1c} reduction, fasting plasma glucose reduction, and weight loss compared with agents associated with weight gain. ALBI was generally well tolerated. Here we present post-hoc pooled analyses of the relationship between renal function and least squares mean change in HbA_{1c} (from baseline to end-of-trial) in HARMONY 1-7.
Materials and methods: These trials of ALBI 30-50 mg/week had placebo and/or active antihyperglycemic controls, ranged from 26 to 104 weeks in length, and prescribed different concomitant medications.
Results: Patients at baseline were ≥18 years old with HbA_{1c} 53.0-91.3 mmol/mol and estimated creatinine clearance >1.0 mL/s. ALBI reduced HbA_{1c} significantly more than placebo across subgroups based on renal function and was noninferior to active comparators in HbA_{1c} reduction. The relationship between ALBI and active comparators was not affected by renal function (Figure). In addition, there was no overall worsening of renal function (estimated glomerular filtration rate) with ALBI versus pooled comparators.
Conclusion: These results are consistent with efficacy of ALBI in T2DM patients with mild to moderate renal impairment, with no dose adjustment required. Previously presented at ADA 2016, and reprinted with permission.



Clinical Trial Registration Number: NCT00849056; NCT00849017; NCT00838903; NCT00838916; NCT00839527; NCT00976391; NCT01128894
Supported by: GlaxoSmithKline.
Disclosure: **V. Bainbridge:** Employment/Consultancy; GlaxoSmithKline. Stock/Shareholding; GlaxoSmithKline.

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Effects of renal impairment on the pharmacokinetics, safety, and tolerability of ITCA 650

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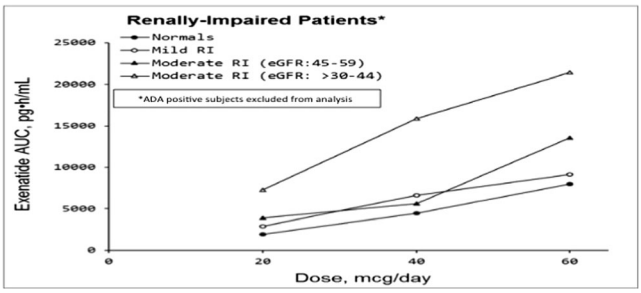
Background and aims: The pharmacokinetics, safety and tolerability of ITCA 650, a subdermally placed osmotic mini-pump that delivers continuous s. c. exenatide (EX) for extended periods of time, was studied in subjects with varying degrees of renal impairment (RI).
Materials and methods: Escalating doses of ITCA 650 20, 40 and 60 mcg/day were placed for consecutive periods of up to 20 days each in 38 subjects (mean age, 57), stratified by estimated GFR (mL/min/1.73 m2) into: normal (≥90), mild RI (60-89), moderate RI (45-59) and moderate RI (>30-44). The primary endpoint was steady state plasma AUC for each dose, derived from 7 time points over 24 hours. Safety and anti-drug antibodies (ADA) were monitored.
Results: EX AUC increased 2.6-3.7 fold, and clearance decreased 1.1-3 fold with increasing RI as previously reported for exenatide. Steady state AUC and EX concentrations (Css) increased 3 to 4-fold over the dose range studied. ITCA 650 was well tolerated. The most common adverse event (AE) was nausea. There were no renal serious AEs or study drug-related renal AEs.
Conclusion: Ex AUC and Css increased with dose and with increased renal impairment. The overall safety and gastrointestinal tolerability of ITCA 650 in mild and moderate renal impaired subjects were consistent with previous studies involving ITCA 650.



Clinical Trial Registration Number: NCT02320045
Supported by: Intarcia Therapeutics, Inc.
Disclosure: **L. Kjems:** Employment/Consultancy; Intarcia Therapeutics.

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Effects of linagliptin on glycaemic control and albuminuria in type 2 diabetes: the MARLINA-T2D™ trial

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Background and aims: Previous evidence suggests that the dipeptidyl peptidase-4 inhibitor linagliptin may exert anti-albuminuric effects beyond glycaemic control. MARLINA-T2D™, a multicentre, double-blind, placebo-controlled clinical trial, investigated glycaemic and renal effects of linagliptin in patients with type 2 diabetes, albuminuria and estimated GFR ≥30 ml/min/1.73 m^2.
Materials and methods: In total 360 patients with type 2 diabetes (HbA_{1c} 6.5-10%) and persistent albuminuria (urinary albumin-to-creatinine ratio [UACR] 30-3000 mg/gCr) despite stable background of single renin-angiotensin system blockade were randomised to either linagliptin (n=182) or placebo (n=178) for 24 weeks. Primary glycaemic and key secondary renal surrogate endpoints were HbA_{1c} and UACR change from baseline over 24 weeks, respectively.
Results: Overall mean (SD) baseline HbA_{1c} and geometric mean (gMean) UACR were 7.8% (0.9) and 126 mg/gCr (microalbuminuria, 73.7%; macroalbuminuria, 20.3%), respectively. At week 24, placebo-adjusted mean HbA_{1c} change from baseline was -0.60% (95% CI -0.78, -0.43; p<0.0001; Table). Placebo-adjusted gMean for time-weighted average of % change from baseline in UACR over 24 weeks was -6.0% (95% CI -15.0, 3.0; NS; Table). Linagliptin was well tolerated with a renal safety profile consistent with previous clinical trials.
Conclusion: Linagliptin significantly improved glycaemic control without a significant effect on UACR. Whether a renoprotective effect of linagliptin could emerge from chronic intervention in more advanced diabetic kidney disease is currently being explored in the ongoing CARMELINA™ trial.

Table. Efficacy results

	Placebo	Linagliptin
HbA_{1c} (%)*	***n*=171**	***n*=172**
Baseline, mean (SE)	7.87 (0.07)	7.79 (0.06)
Change from baseline to week 24, adjusted mean (SE)^	−0.03 (0.06)	−0.63 (0.06)
Comparison *vs* placebo (diff. linagliptin – placebo), adjusted mean (SE)^		−0.60 (0.09)
95% CI		−0.78, −0.43
p-value		*p*<0.0001
Time-weighted average of % change in UACR over 24 weeks (%)**	***n*=173**	***n*=178**
Baseline§, gMean (gCV) (mg/gCr)	132 (167)	121 (153)
Week 24, adjusted gMean†	−5.1	−11.0
95% CI	−11.4, 1.6	−16.8, −4.7
Comparison *vs* placebo (diff. linagliptin – placebo), adjusted gMean†		−6.0
95% CI		−15.0, 3.0
p-value		*p*=0.1954

* Full analysis set (FAS, all randomised patients treated with ≥1 dose of the study drug and with a baseline HbA_{1c}, a baseline UACR and ≥1 on-treatment HbA_{1c} or UACR measurement) using observed cases.
** FAS using last observation carried forward method.
§ Baseline UACR data were available for 354 patients (linagliptin, n=180; placebo, n=174).
^ Adjusted mean based on a mixed model for repeated measurements.
† Adjusted gMean based on an analysis of covariance.

Clinical Trial Registration Number: NCT01792518
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance
Disclosure: **G. Schernthaner:** Employment/Consultancy; Rudolfstiftung Hospital Vienna. Lecture/other fees; Boehringer Ingelheim.

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Sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes and advanced chronic kidney disease

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Background and aims: Type 2 diabetes (T2DM) shows impaired mobilization of hematopoietic and endothelial progenitor cells (EPCs). These

alterations may hamper tissue repair capacity and favor the development of cardiovascular complications. By upregulation of SDF-1α, the dipeptidyl peptidase 4 (DPP4) inhibitor sitagliptin increases circulating EPCs and might expand EPCs pool and improve their functions. We aimed to evaluate the effects of sitagliptin on circulating EPCs and on their functional properties in T2DM with advanced chronic kidney disease (CKD), a subgroup at very high cardiovascular risk.

Materials and methods: Fourty-nine T2DM, 18 with CKD stage 3b, 17 with CKD stages 4-5 and 14 subjects undergoing hemodialysis (HD) have been enrolled in a prospective trial. Their mean age was 73±8 years (IQR 69-78), diabetes duration (DD) 18±14 years (10-30), HbA1c 7.4±1.4% (6.5-8.1), eGFR (CKD-EPI) 23.4±14.6 ml/min/1,73m2 (range 3.8-51.3). These subjects have been randomized 2:1 to sitagliptin (SITA; n. 32) or insulin treatment (INS; n. 17). Circulating levels (flow cytometry) of stem and progenitor cells and function of EPCs (adhesion to fibronectin and migratory capacity) have been evaluated at baseline and after 4, 13, 26 and 52 weeks of treatment. The same trained operators, blind to the clinical status of each subject, performed the tests throughout the entire study.

Results: The three groups did not differ for age, DD, BMI, systolic and diastolic BP, lipids, HbA1c, CV events, retinopathy or lipid- and BP-lowering treatments. CD34+ and CD34+CD133+ cells decreased from CKD3b to CKD4-5 (p=0.023 e p=0.033, respectively) with a small increase of CD34+ cells (p=0.058) in HD; no differences between groups were observed for CD34+KDR+ and CD34+KDR+CD133+. eGFR (CKD-EPI; OR 1.077), HD (OR 12.800) and BMI (OR 1.185) independently contributed to variability of CD34+ cells level (and also to CD34+ CD133+ cells level). No differences in EPCs adhesion and migration have been observed. INS subjects had longer DD, higher HbA1c (8.2±1.9 vs 7.0±1.0%, p=0.025), lower eGFR (18.5±11.8 vs 27.2±15.2 ml/min/1,73m2, p=0.034) and higher rate of patients on HD. Through follow-up BMI, BP, lipids and eGFR did not change in both INS and SITA. HbA1c decreased in INS (from 8.2±1.9% at baseline to 7.3±1.4% at 52-week, p=0.058) and was steady in SITA (7.1±1.0 to 6.9±0.8%). CD34+ cells levels did not change in both groups during treatment. Whereas CD34+ CD133+, CD34+KDR+ e CD34+KDR+CD133+ did not change in INS, CD34+CD133+ (618±434 to 997±913 cell/ml; p=0.029), CD34+KDR+ (239±147 to 391±287 cell/ml; p=0.022) and CD34+KDR+CD133+ (116 ±94 to 206±158 cell/ml; p=0.021) increased significantly in SITA. Both treatments had no effects on EPCs adhesion and migration properties.

Conclusion: In T2DM subjects with advanced chronic kidney disease, sitagliptin but not insulin treatment increases circulating pool of stem/progenitor cells independently of glycaemic control. Both treatments did not affect EPCs functional properties. These data might have implications in the treatment of subjects with T2DM and any stage of advanced chronic kidney disease.

Supported by: Regione Toscana, Grant n. D55E11002680005

Disclosure: **R. Bellante:** None.

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Assessing the safety of sitagliptin in patients with type 2 diabetes and chronic kidney disease in the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS)

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Background and aims: Chronic kidney disease (CKD) is a common sequela of type 2 diabetes (T2DM), and is evident in up to 40% of patients with T2DM. TECOS, a randomized, double-blind, placebo-controlled trial that assessed the impact of sitagliptin on cardiovascular outcomes, provides an opportunity to examine comparative safety-related outcomes in patients with T2DM and CKD.

Materials and methods: Baseline demographic, diabetes-related and cardiovascular characteristics of patients with chronic kidney disease (CKD), defined as an eGFR <60 ml/min/1.73m^2, were summarized. The incidences of severe hypoglycemia and diabetes-related complications were examined for sitagliptin- and placebo-assigned patients in the Intent to Treat population.

Results: TECOS included 3,324 CKD patients (1,667 sitagliptin, 1,657 placebo) with mean (SD) age 68.8 (7.9) years and diabetes duration 13.7 (9.0) years; 62% were male. Over ~2.8 median years' follow-up, sitagliptin-assigned patients, compared with placebo-assigned patients, had generally similar rates of diabetic eye disease, diabetic neuropathy, renal failure, malignancy, bone fracture and pancreatitis (Table). The proportions of patients with hypoglycemia requiring assistance was 3.4% and 3.3% in the sitagliptin and placebo groups, respectively.

Conclusion: In TECOS, no specific safety concerns were identified with the use of sitagliptin in T2DM patients with CKD.

Proportions of CKD patients in TECOS with:	Sitagliptin N=1667	Placebo N=1657
Any diabetes complication	40.1%	42.1%
Diabetic eye disease	3.1%	3.1%
Diabetic neuropathy	3.9%	3.6%
Renal failure	3.3%	3.6%
Malignancy	4.3%	5.1%
Bone fracture	3.7%	3.3%
Pancreatitis	0.1%	0.1%

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Efficacy and safety of gemigliptin in type 2 diabetes patients with moderate to severe renal impairment (GUARD study)

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Background and aims: Renal impairment in type 2 diabetes mellitus (T2DM) limits the available glucose-lowering medication and requires frequent monitoring of renal function. Gemigliptin has balanced elimination between urinary/fecal excretion and hepatic metabolism. Thus, it needs no dose adjustment in patient with moderate to severe renal impairment. This study evaluated the efficacy and safety of gemigliptin in type 2 diabetic patients with moderate to severe renal impairment

Materials and methods: This randomized, double blind, parallel group Phase 3b study comprised a 12-week, placebo-controlled phase followed by a 40-week, double blind active-controlled extension phase. Patients (mean HbA1c 8.4%; age 62.0 years; BMI 26.2 kg/m^2, duration of T2DM 16.3 years; eGFR 33.3 mL/min/1.73 m^2) treated with gemigliptin (n=66) or placebo (n=66) for 12 weeks, then placebo group was switched to linagliptin 5 mg q.d and treatment continued to Week 52. Primary endpoint was HbA1c change from baseline at Week 12.

Results: At Week 12, adjusted mean ± SE change HbA1c with gemigliptin was -0.83 ± 0.14% (change with placebo 0.38 ± 0.14%; difference -1.21, 95% CI -1.54 to -0.89; p<0.0001). After 52 weeks, adjusted mean ± SE change from baseline in HbA1c was -1.00 ± 0.21% and -0.65 ± 0.22% in the gemigliptin and linagliptin groups, respectively. Urinary albumin creatinine ratio (UACR) at week 12 was reduced by 28.0% (95%CI -40.2 to -13.3) with gemigliptin compared with 4.3% (95%CI -19.7 to 14.2) with placebo, with a between-group difference of 24.8% (95%CI -41.8 to -2.9; p=0.0294). During the 40-week extension, adverse events (AEs) were reported in 68.0% and 73.1% of subjects on gemigliptin and linagliptin, respectively. The incidence of hypoglycemia was similar among treatment groups (gemigliptin, 20.0%; linagliptin, 28.8%). There was no meaningful change from baseline in body weight (gemigliptin, 0.28 kg; linagliptin 0.33 kg).
Conclusion: In conclusion, gemigliptin was efficacious and well tolerated in T2DM patients with moderate to severe renal impairment.
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Supported by: LG Life Sciences
Disclosure: **S. Yoon:** None.

PS 069 GLP-1 RA: new agents

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ITCA 650 provides a novel therapeutic approach to treating patients with type 2 diabetes

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Background and aims: ITCA 650 is a novel delivery system and glucagon-like peptide-1 (GLP-1) receptor agonist that can provide continuous subcutaneous (SC) exenatide for up to 12 months after subdermal placement of a small, 44 mm titanium osmotic mini-pump. Three and 6-month mini-pumps were studied in clinical trials of 39 weeks or more that showed significant reductions in HbA1c and body weight, and were well tolerated in patients with T2D who were uncontrolled on anti-diabetes medications. This novel drug/device can ensure maintenance of therapeutic exenatide levels and virtually ensures treatment adherence. We present the procedure experience from the FREEDOM Phase 3 program.
Materials and methods: Placement and removal of ITCA 650 is performed by trained healthcare professionals as a simple brief office procedure. The sterile mini-pump is placed in the sub-dermis of the abdominal wall using a placement tool, is removed or replaced through a small incision (~5 mm) and closed with Steri-Strips. Site personnel are provided with a kit containing all supplies and are trained on all procedures via an online and hands-on training program.
Results: As of November 2015, over 18,383 ITCA 650 placements, replacements, and removals were performed in 5,200 patients by physicians, nurse practitioners, and physician assistants at 493 clinical sites in 28 countries. Procedure adverse events (AEs) were generally mild, transient, and reflect the normal healing process. Superficial skin infection occurred in 0.4% of all procedures. Approximately 1% of procedures were initially unsuccessful and required further assistance to complete; this number that continues to decrease due to an optimized training and user experience program. Next generation placement aids will support continuous improvements. To date, only 0.19% of procedure AEs (0.7% of patients) resulted in treatment discontinuation. There were no procedure serious AEs.
Conclusion: Once or twice-yearly dosing of ITCA 650 has the potential to improve therapeutic outcomes. Procedures to place, replace, and remove the mini-pumps are simple, well tolerated, and have been performed safely by a wide variety of healthcare practitioners.
Supported by: Intarcia Therapeutics, Inc.
Disclosure: **A. Whitson:** Employment/Consultancy; Intarcia Therapeutics.

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Semaglutide reduces appetite and energy intake, improves control of eating and provides weight loss in subjects with obesity

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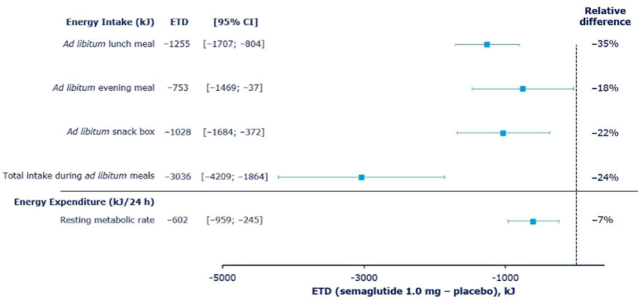
Background and aims: Glucagon-like peptide-1 (GLP-1) therapy has the potential to decrease body weight. Semaglutide is a human GLP-1 analogue in development for the treatment of type 2 diabetes. This study examined the mechanisms of body weight loss compared with placebo.
Materials and methods: This double-blind, crossover study compared once-weekly s.c. semaglutide (dose-escalated to 1.0 mg) with placebo in 30 subjects with obesity and without type 2 diabetes. The primary endpoint was ad libitum energy intake during lunch (5 h after standardised breakfast) after 12 weeks of treatment.

Results: Ad libitum energy intake at lunch and subsequent meals (evening meal, snacks) was lower with semaglutide versus placebo, as was resting metabolic rate, but to a lesser extent. Fasting overall appetite score (visual analogue scale) indicated reduced appetite with semaglutide versus placebo (p=0.0023), while nausea ratings were similar. For semaglutide versus placebo, the Control Of Eating Questionnaire indicated less hunger and food cravings and better control of eating; the Leeds Food Preference Task indicated a relatively lower preference for high-fat versus low-fat foods. Body weight (overall mean at baseline 101.3 kg; mean BMI 33.8 kg/m^2) was reduced by 5.0 ± 2.4 (SD) kg with semaglutide treatment compared with a body weight increase of 1.0 ± 2.4 kg with placebo, with proportionally more fat than lean body mass lost.

Conclusion: Semaglutide-induced body weight loss was confirmed. Possible mechanisms are: reduced energy intake, appetite and food cravings; better control of eating; and lower relative preference for fatty, energy-dense foods.

Figure: Energy intake during *ad libitum* meals and expenditure



CI, confidence interval; ETD, estimated treatment difference.

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Semaglutide improves postprandial glucose and lipid metabolism and delays first-hour gastric emptying in subjects with obesity

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Background and aims: Glucagon-like peptide-1 (GLP-1) therapies may delay gastric emptying and thus influence postprandial glucose and lipid responses. Semaglutide is a human GLP-1 analogue in development for the treatment of type 2 diabetes.This study investigated the effect of semaglutide on gastric emptying, and postprandial glucose and lipid responses, compared with placebo.
Materials and methods: This double-blind, crossover study compared once-weekly s.c. semaglutide (dose-escalated to 1.0 mg) with placebo in 30 subjects with obesity and without type 2 diabetes (mean BMI 33.8 kg/m^2). After each 12-week treatment period, subjects were given a standardised breakfast or standardised fat-rich breakfast. Fasting glucose metabolism was assessed prior to standardised breakfast, and postprandial glucose metabolism and gastric emptying were assessed after. Fasting lipid metabolism was assessed prior to standardised fat-rich breakfast, and postprandial lipid metabolism after.
Results: After 12 weeks of treatment, fasting concentrations of insulin and C-peptide were higher with semaglutide versus placebo, while glucose, glucagon, triglycerides and very low density lipoprotein were lower. After a standardised breakfast, postprandial glucose, insulin and C-peptide levels were lower with semaglutide versus placebo. After a standardised fat-rich breakfast, triglycerides, very low density lipoprotein and apolipoprotein B48 (which facilitates lipid absorption in the intestine) levels were lower for semaglutide versus placebo. No statistical difference between treatments was shown for the overall rate of postprandial gastric emptying ($AUC_{0\text{-}5h}$); however, for semaglutide versus placebo, gastric emptying was delayed during the first hour.
Conclusion: Semaglutide improves fasting and postprandial glucose levels, as well as fasting and postprandial lipid metabolism, versus placebo, and delays gastric emptying during the first hour.

Table: Fasting and postprandial analysis of glucose and lipid metabolism endpoints after 12 weeks of treatment with semaglutide versus placebo in subjects with obesity and without type 2 diabetes

Glucose metabolism parameters	Fasting values		Postprandial values ($iAUC_{0\text{-}5h}$)		
	ETR	95% CI	ETD	95% CI	Relative difference[a]
Glucose	0.95[b]	[0.91; 0.98]	−1.34[b] mmol*h/L	[−2.42; −0.27]	−38.5%
Insulin	1.45[b]	[1.20; 1.75]	−921[b] pmol*h/L	[−1461; −381]	−43.4%
Glucagon	0.86[b]	[0.75; 0.98]	−1.65 pg*h/mL	[−24.16; 20.85]	−5.0%
C-peptide	1.35[b]	[1.20; 1.52]	−1.42[b] nmol*h/L	[−2.33; −0.51]	−28.7%
Lipid metabolism parameters	**Fasting values**		**Postprandial values ($iAUC_{0\text{-}8h}$)**		
	ETR	**95% CI**	**ETD**	**95% CI**	**Relative difference[a]**
Triglycerides	0.88[b]	[0.80; 0.98]	−4.51[b] mmol*h/L	[−6.15; −2.87]	−40.7%
VLDL	0.79[b]	[0.66; 0.95]	−1.17[b] mmol*h/L	[−2.03; −0.32]	−42.8%
Apo B48	1.02	[0.86; 1.21]	−0.0455[b] g*h/L	[−0.0690; −0.0220]	−49.6%
FFA	0.99	[0.88; 1.11]	0.052 mmol*h/L	[−0.060; 0.163]	15.6%

ETR: Estimated treatment ratio; Semaglutide/placebo, ETD: Estimated treatment difference; Semaglutide – placebo
[a]Estimated treatment difference/estimated mean for placebo x100%.
[b]p<0.05.
Apo B48, serum apolipoprotein B48; FFA, free fatty acids; $iAUC_{0\text{-}5h}$, incremental area under the 0–5-hour curve; $iAUC_{0\text{-}8h}$, incremental area under the 0–8-hour curve; VLDL, very low density lipoprotein.

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Disclosure: **J. Hjerpsted:** Employment/Consultancy; Novo Nordisk A/S employee.

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Effect of once-weekly semaglutide on the counter-regulatory response to hypoglycaemia in subjects with type 2 diabetes

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Background and aims: Preservation of the counter-regulatory hormone response is important in patients with type 2 diabetes (T2D) treated with glucose-lowering agents associated with hypoglycaemia. The aim of this trial was to characterise the effect of semaglutide, a human glucagon-like peptide-1 analogue, on the hypoglycaemic response to ensure that safety with regards to counter-regulation is not compromised during hypoglycaemia in subjects with T2D treated with semaglutide.
Materials and methods: In this double-blind, crossover trial, 38 subjects with T2D on metformin alone were randomised to one of two treatment sequences: semaglutide/placebo or placebo/semaglutide. Each sequence comprised a 12-week period of once-weekly semaglutide s.c. (dose escalated to 1.0 mg) or placebo, with a washout period in between. After the last dose of treatment, a stepwise hypoglycaemic clamp was performed at plasma glucose (PG) target levels of 5.5, 3.5, 2.5 (nadir) and 4.0 (recovery) mmol/L via controlled i.v. insulin/glucose infusion. The day before the clamp, sampling for fasting glucagon and PG was performed (ambient level). During the clamp, counter-regulatory hormones (glucagon, adrenaline, noradrenaline, cortisol, growth hormone) and C-peptide were measured at each

PG target level. Glucose infusion rate (GIR) was also assessed, and hypoglycaemic symptoms scores (HSS) and cognitive function tests were performed. The primary endpoint was change in glucagon from PG target level 5.5 mmol/L to nadir.

Results: Baseline characteristics (mean) were: age 54.2 years, BMI 29.4 kg/m^2, HbA_{1c} 7.7%, T2D duration 4.5 years. During hypoglycaemia, there was a similar absolute change in glucagon response from PG target level 5.5 mmol/L to nadir with semaglutide vs placebo. Treatment with semaglutide vs placebo resulted in mean glucagon concentrations that were lower at PG target level 5.5 mmol/L (estimated treatment ratio, ETR [95% CI]: 0.77 [0.63;0.93]) and 3.5 mmol/L (0.71 [0.58;0.89]), but comparable at nadir (0.98 [0.86;1.12]). A trend towards a smaller increase in adrenaline, noradrenaline and cortisol levels from PG target level 5.5 mmol/L to nadir was seen with semaglutide vs placebo, with no difference in increase in growth hormone level. Semaglutide did not compromise the PG-dependent decrease in C-peptide level during hypoglycaemia and AUC_{GIR} during the clamp was similar with semaglutide vs placebo, indicating overall comparable counter-regulation. HSS and awareness at PG target level 3.5 mmol/L and nadir were lower with semaglutide vs placebo, with no difference in cognitive function tests. No new safety or tolerability issues were observed for semaglutide.

Conclusion: Treatment with semaglutide did not compromise the glucagon response or the glucose-dependent decrease in insulin secretion vs placebo during induced hypoglycaemia in subjects with T2D.

Table: Summary of change from PG target level 5.5 mmol/L to nadir[a]

	Semaglutide 1.0 mg	Placebo	ETD [95% CI] Semaglutide vs placebo
Ambient[b] fasting PG, mmol/L [mean]	6.6	8.8	N/A
Ambient[b] fasting glucagon, pg/mL [mean]	41.37	52.16	N/A
Primary endpoint Absolute glucagon change, pg/mL	88.28	83.07	5.21 [−7.72; 18.14]
Relative glucagon change*	5.39	4.23	ETR: 1.28 [1.04; 1.56]
Adrenaline, ng/mL	667.7	795.9	−128.2 [−279.0; 22.6]
Noradrenaline, ng/mL	246.0	316.4	−70.4 [−136.8; −4.1]
Cortisol, ng/mL	125.5	172.7	−47.2 [−80.7; −13.7]
Growth hormone, pg/mL	6.15	4.87	1.28 [−0.14; 2.70]
C-peptide, nmol/L	−0.68	−0.28	−0.4 [−0.49; −0.31]
AUC_{GIR} at nadir[a], mg/kg	29.4	30.9	−1.5 [−10.5; 7.5]

Note: Data are presented as estimated means unless otherwise stated
[a]Nadir: PG target level = 2.5 mmol/L. [b]Ambient = glucose and glucagon level the day before clamp, fasting conditions
AUC_{GIR} measured at nadir (not as a change from PG target level 5.5 mmol/L)
Abbreviations: ETD, estimated treatment difference ETR, estimated treatment ratio (*relative glucagon change is a ratio, no units); GIR, glucose infusion rate; N/A, not applicable; PG, plasma glucose

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Supported by: Novo Nordisk A/S
Disclosure: **S. Korsatko:** Other; Study was sponsored by Novo Nordisk.

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Efficacy and safety of once-weekly semaglutide monotherapy versus placebo in subjects with type 2 diabetes (SUSTAIN 1)

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Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). This study evaluated the efficacy, safety and tolerability of s.c. semaglutide monotherapy vs placebo in drug-naïve subjects with T2D.

Materials and methods: In this phase 3, double-blind study, 388 adults with T2D (HbA_{1c} 7-10%) were randomised to s.c. semaglutide 0.5 mg or 1.0 mg once weekly or placebo for 30 weeks, including 4-8 weeks of dose escalation. The primary endpoint was change in HbA_{1c} from baseline to Week 30. Secondary efficacy endpoints included body weight (BW), BP and other glycaemic parameters.

Results: Mean HbA_{1c} (baseline 8.1%) was reduced with semaglutide 0.5 mg and 1.0 mg by 1.5% and 1.6%, respectively, vs <0.1% in the placebo group (estimated treatment difference vs placebo [ETD] -1.4% and -1.5%; p<0.0001 for both). HbA_{1c} <7% was achieved by 74% and 72% of 0.5 mg and 1.0 mg semaglutide-treated subjects, vs 25% in the placebo group. The corresponding proportion of subjects achieving HbA_{1c} ≤6.5% was 59%, 60% and 13%. Mean fasting plasma glucose (baseline 9.8 mmol/L) was reduced with semaglutide 0.5 mg and 1.0 mg by 2.5 mmol/L and 2.3 mmol/L, respectively, vs 0.6 mmol/L with placebo (ETD -2.0 mmol/L and -1.8 mmol/L; p<0.0001 for both). Mean BW (baseline 91.9 kg) was significantly decreased with semaglutide 0.5 mg and 1.0 mg by 3.7 kg and 4.5 kg, respectively, vs 1.0 kg in the placebo group (ETD -2.8 kg and -3.6 kg; p<0.0001 for both). Changes in BP (baseline 128.8/79.3 mmHg) were comparable between the semaglutide 0.5 mg, 1.0 mg and placebo groups. Systolic BP decreased by -2.6 mmHg, -2.7 mmHg and -1.7 mmHg, respectively (ETD -0.9 mmHg and -1.0 mmHg). Corresponding changes in diastolic BP were -0.5 mmHg, 0.2 mmHg and 0.4 mmHg (ETD -0.9 mmHg and -0.2 mmHg). Pulse (baseline 74.2 beats per minute [bpm]) increased with semaglutide 0.5 mg and 1.0 mg by 2.5 bpm and 2.4 bpm, respectively, vs a 0.5 bpm decrease with placebo (ETD 2.9 bpm and 3.0 bpm). Adverse event (AE) and serious AE (SAE) rates were comparable between groups: 64.1%, 56.2% and 53.5% of patients reported AEs with semaglutide 0.5 mg, 1.0 mg and placebo, and 5.5%, 5.4% and 3.9% reported SAEs. The proportions of patients discontinuing due to AEs were low and similar for semaglutide 0.5 mg (6.3%) and 1.0 mg (5.4%), although both were higher than placebo (2.3%). The most frequent AEs with semaglutide were gastrointestinal (GI), which were mainly mild or moderate. The proportions of subjects reporting GI AEs in the 0.5 mg, 1.0 mg and placebo groups were 20.3%, 23.8% and 7.8% for nausea, 3.9%, 6.9% and 1.6% for vomiting, and 12.5%, 10.8% and 2.3% for diarrhoea. The rate of nausea diminished over time.

Conclusion: Semaglutide monotherapy, s.c. once-weekly doses of 0.5 mg and 1.0 mg, significantly improved glycaemic control and reduced BW vs placebo in patients with T2D. Semaglutide was well tolerated, with a safety profile similar to that of other GLP-1 receptor agonists.

Clinical Trial Registration Number: NCT02054897
Supported by: Novo Nordisk A/S
Disclosure: **C. Sorli:** Employment/Consultancy; Eli Lilly, Novo Nordisk. Lecture/other fees; Janssen, Novo Nordisk.

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Efficacy and safety of semaglutide once-weekly vs placebo as add-on to basal insulin alone or in combination with metformin in subjects with type 2 diabetes (SUSTAIN 5)

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Background and aims: Semaglutide is a glucagon-like peptide-1 (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). This study evaluated the efficacy and safety of once-weekly s.c. semaglutide (0.5 and 1.0 mg) vs placebo (PBO) in subjects with T2D on stable treatment with basal insulin ± metformin.

Materials and methods: In this phase 3a, double-blind study, 397 adults with T2D (HbA_{1c} 7-10%; mean duration 13.3 years; overall mean insulin dose at baseline 37.7 IU/day) were randomised 2:2:1:1 to semaglutide 0.5 or 1.0 mg or PBO 0.5 or 1.0 mg (PBO arms were pooled in the analyses)

once-weekly for 30 weeks, as an add-on to basal insulin ± metformin. Subjects with HbA_{1c} ≤8.0% at screening reduced their basal insulin dose by 20% at start of trial product, with up-titration (not to exceed pre-trial dose) permitted during weeks 10-16. The primary endpoint was change in HbA_{1c} from baseline to Week 30.

Results: Mean HbA_{1c} (overall baseline mean 8.4%) was reduced with semaglutide 0.5 and 1.0 mg by 1.4% and 1.8%, respectively, vs 0.1% with PBO (estimated treatment difference vs PBO [ETD] -1.36% and -1.76%; both p<0.0001). HbA_{1c} <7% was achieved by 61% and 79% of 0.5 and 1.0 mg semaglutide-treated subjects vs 11% with PBO; 41%, 61% and 5% achieved HbA_{1c} ≤6.5%, respectively. Mean body weight (BW; overall baseline mean 91.7 kg) was reduced by 3.7 kg, 6.4 kg and 1.4 kg with semaglutide 0.5 and 1.0 mg and PBO, respectively (ETD -2.28 kg and -5.03 kg; both p<0.0001). Other secondary endpoints are included in Table 1. Adverse events (AEs) were reported by 68.9%, 64.1% and 57.9% of subjects with semaglutide 0.5 and 1.0 mg, and PBO, respectively; 6.1%, 9.2% and 6.8% reported serious AEs spread over multiple system organ classes. Discontinuation due to AEs occurred in 4.5%, 6.1% and 0.8% of subjects, respectively. The majority of discontinuations with semaglutide were due to gastrointestinal (GI) AEs; all GI AEs were mild to moderate in severity. Severe/blood glucose-confirmed (plasma glucose value <3.1 mmol/L [56 mg/dL]) hypoglycaemia was reported by 8.3%, 10.7% and 5.3% of subjects, respectively.

Conclusion: Semaglutide (s.c. 0.5 and 1.0 mg once-weekly) provided superior glycaemic control (change in HbA_{1c}) and superior weight loss vs PBO in subjects with T2D on basal insulin. Semaglutide was well tolerated and had a safety profile similar to that of other GLP-1 receptor agonists.

Table 1. Key secondary outcomes from the SUSTAIN 5 study

	Overall baseline, mean	PBO n=133*	Semaglutide 0.5 mg n=132*		Semaglutide 1.0 mg n=131*	
		Change at Week 30	Change at Week 30	ETD vs PBO (95% CI)	Change at Week 30	ETD vs PBO (95% CI)
Fasting plasma glucose, mmol/L	8.6	−0.5	−1.6	−1.14 (−1.74;−0.54)[a]	−2.4	−1.87 (−2.47; −1.27)[b]
7-point SMPG: mean, mmol/L	11.2	−0.8	−2.5	−1.75 (−2.31;−1.18)[b]	−3.0	−2.27 (−2.83; −1.70)[b]
Postprandial increment of 7-point SMPG, mmol/L	3.1	−0.2	−1.2	−0.64 (−1.08;−0.21)[c]	−0.8	−0.99 (−1.42;−0.56)[b]
Systolic blood pressure, mmHg	134.8	−1.1	−4.3	−3.24 (−6.85;0.37)[d]	−7.3	−6.22 (−9.84;−2.60)[a]
Pulse rate, beats/min	73.5	−0.8	0.8	1.63 (−0.62;3.88)[f]	4.0	4.74 (2.48; 7.01)[b]
Overall treatment satisfaction (DTSQs)	28.3	1.2	2.7	1.48 (0.14;2.82)[g]	3.5	2.22 (0.87; 3.56)[h]

CI, confidence interval; DTSQs, Diabetes Treatment Satisfaction Questionnaire; ETD, estimated treatment difference; PBO, placebo; SMPG, self-measured plasma glucose. *Overall, 397 subjects were randomised; 396 subjects were exposed to treatment; inferential statistics are from a mixed model for repeated measurements; [a]p=0.0002; [b]p<0.0001; [c]p=0.0039; [d]p=0.0782; [e]p=0.0008; [f]p=0.1556; [g]p=0.0303; [h]p=0.0013

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Efficacy and safety of once-weekly semaglutide vs sitagliptin as add-on to metformin and/or thiazolidinediones after 56 weeks in subjects with type 2 diabetes (SUSTAIN 2)

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Background and aims: Semaglutide is a glucagon-like peptide (GLP-1) analogue in development for the treatment of type 2 diabetes (T2D). This study evaluated the efficacy, safety and tolerability of once-weekly subcutaneous (s.c.) semaglutide vs the DPP-4 inhibitor sitagliptin in subjects with T2D inadequately controlled on metformin (MET) and/or thiazolidinediones (TZD).

Materials and methods: In this phase 3, double-blind, double-dummy study, 1231 subjects with T2D (HbA_{1c} 7-10.5%) on MET and/or TZDs were randomised 2:2:1:1 to once-weekly semaglutide 0.5 mg or 1.0 mg or once-daily sitagliptin 100 mg (sitagliptin/semaglutide placebo arms were pooled in the analyses) for 56 weeks, including 4-8 weeks of dose escalation for semaglutide-treated subjects. Primary endpoint was change in HbA_{1c} from baseline to Week 56. Secondary efficacy endpoints included change in body weight, blood pressure and other glycaemic parameters.

Results: Mean HbA_{1c} (baseline 8.1%) was reduced by 1.3% and 1.6% with semaglutide 0.5 mg and 1.0 mg, respectively, vs 0.5% with sitagliptin (estimated treatment difference vs sitagliptin [ETD] -0.77% and -1.06%; p<0.0001 for both). HbA_{1c} <7% was achieved by 69% and 78% of 0.5 mg and 1.0 mg semaglutide-treated subjects vs 36% with sitagliptin; corresponding proportions of subjects achieving HbA_{1c} ≤6.5% were 53%, 66% and 20%. Mean body weight (baseline 89.5 kg) was reduced by 4.3 kg and 6.1 kg with semaglutide 0.5 mg and 1.0 mg vs 1.9 kg with sitagliptin (ETD -2.37 kg and -4.22 kg; p<0.0001 for both). Other secondary endpoints, including fasting plasma glucose and self-measured plasma glucose, also improved (Table 1). Proportions of subjects reporting adverse events (AEs) and serious AEs (SAEs) were comparable between groups: 74.8%, 71.4% and 71.7% of subjects reported AEs and 7.3%, 7.3% and 7.1% reported SAEs with semaglutide 0.5 mg, 1.0 mg and sitagliptin, respectively. Six fatal events occurred (2, 1 and 3 in the semaglutide 0.5 mg, 1.0 mg and sitagliptin arms, respectively). Proportions of subjects discontinuing treatment due to AEs were 8.1% for semaglutide 0.5 mg, 9.5% for semaglutide 1.0 mg and 2.9% for sitagliptin. The most frequent AEs were gastrointestinal (mainly mild or moderate in severity), and were reported by 43.5%, 39.9% and 23.6% of subjects in the semaglutide 0.5 mg, 1.0 mg and sitagliptin groups.

Conclusion: Semaglutide (s.c. once weekly) 0.5 and 1.0 mg was superior to sitagliptin in improving glycaemic control and reducing body weight in subjects with T2D inadequately controlled on MET and/or TZDs. Semaglutide was well tolerated with a safety profile similar to other GLP-1 receptor agonists.

Table 1. Key secondary outcomes from the SUSTAIN 2 study

	Overall baseline, mean	Sitagliptin 100 mg n=407*	Semaglutide 0.5 mg n=409*		Semaglutide 1.0 mg n=409*	
		Change at Week 56	Change at Week 56	ETD vs sitagliptin (95% CI)	Change at Week 56	ETD vs sitagliptin (95% CI)
Fasting plasma glucose, mmol/L	9.4	−1.1	−2.1	−0.97 (−1.25; −0.68)[†]	−2.6	−1.48 (−1.77; −1.20)[†]
24 h 7-point SMPG: mean, mmol/L	10.8	−1.1	−2.1	−0.97 (−1.23; −0.72)[†]	−2.4	−1.33 (−1.59; −1.07)[†]
Postprandial increment of 7-point SMPG, mmol/L	2.8	−0.6	−0.8	−0.18 (−0.39; 0.03)[‡]	−1.0	−0.38 (−0.59; −0.17)[§]
Systolic blood pressure, mmHg	132.6	−2.4	−5.1	−2.70 (−4.51; −0.89)[‡]	−5.6	−3.24 (−5.04; −1.44)[§]
Pulse rate, beats/min	75.7	0.6	1.6	1.02 (−0.12; 2.17)[¶]	1.8	1.27 (0.11; 2.42)**
Overall treatment satisfaction (DTSQs)	26.6	4.4	5.3	0.83 (0.18; 1.48)[††]	5.9	1.46 (0.81; 2.11)[†]
Mental component summary (SF-36v2)[¶¶]	48.7	0.5	2.2	1.72 (0.54; 2.89)[††]	2.2	1.64 (0.47; 2.81)[§§]

CI, confidence interval; DTSQs, Diabetes Treatment Satisfaction Questionnaire Status Version; ETD, estimated treatment difference; SF-36v2, Short-Form 36 Version 2; SMPG, self-measured plasma glucose

*Overall, 1231 subjects were randomised; 1225 subjects were exposed to treatment; inferential statistics are from a mixed model for repeated measurements; [†]p<0.0001; [‡]p=0.0926; [§]p=0.0004; [¶]p=0.0034; [¶]p=0.0806; **p=0.0314; [††]p=0.0118; [††]p=0.0042; [§§]p=0.0059; [¶¶]No significant difference was observed between treatment arms for the Physical Component Summary of the SF-36v2 questionnaire

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PS 070 Incretins and the heart

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Mortality risk following a non-fatal CV event in patients with type 2 diabetes: findings from the EXAMINE trial

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Background and aims: The EXAMINE trial patients had elevated cardiovascular (CV) risk due to type 2 diabetes and a recent (15-90 days) acute coronary syndrome (ACS). We evaluated the risk of CV death in patients randomized to treatment with alogliptin or placebo and following major non-fatal CV events that occurred during the trial.
Materials and methods: 5380 patients with type 2 diabetes were randomly assigned to alogliptin or placebo within 15 to 90 days of an acute coronary syndrome (ACS). Deaths and non-fatal CV events (myocardial infarction (MI), stroke, hospitalized heart failure (HHF), and hospitalization for unstable angina (UA) were adjudicated. Patients were followed until censoring or death, regardless of a prior post-randomized non-fatal CV event. Time-updated multivariable Cox models were used to estimate the risk of death in the absence of or following each non-fatal event.
Results: Overall rates of CV death were 4.1% for alogliptin and 4.9% for placebo (HR = 0.85, 95% CI, 0.66-1.10). There were a total of 736 patients (13.7%) who experienced at least one first non-fatal CV event (5.9% MI, 1.1% stroke, 3.0% HHF, and 3.8% UA). CV death occurred subsequently in 8.2% of those experiencing an MI event, 20.1% of those experiencing a HHF event, 8.8% of those experiencing a stroke, and 3.4% of those experiencing UA, versus 3.7% (n = 172) of the 4644 patients without a non-fatal CV event. Compared with patients who did not experience a non-fatal event, the adjusted hazard ratio for CV death was 3.12 (95% CI, 2.13-4.58, p <0.0001) after MI, 4.96 (95% CI, 3.29-7.47, p < 0.0001) after HHF, 3.08 (95% CI, 1.29-7.37, p = 0.011) after stroke, and 1.66 (95% CI, 0.81-3.37, p = 0.164) after admission for UA. Mortality rates following a non-fatal event were comparable on alogliptin and placebo.
Conclusion: In EXAMINE, the majority of deaths occurred in patients who did not experience a non-fatal CV event, although the risk of death was markedly higher following a non-fatal event, particularly HHF. These findings illustrate ongoing opportunities to reduce mortality in patients with type 2 diabetes and CV diseases.
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Disclosure: **W.B. White:** Employment/Consultancy; Takeda Development Center; EXAMINE Steering Committee. Honorarium; Takeda Development Center; EXAMINE Steering Committee.

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Haemodynamic effects of combination therapy with the DPP-4 inhibitor linagliptin and renin-angiotensin system inhibitors in patients with type 2 diabetes

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Background and aims: People with type 2 diabetes often require multidrug therapy for adequate vascular risk factor management. Previous mechanistic clinical studies hypothesised an unfavourable potential drug-drug interaction between dipeptidyl peptidase (DPP)-4 inhibitors and angiotensin-converting enzyme inhibitors (ACEi) by postulating a clinically relevant increase in sympathetic activity and rise in blood pressure. We therefore investigated whether initiation of the DPP-4 inhibitor linagliptin on a background of renin-angiotensin system (RAS) blockade may alter clinical haemodynamic conditions.
Materials and methods: MARLINA-T2D™, a multicentre, double-blind, placebo-controlled clinical trial, randomised 360 patients with type 2 diabetes on a stable background of single RAS blockade (ACEi 33.3% [*n*=120]; angiotensin receptor blocker [ARB] 66.7% [*n*=240]) to either linagliptin (*n*=182) or placebo (*n*=178). Twenty-four-hour ambulatory blood pressure monitoring (ABPM) was considered as an exploratory safety endpoint and was conducted at baseline (BL) and after 24 weeks of treatment.
Results: In the treated set population, ABPM data were available for 298 patients (linagliptin, *n*=155; placebo, *n*=143) at baseline. Overall mean (SE) BL 24-h systolic blood pressure (SBP), diastolic BP (DBP), and pulse rate were 132.7 (0.7) mmHg, 75.9 (0.5) mmHg, and 77.3 (0.6) bpm, respectively. At week 24, treatment with linagliptin was not associated with any clinically relevant haemodynamic changes: placebo-adjusted mean (SE) 24-h SBP, DBP, and pulse rate changes from BL were 0.0 (1.4) mmHg (95% CI -2.7, 2.7; NS), 0.0 (0.8) mmHg (95% CI -1.5, 1.5; NS), and 0.8 (0.7) bpm (95% CI -0.7, 2.2; NS), respectively. The presence of either ACEi or ARB background therapy at BL provided similar results (Table).
Conclusion: Adding linagliptin to stable RAS blockade background therapy was not associated with systemic haemodynamic changes. Our study supports its concomitant use for dual enzyme blockade of DPP-4 and ACE in patients with type 2 diabetes.

Table. Change from baseline in 24-hour blood pressure and 24-hour pulse rate at week 24 by background ARB or ACEi therapy at baseline: treated set (OC-H)*

Background therapy at baseline	Treatment	*n*	Adjusted[§] mean (SE) change at week 24	Treatment difference (linagliptin – placebo)	*p*-value
24-h systolic BP, mmHg					
ARB	Linagliptin	81	0.4 (1.1)	0.9 (1.6)	0.5825
	Placebo	69	−0.5 (1.2)		
ACEi	Linagliptin	30	−1.4 (1.8)	−2.3 (2.6)	0.3789
	Placebo	28	0.9 (1.9)		
24-h diastolic BP, mmHg					
ARB	Linagliptin	81	−0.2 (0.6)	0.2 (0.9)	0.8260
	Placebo	69	−0.4 (0.7)		
ACEi	Linagliptin	30	−0.9 (1.0)	−0.6 (1.4)	0.6712
	Placebo	28	−0.3 (1.0)		
24-h pulse rate, bpm					
ARB	Linagliptin	81	1.4 (0.6)	1.3 (0.9)	0.1351
	Placebo	69	0.1 (0.6)		
ACEi	Linagliptin	30	1.8 (1.0)	−0.5 (1.4)	0.7388
	Placebo	28	2.3 (1.0)		

OC-H=observed cases without values following a change in antihypertensive therapy; BP=blood pressure; ARB=angiotensin receptor blocker; ACEi=angiotensin-converting enzyme inhibitor; UACR=urinary albumin-to-creatinine ratio.
* Data were available for 208 patients (linagliptin, *n*=111; placebo, *n*=97).
§ ANCOVA model includes baseline mean 24-hour of the relevant parameter, baseline $\log_{10}$ (UACR), and baseline HbA_{1c} as linear covariates and treatment, relevant background therapy at baseline, and relevant background therapy at baseline by treatment interaction as fixed effects.

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Disclosure: **M.E. Cooper:** Employment/Consultancy; Baker IDI Heart & Diabetes Institute. Lecture/other fees; Boehringer Ingelheim.

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Effect of lixisenatide on heart rate: a pooled analysis of eight GetGoal studies

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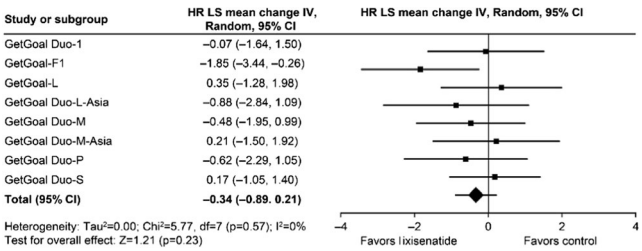
Background and aims: Accelerated heart rate (HR) may be associated with increased cardiovascular disease, prompting attention to possible glucagon-like peptide-1 receptor agonist (GLP-1 RA) effects on this parameter. This exploratory analysis assessed HR in patients with type 2 diabetes from eight Phase III trials of lixisenatide, a short-acting GLP-1 RA, in the GetGoal clinical trial programme.
Materials and methods: HR (pulse) data were collected from routine vital signs recordings prior to dosing (24h post previous injection).
Results: Using pooled data from the eight studies (N=3687), no significant difference was seen with lixisenatide vs placebo in vital sign HR data using within-study least squares mean difference (Figure). Furthermore, data were retrospectively pooled, grouped by baseline HR (≥90, 60-89, <60 bpm) and changes in HR group at Week 24 were analysed (Table). The majority of lixisenatide and placebo patients experienced a change in HR of +/-5 bpm from baseline to Week 24. No difference in HR was seen at Week 24 with lixisenatide vs placebo across the baseline HR patient categories. In addition, a comprehensive sensitivity analysis involving regression and meta-analyses considered whether preselected predictive factors correlated with HR change from baseline to Week 24. No significant difference was observed between the treatment groups.
Conclusion: Lixisenatide had no substantial effect on HR in patients with type 2 diabetes, with baseline HR not serving as a predictor in most patients in these eight studies.



Baseline HR, bpm	Patients with HR in specified ranges at Week 24, n (%)*					
	Lixisenatide			Placebo		
	≥90	60–89	<60	≥90	60–89	<60
≥90 (N, lixisenatide 124; placebo 77)	38 (30.7)	86 (69.4)	0 (0.0)	28 (36.4)	49 (63.6)	0 (0.0)
60–89 (N, lixisenatide 2068; placebo 1221)	90 (4.4)	1908 (92.3)	70 (3.4)	64 (5.2)	1129 (92.5)	28 (2.3)
<60 (N, lixisenatide 118; placebo 79)	1 (0.9)	62 (52.5)	55 (46.6)	1 (1.3)	38 (48.1)	40 (50.6)

*Data pooled from 8 studies listed above
bpm=beats per minute; CI=confidence interval; HR=heart rate; IV=independent variable: LS=least squares

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The effect of liraglutide on body composition among patients with heart failure with and without type 2 diabetes: a sub-study from the LIVE randomised clinical trial

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Background and aims: Glugagon-like peptide-1 receptor agonists (GLP-1RA) are increasingly used in heart failure (HF) patients with type 2 diabetes (T2D) and obesity. The weight reduction effect however, is questionable in HF patients, as available evidence suggests that low BMI is predictive of poor outcome. The most likely explanation of this obesity paradox is underlying cardiac wasting with loss of muscle mass, instead of reduction in metabolically active abdominal fat mass, which supposedly should be beneficial in these patients. The aims of the present study were to investigate the impact of liraglutide on body composition in HF patients with and without T2D.
Materials and methods: Double-blinded placebo controlled trial randomizing 102 patients with HF and reduced left ventricular ejection fraction (LVEF) ≤ 45% in a pre-specified sub-study from the LIVE trial. Patients were randomized 1:1 to liraglutide 1.8 mg or placebo once daily for 24 weeks. At baseline and after 24 weeks dual energy absorptiometry (DXA) scanning were obtained measuring whole body composition and fat distribution, divided into abdominal and gyneoid fat, and with measurements of visceral adipose tissue (VAT) volume using a 5 cm high transverse slice at L4/L5 level.
Results: A total of 32 patients had T2D by history or newly diagnosed by HbA1c. Mean (± SD) age was 66.9 (±10.6) years, BMI: 28.5 (±4.5) kg/m2, LVEF: 35 (±7) % and the majority (86%) were male. Patients with T2D were well regulated with HbA1c of 6.6% (±1.0). Intervention with liraglutide for 24 weeks decreased total body weight compared with placebo (mean difference: -2.1 kg (95% CI -3.5, -0 6 kg; p= 0.005) and the mean difference between groups in HbA1c was -0.2% (95% CI -0.5, 0.2; p=0.08). Treatment with liraglutide reduced total body fat mass compared with placebo with a mean difference of -1.1 kg (-2.1, - 0.5; p= 0.04), and a significant difference in change of abdominal fat -0.23 kg (-0.43, -0.02; p= 0.03) was observed. There was no change in gyneoid fat after 24 weeks in neither group. Visceral abdominal fat volume decreased on liraglutide therapy (728 ± 316 to 676 ± 323 cm3, p< 0.001), with no change in VAT volume in the placebo group (p= 0.67), and no significant difference between groups (p=0.17). Lean tissue mass did not change significantly during treatment with liraglutide (57.4 ± 11.7 vs 56.3 ± 11.8 kg; p= 0.13) or with placebo (58.9 ± 10.5 vs 59.6 ± 11.0 kg; p= 0.15) although, a significant difference between groups was observed (mean difference: -1.7 kg (-3.3, -0.76; p=0.04). In patients with T2D a comparable difference in change of mean lean tissue mass was observed during treatment with liraglutide: -2.7 kg (-4.7, -0.8; p=0.008) while, no differences between groups with respect to other parameters of body composition were demonstrated in T2D patients.
Conclusion: Treatment with liraglutide in HF reduces weight, predominantly due to a loss of abdominal fat and VAT. However, also a significant difference in change of lean tissue mass during treatment with liraglutide in both HF patients with and without T2D was observed, indicating loss of muscle mass, which may well be a concern in these patients.
Clinical Trial Registration Number: NCT01472640
Supported by: Novo Nordisk A/S
Disclosure: **C. Kistorp:** Grants; Unristricted grant Novo Nordisk.

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Treatment with liraglutide increases 24-hour heart rate in chronic heart failure patients with and without type 2 diabetes: a sub-study from the LIVE randomised clinical trial

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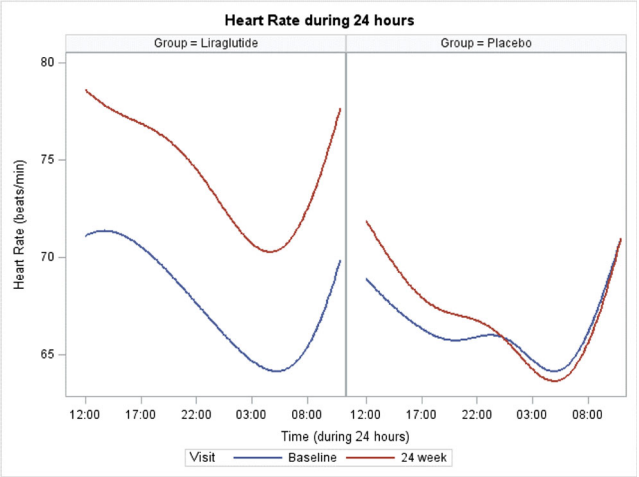
Background and aims: Glucagon-like peptide-1 receptor agonist (GLP-1RA) has been reported to increase heart rate (HR) and decrease blood pressure (BP), which could be of clinical importance in patients with chronic heart failure (CHF) with reduced ejection fraction and linked with adverse outcome. Therefore, we investigated the effect of Liraglutide on 24-hour BP and HR in CHF patients on optimal dose of ACE-inhibitors and beta blockers with and without type 2 diabetes (T2D).
Materials and methods: A randomized double-blind, placebo-controlled trial. A total of 31 patients with CHF (NYHA I-III), 32% with T2D were randomized 1:1 to either liraglutide or placebo 1.8 mg once daily for 24 weeks. At baseline and after 24 weeks 24-hour BP and HR were measured. Daytime was defined as 06:00 AM to 11:00 PM and night from 11:00 PM to 6:00 AM. Blood pressure was measured every 20-minute during daytime and every 60-minute during the night, using IEM Mobil-O-Graph New Generation 24h ABPM Classic.
Results: Thirty-one patients with CHF were included in the LIVE sub-study (15 in the liraglutide group and 16 in the placebo group). Five participants withdrew from the study due to side-effects (four treated with liraglutide and one with placebo, p=0.17). Participants were predominantly (n=30) male, with a mean (±SD) age of 64 (±8) years. At baseline there was no difference between treatment groups in mean 24-hour systolic BP (SBP): liraglutide 118 vs placebo 117mmHg, p=0.88; diastolic BP (DBP): liraglutide 76 vs placebo 73 mmHg, p =0.46 or HR: liraglutide 68 vs placebo 67 bpm, p=0.63. An absolute increase in mean 24-hour HR was observed in the liraglutide group after 24 weeks (75 bpm (95% CI 70-80), p= 0.02), whereas no change was observed in the placebo group at week 24 (68 bpm (95% CI 63-73), p=0.26). The change in mean 24-hour HR differed significantly between the two groups, mean difference at week 24: 7 bpm (95% CI: 1-13, p=0.053). The diurnal monitoring revealed that the HR increase observed in the liraglutide group was persistent during night (Figure). We did not observe any impact of liraglutide on mean 24-hour SBP liraglutide 114 mmHg vs placebo 116 mmHg, p=0.70 or DBP liraglutide 74 mmHg vs placebo, 72 mmHg, p=0.62.
Conclusion: The GLP-1 agonist liraglutide increases mean 24-hour HR by 7 bpm among patients with CHF with reduced ejection fraction despite optimal beta-blocker therapy with a significant difference in change from baseline as compared with controls. No effect on 24-hour BP was shown. The clinical consequences of this increased HR in patients with HF and T2D could be a concern.



Clinical Trial Registration Number: NCT01472640
Supported by: Novo Nordisk A/S
Disclosure: **M.L. Johansen:** None.

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Effect on cardiac function of exercise combined with glucagon-like peptide-1 receptor agonist treatment: a randomised double-blind placebo-controlled clinical trial

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Background and aims: In patients with type 2 diabetes, supervised exercise may improve cardiac function. We evaluated the effect of supervised exercise combined with glucagon-like peptide-1 receptor agonist (GLP-1RA) treatment on cardiac function in sedentary patients with type 2 diabetes.
Materials and methods: Thirty-three dysregulated (HbA1c: 65±14 mmol/mol), and overweight (BMI: 32±4 kg/m2) patients with type 2 diabetes on diet and/or metformin treatment were randomly assigned to exercise (3 supervised 60-minute training sessions per week) in addition to either liraglutide (1.8 mg once-daily) or placebo for 16 weeks. All patients underwent echocardiography including color tissue Doppler and 2D speckle tracking.
Results: Results are shown in the table. Measures of left ventricular (LV) diastolic function (assessed as early diastolic myocardial velocity (e')) were improved in the placebo group (-7.1±1.6 (mean±SD) to -7.7±1.8 cm/s, P=0.01), but not in the liraglutide group (-7.1±1.4 to -7.0±1.4 cm/s, P=0.60; between groups: P=0.02). Similarly, the ratio of early and atrial LV myocardial filling velocities (e'/a') improved in the placebo group (1.0±0.4 to 1.2±0.4, P=0.003), but not in the liraglutide group (1.0±0.3 to 1.0±0.3, P=0.87; between groups: P=0.03). No changes in heart rate were observed in any of the groups (placebo: 70±12 to 69±13 bpm, P=0.50; liraglutide: 70±9 to 71±9 bpm, P=0.82; between groups: P=0.32). We found no significant differences in LV structural adaptations including LV wall diameters, internal dimensions and mass within or between the placebo and the liraglutide groups. LV systolic function, i.e. ejection fraction and global longitudinal strain, were also similar within and between the two groups.
Conclusion: Addition of the GLP-1RA liraglutide to exercise in sedentary patients with dysregulated type 2 diabetes apparently seems to blunt the beneficial effect of exercise on LV diastolic function. Further research is needed to ascertain these findings and illuminate their clinical implications.

		Before exercise	After exercise	*P* value Before/after	*P* value Between group
Systolic measurements					
Peak systolic velocity (cm/s)	Liraglutide	5.9 (1.3)	5.9 (1.2)	0.92	0.67
	Placebo	6.0 (0.9)	5.9 (0.7)	0.63	
Global longitudinal strain (%)	Liraglutide	-17.8 (2.7)	-17.5 (2.4)	0.71	0.57
	Placebo	-18.1 (2.0)	-18.1 (1.7)	0.97	
Left ventricular ejection fraction (%)	Liraglutide	59.2 (6.1)	59.7 (5.1)	0.79	0.81
	Placebo	60.7 (6.6)	60.8 (6.9)	0.93	
Diastolic measurements					
E/e'	Liraglutide	8.1 (1.9)	7.5 (2.3)	**0.04**	0.08
	Placebo	8.2 (2.3)	8.5 (2.9)	0.45	
e' (cm/s)	Liraglutide	**-7.1 (1.4)**	**-7.0 (1.4)**	0.60	**0.02**
	Placebo	**-7.1(1.6)**	**-7.7 (1.8)**	**0.01**	
a' (cm/s)	Liraglutide	-7.4 (1.6)	-7.13 (1.2)	0.34	0.23
	Placebo	-7.2 (1.1)	-6.6 (1.0)	0.11	
e'/a'	Liraglutide	**1.0 (0.3)**	**1.0 (0.3)**	0.87	**0.03**
	Placebo	**1.0 (0.4)**	**1.2 (0.4)**	**0.003**	

E= peak early mitral inflow velocity, e' = Peak early diastolic myocardial velocity, a' = Peak atrial diastolic myocardial velocity.
Bold text indicates p<0.05

Clinical Trial Registration Number: NCTO1455441
Disclosure: **P.G. Jørgensen:** None.

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Impact of sitagliptin on cardiovascular and safety-related outcomes in insulin-treated type 2 diabetes: the TECOS experience

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Background and aims: The Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS) was a randomized, double-blind, placebo-controlled trial that assessed the cardiovascular (CV) safety of sitagliptin when added to existing glucose-lowering therapies in 14,671 patients with type 2 diabetes and prior CV disease. The large subset of patients treated with insulin at baseline (N=3,408) provides an opportunity to examine the impact on glycaemic, cardiovascular, and safety-related outcomes when sitagliptin is added to insulin therapy.
Materials and methods: Baseline demographic, diabetes-related and cardiovascular characteristics were summarized for patients on insulin compared with other glucose-lowering therapies. Among insulin-treated patients, HbA_{1c} and estimated GFR (eGFR) changes over time, and rates of key cardiovascular endpoints, severe hypoglycaemic episodes and diabetes-related complications were examined in the intent-to-treat population for sitagliptin vs. placebo-treated patients. Serious adverse events (SAEs) were also examined in the randomized population that received ≥1 dose of study medication.
Results: Those treated with insulin at baseline, compared with non-insulin-treated patients, were slightly older (66.1 vs. 65.3 years), had longer diabetes duration (17.1 vs. 9.9 years), higher HbA_{1c} (7.4% vs. 7.2%), lower eGFR (71.0 vs. 76.1 ml/min/1.73m^2), and were more likely to have prior heart failure (22.6% vs. 16.6%). A lower HbA_{1c} was seen at 4 months in patients using insulin at baseline allocated to sitagliptin (0.39%) compared with placebo, and over the study duration (0.30%, p<0.001 for both). Similar rates were observed for severe hypoglycemia (3.5% vs. 4.0%), a composite of major adverse cardiovascular events (12.4% vs. 12.3%), the individual components of the composite [CV-related death (4.4% vs. 4.6%), nonfatal MI (5.5% vs. 5.7%) and nonfatal stroke (2.4% vs. 2.0%)], and hospitalizations for heart failure (3.9% vs. 4.8%) in the sitagliptin and placebo groups respectively. Rates of diabetes-related complications and SAEs were also similar in the two treatment groups (Table).
Conclusion: In TECOS, among patients treated with insulin at baseline, similar rates of cardiovascular outcomes, diabetes-related complications and SAEs were observed in the sitagliptin and placebo treatment groups. The slightly greater reduction in HbA_{1c} seen with the addition of sitagliptin did not result in an increase in the incidence of severe hypoglycemia.

Proportion of Insulin-treated Patients with Incident Complications of:	Insulin + Sitagliptin (N=1,724)	Insulin + Placebo (N=1,684)
Serious Adverse Events	14.3%	13.1%
Diabetic eye disease	5.6%	4.2%
Diabetic nephropathy	8.7%	9.0%
Renal failure	1.8%	2.3%
Diabetic neuropathy	5.4%	5.0%
Peripheral arterial disease	3.8%	3.3%
Amputation	1.5%	1.9%
Gangrene	1.0%	1.4%

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Effectiveness of lixisenatide in nonalcoholic fatty liver disease in patients with type 2 diabetes after an acute coronary syndrome compared to sitagliptin and pioglitazone

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Background and aims: NAFLD shows a wide disease spectrum ranging from simple steatosis to steatohepatitis and finally cirrhosis while it is also associated with the presence and morphology of subclinical coronary atherosclerosis. Lixisenatide is a short acting glucagon-like peptide-1 (GLP-1) which improves glycaemic control and beta-cell function and reduces body weight. Sitagliptin is a widely used DPP-4 inhibitor while pioglitazone already has several clinical evidence in the treatment of NAFLD. The aim of the study was to examine the effectiveness of lixisenatide in NAFLD patients with type 2 Diabetes Mellitus (T2DM) after an Acute Coronary Syndrome (ACS) compared with sitagliptin and pioglitazone.
Materials and methods: 117 T2DM patients with NAFLD and a medical history of ACS were included in the study. 39 patients were under treatment with lixisenatide, 36 under treatment with pioglitazone and 42 under treatment with sitagliptin. All patients also received metformin. The patients' somatometric and laboratory characteristics were recorded, as well as metabolic comorbidities for the duration of the study (mean follow up period was 9±2 months). The evaluation of liver fibrosis depended on calculation of aspartase aminotransferase (AST) to platelet counts ratio (APRI) index. APRI over 1.5 was considered as bridging fibrosis and over 2.0 as liver cirrhosis. All patients went through an ultrasonography before being included in the study and after the end of the study.
Results: The study's patients were aged 60.8 ± 8.6 years without differences between groups under study and mean duration of T2DM was 5.7 ±3.2 years without differences between treatment (p=0.285) while there were also no differences in Body Mass Index (BMI) (p=0.447) and HbA1c (p=0.204) with mean value 7.72±0.92%. HbA1c values improved in all three groups with most patients reaching the therapeutic goal in the lixisenatide (p=0.036) group. APRI index's improvement was also higher in the lixisenatide group (1.05 (0.48-1.21) vs 0.79 (0.37-0.94), p<0.01) compared to that in the pioglitazone group (1.09 (0.52-1.29) vs 0.88 (0.48-1.01), p=0.011) while there was no improvement in the sitagliptin group (0.98 (0.36-1.06) vs 0.95 (0.37-1.08) p=0.376). APRI index's improvement was accompanied by a significant change of fatty liver in ultrasonography. The decrease of body weight in the lixisenatide group was statistically significant (p5% decrease.

Conclusion: Administration of lixisenatide led not only to good control of T2DM but also improvement of liver inflammation, alteration of liver fibrosis, and reduction of body weight, which are particularly important factors in patients with T2DM after an ACS. Aggravation of liver fibrosis score might lead to future liver cirrhosis, and body weight gain, observed in the pioglitazone group, could exacerbate liver inflammation and other metabolic disorders. As far as we know, this study is the first to be carried out on the effect of lixisenatide (a short acting GLP-1 agonist) on liver fibrosis which indicates its positive impact in T2Dm with NAFLD after an ACS. Particularly, body weight reduction was a favorable outcome of applying lixisenatide in NAFLD patients with T2DM after an ACS.
Disclosure: **A. Koutsovasilis:** None.

PS 071 DPP-4 inhibitors

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Characteristics of elderly patients initiating sitagliptin and non-DPP-4i oral antihyperglycaemic agents: a cross-sectional US claims database analysis

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Background and aims: Previous analyses of sitagliptin prescribing patterns concluded that new users of sitagliptin <65 years of age were older and had more comorbidities and complications than new users of other oral antihyperglycemic agents (OAHAs). However, treatment patterns in older patients (≥65 years), who tend to have more chronic problems, have not been analyzed. This study sought to determine the differences in baseline characteristics and comorbidities of elderly patients with type 2 diabetes mellitus (T2DM) initiating sitagliptin vs. non-DPP-4i OAHA.
Materials and methods: Patients ≥65 years of age with T2DM were identified from MarketScan® Medicare Supplemental Database between 2006 and 2014. Eligible patients were categorized according to their complexity of antihyperglycemic treatment: initiating monotherapy, escalating to dual combination therapy and escalating to triple therapy. Within each category, the comparison between sitagliptin and non-DPP-4i OAHA was made within 3 different age groups: 65-74 years, 75-84 years, and ≥85 years. Gender and comorbidity recorded within the 12 months prior to the index date (defined as date of initiation/escalation of antihyperglycemic treatment) were assessed as baseline characteristics in each group. Differences in each covariate between those initiating sitagliptin and non-DPP-4i OAHA were compared using standardized differences, with a 10% difference indicating a meaningful difference.
Results: Patients with T2DM who initiated treatment with sitagliptin tended to be older than those initiating non-DPP-4i OAHAs. The results of this analysis were stratified by age and treatment complexity and are presented in Table 1. In general, patients initiating treatment with sitagliptin were more likely to have a pre-treatment history of arrhythmia, congestive heart failure, peripheral vascular disease, renal failure, and stroke. However, this pattern varied across subgroups defined by age and treatment complexity, with the most pronounced differences observed between patients initiating monotherapy in all 3 age groups. As treatment complexity advanced to dual combination therapy, the differences were attenuated and mostly observed in the 75-84 and ≥85 age groups. In patients aged 65-74 years initiating triple therapy, no differences were observed between groups.
Conclusion: Similar to prior observations in patients <65 years of age, elderly patients (≥65 years) with T2DM initiating sitagliptin tend to be older and have more comorbidities than those prescribed other classes of OAHA. The differences in baseline characteristics between treatment groups vary by age and the complexity of antihyperglycemic treatment, the latter likely reflecting the limitation of additional treatment choices with increasing treatment complexity. Appropriate adjustment is required to minimize the impact of potential confounding and channeling bias in any comparative analyses that include users of sitagliptin.

Table 1. Baseline characteristics of patients with T2DM up to 1 year before initiating treatment with sitagliptin or non-DPP-4i oral antihyperglycemic agent: standardized difference stratified by treatment complexity and age

	Mono therapy			Dual combination therapy			Triple therapy		
Comobidity[1]	65-74 yr	75-84 yr	≥85 yr	65-74 yr	75-84 yr	≥85 yr	65-74 yr	75-84 yr	≥85 yr
Arrhythmia	**16.1%**	**14.5%**	**15.4%**	2.6%	**13.2%**	7.6%	0.7%	6.1%	4.8%
Congestive heart failure	**16.5%**	**18.3%**	**14.9%**	5.4%	**12.5%**	**11.6%**	5.6%	4.7%	2.6%
Cognitive impairment	5.2%	5.4%	-7.9%	0.6%	2.0%	1.4%	-3.1%	2.1%	**-13.8%**
Hearing loss	7.4%	1.4%	1.3%	2.0%	0.2%	7.4%	1.4%	-5.1%	**12.5%**
Hypertension	6.5%	**15.4%**	9.0%	**11.6%**	**12.0%**	6.6%	9.1%	**12.6%**	8.0%
Myocardial Infarction	6.0%	11.5%	9.7%	3.2%	4.8%	1.5%	-1.9%	2.6%	7.0%
Neuropathy	4.5%	8.8%	0.5%	1.8%	1.1%	3.8%	-2.7%	3.4%	**10.9%**
Peripheral vascular disease	**14.2%**	**10.1%**	**16.7%**	0.3%	5.9%	7.6%	4.6%	1.7%	-1.0%
Renal failure	**13.8%**	**12.7%**	**16.2%**	7.2%	**12.3%**	**20.2%**	1.8%	1.3%	-2.9%
Stroke	**14.9%**	**12.3%**	**10.8%**	2.0%	8.7%	**10.0%**	4.1%	3.9%	3.7%

1. There were no differences between treatment groups in the following baseline characteristics: gender, blindness, cataract, fracture, hypoglycemia, proteinuria, and retinopathy.

Disclosure: **T. Wang:** Employment/Consultancy; Merck & Co., Inc. Stock/Shareholding; Merck & Co., Inc.

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Safety and tolerability of alogliptin in patients with type 2 diabetes: pooled analysis of 20 double-blind randomised controlled clinical studies

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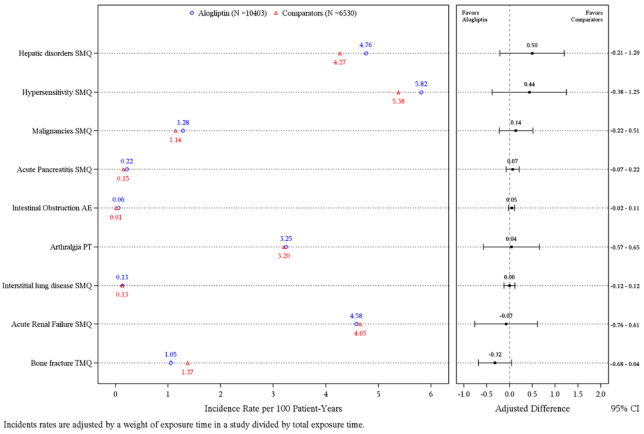
Background and aims: Alogliptin is an orally available, highly selective, and potent inhibitor of the dipeptidyl-peptidase-4 (DPP-4) enzyme, developed as a novel antihyperglycemic agent. It is approved for use in the treatment of type 2 diabetes mellitus (T2DM) in many countries worldwide. This analysis provides a safety and tolerability assessment of alogliptin using pooled data from 20 double-blind clinical studies.

Materials and methods: The analysis includes data from 16933 T2DM patients in 20 studies who received either alogliptin (n=10403, alogliptin group) or a comparator agent (n = 6530, non-exposed group consisting of placebo and other antidiabetic agents). The studies were, double-blind, randomized, controlled trials conducted by the Sponsor that included patients treated with the alogliptin between 12 weeks and 4.5 years. The studies assessed alogliptin versus comparator, taken as monotherapy, initial combination therapy with metformin or thiazolidinediones or as add-on combination therapy with other antihyperglycemic agents (metformin, sulfonylurea, thiazolidinediones, insulin, thiazolidinediones + metformin, thiazolidinediones + sulfonylurea, insulin + metformin, or standard of care). The primary assessment was based on overall safety data and analysis of AEs of special interest (AESIs) based on SMQs, including: hepatic, hypersensitivity, malignancies, acute pancreatitis, and acute renal failure. Other safety endpoints included arthralgia (preferred term ([PT]), bone fracture (sponsor defined custom MedDRA query [TMQ]), intestinal obstruction (PT), and interstitial lung disease (SMQ). Additionally, hypoglycemia was assessed using special CRFs. Cardiovascular events and heart failure events were assessed in a long term cardiovascular study using adjudicated events. To account for potential differences between groups in duration of exposure to treatment, reports of AEs were expressed as exposure-adjusted incidence rates (numbers of patients with events per 100 patient-years). Differences between treatment groups and the associated 95% CI were calculated using the Miettinen and Nurminen method, stratified by study. Cardiovascular events and heart failure were assessed using the Cox model.

Results: Overall, the incidence rates of AESIs, additional safety endpoints, and hypoglycemia incidence were similar between the alogliptin and comparator group in terms of overall incidence, relatedness, and severity. Treatment with alogliptin was not associated with an increased risk of adverse events. In the large cardiovascular outcome study, there was no evidence of an increase in cardiovascular events or heart failure.

Conclusion: In this pooled safety analysis of data from 16933 patients with T2DM, alogliptin was generally well tolerated in clinical trials of up to 4.5 years in duration. The large cardiovascular outcome study did not show any evidence of an increase in cardiovascular events or heart failure AESIs



Incidents rates are adjusted by a weight of exposure time in a study divided by total exposure time.

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Disclosure: **M.S. Munsaka:** Employment/Consultancy; Fulltime employee of Takeda, Deerfield, IL.

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Linagliptin (LINA) as add-on to empagliflozin (EMPA) and metformin in patients with type 2 diabetes (T2DM): two 24-week randomised, double-blind, parallel-group trials

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Background and aims: Two Phase III studies investigated the efficacy and safety of LINA 5 mg vs placebo (PBO) as add-on to EMPA 10 mg or EMPA 25 mg, respectively, and metformin in patients with T2DM.

Materials and methods: Patients with HbA_{1c} ≥8.0 and ≤10.5% while receiving stable-dose metformin received open-label EMPA 10 mg (study 1; n=352) or EMPA 25 mg (study 2; n=354) for 16 weeks (n=706). Subsequently, patients with HbA_{1c} ≥7.0 and ≤10.5% were randomised to 24 weeks' double-blind, double-dummy treatment with a single-pill combination of LINA 5 mg/EMPA 10 mg (n=126) or PBO + EMPA 10 mg (n=130) in study 1, and a single-pill combination of LINA 5 mg/EMPA 25 mg (n=114) or PBO + EMPA 25 mg (n=112) in study 2. Endpoints included changes from baseline in HbA_{1c} (primary endpoint), fasting plasma glucose (FPG; key secondary endpoint) and weight (further endpoint) after 24 weeks of double-blind treatment.

Results: At week 24, LINA 5 mg significantly reduced HbA_{1c} and FPG from baseline vs PBO as add-on to EMPA 10 mg (study 1) or 25 mg (study 2) and metformin (Table). There were no significant changes in body weight with LINA 5 mg vs PBO. More patients reported adverse events with PBO than LINA 5 mg in both studies (Table).

Conclusion: LINA 5 mg as add-on to EMPA 10 mg or EMPA 25 mg and metformin was associated with clinically relevant improvements in glycaemic control vs PBO and was well tolerated in patients with T2DM.

	EMPA 10 mg and metformin (study 1)		EMPA 25 mg and metformin (study 2)	
	PBO	LINA 5 mg	PBO	LINA 5 mg
HbA_{1c} (%)				
Baseline*	8.03 (0.08)	8.04 (0.09)	7.88 (0.09)	7.82 (0.07)
Difference vs. PBO at week 24†	–	−0.32	–	−0.47
(95% CI)		(−0.52, −0.13)		(−0.66, −0.28)
p-value		0.0013		<0.0001
FPG (mmol/L)				
Baseline*	8.6 (0.2)	8.8 (0.2))	8.6 (0.2)	8.5 (0.2)
Difference vs. PBO at week 24†	–	−0.7	–	−0.4
(95% CI)		(−1.2, −0.2)		(−0.9, −0.0)
p-value		0.0103		0.0452
Body weight (kg)				
Baseline*	85.6 (1.6)	88.5 (1.5)	89.9 (1.6)	85.9 (1.6)
Difference vs. PBO at week 24†	–	0.6	–	0.1
(95% CI)		(−0.1, 1.3)		(−0.6, 0.8)
p-value		0.0945		0.8008
Adverse events, n (%)‡				
Any adverse event	71 (55.5)	61 (48.4)	66 (58.9)	59 (52.7)
Confirmed hypoglycemia§	0	0	3 (2.7)	0

*Mean (SE). †Adjusted mean difference in change from baseline based on a mixed model repeated measures analysis in patients who received ≥1 dose of study drug during the double-blind period and had baseline and ≥1 on-treatment HbA_{1c} measurements. The model included treatment, baseline estimated glomerular filtration rate, region, visit, and visit by treatment as fixed effects and baseline values for HbA_{1c} and the endpoint in question as linear covariates (observed cases, excluding values after initiation of rescue therapy). ‡Patients who received ≥1 dose of study drug during the double-blind period. §Plasma glucose ≤3.9 mmol/L and/or requiring assistance.

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Efficacy and safety of saxagliptin plus metformin versus acarbose plus metformin in type 2 diabetes patients inadequately controlled with metformin monotherapy

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Background and aims: This is a multicentre, randomized, open-label, parallel group, active controlled Phase IV study to assess the efficacy, safety and tolerability of saxagliptin plus metformin combination therapy compared with acarbose plus metformin in patients with Type 2 Diabetes Mellitus (T2DM) who are inadequately controlled with metformin monotherapy over 24 weeks.
Materials and methods: A total of 488 patients were involved in this study. Patients were randomized to saxagliptin (5mg daily, given as once daily, n=244) add on to metformin or acarbose (up to 300mg daily, given as three equally divided doses, n=244) add-on to metformin.
Results: 12-week results show that saxagliptin add-on to metformin is significant superior to acarbose add-on to metformin in HbA1c (-0.78% versus -0.61%, p=0.0410) and in fasting plasma glucose (-1.06 mmol/L versus -0.63 mmol/L, p=0.0086). 24-week results show that saxagliptin add-on to metformin is non-inferior to acarbose add-on to metformin in glycaemic control in HbA1c (-0.86% versus -0.76%, p=0.6323), fasting plasma glucose (-0.99 mmol/L versus -1.01 mmol/L, p=0.8909), and 120 min postprandial glucose (-0.79 mmol/L versus -1.06 mmol/L, p=0.1169), respectively. The proportion of patients experiencing gastrointestinal (GI) adverse events (AEs) were significantly more frequent with acarbose plus metformin than saxagliptin plus metformin (24.7% vs 5.5%, P< 0.0001). The proportion of hypoglycaemia was similar for both groups (1.2% for saxagliptin plus metformin and 1.6% for acarbose plus metformin), no severe hypoglycaemia was reported for either group.
Conclusion: In conclusion, saxagliptin add-on to metformin is non-inferior to acarbose add-on to metformin in glycaemic control but with better GI tolerability in T2DM patients who have failed initial metformin therapy. The findings from this study will add further clinical evidence to physicians for selection of second line treatments for T2DM patients failed on metformin monotherapy.
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Disclosure: **J. Du:** None.

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Sitagliptin and risk of fractures in type 2 diabetes: results from the TECOS trial

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Background and aims: Type 2 diabetes is associated with an increased risk of fractures, and some diabetes treatments, such as thiazolidinediones (TZDs) and sodium-glucose co-transporter 2 (SGLT2) inhibitors, may further elevate this risk. Data regarding the association of dipeptidyl peptidase-4 inhibitors and fractures are mixed. In the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS) we examined in a prespecified intent-to-treat analysis, the self-reported incidence of clinician- or radiograph-verified fractures.
Materials and methods: The TECOS prospective double blind study randomized 14,671 patients to the addition of sitagliptin (N=7,332) vs. placebo (N=7,339) to existing diabetes treatments. Open-label use of antihyperglycaemic therapy was encouraged with individually appropriate glycaemic targets, aiming to achieve between-group glycaemic equipoise.
Results: At baseline, mean (SD) age was 65.5 (8.0) years, diabetes duration 11.6 (8.1) years, and HbA1c 7.2% (0.5%). 29.3% were women and 32.1% were non-White. Over median 3.0 years, 3.9% of patients had a non-fatal myocardial infarction, 3.1% were hospitalized for heart failure, and 2.1% had a non-fatal stroke. Over 43,222 person years of follow-up, 375 patients (2.6%) had a fracture, including 146 major fractures (hip: n=34; upper extremity: n=81; spine: n=31). An increased fracture risk was associated independently, in adjusted analyses with older age (p<0.001), female sex (p<0.001), White race (p=0.001), lower diastolic blood pressure (p<0.001), diabetic neuropathy (p=0.003), and insulin therapy (p=0.021). A lower risk was associated with metformin therapy (p=0.038). In patients on sitagliptin, 189 fractures (8.7 per 1000 person-years) occurred vs. 186 (8.6 per 1000 person-years) with placebo (hazard ratio 1.01 [95% CI 0.82-1.23], p=0.94). Sitagliptin was also not associated with major fractures (p=0.78) or hip fracture specifically (p=0.75).
Conclusion: In TECOS, fractures were not uncommon, especially in older patients, and occurred at rates similar to heart failure hospitalization or stroke. There was no significant difference in fracture rates between sitagliptin and placebo. This finding can help clinicians when considering their choice of second-line diabetes treatments in patients at high risk for fractures.
Clinical Trial Registration Number: NCT00790205
Supported by: MSD
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Combination therapy with linagliptin and empagliflozin improves alpha- and beta cell function in patients with type 2 diabetes

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Background and aims: Recently it has been suggested that SGLT-2 inhibitors and DPP-IV inhibitors might evolve complementary effects on alpha- and beta cell function in patients with type 2 diabetes mellitus (T2DM). We investigated the effect of sequential treatment escalation with empagliflozin and linagliptin on alpha- and beta cell function in T2DM insufficiently controlled on metformin monotherapy (baseline HbA1c 7.0 - 9.9%).
Materials and methods: Fourty-four patients with T2DM (40 male, mean age 63.8±7.4 years; mean duration of diabetes 9.7±5.1 years; mean HbA1c 8.1±0.8; mean±SD) received 25 mg empagliflozin as add on to their existing metformin therapy for a duration of 4 weeks in an open-label fashion (treatment period (TP1)). Thereafter, patients were randomised to a double-blind add-on therapy with linagliptin 5 mg or placebo (TP2). At baseline and at the end of both TP1 and TP2 alpha- and beta cell function were assessed with a) a standardised liquid meal test (LMT) and b) an intravenous (iv) glucose challenge (150 mg/kg) followed by a hyperglycaemic glucose clamp (target 16.7 mmol/l) on two consecutive in-patient days. Efficacy parameters comprised of the area under the curve (AUC) for glucose, insulin, proinsulin and glucagon after the LMT and the assessment of first- and second-phase insulin and glucagon response after the iv. glucose load/clamp.
Results: Compared with baseline empagliflozin reduced postprandial (pp) AUC_Glucose by -1.14 (-0.5, -1.8) mmol*h/L [mean (95%-CI)], AUC_Insulin by -8.9 (-13.4, -4.4) mU/pmol, and AUC_Proinsulin by -5.9 (-3.6, -8.2) pmol*h/L in TP1. Pp AUC_Glucagon increased non-significantly by 3.33 (-0.80, 7.50) pmol*h/L. In TP2 addition of linagliptin resulted in a further reduction in pp AUC_Glucose by -1.93 (-2.74, -1.12) mmol*h/L and a pronounced reduction in pp AUC_Glucagon by -10.45 (-15.63, -5.28) pmol*h/L without relevant change in pp insulin levels. In contrast, treatment with placebo only increased AUC_Proinsulin by 4.14 (1.36, 6.93) pmol*h/L without significant changes in the other parameters. First phase insulin response improved by 0.44 (0.27, 0.61) mU*h/l with empagliflozin in TP1 and further increased by 0.39 (0.13, 0.64) mU*h/L by the addition of linagliptin in TP2. No change in first phase insulin response could be observed with placebo -0.01 (-0.27, 0.25) in TP2.
Conclusion: Empagliflozin improves pp glucose control with a reduction in beta cell stress and an improvement in beta cell function (TP1). Moreover, addition of linagliptin further enhances the improvement in pp glucose due to an enhanced first phase insulin response and an attenuated release of glucagon from the alpha cell (TP2). Due to the improvements in both alpha- and beta cell function, the combination therapy with empagliflozin and linagliptin might be an attractive treatment option in patients with T2DM insufficiently controlled with metformin alone.
Clinical Trial Registration Number: NCT02401880
Supported by: Boehringer Ingelheim
Disclosure: **T.A. Forst:** Employment/Consultancy; Boehringer Ingelheim, Eli Lilly, Astra Zeneca, Sanofi. Lecture/other fees; Boehringer Ingelheim, Eli Lilly, Astra Zeneca, Sanofi, Berlin Chemie, Novartis, Takeda.

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Behaviour of free fatty acid by mixed meal tests along with incretin therapies

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Background and aims: Serum free fatty acid is regulated by several mechanisms including insulin and glucagon, and affect the metabolism of glucose and lipids. Recently,mechanisms of glucagon regulation through which diabetes develops have been closed up as therapeutic targets. To elucidate the fatty acid regulation along these processes would also bring better understandings of therapies. We intended to evaluate the effect of DPP-4 inhibitors and elucidate the mechanisms through which to attain HbA1c reduction on the focus of free fatty acids.
Materials and methods: We performed mixed meal tests for patients initiating DPP-4 inhibitor use before and after 1 month administration. We checked plasma glucose (PG), insulin (IRI), CPR, glucagon (IRG), and free fatty acids (FFA).
Results: 66 patients (39 men and 27 women; mean age 63.1 years and HbA1c 8.15±1.51%) were analyzed. The breakdown of DPP-4 inhibitors was 32 of sitagliptin, 13 of vildagliptin, 13 of linagliptin, 5 of teneligliptin, and 3 of anagliptin. At the baseline, fasting FFA was significantly higher in women. Fasting FFA showed a tendency of negative correlation to BW, BH, or BMI. Fasting FFA and AUC of FFA positively correlated to HbA1c, respectively. By 1 month of administration, HbA1c reduced to 7.22±0.73%. MMTs showed significant reduction of PG, and FFA at each time point (except for FFA at 60 min), and so did the AUCs. Whereas IRI and CPR exhibited no significant differences, IRG significantly reduced at 30, 60, and 120 minutes respectively, and so did the AUC. AUC of FFA positively correlated to baseline AUC of FFA, suggesting individual predisposition for the FFA level. AUC of FFA was positively correlated to baseline BW, or HOMA-IR. While AUC of FFA remained correlated to HbA1c at 1 month, fasting FFA was not correlated. Insulinogenic index showed a negative correlation to fasting FFA value. On the parameters at fasting, difference(Δ) in FFA showed no significant correlations to ΔPG, ΔIRI, ΔCPR, ΔIRG, ΔIRG/IRI nor changes in indices of insulin resistance such as ΔHOMA-IR and ΔISI (Matsuda Index), but positive correlation to ΔHbA1c. ΔPG positively correlated to ΔIRI or ΔCPR, suggesting the secondary change in insulin secretion. The reduction in HbA1c was positively correlated to baseline fasting FFA and AUC of FFA. The reduction in HbA1c showed significant correlation to ΔAUC of FFA and IRG/IRI , but not of IRI, CPR, IRG. ΔAUC of FFA correlated positively to that of IRG, but not PG, IRI, nor IRG/IRI. It also showed a positive correlation to BMI.
Conclusion: FFA reduction was correlated to HbA1c improvement, which suggest the effect of DPP-4 inhibitors via FFA driven by IRG change. FFA would be regarded as a marker for glucagon effect, and a predictor for the effect of DPP-4 inhibitors. However, sustained level of FFA suggested predisposition factor including insulin resistance, which might diminish the effect of DPP-4 inhibitors. Further investigation would be required as to the behavior of FFA along with diabetes therapies.
Disclosure: **K. Sakamoto:** None.

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GLP1R gene variant is associated with glycaemic response to treatment with DPP-4 inhibitors

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Background and aims: DPP-4 inhibitors (gliptins) have been used for the treatment of type 2 diabetes (T2DM) for the last decade. There is a considerable variability in the glycemic response to gliptins, which could be partially explained by genetic factors. Gene variants in TCF7L2, GLP1R, GIPR and KCNQ1 were shown to be associated with GLP-1 stimulated insulin response in physiogenetic studies. The aim of the present pharmacogenetic study was to examine associations of the mentioned gene variants with the glycemic response to gliptin treatment.

Materials and methods: One hundred and forty consecutive patients with T2DM (72 males/68 females) followed at five outpatient clinics with an average (±SEM) age of 57.0±0.9 years were included to the present study. Sitagliptin (n=92) or vildagliptin (n=48) were given as add-on treatments to metformin (n=100), or metformin and sulfonylurea combination (n=40). Genotyping of TCF7L2 rs7903146, GLP1R rs6923761, GIPR rs10423928 and KCNQ1 rs151290 was performed using high-resolution melting curve analysis after real-time PCR. All four genotypes followed Hardy-Weinberg equilibrium. The main study outcome was reduction in HbA1c (ΔHbA1c) after 6-month treatment with gliptins. Data are displayed as mean±SEM. The statistical analysis was performed using additive and recessive genetic models, final models adjusted for baseline HbA1c and creatinine values as covariates. Since four variants were examined, statistical significance was predefined after Bonferroni correction as p=0.0125.

Results: In the entire study group, the mean HbA1c was reduced from baseline 8.04±0.09% (64.4±1.0 mmol/mol) to 7.33±0.09% (56.6±1.0 mmol/mol) after 6-month gliptin treatment, which corresponded to the mean reduction ΔHbA1c of 0.71±0.10% (7.7±1.1 mmol/mol). Only GLP1R rs6923961 G>A (Ser168Gly) variant was significantly associated with ΔHbA1c in adjusted additive model (β=-0.33, p=0.011). The average ΔHbA1c in Gly/Gly homozygotes was significantly lower than in Ser-allele carriers [0.12±0.23% vs. 0.80±0.09% (1.3±2.5 vs. 8.7±1.0 mmol/mol), p=0.008]. ΔHbA1c significantly correlated with baseline HbA1c (r=0.50, p<0.0001) and baseline creatinine values (r=0.17, p=0.042), but not with age, BMI or gender.

Conclusion: In the present pilot study we observed a significant, by 0.68% smaller reduction in ΔHbA1c after 6-month gliptin treatment in homozygous AA (Gly/Gly) carriers of rs76923961 variant in GLP1R (Ser168Gly) in comparison with G-allele (Ser) carriers. This finding is in accordance with a previous physiogenetic study. The substitution of an amino acid in the GLP-1 receptor molecule might lead to a decreased sensitivity of receptors to endogenous GLP-1, and hence also to smaller effect of gliptin treatment. Since the average reduction after gliptin treatment in our entire study group was 0.71%, our observation could have a translational implication for precision medicine and genotype-directed treatment.

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Differential efficacy of a GLP-1R/GCGR agonist versus a GLP-1R agonist in improving hepatic insulin sensitivity and reducing steatosis in obese mice

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Background and aims: The increasing incidence of obesity and type 2 diabetes worldwide has prompted the need for new therapies. We have designed a pharmacological intervention protocol with a single GLP-1R or GLP-1R/GCGR dual agonist aimed to investigate their differential effects in preventing diet-induced steatosis and hepatic insulin resistance in mice.

Materials and methods: 8 week-old male C57 Bl/6 mice were fed chow (group 1) or HFD for 10 weeks. HFD-fed mice were subsequently s.c. injected every 2 days with vehicle (group 2) or a GLP-1R agonist (10 nmol/kg; group 3) or G49 a GLP-1R/GCGR dual agonist (4.4 mg/kg; group 4) for 6 weeks. Parameters that assess adiposity, glucose homeostasis and hepatic insulin sensitivity were analyzed following 3 and 6 weeks of treatment

Results: Following 3 weeks of treatment, body weight loss was higher in mice injected with the dual agonist G49 (-15.62%±4.57) vs HFD animals (+9.824%±4.14;p<0.001) and also vs GLP-1R agonist treated animals (3.88%±3.53: p<0.001). GTT (AUC 23963±542) and ITT (AUC 11594 ±1625) showed enhanced effects of the dual agonist on improving insulin sensitivity as compared with HFD animals (GTT AUC 46322±1250; p<0.001. ITT AUC 18671±1625; p<0.001) and also with GLP-1R agonist treated animals (GTT AUC 31237 ±641; p<0.01.ITT AUC 13305±678; p<0.05) we also observed this beneficial effects in whole body glucose homeostasis. These differential effects were maintained following 6 weeks of treatment, at which time the effect of G49 on reducing adiposity (eWAT weight 1.22 ±0.576 g; iWAT weight 0.94±0.732 g) and hepatic steatosis (liver triglyceride 8.28 ±1.850 mg/g tissue) was higher than that of the GLP-1R agonist (eWAT weight 1.88 ±0.635 g, p<0.01. iWAT weight 1.47 ±0.648 g, p<0.05. Liver triglyceride 17.41 ±8.696 mg/g tissue, p<0.01). The efficacy of GLP-1R/GCGR dual agonist was also demonstrated by its ability to enhance insulin signalling in the liver compared with HFD animals (p<0.001) and also when compared with GLP-1R agonist (p<0.01). Moreover, we also observed primary hepatocytes from HFD mice and also from control animals treated with palmitate and G49 (p<0.01).

Conclusion: Our results strongly suggest a novel role of an oxyntomodulin-like GLP-1R/ GCGR dual agonist in reducing steatosis and hepatic insulin resistance in mice that may offer a potential therapeutic application in humans.

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Disclosure: **P. Valdecantos:** None.

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Liraglutide protects against the development of atherosclerosis in obese pro-atherosclerotic LDLr-/- mice

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Background and aims: Atherosclerosis leading to coronary artery disease is a huge medical problem, particularly in connection with diabetes. Glucagon-Like Peptide 1 (GLP-1) based therapy effectively lowers blood glucose and body weight in type 2 diabetes and also lowers systolic blood pressure. Recently, it has been demonstrated that GLP-1 therapy may exert additional beneficial effects on the vasculature beyond its well-known anti-diabetic and weight lowering effects. In this study we evaluated the effect of liraglutide in obese, pro-atherosclerotic LDLr-/- on the development of atherosclerosis and inflammation.

Materials and methods: Male LDLr-/- mice (6-8 weeks, JAX, USA) on western diet D12049B, Research Diets, USA) were divided into two groups (n=15-20/group) having a control vehicle (VEH) and a group treated with liraglutide (LIRA) 1mg/kg, SC once daily for 17 weeks. At termination a predefined section of thoracic aorta was excised and plaque lesion area was quantified by the En Face method (VisioMorph, Denmark). Subsequently, quantitave gene expression analysis was performed on N2 frozen aortic tissue applying the Nanostring technology. Plasma cholesterol lipoproteins and triglycerides (TG) were determined by HPLC. Body weight and 24 hour food intake was monitored throughout the study. Data are presented as mean±sem.
Results: Treatment with LIRA showed a lower body weight at all-time points as compared to the VEH group. At termination the vehicle control animals had gained 65% in body weight whereas the LIRA group only gained 17%, resulting in a significant lower body weight at termination (VEH: 43.5±1.1g vs. LIRA: 30.9±0.9g; p<0.0001). Feeding with WD showed a robust acceleration in aortic plaque lesion in the VEH whereas treatment with LIRA resulted in a significant suppression of plaque lesion development (VEH: 13±1.0% vs. LIRA: 2.8±0.9%; p<0.0001). These beneficial effects by LIRA were also reflected on plasma lipids showing a significant reduction in plasma TG (VEH: 11.5±0.7mM vs. LIRA: 7.1±1.0mM; p<0.0001), vLDL (VEH: 25.9±2.2mM vs. LIRA: 9.6±2.4mM; p<0.0001), LDL (VEH: 36.3 ±18.3mM vs. LIRA: 18.3±2.1%; p<0.0001) and an increase in HDL (VEH: 1.9±0.2mM vs. LIRA: 3.5±0.2; p<0.0001). The 10 most down regulated genes by LIRA were, Interleukin-6, Matrix metalloproteinase 12 and 13, CD68, Cathepsin S, Neutrophil gelatinase-associated lipocalin, interleukin 1 Receptor Antagonist, macrophage scavanger 1, serum amyloid A3 and secreted Phosphoprotein 1, predominantly genes related to inflammation.
Conclusion: These data supports the hypothesis that treatment with liraglutide may protect against the development of atherosclerosis. Our data suggest that the beneficial effects of liraglutide may also comprise anti-inflammatory mechanisms.
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Disclosure: **G. Rakipovski:** Employment/Consultancy; Novo Nordisk A/S. Stock/Shareholding; Novo Nordisk A/S.

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Liraglutide exerts anti-steatotic and anti-fibrotic action in a diet-induced obese mouse model of biopsy-confirmed nonalcoholic steatohepatitis

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Background and aims: The long-acting GLP-1 analogue liraglutide is currently approved for the treatment of type 2 diabetes and obesity. In addition, liraglutide treatment was recently demonstrated to improve liver function and histopathology in biopsy-proven NASH patients. Here we evaluated the treatment efficacy of liraglutide on metabolic parameters, steatohepatitis and liver fibrosis in a novel diet-induced obese mouse model with liver biopsy-confirmed NASH
Materials and methods: Male C57BL/6J mice were fed a modified Amylin Liver NASH diet high in trans-fat, fructose and cholesterol for 26 weeks. After diet-induction, a liver biopsy was performed for histological evaluation of individual disease progression and randomization into treatment groups receiving once daily subcutaneous administration with vehicle or liraglutide (0.4 mg/kg) for a further 8 weeks. Endpoints included a blinded histological assessment for individual and combined components of the clinically derived NAFLD Activity Score (NAS) (steatosis, inflammation, ballooning degeneration) and Fibrosis Stage. In addition, levels of hepatic total cholesterol (TC), triglyceride (TG) and collagen (hydroxyproline) content were measured. Metabolic parameters included body weight, body composition, blood glucose and plasma levels of lipids (TC and TG) and plasma transaminases (ALT and AST).
Results: The modified AMLN diet mouse model exhibited hallmark features of NASH (hepatosteatosis, inflammation and fibrosis) and metabolic disease. As expected, liraglutide reduced body weight and adiposity, decreased plasma levels of lipids (TC) and concomitantly improved glucose tolerance following 8 weeks of treatment. In addition, liraglutide improved liver function by significantly reducing plasma liver enzymes ALT and AST. Importantly, liraglutide significantly decreased hepatosteatosis by approximately 50% (p<0.001), and concomitantly reduced total NAFLD Activity Score by ~2 points from baseline (p<0.05). Finally, liraglutide decreased liver hydroxyproline (collagen) content by approximately 30% (p<0.05) and concomitantly reduced liver fibrosis stage by approximately 0.7 from baseline (p<0.01).
Conclusion: In conclusion, the GLP-1 analogue liraglutide improved metabolic parameters and alleviated key hallmarks of nonalcoholic steatohepatitis including reversal of liver fibrosis in a novel diet-induced obese mouse model of biopsy-confirmed fibrotic NASH.
Disclosure: **N. Vrang:** Stock/Shareholding; Owner of Gubra.

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A long-acting glucagon-like peptide 1 and 2 co-agonist improves glycaemic control in db/db mice

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Background and aims: Bariatric surgery is currently the most effective treatment modality for obesity and often with a concomitant resolution of type 2 diabetes. Roux-en-Y gastric bypass is manifested by intestinal growth and increased plasma levels of a number of circulating gut hormones, including glucagon-like peptide 1 (GLP-1) and GLP-2. Here, we aimed to explore whether a dual analog of the insulinotropic hormone, GLP-1, and the intestinotropic hormone, GLP-2, may show potential as a RYGB mimetic by reducing body weight and improving glucose homeostasis.
Materials and methods: A long-acting lipidated GLP-1/2 co-agonist (GUB09-145) with potent agonistic effect on both the GLP-1 and GLP-2 receptor was generated using solid-phase peptide synthesis. Next, the effects of bi-daily subcutaneously administration for 21 days of GUB09-145 (50 nmol/kg) were evaluated in male db/db mice and compared to vehicle and liraglutide (50 nmol/kg) treated controls. Main endpoints included effects on body weight, glucose homeostasis and gastric emptying.
Results: The data demonstrate that chronic administration of GUB09-145 and liraglutide to male db/db mice has equipotent effects on food intake and body weight, with no lasting effects on gastric emptying. In contrast, GUB09-145 showed superior effects on weekly fed glucose levels and glucose homeostasis, ultimately reflected by a significant reduction in HbA1c compared to vehicle (5.18±0.15 GUB09-145 vs 7.01±0.23 vehicle, mean%±SEM) and compared to liraglutide (6.27±0.18%). Terminal gut weight showed a marked intestinotrophic effect of GUB09-145 in the small intestine only (>70% increase), with no changes in the colon
Conclusion: In conclusion, we demonstrate that a long-acting GLP-1/GLP-2 co-agonist exerts beneficial effects on body weight and glucose homeostasis. The combination of insulinotrophic an intestinotrophic mechanism of action may show promise as therapeutic option for obesity and diabetes.
Disclosure: **J. Jelsing:** Stock/Shareholding; Owner of Gubra.

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Investigation of blood-brain barrier penetration of albiglutide in mice

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Background and aims: The gastrointestinal adverse events associated with glucagon-like peptide 1 receptor agonists (GLP-1RAs) could be triggered by its crossing the blood-brain barrier and stimulating specific brain centers. The GLP-1RA albiglutide (ALBI) is a fusion product of a dimer of a dipeptidyl peptidase-4-resistant analog of GLP-1 with human serum albumin. The potential for ALBI to act centrally by crossing the blood-brain barrier was assessed in a pharmacokinetic (PK) radiolabeling study in mice.
Materials and methods: ^{125}I-ALBI or comparator ^{125}I-albumin or ^{125}I-transferrin were administered subcutaneously. Quantitation of radioactivity

in plasma and brain homogenates allowed systemic PK parameters to be calculated along with distribution to the brain parenchyma. Because proteins such as ALBI are expected to have a relatively low distribution in the brain, blood contamination was background-subtracted using a ^{123}I-albumin tracer that was administered just prior to sacrifice in addition to a capillary depletion method that was used to remove ^{125}I-ALBI that was sequestered in the brain microvasculature and not transcytosed into the brain parenchyma.

Results: Overall, systemic PK parameters after a 5 mg/kg dose of ^{125}I-ALBI were in agreement with previous data for ALBI in mice, with a maximum concentration (C_{max}) in plasma of 8.4 μg/g (similar to 30-mg steady state C_{max} in humans), time at maximum (T_{max}) of 16 h, and half-life ($t_{1/2}$) of 25 h. The overall radioactivity measured in the brain after administration of ^{125}I-ALBI was very low (<0.02% irradiated dose/g), with an overall exposure ratio ($AUC_{0\text{-}24}$ brain/plasma) of 0.00034, which was similar to the albumin control ($AUC_{0\text{-}24}$ brain/plasma ratio of 0.00018) and approximately 4 times lower than the transferrin control (brain/plasma ratio of 0.00139). In addition, ALBI did not accumulate in the brain, achieving T_{max} concentrations at 24 h, with a noticeable reduction in concentration at 48 h.

Conclusion: The potential for albiglutide to cross the blood-brain barrier is low and similar to albumin. Previously presented at ADA 2016 and adapted with permission.

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Disclosure: **C.S. Hottenstein:** Employment/Consultancy; GlaxoSmithKline.

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Comparative effects of proximal and distal small intestinal glucose on glycaemia, incretin hormone secretion and incretin effect in healthy males

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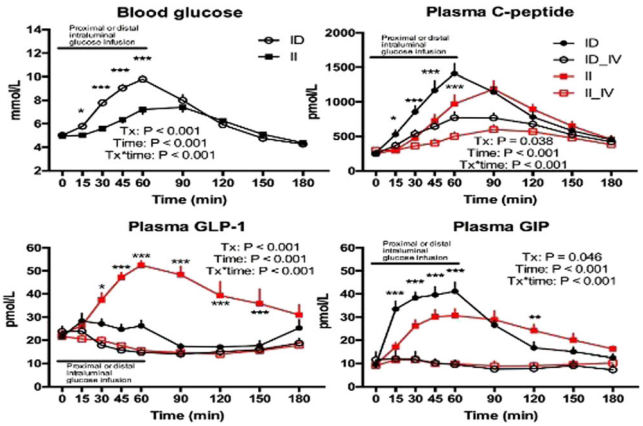
Background and aims: Glucose-dependent insulinotropic polypeptide (GIP)- and glucagon-like peptide-1 (GLP-1)-releasing cells are distributed predominately in the proximal and distal gut, respectively. Accordingly, the region of the gut exposed to nutrients is likely to be a determinant of the secretion of these two incretin hormones, as well as the incretin effect (IE) and gastrointestinal-mediated glucose disposal (GIGD). In the present study, we have determined the glycaemic and incretin responses to glucose administered directly into the proximal or distal small intestine, and quantified IE and GIGD in the two conditions, using intravenous (IV) "isoglycaemic" clamp in healthy subjects.

Materials and methods: Ten healthy males (age: 45 ± 8 years; BMI: 25.3 ± 1.3 kg/m2) were studied on four days (≥7 days apart) in single-blind fashion. On the two enteral infusion days, a catheter was positioned with proximal and distal infusion ports opening 10cm and 190cm beyond the pylorus. A solution containing 30g glucose mixed with 3g 3-O-methylglycose (3-OMG, a marker of glucose absorption), dissolved in water to 120mL, was infused into either the proximal or distal small intestine over 60 min (ie. 2kcal/min) in randomised order, while 0.9% saline was infused into the alternate site. "Arterialised" venous blood was sampled frequently over 180 min for measurements of blood glucose, serum 3-OMG, plasma C-peptide, total GLP-1 and GIP. Subsequent to each enteral infusion study, an IV isoglycaemic clamp was performed. IE was calculated from the incremental area under the curve (iAUC) for plasma C-peptide as [(iAUC - iAUC_IV)/iAUC] × 100. GIGD was calculated as 100 × (30 - IV glucose (g))/30.

Results: Both blood glucose and serum 3-OMG concentrations were higher in response to proximal than distal glucose infusion (P<0.001 each). Plasma GLP-1 increased minimally with proximal glucose infusion, but substantially with distal infusion, with a significant difference between them (P<0.001). Plasma GIP increased promptly with both infusions, with concentrations being greater initially, but less sustained, with proximal vs. distal glucose infusion (treatment × time interaction: P < 0.001). Both IE (42 ± 3% vs. 67 ± 3%, P < 0.001) and GIGD (27 ± 5% vs. 47 ± 3%, P = 0.009) were less with proximal vs. distal glucose infusion.

Conclusion: In healthy humans, proximal glucose is associated with a higher blood glucose excursion, faster glucose absorption, profoundly less plasma GLP-1 response, slightly greater but less sustained GIP release, and less IE and GIGD, compared with distal glucose infusion. These observations provide a rationale for diverting nutrients from the proximal to the distal gut, such as by nutritional (complex carbohydrates), pharmacological (acarbose), or surgical (Roux-en-Y bypass) means, for the management of type 2 diabetes.



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Disclosure: **X. Zhang:** None.

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Effect of GLP-1 receptor agonist on digestive tract movement evaluation using capsule endoscopy

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Background and aims: GLP-1 receptor agonists (GLP-1 RAs) exhibit physiological actions, such as incretin actions, gastric emptying-delaying effects, and appetite-reducing actions through the suppression of digestive tract movement. In humans, the gastric emptying-delaying or digestive tract movement-reducing actions have been quantitatively assessed using the acetaminophen loading test. However, it is impossible to evaluate the effects on the movement of each digestive tract using this method. In this study, we quantitatively evaluated the actions of a GLP-1 RA on digestive tract movement by examining the gastrointestinal transit time using capsule endoscopy before and after administration.

Materials and methods: The subjects were 15 patients with type 2 diabetes admitted to our hospital for the treatment of diabetes who had not received any GLP-1 RA or dipeptidyl peptidase-IV (DPPIV) inhibitor (male:female=10:4, mean age: 60.4±13.1 years, mean HbA1c: 9.9 ±2.4%). To examine the gastrointestinal transit time, capsule endoscopy was performed before the administration of a GLP-1 RA, liraglutide, and more than 1 week after increasing the dose to 0.9 mg.

Results: After liraglutide administration, the gastric transit time increased from 3,840±3,509 to 12,181±17,840 seconds (P=0.083), and the small intestinal transit time increased from 16,541±5,330 to 26,175±11,298 seconds (P=0.017). Furthermore, there were no new gastrointestinal mucosal changes after liraglutide administration.

Conclusion: Previous studies have suggested that gastric emptying-delaying effects primarily contribute to the digestive tract movement-reducing action mechanism of GLP-1 RAs. However, this study showed

that the GLP-1 RA more significantly delayed small intestinal movement rather than gastric emptying.
Clinical Trial Registration Number: UMIN000021686
Supported by: AstraZeneca
Disclosure: **Y. Nakatani:** None.

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The rate of intraduodenal glucose delivery affects the lowering of blood glucose by vildagliptin in type 2 diabetes

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Background and aims: The rate of gastric emptying, which exhibits a substantial inter-individual variation, is a major determinant of the secretion of the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). Accordingly, it may modulate the magnitude of glucose-lowering by dipeptidyl peptidase-4 (DPP-4) inhibitors, which improve glycaemic control by reducing degradation of endogenously released GLP-1 and GIP. In the present study, we compared the effects of the DPP-4 inhibitor, vildagliptin, during intraduodenal (ID) glucose infusion at two different rates within the physiological range of gastric emptying, in patients with type 2 diabetes.
Materials and methods: 16 patients with type 2 diabetes, managed by diet alone [65.5 ± 2.4 years; BMI 30.4 ± 1.5 kg/m2; HbA1c 6.3 ± 0.1% (45.6 ± 1.6 mmol/mol)], were studied on four separate days in double-blind, randomised, fashion. On each day, either 50 mg vildagliptin (VILD) or placebo (PLBO) was given 60 min prior to an ID glucose infusion (2 or 4 kcal/min, both 4 mL/min, with osmolality of 1390 mOsmol/L). Plasma glucose, insulin, C-peptide, glucagon, and incretin hormones (total and intact forms; however, intact GIP was not measured due to technical issues) were measured at frequent intervals, and the incremental areas under the curve (iAUCs) during t = 0-120 min were compared using two-factor repeated measures ANOVA, with the rate of ID glucose and treatment with VILD or PLBO as factors.
Results: iAUCs for plasma glucose, insulin, C-peptide, glucagon, total GIP, and total and intact GLP-1 were higher during ID glucose at 4 vs. 2 kcal/min. VILD was associated with higher intact GLP-1, insulin and C-peptide, and lower glucose, total GIP and total GLP when compared with PLBO, without affecting plasma glucagon. There were significant interactions between the rate of ID glucose and VILD on the peak plasma glucose concentration and the iAUC for plasma glucose, intact GLP-1, and total GLP-1 and GIP, but not insulin, C-peptide or glucagon. The reductions in peak and iAUC for glucose and the increment in iAUC for intact GLP-1 after vildagliptin were more than 3 fold greater during ID glucose at 4 kcal/min than 2 kcal/min.
Conclusion: In type 2 diabetes, there is synergy between the rate of glucose delivery to the small intestine and DPP-4 inhibition to reduce plasma glucose. The implication is that improvement in postprandial glycaemia with DPP-4 inhibition will be greater in type 2 patients with relatively more rapid gastric emptying.

	ID glucose (2 kcal/min)		ID glucose (4 kcal/min)		P-value		
	PLBO	VILD	PLBO	VILD	ID glucose	VILD	Interaction
Peak glucose (mmol/L)	12.9 ± 0.5	12.2 ± 0.6 *	15.4 ± 0.8	13.1 ± 0.6 ***	<0.001	<0.001	0.003
Glucose iAUC (mmol/L × h)	7.3 ± 0.6	6.7 ± 0.6 *	10.1 ± 0.8	8.2 ± 0.7 ***	<0.001	<0.001	0.026
Insulin iAUC (mU/L × h)	51.0 ± 8.0	71.7 ± 11.0	139.7 ± 12.3	166.3 ± 12.7	<0.001	0.001	0.678
C-peptide iAUC (pmol/L × h)	1927.8 ± 237.7	2550.6 ± 235.1	3910.5 ± 236.6	4238.3 ± 215.4	<0.001	0.001	0.216
Glucagon iAUC (pg/mL × h)	-6.5 ± 4.2	-5.6 ± 4.9	23.7 ± 8.3	16.0 ± 10.3	<0.001	0.275	0.178
Total GIP iAUC (pmol/L × h)	56.3 ± 3.9	55.5 ± 3.9	71.5 ± 4.4	62.5 ± 4.5 *	<0.001	0.062	0.010
Total GLP-1 AUC (pmol/L × h)	17.6 ± 3.6	15.3 ± 4.5	71.9 ± 10.7	54.6 ± 11.1 **	<0.001	0.008	0.013
Intact GLP-1 AUC (pmol/L × h)	0.1 ± 1.5	5.0 ± 2.3	14.4 ± 5.8	32.8 ± 9.0 **	0.003	0.004	0.002

Data were analysed using two-factor repeated measures ANOVA, with ID glucose and treatment with VILD/PLBO as factor. Data are means ± SEM.

Clinical Trial Registration Number: ACTRN12613001158752
Supported by: Novartis.
Disclosure: **T. Wu:** Grants; This investigator-initiated study was funded by Novartis.

PS 073 Incretin therapies: metabolic and physiological effects

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Metabolic effects of dapagliflozin QD and exenatide QW in obese adults without diabetes: a 24-week randomised placebo-controlled phase 2 study

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Background and aims: GLP-1 receptor agonists and SGLT-2 inhibitors have rarely been studied in combination. A blinded, placebo (PBO)-controlled Phase 2 study in obese adults with or without impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) investigated the effects of dapagliflozin (DAPA) combined with exenatide (EXE). Body weight (BW)-related results were presented (ADA June 2016). This work focuses on glucose and lipid metabolism.
Materials and methods: In a single center, randomized, 24-week double-blind study, 50 nondiabetic obese adults (age 18-70 y; BMI 30-45 kg/m^2) were randomized 1:1 to oral DAPA 10 mg QD + sc EXE 2 mg QW (n=25) or PBO pills + injections (n=25). Assessments (full analysis set, N=49) included anthropometry, whole body MRI, fasting blood chemistry and 3h oral glucose tolerance test (OGTT).
Results: Baseline mean BW for DAPA+EXE / PBO was 106.4 / 102.7 kg. Mean BW reduction with DAPA+EXE vs PBO was -4.11 kg (P=0.0007), with a mean loss of -4.5% with DAPA+EXE vs -0.3% with PBO. This was mainly accounted for by loss of adipose tissue, both visceral and subcutaneous (P<0.05). Reductions in HbA1c and FPG, baseline to week 24, were significantly greater with DAPA+EXE than PBO (Table). Among per protocol completers, IFG ([FPG ≥5.6 mmol/L)/IGT significantly declined from 15/11 to 8/4 out of 22 subjects (P<0.05) for DAPA+EXE. IFG/IGT did not change for PBO; from 15/7 to 17/8 out of 20 subjects. The area under the curve (AUC) of glucose during OGTT significantly decreased, whereas FFA AUC increased by 19% from baseline with DAPA+EXE, but these parameters did not change with PBO. Insulin and glycerol AUC and β-OH-ketones did not display significant changes (Table). Further, there were no significant treatment differences in insulin secretion (30 min insulinogenic index) or insulin sensitivity (Matsuda index corrected for urinary glucose) assessed by OGTT. Triglycerides and total, low-density, and high-density cholesterol did not change with either treatment. At week 24, mean (SD) urine glucose excretion following OGTT was 50.5 (31.4) mmol/3h for DAPA+EXE vs 0.3 (0.8) in the PBO group (P<0.001). Among adverse events (AEs), gastrointestinal were most common. No confirmed hypoglycaemia occurred. Two participants on DAPA+EXE and one on PBO experienced urinary tract infection; one on DAPA+EXE had vaginal infection. Two participants had serious AEs, one in each treatment group, deemed unrelated to study drugs.
Conclusion: In this Phase 2 study in obese patients without diabetes, the combination of DAPA+EXE vs PBO significantly reduced BW, HbA1c, and post-challenge glucose. IFG and IGT were largely normalized. These effects occurred without any significant change in estimated insulin sensitivity and beta cell function. Increased lipid mobilization providing more fatty acids relative to glucose as energy substrate was suggested by increased FFA levels.

Table. Adjusted Mean Change from Baseline and Treatment Differences (Least Squares Estimates), DAPA+EXE (n=25) vs PBO (n=24; Full Analysis Set)

Variable	Baseline DAPA+ EXE / PBO	Change from baseline DAPA+ EXE	Change from baseline PBO	Treat-ment diff, *P*-value
HbA1c (mmol/mol)	37.2 / 38.1	−3.9	−1.6	−2.3 *P*<0.001
FPG (mmol/L)	5.90 / 5.83	−0.41	0.25	−0.66 *P*<0.001
Glucose* AUC_{0-3h} (mmol/L x min)	1427 / 1289	−187	37	−223 *P*=0.003
F-Insulin (mU/L)	13.47 / 15.11	1.79	1.42	0.37 *P*=0.904
Insulin* AUC_{0-3h} (mU/L x min)	12942 / 15656	−1619	285	−1905 *P*=0.259
Glycerol* AUC_{0-2h} (µmol/L x min)	9565 / 9314	831	−244	1075 *P*=0.355
FFA* AUC_{0-2h} (µmol/L x min)	13087 / 11370	2433	−439	2871 *P*=0.002
F-β-OH-ketones (mmol/L)	0.23 / 0.21	0.01	−0.03	0.05 *P*=0.149
β-OH-ketones* 120 min (mmol/L)	0.17 / 0.16	0.01	−0.02	0.02 *P*=0.284
F-Glucagon (pmol/L)	9.99 / 8.34	2.72	5.00	−2.29 *P*=0.215

*Assessed during OGTT (oral glucose tolerance test). AUC, area under the curve; F, fasting; FFA, free fatty acids; FPG; fasting plasma glucose; OGTT, oral glucose tolerance test.

Clinical Trial Registration Number: NCT02313220
Supported by: AZ
Disclosure: **P. Lundkvist:** Grants; AstraZeneca.

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Exenatide plus metformin and glibenclamide compared with metformin plus glibenclamide on beta cell and alpha cell function and adipocytokines in patients with type 2 diabetes

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Background and aims: To quantify how much exenatide added to metformin and glibenclamide improves α- and β-cell function, and to evaluate the impact on glycaemic control, insulin resistance and adipocytokines levels compared with metformin plus glibenclamide.
Materials and methods: A total of 45 Caucasian patients with type 2 diabetes with poor glycaemic control were instructed to take metformin at a mean dose of 2000± 500 mg/day plus glibenclamide at a mean dose of 11.5 ± 3.5 mg/day for 10±2 months, then they were randomly assigned to take exenatide (5 µg twice a day for the first 4 weeks and 10 µg twice a day thereafter) or metformin plus glibenclamide at the doses remaining unchanged (control group). Active group (30 patients): average age - 59 [53, 63] years, DM2T duration - 8 [6, 10] years, body mass index (BMI) -33.2 [30.4, 38.4] kg/m^2, glycated haemoglobin (HbAlc) level-8.5 [7.9, 9.3]%. Control group (15 patients): average age - 55 [50,60] years, DM2T duration - 7 [6,10] years, BMI -35 [30.5,38.0] kg/m^2,HbAlc-8.8 [8.2, 9.0] %. We evaluated the following parameters at baseline and after 6 months: body mass index (BMI), glycated haemoglobin (HbA1c),fasting plasma glucose, proinsulin / fasting plasma insulin ratio (Pr/FPI ratio), adiponectin (AND), resistin (RES),homeostasis model assessment insulin resistance index (HOMA-IR), homeostasis model assessment β-cell function index (HOMA-β). A 75-gram glucose tolerance test was performed for all patients (0 to 180 minutes), and areas under the curve for glucose, insulin, proinsulin, GLP-1 and glucagon were calculated. Statistical analysis was performed using Excel 2010 (Microsoft), Statistica 8.0 (StatSoft, Inc.); test parameters were expressed as Me [25; 75](Me - median; 25 and 75 - 1stand 3d quartiles); a significance level (P) of 0.05 was used for comparison.
Results: Intensified therapy with exenatide was associated with the area under the curve reduced by 27%($P \leq 0.0001$) for glucose, by 13.1% ($P \leq 0.05$) for glucagon, by 17.5% ($P = 0.01$) for proinsulin, and insignificantly increased by 11% ($P = 0.06$) for insulin. The absolute reduction of HвA1c was Δ 1.6 [1.2; 1.9]% ($P < 0.001$). HOMA-β was 34.8 [23.7; 48.3] at baseline and 53.8 [38.0; 88.1]($P < 0.001$) at 6 months. HOMA-IR was 7.0 [5.0; 8.9] at baseline and 5.6 [4.8; 8.8] ($P^1=0.07$) at 6 months. The Pr/FPI ratio decreased by 27% towards the reduction of synthesis and secretion of fasting proinsulin ($P = 0.01$).The weight reduced by 4.4 kg, AND level did not change and RES value reduced by 29% ($P < 0.001$). By the end of the trial, the target HbA1c level of ≤ 7% was achieved in 15 patients (50%). In the control group the area under the curve reduced by 4.1% ($P \leq 0.1$) for glucose, by 4% ($P = 0.8$) for glucagon, by 3.7% ($P = 0.9$) for proinsulin and by 3.4% ($P = 0.6$) for insulin. The absolute reduction of HвA1c was Δ 0.5 [0.2; 0.7] %($P < 0.1$),Pr/FPI ratio, AND,RES, HOMA-β and HOMA-IR did not change ($P = 0.7$).By the end of the trial, the target HbA1c level of ≤ 7% was achieved in 1 patient (6.7%).
Conclusion: Addition of exenatide to the therapy results in the improved glycaemic control associated with a better functional activity of pancreatic β-cells, enhanced insulin synthesis, lower functional activity of pancreatic α-cells and reduced IR due to decreased levels of resistin and glucagon.
Disclosure: **O. Koteshkova:** None.

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Combination therapy with metformin/pioglitazone/exenatide is superior to sequential add-on therapy in new onset diabetes: 3-year follow-up results of the EDICT study

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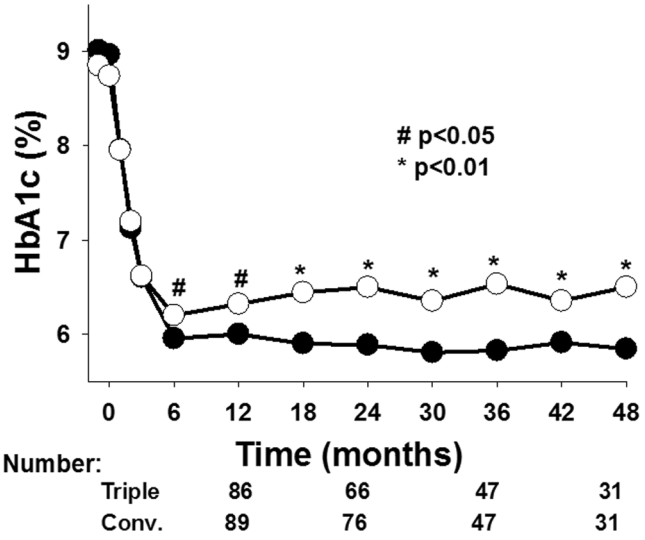
Background and aims: We previously have shown that initial combination therapy with metformin/pioglitazone/ exenatide produces greater and

more durable reduction in HbA1c with lower rates of hypoglycemia than the sequential add-on recommended therapy in subjects with new onset diabetes

Materials and methods: The EDICT study is a randomized open label study in which subjects with new onset diabetes were randomized to receive metformin/pioglitazone/exenatide (Triple Therapy) (n=86) or escalating dose of metformin followed by sequential addition of glipizide and then glargine insulin (Conventional Therapy) (n=89) to maintain HbA1c< 6.5. Here we report the 3-year follow-up results.

Results: Subjects receiving Triple Therapy experienced a significantly greater reduction in HbA1c after a mean follow-up of 3 years versus Conventional Therapy (5.8% vs 6.50%, p<0.001) (Figure 1). Despite lower bA1c, subjects receiving Triple Therapy experienced 7-fold lower rate of hypoglycemia versus subjects receiving Conventional Therapy.

Conclusion: The results of this study demonstrate that combination therapy with metformin/pioglitazone/ exenatide in newly diagnosed T2DM patients produces a durable and greater reduction in HbA1c than sequential add-on therapy with metformin, sulfonylurea, and basal insulin with lower rates of hypoglycemia.



Clinical Trial Registration Number: NCT01107717

Supported by: American Diabetes Association

Disclosure: **R.A. DeFronzo:** Grants; Astrazeneca, Boehringer-Ingelheim, Takeda, Janssen. Other; Speaker's Bureau - Novo-Nordisk, Astra Zeneca, Advisory Board: AstraZeneca, Novo Nordisk, Janssen, Boehringer Ingelheim, Intarcia.

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The efficacy and durability of exenatide in combination with pioglitazone versus basal bolus insulin in poorly controlled type 2 diabetic patients: the QATAR study

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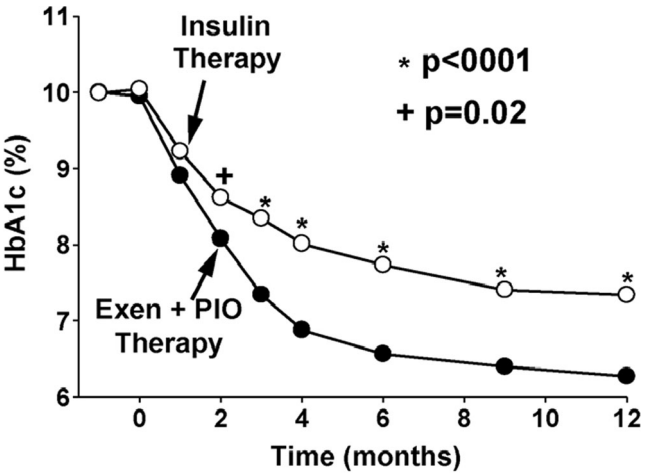
Background and aims: We previously have shown that addition of exenatide (EXEN) plus pioglitazone (PIO) to metformin at the time of diagnosis of T2DM produces greater HbA1c reduction compared to sequential addition of sulfonylurea (SU) to metformin followed by basal insulin (Diab Obes, Metab 17:268-75, 2015). This study aims to examine the efficacy of adding exenatide plus pioglitazone versus basal-bolus insulin to poorly controlled T2DM patients on maximal dose metformin/SU therapy.

Materials and methods: 226 poorly controlled T2DM patients (age=56y; BMI=31; Diabetes duration=11 y, HbA1c=10.1%) on maximal doses of both metformin plus SU were randomized to receive: (1) once weekly exenatide plus pioglitazone (n=112) or (2) basal (glargine)-bolus (aspart) insulin (n=114) to maintain HbA1c below 7%.

Results: In subjects receiving EXEN + PIO, A1c decreased to 6.5%, and 6.2% at 6 and 12 mo, respectively. With Insulin Therapy, A1c declined to 7.5% and 7.3% at 6 and 12 mo, respectively (Figure 1). More subjects receiving Insulin Therapy failed to achieve the A1c goal <7% (63 vs 17%, p<0.0001). Despite significantly lower A1c, subjects receiving EXEN + PIO had 3.1-fold lower rate of hypoglycemia compared to subjects receiving Insulin Therapy (0.21 vs 0.67 events/patient year). Lastly, subjects treated with Insulin gained 3.1 kg vs 0.7 kg (p=0.0002) versus subjects treated with EXEN + PIO.

Conclusion: Addition of EXEN + PIO in poorly controlled T2DM patients on MET/SU produces greater HbA1c reduction with lower rate of hypoglycemia compared to Insulin Therapy.



Supported by: Qatar Foundation NPRP 5-273-3-079

Disclosure: **M. Abdul-Ghani:** None.

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Glycaemic lowering with albiglutide: effective at 1 week and efficacy maintained for 1 year

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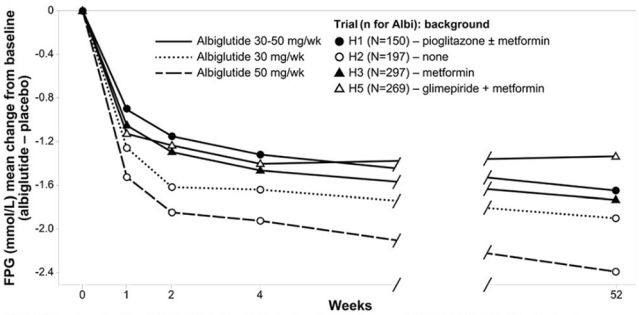
Background and aims: Albiglutide is a long-acting, GLP-1 receptor agonist composed of a dipeptidyl peptidase-4-resistant dimer fused to human albumin. The HARMONY program of eight Phase 3 trials evaluated the efficacy and safety of albiglutide 30-50 mg/week in type 2 diabetes mellitus (T2DM). The aim of this post hoc analysis was to compare placebo-subtracted mean fasting plasma glucose (FPG) across HARMONY trials.

Materials and methods: Four HARMONY trials (H1-3 and 5) were double-blind and placebo-controlled, with or without additional active controls (sitagliptin, glimepiride, or pioglitazone). FPG mean change from baseline was a secondary endpoint calculated using an analysis of covariance model.
Results: At baseline, participants were required to be ≥18 years old, with HbA1c 7.0-10.0% (53.0-85.8 mmol/mol) and creatinine clearance >60 mL/min (Cockcroft-Gault formula). HbA1c decreased significantly versus placebo in each trial (differences from placebo across trials ranging from −0.8% to −1.04% [−8.8 to −11.4 mmol/mol]). Baseline FPG (standard deviation) ranged from 9.1 (2.3) mmol/L (HARMONY 3, placebo arm) to 9.9 (3.2) mmol/L (HARMONY 5, pioglitazone arm). In all four trials, placebo-subtracted FPG levels for albiglutide decreased within 1 week by >0.8 mmol/L (Figure, $P<0.0001$ vs baseline for all). At 1 year, placebo-subtracted FPG (95% CI) ranged from −1.3 (−1.9,−0.8) mmol/L for albiglutide 30-50 mg/week in HARMONY 5 to −2.4 (−3.1, −1.7) mmol/L for albiglutide 50 mg in HARMONY 2. The proportion of participants experiencing any adverse event was similar between albiglutide and comparators.
Conclusion: In adults with T2DM, albiglutide lowered FPG from baseline as early as Week 1, and levels remained reduced for 1 year. Previously presented at ADA 2016 and adapted with permission.

Placebo-subtracted mean change from baseline in fasting plasma glucose (ITT population – LOCF) 1–4 and 52 weeks*



*$P<0.0001$ vs baseline for all; Albi, albiglutide; FPG, fasting plasma glucose; H, HARMONY; ITT, intent-to-treat; LOCF, last observation carried forward.

Clinical Trial Registration Number: NCT00849056; NCT00849017; NCT00838903; NCT00839527
Supported by: GlaxoSmithKline.
Disclosure: **S. Forero-Schwanhaeuser:** Employment/Consultancy; GlaxoSmithKline. Stock/Shareholding; GlaxoSmithKline.

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Liraglutide increases circulating miR-27b, miR-130a and miR-210 in patients with type 2 diabetes: a 4-month pilot study

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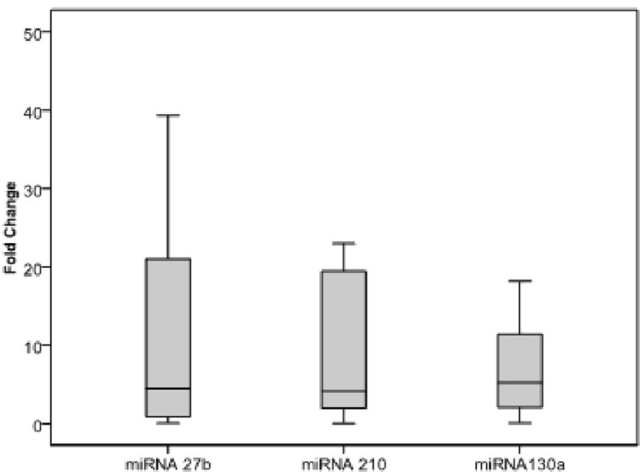
Background and aims: Liraglutide has been shown to have significant effects on several cardiovascular risk markers, beyond the modulation of glucose metabolism. MicroRNAs (miRs), endogenous 21-25 nucleotides noncoding RNA, are regulators of gene expression that postranscriptionally modify cellular responses and function. Specific miRs, including miR-130a, miR-27b, and miR-210, appear to play a role in cardio-metabolic diseases, since they are involved in molecular mechanisms maintaining endothelial cell homeostasis and preventing vascular inflammation and metabolism dysfunction. Yet, the impact of liraglutide treatment on specific miRs is still unknown.
Materials and methods: Ten subjects (7 men and 3 women; age: 63±10 years), with T2DM naïve to incretin-based therapies, were treated with liraglutide at a fixed dose of 1.2 mg/day as add-on therapy to metformin (1500-3000 mg/day) for 4 months. miRs were isolated from sera using the mirVana miRNA Isolation Kit (Ambion, Waltham, MA, USA), and then quantificated by SYBR Green Real-Time (RT) polymerase chain reaction (PCR).
Results: Statistical analysis was performed by Wilcoxon test and Spearman correlation method. After 4 months of liraglutide therapy, it was observed a significant reduction in fasting glycaemia (from 12.4 ±6.8 to 6.3±1.4 mmol/L, p=0.012), A1c (from 8.7±1.8 to 6.4±1.1%, p=0.011), total-cholesterol (from 5.2±1.1 to 3.9±0.5 mmol/L, p=0.017) and LDL-cholesterol (from 3.3±1.0 to 2.3±0.6 mmol/L, p=0.037). As shown in the box plot, levels of miR-27b, miR-210 and miR-130a were significantly increased after liraglutide therapy (4.4, 4.1 and 5.2 median fold change, respectively). No correlation was found between changes in the evaluated miRs and those in any other evaluated metabolic parameter.
Conclusion: We provide evidence, for the first time, showing that liraglutide may exert epigenetic effect in patients with T2DM by regulating miRs involved in the maintenance of endothelial cell homeostasis.



Clinical Trial Registration Number: NCT01715428
Disclosure: **R.V. Giglio:** Other; RVG has participated in clinical trials sponsored by AstraZeneca and Novo Nordisk.

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Effects of exenatide once weekly on dynamics of 24-h glucose as a function of baseline age and mean daily glucose in patients with type 2 diabetes

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Background and aims: Reducing glycaemic fluctuations is an important component of glucose control for patients with type 2 diabetes (T2D). This randomized, controlled, double-blind, phase 4 clinical trial used data obtained from continuous glucose monitoring (CGM) to investigate the effects of the glucagon-like

peptide-1 receptor agonist exenatide once weekly (EQW) compared with placebo (PBO) on glucose fluctuations in patients with T2D. Here, our objective was to analyse the total energy of daily glucose fluctuations and the effects of treatment, age, and mean daily glucose on this parameter.

Materials and methods: Patients with T2D uncontrolled on background metformin (MET) were randomised to EQW 2 mg or PBO for 10 weeks. Glucose concentration was measured over 7 days at lead-in, Week 4, and Week 10 using CGM (Dexcom G4). An exploratory outcome was total energy of glucose fluctuation in each treatment group, which was calculated as the sum of squared frequency times amplitude of Fourier coefficients for the 24-h individual average glucose curves averaged for a week over 24 h. This measure provides additive qualitative data in addition to mean amplitude of glucose excursions (MAGE). To investigate the effects of age and glucose concentration at baseline on glucose fluctuation, total energy was analysed by baseline covariates of age (45, 56, and 65 y) and 24-h mean glucose (140, 182, and 220 mg/dL) at baseline, Week 4, and Week 10. This was a Bayesian linear model analysis of the total energy together with the components of energy within the frequency domain. The linear model included study arm as well as baseline age and 24-h mean glucose. Prior distributions on the regression coefficients were chosen to be relatively flat.

Results: In the EQW (N=60)/PBO (N=56) groups (modified intent-to-treat population), mean baseline age was 55/56 y, HbA1C was 8.2/8.0%, and 24-h mean weighted glucose was 186/184 mg/dL. EQW reduced total energy versus PBO [Week 10: −2.5 vs 2.4 $(mg/(dL*h))^2/1000$ over 24 h; treatment difference: −4.9 $(mg/(dL*h))^2/1000$ over 24 h, P=0.003] from a baseline of 12.3/10.0 $(mg/(dL*h))^2/1000$ over 24 h in the EQW/PBO groups. Energy spectrum analysis at Week 10 showed reductions of slower glucose fluctuations rather than fast fluctuations. At Week 10, EQW numerically reduced total energy from baseline across almost all baseline subgroups, with substantial differences depending on age and mean glucose at baseline (Table). Energy spectrum results analysed by baseline covariates were similar at Week 4.

Conclusion: In patients with T2D treated with background MET, EQW significantly reduced glucose fluctuations, as measured by total energy, compared with PBO. Effects on glucose fluctuations with EQW differed by baseline age and glucose. This energy analysis provided insight into the quality of glycaemic control that is additive to that of MAGE and other more common measures.

Table. Total Energy Over 24 h as a Function of Baseline Age and 24-h Mean Glucose, and Probability Bounds of the Treatment Difference (EQW − PBO)

Baseline Age (y) / 24-h Mean Glucose (mg/dL) Subgroup		Baseline EQW N=61 PBO N=56		Week 10 EQW N=51 PBO N=48	
Age	/ Glucose	Total Energy EQW/ PBO $(mg/(dL*h))^2$ /1000	95% Probability bounds (EQW−PBO)	Total Energy EQW/ PBO $(mg/(dL*h))^2$ /1000	95% Probability bounds (EQW−PBO)
45	140	6.8 / 8.0	−3.7 to 2.6	7.9 / 10.5	−5.7 to 2.5
	182	9.1 / 9.6	−3.3 to 3.2	8.9 / 12.2	−6.2 to 0.9
	220	11.7 / 11.4	−3.5 to 5.9	9.8 / 13.9	−7.7 to 1.6
56	140	6.9 / 7.2	−2.4 to 2.6	6.4 / 8.6	−4.3 to 0.9
	182	9.2 / 8.7	−1.5 to 3.0	7.2 / 9.9	−4.5 to −0.6
	220	11.9/ 10.3	−1.6 to 6.0	7.9 / 11.3	−5.9 to 0.2
65	140	7.0 / 6.7	−2.1 to 3.9	5.4 / 7.3	−3.9 to 1.2
	182	9.3 / 8.0	−1.4 to 5.0	6.0 / 8.4	−4.2 to 0.2
	220	12.0 / 9.4	−1.4 to 8.6	6.7 / 9.6	−5.4 to 1.0
EQW, exenatide once weekly; PBO, placebo.					

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The effect of glucagon-like peptide-1 receptor agonists on postprandial glucagon secretion independent of the gastric emptying rate

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Background and aims: Glucagon-like Peptide-1 (GLP-1) delays gastric emptying (GE) and inhibits glucagon secretion. The emptying effect rapidly shows tachyphylaxis during liraglutide treatment whereas it is sustained with exenatide. The effect of GLP-1 on glucagon secretion independent of the GE rate is largely unknown. We investigated the glucagonostatic effect of the short- and long-acting glucagon-like peptide-1 receptor agonists (GLP-1RA), exenatide and liraglutide, respectively, independent of the GE rate to see whether the glucagonostatic effect would show tachyphylaxis with either drug

Materials and methods: We conducted a randomised study, in which 8 persons with type 1 diabetes (T1D) (mean±SD; age 39±6 years, BMI 24.5 ±3.5 kg/m2, HbA1c 68±11 mmol/mol, diabetes duration 19±10 years, and C-peptide negative) were given a liquid meal (Boost, Nestlé, 240 Kcal) administered by gastroscopy directly into duodenum over 3 minutes at baseline and after two weeks´ crossover treatment with liraglutide 1.2 mg once daily/exenatide 10 μg twice daily as add-on to insulin treatment. The primary outcome was postprandial glucagon responses. Secondary outcomes included glucose-dependent insulinotropic peptide (GIP), glicentin, GLP-1, and somatostatin

Results: Postprandial glucagon total areas-under-the-curve (AUCs) did not differ between baseline (1735 pM x 240 min), or during liraglutide (1552 pM x 240 min; p=0.45 vs. baseline) or exenatide treatment (1663 pM per 240 min; p=0.62 vs. baseline), respectively. Postprandial glucose incremental AUCs were similar on the experimental days; 1112 mM x 240 min at baseline, 1082 mM x 240 min with liraglutide (p=0.18 vs. baseline) and 1235 mM x 240 min with exenatide (p=0.64 vs. baseline). Peak postprandial concentrations of GIP increased significantly with liraglutide compared with exenatide (p=0.0374) and baseline (p=0.0421). Liraglutide significantly suppressed peak postprandial GLP-1 compared with baseline (p=0.022). Glicentin increased and somatostatin did not change on all experimental days with no treatment-related differences (table)

Conclusion: Neither liraglutide nor exenatide suppressed postprandial glucagon secretion or altered plasma glucose excursions when differences in GE rates were prevented, suggesting that neither short nor long-acting GLP-1RA´s act directly upon duodenally stimulated glucagon secretion in C-peptide negative patients with T1D. All the gastrointestinal hormones increased substantially, probably caused by rapid entry of nutrients to the gut. Liraglutide significantly increased GIP and suppressed GLP-1 secretion

Key results: Peak postprandial concentrations.

Hormone/intervention	Exenatide	Liraglutide	Placebo
Glucagon (pM)	24.8 ± 3.9	24.0 ± 2.9	22.5 ± 3.8
GIP (pM)	97.3 ± 12.0	162.8 ± 38.7*	99.1 ± 12.0
Glicentin (pM)	391.6 ± 54.1	412.2 ± 50.7	423.4 ± 33.9
GLP-1 (pM)	212.0 ± 39.8	146.3 ± 27.9**	241.6 ± 38.9

Data are mean±SEM
*p=0.0421 vs. baseline; p=0.0374 vs. exenatide
**p=0.022 vs. baseline; p=0.098 vs. exenatide

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PS 074 GLP-1 RA: combination with insulin

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Effect of exenatide after short-time intensive insulin therapy on maintenance of 2-year drug-free remission in patients with newly diagnosed type 2 diabetes

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Background and aims: Short-term intensive insulin (STII) therapy can induce long term drug-free remission in newly diagnosed type 2 diabetes mellitus (T2DM) patients, but more than half of them will suffer hyperglycemia relapse within 1-2 year. Whether glucagon-like peptide-1 receptor agonist (GLP-1RA) following STII therapy will induce higher long-term glycaemic remission is currently unknown. We did a randomised controlled trial to assess the effect of STII+exenatide therapy, compared with STII only, on maintenance of 2-year drug-free glycaemic remission in newly diagnosed T2DM patients.
Materials and methods: 157 newly diagnosed T2DM patients were screened in our university hospital. Of the 129 eligible patients (66 in STII+exenatide group and 63 in STII only group), all received insulin with an insulin pump for 2 weeks after the glycaemic remission target was reached, then patients in STII+exenatide group was treated with exenatide for 12 weeks further, and no further treatment for those in STII only group. All patients were then instructed to continue diet and physical exercise only. Systematic glycaemic monitoring (FPG and 2-h postprandial plasma glucose tests) was conducted monthly during the initial 3 months and at a 3-month interval thereafter. The primary endpoint was maintenance of 2-year glycaemic remission after STII.
Results: Within 24-month duration of follow-up after STII therapy, 37 patients in STII+exenatide group and 22 patients in STII only group sustained in glycaemic remission goal without any further anti-diabetic medication. The overall cumulative probability of maintenance of 2-year drug-free remission in the STII+exenatide group was 53.0±6.1%, which was significantly higher than that of STII only group (31.8±5.9%) (log-rank test: $\chi2=460.11$, $p< 0.001$). Further analyses showed that during the first 12 weeks of follow-up (duration of exenatide treatment for STII+exenatide group), 60 patients in STII+exenatide group and 37 patients in STII only group sustained in glycaemic remission with the cumulative probabilities of maintenance of drug-free remission of 90.9±3.5% for the STII+exenatide group and 60.3±6.2% for the STII only group (log-rank test: $\chi2=343.92$, $p< 0.001$); while during the remaining period of follow-up, the cumulative probabilities of maintenance of drug-free remission were not statistically significant between the two groups (log-rank test: $\chi2=0.063$, $P =0.802$).
Conclusion: As compared to STII only therapy, sequential exenatide therapy for 12 weeks can induce significantly higher maintenance of overall 2-year drug-free glycaemic remission. However, the protective effect of exenatide on glycaemia control, body fat reduction and maintenance of glycaemic remission disappeared upon cessation of exenatide treatment.
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Disclosure: **X. Shi:** None.

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Postprandial glycaemic outcomes of a fixed-ratio combination of insulin glargine and lixisenatide in the LixiLan-L trial

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Background and aims: In patients with type 2 diabetes (T2D) inadequately controlled on basal insulin, the LixiLan-L trial demonstrated the efficacy and safety of LixiLan (iGlarLixi), a novel titratable fixed-ratio combination of insulin glargine 100U (iGlar) and lixisenatide (Lixi), compared with iGlar alone, with up to two oral glucose-lowering drugs. By itself, Lixi is a once-daily, prandial glucagon-like peptide-1 receptor agonist with a predominant postprandial plasma glucose (PPG)-lowering effect brought about mainly by delaying gastric emptying. This mechanism is complementary to the fasting plasma glucose-lowering effect of iGlar, and, therefore, the combination of the two agents as iGlarLixi could potentially lead to better glycaemic control compared with individual agents in patients with T2D. Here we report an exploratory analysis of iGlarLixi PPG outcomes from the LixiLan-L trial.
Materials and methods: LixiLan-L had a 6-week run-in when iGlar was introduced or optimized; participants were then randomized to iGlarLixi or iGlar. Post hoc analyses were performed to assess the percentage of patients reaching PPG <7.8 (AACE target) or <10 mmol/L (ADA/EASD target) at 0.5, 1 and 2 h after a standardized liquid meal at baseline and Week 30, PPG 0-2 h area under the curve (AUC0-2h; after breakfast liquid meal) change at Week 30 (end of treatment), and 7-point self-monitored plasma glucose (SMPG) at selected times. P-values were calculated between treatment groups for all parameters analysed.
Results: At Week 30, the percentage of patients with PPG <7.8 mmol/L and <10 mmol/L at 0.5, 1 and 2 h post standardized liquid meal (Table) was greater in the iGlarLixi group versus the iGlar group; this difference was greatest at later time points post-meal. Compared with iGlar, treatment with iGlarLixi also resulted in a significantly greater reduction in PPG AUC0-2h (Figure). SMPG profiles at time points after three daily meals showed larger percentages of iGlarLixi patients at the PPG targets compared with iGlar, with the difference decreasing post-dinner (Table).
Conclusion: In patients with T2D uncontrolled with basal insulin in LixiLan-L, iGlarLixi demonstrated greater postprandial glycaemic control compared with iGlar, with consistently more patients reaching PPG targets after all meals throughout the day.

Table. PPG outcomes from the LixiLan-L trial

			LixiLan (iGlarLixi)	Insulin glargine 100U (iGlar)	p-value†
			n=335*	n=342*	
% pts PPG <7.8 mmol/L (meal test)	Baseline	0.5 h	11.7	12.8	0.654
		1 h	4.9	3.9	0.530
		2 h	3.1	1.2	0.096
	Week 30	0.5 h	41.4	25	<0.0001
		1 h	30.7	8.3	<0.0001
		2 h	33.6	5.4	<0.0001
% pts PPG <10 mmol/L (meal test)	Baseline	0.5 h	39.0	39.9	0.808
		1 h	15.5	14.3	0.660
		2 h	8.0	8.7	0.742
	Week 30	0.5 h	71.2	59.5	0.002
		1 h	55.6	27.7	<0.0001
		2 h	58.1	17.3	<0.0001
			n=294*	n=298*	
% pts PPG <7.8 mmol/L (SMPG)	Week 30	10 am	61.2	28.7	<0.0001
		3 pm	41.1	24.6	<0.0001
		10 pm	45.9	33.7	0.005
% pts PPG <10 mmol/L (SMPG)	Week 30	10 am	86.7	62.9	<0.0001
		3 pm	78.7	54.7	<0.0001
		10 pm	74.9	65.5	0.019
			n=320	n=326	
Mean change ± SE in PPG AUC_{0-2h} (mmol/L)	Week 30		–6.28±0.35	–2.78±0.35	<0.0001

*n=number of patients with data at baseline and Week 30 at time 0 h
†p-values for % pts PPG are from Chi-square test; p-values for PPG AUC_{0-2h} are from trapezoid method with two-sample *t*-test
AUC=area under the curve; iGlar=insulin glargine 100U; iGlarLixi=insulin glargine 100U/lixisenatide fixed-ratio combination; PPG=postprandial plasma glucose; pts=patients; SE=standard error; SMPG=self-monitored plasma glucose

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Insulin glargine/lixisenatide fixed ratio combination improves glycaemic variability in type 2 diabetes

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Background and aims: iGlarLixi is a once-daily titratable, single injection of a fixed-ratio combination of insulin glargine 100 U/mL (Gla-100) and lixisenatide, and is in development for the treatment of type 2 diabetes.
Materials and methods: This post-hoc analysis compared glycemic variability (GV) as measured by the high blood-glucose index (HBGI) and area under the curve (AUC) of patient self-monitored plasma glucose (SMPG) 7-point profile data from the Phase 3, 30-week LixiLan-O trial comparing iGlarLixi, Gla-100, and lixisenatide in 1,170 patients uncontrolled on metformin ± 1 other oral antidiabetes drug [OAD], and the LixiLan-L trial comparing iGlarLixi with Gla-100 in 736 patients uncontrolled on basal insulin ± 1 or 2 OADs. In both trials, only metformin was continued upon study initiation and dosing was either optimized up to 2,000 mg/day or stabilized ≥ 1,500mg/day.
Results: Compared with Gla-100 or lixisenatide alone, iGlarLixi resulted in a statistically significant improvement in GV profiles as indicated by the HBGI and AUC metrics (see Table), without a clinically significant change in the low blood-glucose index as a proxy for hypoglycemia (remaining < 1.0 for all). In addition, statistically significant mean blood-glucose level reductions were achieved.
Conclusion: In conclusion, iGlarLixi demonstrated a cumulative decrease in GV, greater than each of its components (Gla-100 and lixisenatide), in both the LixiLan-O and LixiLan-L trials.

Table: Patient characteristics and glycemic variability outcomes.

	LLO Study			LLL Study	
	iGlarLixi n = 300	Gla-100 n = 284	Lixisenatide n = 144	iGlarLixi n=246	Gla-100 n = 238
Baseline characteristics					
Age, years	57.9	58.5	59.3	58.9	60.4
Female, %	56	53	44	57.3	52.9
A1C, %	8	8.1	8.2	8.0	8.1
FPG, mmol/L	9.8	9.6	9.8	7.5	7.3
Mean SMPG,[a] mmol/L					
Baseline	10.4	10.2	10.4	9.2	9.0
Week 30	7.0	7.7	8.6	7.7	8.6
Change vs baseline	-3.4	-2.5	-1.9	-1.5	-0.4
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.002
Mean change vs iGlarLixi		0.9	1.5		1.1
P value		< 0.0001	< 0.0001		< 0.0001
HBGI[a]					
Baseline	10.3	9.8	10.4	7.0	6.3
Week 30	2.0	3.4	5.1	3.7	5.7
Change vs baseline	-8.3	-6.3	-5.3	-3.3	-0.56
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.116
Mean change vs iGlarLixi		2.0	3.0		2.79
P value		< 0.0001	< 0.0001		< 0.0001
AUCn[a]					
Baseline	2633	2592	2641	2355	2294
Week 30	1775	1973	2149	1954	2205
Change vs baseline	-858	-619	-492	-401	-90
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.004
Mean change vs iGlarLixi		239	366		312
P value		< 0.0001	< 0.0001		< 0.0001

[a]Based on 7-point blood SMPG profiles values (pre-injection fasting glucose, 2 hours after breakfast, before lunch, 2 hours after lunch, before dinner, 2 hours after dinner, at bedtime). AUCn, area under curve calculated for each subject. Comparisons used two sample t-tests.

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Patient-reported outcomes with once weekly dulaglutide versus placebo, both in combination with once daily insulin glargine (+/- metformin) in type 2 diabetes (AWARD-9)

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Background and aims: This was a 28-week (wk), randomised, double-blind study that compared once weekly injection of dulaglutide (DU) 1.5 mg to placebo (PL), both added to titrated once daily insulin glargine (±metformin) in patients with type 2 diabetes and inadequate glycaemic control (HbA1c ≥7% [53 mmol/mol] and ≤10.5% [91 mmol/mol). Patient reported outcomes (PRO) were assessed as an exploratory objective to further understand patients' physical, psychological, and social aspects of well-being as well as patient experience with injection device.
Materials and methods: Patients (N = 300; mean baseline [BL] characteristics: age 60.4 y; HbA1c 8.4% [68 mmol]; BMI 32.7 kg/m^2; glargine dose 39 U [0.42 U/kg]) were randomised (1:1) to DU 1.5 mg, or PL; glargine was titrated to fasting plasma glucose target (71 to 99 mg/dl [3.9 to 5.5 mmol/l]). Impact of Weight on Self-Perceptions (IW-SP), EQ-5D-5L, 18-item Diabetes Health Profile (DHP-18), and Medication Device Delivery Assessment (MDDAB) for the DU Single Use Pen (SUP) and glargine delivery device were administered at baseline and 28 wks; and 6 wks or 12 wks for some PRO instruments. A mixed model for repeated measures was used to analyse change from baseline scores.

Results: DU resulted in significantly (P<0.001) greater reductions than PL in HbA1c and fasting serum glucose; body weight decreased with DU and increased with PL (P <0.001). Hypoglycaemia events were similar between groups, and nausea and diarrhoea were more common with DU vs PL. Improvement observed with DU for the IW-SP Transformed Total Score at 28 wks was significantly better compared to PL (LSM difference 6.06; P=0.019). There were no significant overall between-treatment differences in quality of life as measured by EQ-5D-5L change from baseline at 28 wks. There were no significant between-treatment differences in changes from baseline at 28 wks in the Barriers to Activities or Psychological Distress Domains of the DHP-18. Improvement with DU in the DHP-18 Disinhibited Eating Domain Transformed Score was significantly better compared to PL at 28 wks (LSM difference -4.50 ; P=0.017). For all patients, 95% of patients reported that overall, the SUP was "Easy" or "Very Easy" to use at 28 wks. Device features scores showed the majority of patients liked the SUP features, with the 3 highest rated items relating specifically to features of the needle (not having to touch the needle, not having to attach the needle, the automatic insertion). The majority of patients (~90%) "Agreed" or "Strongly Agreed" that they were satisfied with the overall SUP injection experience at 28 wks.

Conclusion: DU-treated patients had greater improvements in weight-related quality of life measures compared to PL-treated. These PRO results were consistent with clinical results given significant weight reduction observed for DU compared to weight gain for PL. Having favourable weight-related PRO results can be viewed as beneficial when evaluating treatment options. Results from the MDDAB indicated overall satisfaction with the DU SUP injection experience, which may be an important factor for some patients when initiating injectable therapy.

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Disclosure: **M. Yu:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Outcomes of LixiLan, a fixed-ratio combination of insulin glargine/lixisenatide, versus insulin glargine and lixisenatide by baseline characteristics: LixiLan-O trial

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Background and aims: LixiLan-O was an open-label trial comparing the efficacy and safety of LixiLan (iGlarLixi), a novel titratable fixed-ratio combination of insulin glargine 100U (iGlar) plus lixisenatide (Lixi), with iGlar alone and Lixi alone over 30 weeks in patients with type 2 diabetes (T2D) inadequately controlled on metformin ± a second oral glucose-lowering drug.

Materials and methods: In this exploratory analysis, participants were split according to baseline characteristics: HbA1c <8%, ≥8%; duration of T2D <7, ≥7 years; and BMI <30, ≥30 kg/m2.

Results: iGlarLixi was shown to be consistently effective in all subgroups, including those with defined obesity and high HbA1c (Table). Glycaemic control was consistently improved with iGlarLixi, with more responders in all subgroups compared with the iGlar or Lixi component subgroups. There was no increase in the incidence of hypoglycaemia in the iGlarLixi versus the iGlar groups, despite better glycaemic control.

Conclusion: iGlarLixi consistently improved glycaemic control compared with iGlar and Lixi, without an increase in hypoglycaemic risk compared with iGlar in all subpopulations tested, and results were consistent with those obtained for the total LixiLan-O cohort population.

		LixiLan (iGlarLixi , QD)			Insulin glargine 100U (iGlar, QD)			Lixisenatide (Lixi, QD)		
Baseline characteristics subgroup (N=iGlarLixi;iGlar;Lixi)	**Parameters**	**HbA$_{1c}$, %**	**%HbA$_{1c}$ <7%***	**Hypo, %† (events/ patient-year)**	**HbA$_{1c}$, %**	**%HbA$_{1c}$ <7%***	**Hypo, %† (events/ patient-year)**	**HbA$_{1c}$, %**	**%HbA$_{1c}$ <7%***	**Hypo, %† (events/ patient-year)**
HbA$_{1c}$ <8%	BL	7.5			7.5			7.5		
(N=222;223;109)	Week 30	6.3	83.8	23.0	6.7	67.7	22.4	7.0	50.5	5.5
for Resp/Hypo	Change	−1.2		(1.3)	−0.8		(1.4)	−0.5		(0.2)
(N=222;223;110)	‡Diff				−0.3±0.1	16.0±4.0		−0.7±0.1	33.3±5.2	
HbA$_{1c}$ ≥8%	BL	8.6			8.6			8.7		
(N=245;241;124)	Week 30	6.7	69.8	27.9	7.0	53.9	24.6	7.6	19.4	7.3
for Resp/Hypo	Change	−1.9		(1.6)	−1.6		(1.1)	−1.1	51.4±4.5	(0.5)
(N=247;244;123)	‡Diff				−0.3±0.1	16.5±4.4		−0.8±0.1		
Duration of diabetes	BL	8.0			8.1			8.0		
<7 years	Week 30	6.5	75.2	21.3	6.9	61.9	19.0	7.2	37.5	7.3
(N=202;210;96)	Change	−1.5		(1.3)	−1.2		(0.8)	−0.8		(0.3)
for Resp/Hypo	‡Diff				−0.3±0.1	13.8±4.5		−0.7±0.1	38.9±5.4	
(N=202;210;96)										
Duration of diabetes	BL	8.1			8.1			8.2		
≥7 years	Week 30	6.6	72.6	28.8	6.8	57.4	27.2	7.4	29.9	5.8
(N=265;254;137)	Change	−1.6		(1.6)	−1.3		(1.6)	−0.8		(0.4)
for Resp (N=266;256; 137) for Hypo (N=267;257; 137)	‡Diff				−0.3±0.1	15.5±4.1		−0.8±0.1	43.2±4.6	
BMI <30 kg/m^2	BL	8.1			8.1			8.1		
(N=173;178;74)	Week 30	6.5	78.6	31.6	6.9	57.9	29.1	7.4	28.4	12.0
for Resp/Hypo	Change	−1.6		(2.2)	−1.2		(1.8)	−0.7		(0.8)
(N=174;179;75)	‡Diff				−0.4±0.1	20.3±4.7		−0.9±0.1	50.4±5.8	
BMI ≥30 kg/m^2	BL	8.1			8.1			8.1		
(N=294;286;159)	Week 30	6.6	75.2	22.0	6.8	62.2	20.1	7.3	36.5	3.8
for Resp/Hypo	Change	−1.5		(1.0)	−1.3		(0.9)	−0.8		(0.1)
(N=295;288;158)	‡Diff				−0.3±0.1	13.3±3.8		−0.7±0.1	38.4±4.3	

All data are mean unless stated otherwise

*Responders are defined as patients who achieved HbA$_{1c}$ <7%, LOCF (HbA$_{1c}$ <8%, ≥8% and BMI <30, ≥30 kg/m^2 subgroups) or at Week 30 (duration of diabetes <7, ≥7 years subgroups).

†Incidence of documented symptomatic hypoglycaemia (% of patients experiencing hypoglycaemia, where hypoglycaemia is defined as an event with typical symptoms accompanied by a measured plasma glucose concentration of ≤3.9 mmol/L)

‡Diff=mean difference iGlarLixi versus iGlar or Lixi±SE

BL=baseline; iGlar=insulin glargine 100U; iGlarLixi=insulin glargine 100U/lixisenatide fixed-ratio combination; Hypo=hypoglycaemia; Lixi=lixisenatide; LOCF=last observation carried forward; QD=once daily; Resp=responders

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Clinical impact of LixiLan, a fixed-ratio combination of insulin glargine plus lixisenatide in type 2 diabetes inadequately controlled on oral agents: LixiLan-O trial

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Background and aims: The efficacy and safety of LixiLan (iGlarLixi), a novel titratable fixed-ratio combination of insulin glargine 100U (iGlar) with lixisenatide (Lixi), was compared with both components separately (iGlar and Lixi) in patients with type 2 diabetes inadequately controlled on metformin (Met) ± a second oral glucose-lowering drug.

Materials and methods: After a 4-week run-in to optimize Met and stop other oral antidiabetic drugs, participants (N=1170) with mean diabetes duration ~9 years and BMI ~32 kg/m2 were randomized (2:2:1) to once-daily iGlarLixi or iGlar titrated to maintain fasting plasma glucose 4.4-5.6 mmol/L (maximum 60 U/day for both groups), or Lixi (10 μg then 20 μg maintenance dose), continuing with Met for 30 weeks.

Results: iGlarLixi showed greater reductions in HbA1c from baseline (8.1%) versus iGlar and Lixi (-1.6%, -1.3% and -0.9%, respectively; p<0.0001), reaching mean HbA1c levels of 6.5%, 6.8% and 7.3%, respectively, at Week 30 (Table). More patients reached target HbA1c <7%

with iGlarLixi (74%) vs iGlar (59%) or Lixi (33%). Mean body weight increased with iGlar (+1.1 kg), and decreased with iGlarLixi (-0.3 kg; difference 1.4 kg, p<0.0001) and Lixi (-2.3 kg). Documented (≤3.9 mmol/L) symptomatic hypoglycaemia was similar with iGlarLixi (1.4 events/year; E/Y) and iGlar (1.2 E/Y), but lower with Lixi (0.3 E/Y). iGlarLixi improved postprandial glycaemic control versus iGlar, and demonstrated considerably fewer nausea and vomiting events than Lixi.
Conclusion: iGlarLixi meaningfully improved glycaemic control with no weight gain and without increase in hypoglycaemic risk compared with iGlar, and with low levels of gastrointestinal side effects compared with Lixi.

Endpoint	LixiLan (iGlarLixi, QD) (n=468)	Insulin glargine 100U (iGlar, QD) (n=466)	Lixisenatide (Lixi, QD) (n=233)
HbA_{1c} (%)			
Baseline	8.1±0.7	8.1±0.7	8.1±0.7
Week 30	6.5±0.8	6.8±0.8	7.3±0.9
LS mean±SE change from baseline*	−1.6±0.04	−1.3±0.04	−0.9±0.05
LS mean±SE difference iGlarLixi vs iGlar*		−0.3±0.05	
95% CI		−0.4; −0.2	
p value		<0.0001	
LS mean±SE difference iGlarLixi vs Lixi*			−0.8±0.06
95% CI			−0.9; −0.7
p value			<0.0001
Proportion of patients reaching HbA_{1c} <7.0% at Week 30 (%)	73.7	59.4	33.0
Difference in proportion (iGlarLixi vs iGlar)†		14.3	
95% CI		8.4; 20.3	
p value		<0.0001	
Difference in proportion (iGlarLixi vs Lixi)†			40.6
95% CI			33.6; 47.6
p value			<0.0001
FPG (mmol/L)			
Baseline	9.9±2.3	9.8±2.3	9.8±2.1
Week 30	6.3±1.5	6.5±1.8	8.3±2.2
LS mean±SE change from baseline*	−3.5±0.1	−3.3±0.1	−1.5±0.1
LS mean±SE difference iGlarLixi vs iGlar*		−0.2±0.1	
95% CI		−0.42; 0.04	
LS mean±SE difference iGlarLixi vs Lixi*			−2.0±0.1
95% CI			−2.2; −1.7
p value			<0.0001
2-hour PPG (mmol/L)			
Baseline	15.2±3.6	14.6±3.6	14.7±3.3
Week 30 (LOCF)	9.2±3.2	11.4±3.1	10.0±3.9
LS mean±SE change from baseline‡	−5.7±0.2	−3.3±0.2	−4.6±0.2
LS mean±SE difference iGlarLixi vs iGlar‡		−2.4±0.2	
95% CI		−2.8; −2.0	
LS mean±SE difference iGlarLixi vs Lixi‡			−1.1±0.3
95% CI			−1.6; −0.6
Body weight (kg)			
LS mean±SE change from baseline*	−0.3±0.2	1.1±0.2	−2.3±0.3
LS mean±SE difference iGlarLixi vs iGlar*		−1.4±0.3	
p value		<0.0001	
LS mean±SE difference iGlarLixi vs Lixi*			2.0±0.3
Documented symptomatic hypoglycaemia (plasma glucose ≤3.9 mmol/L)			
Patients with event, n (%)	120 (25.6)	110 (23.6)	15 (6.4)
Number of events per patient-year	1.4	1.2	0.3
Documented symptomatic hypoglycaemia (plasma glucose ≤3.3 mmol/L)			
Patients with event, n (%)	66 (14.1)	50 (10.7)	6 (2.6)
Number of events per patient-year	0.5	0.3	0.1

Data are mean±SD unless otherwise specified for modified intent-to-treat population
*Mixed-effect model with repeated measures
†Cochran–Mantel–Haenszel method
‡Analysis of covariance model
iGlar=insulin glargine 100U; iGlarLixi=insulin glargine 100U/lixisenatide fixed-ratio combination
Lixi=lixisenatide; LOCF=last observation carried forward; LS=least squares; FPG=fasting plasma glucose; PPG=postprandial plasma glucose; QD, once daily

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PS 075 GLP-1 RA: GDM and real world

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Effects of the GLP-1 analogue liraglutide on ectopic fat distribution and sex hormones in women with PCOS: a randomised, clinical trial

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Background and aims: Polycystic ovary syndrome (PCOS) is associated with insulin resistance, abdominal obesity and high prevalence of non-alcoholic fatty liver disease (NAFLD), and consequently a markedly increased risk of type 2 diabetes. Treatment options are limited, why we investigated the impact of the GLP-1 analogue Liraglutide on ectopic fat, free testosterone and insulin resistance in women with PCOS.
Materials and methods: A total of 67 women with PCOS according to the Rotterdam criteria were randomized 2:1 to 1.8 mg Liraglutide or placebo daily for 26 weeks. Diabetes mellitus was exclusion criterion and the participants received no hormonal or anti-diabetic medication. Liver fat percent was determined by 1H MR Spectroscopy measuring triglyceride content in a 1.1 cm3 in the right liver lope. Visceral (VAT) and subcutaneous fat (SAT) were measured with MR Imaging in a 1 cm thick transverse slice at the L3-level. By DXA scan (Whole body fan mode, Discovery Hologic) the body was divided into two compartments: fat mass and lean mass (including bone mineral density). Blood pressure and heart rate were measured three times in the sitting position. HOMA2-IR was calculated from fasting plasma glucose and insulin. Total testosterone was analyzed with mass spectroscopy. The SAS proc mixed procedure with merged groups at baseline was used for statistical analyses.
Results: Mean (SD) age was 29.7 year (6.16), weight 92.0 kg (13.9), waist circumference 101.5 cm (9.9), BMI 32.9 kg/m2 (4.6), HOMAR2-IR 2.6 (1.2), median (SD) free testosterone 0.0266 nmol/L (0.017). At baseline 26.9% of the PCOS cohort had NAFLD defined as >5% liver fat. Liraglutide treatment vs. placebo reduced prevalence of NAFLD to 8.1% and 30%, respectively (P=0.009). Liraglutide vs. placebo treatment resulted in a reduction of 5.6% in weight (P=<0.001), 17% in VAT (P=0.007), 10% in SAT (P=1.0142), 19% in free testosterone (P=0.058), an increase of 19% in SHBG (sex hormone-binding globulin) (P=0.033) and 6.5% in heart rate (P=0.01). The following did not change: HOMA2-IR, DXA fat percent, LDL cholesterol, triglycerides or blood pressure.
Conclusion: Liraglutide seems as an attractive treatment option in PCOS, reducing ectopic fat in the liver and thus prevalence of NAFLD to one-third, as well as both visceral and subcutaneous fat, with an weight loss of 5,6% in 26 weeks. Further, a reduction in hyperandrogenism was demonstrated.
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Disclosure: **S. Frøssing:** Grants; Herlev Hospital Research Foundation, Novo Nordisk A/S.

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The effect of one-year treatment with a glucagon-like peptide-1 receptor agonist on NAFLD in women with prior gestational diabetes

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Background and aims: Prior gestational diabetes mellitus (GDM) is associated with an increased risk of non-alcoholic fatty liver disease (NAFLD). This study evaluates the effect of one-year treatment with liraglutide on NAFLD in non-diabetic women with prior GDM.
Materials and methods: Non-diabetic women with prior GDM (n=82, age (mean±standard deviation): 38±5 years, body weight: 88±16 kg, BMI: 32±5 kg/m^2) participating in a randomised, double-blinded, placebo-controlled trial evaluating liraglutide 1.8 mg subcutaneously once-daily (n=37) vs placebo (n=45) underwent abdominal ultrasound scanning to diagnose NAFLD at baseline and after one year. A subgroup (n=60) also underwent transient elastography scanning (FibroScan®) with assessment of intrahepatic fat by controlled attenuation parameter (CAP) at baseline and after one year (CAP: 266±48 dB/m).
Results: None of the participants had elevated liver blood tests or cirrhosis at baseline. Based on the abdominal ultrasound and elastography scanning, 18 women had NAFLD at baseline. Eleven women (13%) developed NAFLD during the one-year follow-up. Eight women had NAFLD at baseline, but not after one year. These women had lower alanine aminotransferase levels at the end of follow-up compared to baseline (p=0.042). None of the remaining patient characteristics were associated with resolution of NAFLD. Liraglutide had no effect on the prevention, development or resolution of NAFLD (p=NS), but significantly reduced intrahepatic fat assessed by CAP compared to placebo (-35±52 vs -5±52 dB/m, p=0.003). Liraglutide reduced body weight (4.7±5.2 vs 1.4±5.5 kg, p=0.007), but this effect was not associated with CAP values or resolution of NAFLD in multivariate analyses.
Conclusion: This one-year trial found no effect of liraglutide on the presence of NAFLD in women with prior GDM but a significant greater weight loss and reduced steatosis as measured by CAP was observed in women treated with liraglutide compared to placebo.
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Intervention with a glucagon-like peptide-1 receptor agonist improves glycaemic control in women with prior gestational diabetes: a randomised, placebo-controlled trial

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Background and aims: Women with prior gestational diabetes mellitus (GDM) are at high risk of developing type 2 diabetes and constitutes an opportunity for studying the early stages of the disease as well as therapeutic modulation of a high-risk condition.
Materials and methods: Overweight, non-diabetic women with prior GDM were randomised to the GLP-1RA liraglutide 1.8 mg subcutaneously once-daily for one year (n=49; age: 37.8±5.1 (mean±SD) years; bodyweight (BW): 90.4±19.1 kg; body mass index (BMI): 32.4±5.6 kg/m^2; fasting plasma glucose (FPG): 5.5±0.5 mol/l; HbA_{1c}: 32.8±4.2 mmol/mol; time since index pregnancy: 5.3±2.6 years) or placebo (n=55; age: 38.1±4.9 years; BW: 86.2±10.7 kg; BMI: 31.0±3.1 kg/m^2; FPG: 5.4±0.4 mmol/l; HbA_{1c}: 32.1±4.0 mmol/mol; time since index pregnancy: 4.9±2.5 years). At baseline, all parameters were similar in the two groups. The women underwent a 4-hour 75g oral glucose tolerance test (OGTT) at baseline and after one year (n=83). In addition, after one year, the liraglutide group underwent a second OGTT following one week's wash-out of liraglutide (n=40). The primary endpoint was defined as the change in glucose tolerance (total area under the curve (tAUC) for plasma glucose during OGTT) after one year's intervention.
Results: Glucose tolerance (tAUC) was similar in the two groups at baseline 1,706±202 vs 1,696±212 mmol/l × min (mean±SD), p=0.75) and was improved in the liraglutide group compared to the placebo group at one year (1,477±217 vs 1,691±253 mmol/l × min, p<0.0001). This difference was not sustained after one week's wash-out of liraglutide 1,712±284 vs 1,691±253 mmol/l × min, p=0.76). Accordingly, at one year the glucose tolerance (tAUC) (-218±190 mmol/l × min, p<0.0001) improved during treatment in comparison to placebo. Improvements during treatment were also observed for BW (-4.9±5.5 vs -1.3±5.5 kg, p=0.002); FPG (-0.4±0.4 vs -0.03±0.4 mmol/l, p<0.001) and HbA1c (-2.9±5.9 vs 0.2±6.2 mmol/mol, p=0.041) in comparison to placebo. For FPG, the difference was not sustained after one week's wash-out of liraglutide (-0.0±0.4 mmol/l p=0.96).
Conclusion: Treatment with the GLP-1RA liraglutide in women with prior GDM improved glucose tolerance, BW, FPG and HbA_{1c} compared to placebo, but the effects on FPG and glucose tolerance were not sustained after one week's wash-out of liraglutide.
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Disclosure: **S. Foghsgaard:** None.

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Treatment attributes of GLP-1 receptor agonists important to injection-naïve patients with type 2 diabetes: a multi-national preference study

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Background and aims: GLP-1RAs are a growing class of drugs for T2DM treatment that differ in efficacy, side effects (SEs), and other attributes. Understanding patient preferences for treatment can help inform the development of future treatments and increase patient acceptance, thereby improving adherence and health outcomes. The study objective was to assess the importance of treatment-related attributes in influencing preferences for GLP-1RAs among injection-naïve patients with T2DM in Brazil, China, Germany, Japan, and the UK.
Materials and methods: T2DM patients, who were naïve to injectable treatments and currently on oral treatments, were recruited through market research panels and completed a web-based, discrete choice experiment survey in local languages that elicited choices between hypothetical, blinded treatment profiles reflecting attributes for GLP-1RAs, including exenatide QW and liraglutide QD. Eight attributes were included in the survey: efficacy (improvement in A1c), common GLP-1 RA SEs (GI-related SEs and injection site

nodules), injection device size, needle size, need for titration, required injection preparation (associated with vial/syringe, single-use pen, multi-use pen or auto-injector), evidence of long-term efficacy/safety, and dosing frequency (daily or weekly). Odds ratios (OR) and 95% CIs were calculated using a conditional logit model to indicate likelihood of choosing a treatment with a given attribute level versus a reference attribute level.

Results: 1,482 patients with a mean age of 56 years completed the survey; 68% were male. Mean time since diagnosis was 7.0 years. Pooled and country specific analysis revealed that SEs, efficacy, and dosing frequency were the three most important attributes to patient preference, while needle size, device size, and required injection preparation were the least important. The impact and ranking of these attributes varied by country. Odds ratios comparing different attribute levels were shown in Table 1. Needle size was not a significant attribute for all countries; preparation was not significant for 4 of 5 countries. Pooled data showed that patients favored a profile of GLP-1RA approximating exenatide QW vs a profile approximating liraglutide QD (Overall OR 3.36; p<.001), with 77.0% of the sample preferring the profile approximating exenatide QW. When examined by country, preference was highest among the German sample (OR 4.93) and lowest among the Chinese sample (OR 1.60).

Conclusion: The most influential drivers of treatment preferences were SEs, efficacy, and dosing frequency among injection-naïve T2DM patients across all countries. Preference elicitation can promote patient-centered care as well as inform new generations of T2DM treatments, which can lead to improved patient medication acceptance, adherence, and patient health outcomes.

Table 1: Attribute Preference Results*

Country	Total N=1,482	Brazil n=296	China n=297	Germany n=296	Japan n=296	UK n=297
Mean age (SD)	56.0 (11.4)	51.7 (12.2)	49.9 (9.2)	58.8 (9.8)	57.2 (11.4)	62.3 (9.5)
Mean duration of T2DM in years	7.0	6.7	3.9	7.7	8.5	8.3
Side effects (exenatide QW vs liraglutide QD)	OR=2.14	OR=2.00	OR=1.27	OR= 2.39	OR= 2.55	OR=3.54
Efficacy (-1.5% A1c vs -0.8%)	OR=1.85	OR=1.97	OR=2.67	OR=1.92	OR=1.30	OR=1.75
Dosing Frequency (weekly vs daily)	OR=1.63	OR=1.75	OR=1.19	OR=1.71	OR=2.24	OR=1.69
Preparation Required (most preferred preparation type vs vial/syringe)[a]	Multi-use pen OR=1.09	Multi-use pen OR=1.10 ns	Auto-injector OR=1.06 ns	Multi-use pen OR=1.06 ns	Auto-injector OR=1.02 ns	Multi-use pen OR=1.36
Titration (No titration vs titration)	OR=1.12	OR=1.09	OR=0.99 ns	OR=1.15	OR=1.17	OR=1.23
Evidence of effectiveness and safety (8 years vs 1 year)	OR=1.30	OR=1.41	OR=1.17	OR=1.55	OR=1.14	OR=1.30
Device Size (most preferred size vs single-use pen)[b]	Auto-injector OR=1.04	Vial & syringe OR=1.06	Auto-injector OR=1.02	Auto-injector OR=0.99 ns	Vial & syringe OR=1.14	Auto-injector OR=1.07 ns
Needle Size (shorter/thinner vs longer/thicker)	OR=1.03	OR=1.05 ns	OR=1.02 ns	OR=0.98 ns	OR=1.05 ns	OR=1.07 ns

*All significant at p<.05 unless noted as "ns" indicating not significant
[a] Vial/syringe was used as reference for preparation preference
[b] Single-use pen was used as reference for device-size preference

Disclosure: **S. Chen:** None.

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Higher adherence and persistence observed in patients with type 2 diabetes treated with dulaglutide compared to exenatide once weekly or liraglutide

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Background and aims: There is limited real-world evidence on comparative adherence among the glucagon-like peptide-1 receptor agonists (GLP-1 RAs), including once-weekly dulaglutide.This retrospective observational study compared adherence and persistence over a 6-month follow-up period on a propensity-matched sample of patients who were treated with dulaglutide vs. exenatide once weekly, and with dulaglutide vs. liraglutide.

Materials and methods: The study utilized Early View data from a health research database to identify patients newly initiating GLP-1 RAs between November 2014 and April 2015. Index treatment was identified in hierarchical order (dulaglutide, albiglutide, exenatide once weekly, exenatide twice-a-day, liraglutide) with no index drug claim in the 6-month pre-index period. Patients who were ≥18 years old, had ≥1 claim with a type 2 diabetes diagnosis, and had continuous enrolment 6 months pre- and post-index were included.

Results: The initial cohorts included 2470 patients for dulaglutide, 5022 for exenatide once weekly and 8705 for liraglutide. After adjustment, the matched dulaglutide-exenatide once-weekly cohort had 2414 patients per arm and the matched dulaglutide-liraglutide cohort had 2037. There were no significant differences in baseline characteristics (age, gender, Deyo Charlson Comorbidity Index).

Conclusion: Results showed that patients on dulaglutide had higher medication adherence, were more persistent, and had lower discontinuation compared to patients on either exenatide once weekly or liraglutide.

	Dulaglutide vs. Exenatide Once Weekly			Dulaglutide vs. Liraglutide		
	Dulaglutide (N=2414)	Exenatide Once Weekly* (N=2414)	p-value	Dulaglutide (N=2037)	Liraglutide (N=2037)	p-value
Adherence -Proportion of Days Covered mean (SD)	72% (27)	61% (29)	<.0001	72% (27)	67% (28)	<.0001
Proportion of Days Covered >= 80%	54%	38%	<.0001	54%	44%	<.0001
Patients who discontinued	26%	49%	<.0001	28%	36%	<.0001
Persistence (days) mean (SD)	148.4 (55.4)	122.9 (61.5)	<.0001	146.6 (56.6)	136.1 (61.1)	<.0001
Patients who refilled the index medication	85%	67%	<.0001	84%	73%	<.0001

*72% of patients in the exenatide once weekly cohort used the pen and 28% used vial and syringe.

Disclosure: **L. Fernández Landó:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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Treatment and dosing patterns among patients with type 2 diabetes initiating glucagon-like peptide-1 receptor agonists (GLP-1 RAs) in Europe

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Background and aims: Several new GLP-1 RA therapies have been introduced in the past several years. This study evaluated recent real-world (RW) treatment patterns and average daily dose (ADD) among patients initiating GLP-1 RAs.

Materials and methods: Adult T2D patients initiating exenatide twice daily (exBID), liraglutide once daily (LIRA), exenatide once weekly (exQW) or lixisenatide once daily (LIXI) during 2013 were identified using retail pharmacy data from the IMS LifeLink™ longitudinal prescriptions (LRx) databases in Belgium (BE), France (FR), Germany (GE) and Netherlands (NE) (Notes: LIXI was removed from the German market in 4/2014. The liraglutide product indicated for weight management was specifically excluded from this study). The date of initiation was termed the "index date" and eligible patients had ≥180 days pre-index (with ≥1 OAM and no GLP-1 RA therapy used) and ≥360 days post-index of continuous patient and data stability. LIRA and exBID have variable maintenance dosing (LIRA: 1.2 or 1.8mg daily; exBID: 5 or 10μg twice daily). Treatment modifications and persistence on the index therapy were evaluated at 1-year post-index and over the available follow-up. Treatment modifications included discontinuation (gap between two prescriptions >2x the expected duration), switch and augmentation (i.e., new therapy add-on), and additionally for LIRA and exBID, off-label up-titration and down-titration. Kaplan-Meier (KM) survival curves evaluated time to stopping (first of discontinuation or switch) the index therapy.

ADD (and average weekly dosage [AWD] for exQW) of the index therapy was evaluated over the follow-up while persistent. Results for treatment cohorts with N>100 are reported.

Results: The final study sample comprised 17,512 LIRA, 4,145 exBID, 1,157 exQW and 1,731 LIXI patients (across therapy cohorts: 45-55% male, mean age 57.1-62.9 years, mean follow-up 25.3-30.7 months). Proportion with a first treatment modification by 1-year post-index ranged from 60-81% for LIRA, 87-88% for exBID, 53-73% for exQW and was 97% for LIXI. Proportion persistent at 1-year post-index ranged from 29-61% for LIRA, 29-44% for exBID, 33-51% for exQW and was 4% for LIXI. Over the available follow-up, mean persistence months ranged from 9.4-16.4 for LIRA, 9.2-13.3 for exBID, 9.8-14.4 for exQW and was 5.0 months for LIXI. In the KM analyses, median length of therapy ranged from 129 (GE LIXI) to 566 (NE LIRA) days. Mean ADD ranged from 1.4-1.7 milligrams (mg) for LIRA, 18.7-18.9 micrograms (μg) for exBID and was 19.7μg for LIXI. Mean AWD ranged from 2.1-2.2mg for exQW.

Conclusion: Persistence and appropriately titrating therapy are clinically important to achieve glycemic goals in T2DM. Our results suggest differences exist between patients initiating different GLP-1 RA therapies. Our RW findings suggest that on average, many LIRA and exBID patients are using and benefiting from the higher dose.

	LIRA BE	LIRA FR	LIRA GE	LIRA NE	exBID FR	exBID GE	exQW GE	exQW NE	LIXI-GE
N	666	8,606	6,916	1,324	1,884	2,261	1,035	122	1,731
At 1-Year Post-index									
% Persistent	29.0	51.5	43.1	60.8	44.4	29.0	32.8	50.8	4.2
% With a Treatment Modification	80.5	78.1	73.9	59.5	86.7	87.5	73.4	53.3	96.9
Over the Available Follow-Up									
% Persistent	13.5	35.8	27.4	42.5	28.9	16.8	20.6	36.9	0.3
% With a Treatment Modification	92.2	89.2	87.2	76.8	94.8	95.3	84.6	65.6	99.8
Mean (SD) Persistence Months	9.4 (8.7)	15.2 (11.5)	12.3 (10.2)	16.4 (10.7)	13.3 (11.3)	9.2 (9.1)	9.8 (9.6)	14.4 (11.2)	5.0 (3.7)
Mean (SD) ADD	1.44 (0.19) mg	1.64 (0.13) mg	1.62 (0.46) mg	1.69 (0.16) mg	18.69 (0.74) μg	18.90 (1.94) μg			19.67 (3.12) μg
Mean (SD) AWD							2.05 (0.65) mg	2.15 (0.80) mg	

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Health service utilisation and costs of treatment with either exenatide twice daily or basal insulin for patients with type 2 diabetes: a retrospective UK study

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Background and aims: Type 2 diabetes (T2DM) is a major health problem that places increasing demands on healthcare systems. Alternate therapy regimens are associated with different patterns of adherence, adverse events and mechanisms of action which may impact upon resource use. In this study we estimated resource use following initiation with exenatide twice daily (EBID), compared to basal insulin.

Materials and methods: Retrospective data were extracted from the Clinical Practice Research Datalink (CPRD); a data resource comprising approximately 10% of all patients registered in primary care practices in the United Kingdom. Analysis was restricted to those practices which were part of the CPRD linkage scheme which allowed access to corresponding hospital and mortality data. Patients with T2DM who were naïve to injectable therapies, were selected if they initiated EBID between 2009-2014. Comparison subjects initiated basal insulin-based regimens during the same period. Three analyses were performed: all subjects, subjects matched by propensity score and subjects matched directly. Patients were followed from therapy initiation until end of therapy (defined as 90 days after the last prescription). Primary care contacts, inpatient admissions, glucose lowering drugs and associated costs were compared using adjusted rate ratios (aRRs) estimated from a Poisson regression model accounting for age, gender, baseline HbA1c, weight, and underlying morbidity. Costs were based on standard UK healthcare costing tariffs at 2013-2014 prices.

Results: Overall, 2,180 patients were prescribed EBID and 8,723 were prescribed basal insulin. 1,438 patients were included in each arm of the propensity matched cohorts and 1,192 in the direct matched cohorts. There were differences between the unmatched cohorts at baseline: EBID patients were younger (mean years 56.6 years versus 64.8, p<0.001), had lower HbA1c (mean 9.2% versus 9.7%, p <0.001) and had higher BMI (mean 38.6 kg/m2 versus 30.0; p<0.001). Table 1 shows the results from the propensity matched analysis. Compared with BI based regimens, patients treated with EBID in had significantly lower rates of primary care contacts and inpatient admissions, and consequently lower care-related costs (Table 1). Costs for glucose lowering drugs were higher but total healthcare costs were lower for EBID than for BI (Table 1). Similar reductions in total costs for patients treated with EBID were observed in the directly matched (0.886 (0.884-0.887) and unmatched (0.808 (0.807- 0.809) analyses.

Conclusion: By comparison with BI based regimens, people treated with EBID in the UK had reduced healthcare resource utilisation and reduced total costs. Although the analysis adjusted for key covariates, the possibility of residual confounding should be considered when interpreting these results. Also this study did not account for non-glucose lowering drug costs.

Table 1.

Healthcare utilization and costs incurred per person year (PY) of follow-up for patients treated with exenatide BID versus basal insulin in the propensity matched analysis.

	Exenatide BID		Basal insulin		Adjusted rate ratios
	Total	Per PY	Total	Per PY	
Primary care					
Contacts (n)	30,778	24.1	40,271	31.5	0.754 (0.745- 0.763)
Primary care costs	£1,008,343	789.8	£1,300,414	£1,019	0.762 (0.761- 0.764)
Glucose lowering drugs	£1,270,063	£962	£1,069,315	£838	1.156 (1.153- 1.159)
Secondary care					
Admission (n)	565	0.4	932	0.7	0.534 (0.491- 0.581)
Emergency admission(n)	206	0.2	442	0.3	0.489 (0.426- 0.560)
Total days of stay	1,725	1.4	3,252	2.5	0.492 (0.469- 0.516)
Total inpatient costs	£959,507	751.5	£1,530,100	£1,198	0.588 (0.586- 0.589)
Total costs*	£3,237,913	£2,452	£3,899,829	£3,055	0.819 (0.818- 0.820)

*Total costs for primary care contacts, glucose lowering drugs and secondary care inpatient admissions

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Disclosure: **C.L. Morgan:** Employment/Consultancy; Astra Zeneca.

PS 076 Depression and diabetes

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Effect of weight loss after laparoscopic sleeve gastrectomy in BECK depression scale in diabetic and non-diabetic obese subjects: a 2 years follow-up study

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Background and aims: Obesity has reached epidemic proportions in most countries worldwide and is associated with an increased risk of morbidity and mortality as well as with debilitating psychosocial burdens, such as depression, low self-esteem and social bias. Laparoscopic sleeve gastrectomy (LSG) is one of the bariatric surgical methods that have showed favourable effect on the management of obesity. The aim of the present study was to evaluate the effect of weight loss after LSG in BECK depression scale in diabetic and non-diabetic obese subjects.
Materials and methods: 79 (36 males) morbidly obese subjects were recruited [body mass index (BMI) ±standard deviation (±SD): 42.9±3.2 Kg/m2, aged (±SD): 42.0±10.8 years], after informed consent, who were scheduled to undergo a LSG,from January 2012 to December 2014. Full clinical examination, blood tests plus a standard questionnaire were documented for all participants. Recruits were asked to complete a standard self-report questionnaire consisting of 21 multiple-choice items (BECK depression scale). The same procedure was repeated at 12 and 24 months after LSG.
Results: At baseline, 25.3% (n=20) of study participants had diabetes, 50.6% (n=40) arterial hypertension, 54.4% (n=43) dyslipidemia and 54.4% (n=43) were current smokers. The results from the BECK depression scale are shown in table 1, where statistically significant amelioration of depression was observed from baseline to the end of the study. It is noteworthy that after 24 months there was no study participant with severe depression. Changes in Beck scale were statistically significant between baseline and 24 months (P<0.001), and between 12 and 24 months (p<0.001) after the LSG. BMI significantly changed from baseline to 12 months (30.2±3.5 Kg/m2) and to 24 months (28.5±2.7 Kg/m2) (P<0.001, for both comparisons). At baseline, in the diabetic subgroup, 50% (n=10) had minimal, 30% (n=6) mild, 10% (n=2) moderate, and 10% (n=2) severe depression. After 12 months, 60% (n=12) of the diabetics had minimal, 35% (n=7) mild, and 5% (n=1) moderate depression while after 24 months, 85% (n=17) had minimal, and 15% (n=3) mild depression. It is noteworthy that after 24 months there was no study participant with moderate or severe depression. Changes in Beck scale were statistically significant between baseline and 24 months after LSG (P=0.08).
Conclusion: The results of the present study showed a favorable effect of weight loss after LSG on BECK depression scale in diabetic and non-diabetic obese subjects. However, longer longitudinal follow-up studies are needed to comprehend the life time effects of the bariatric procedures.

BECK Depression Scale Results							
	BASELINE		12 MONTHS		24 MONTHS		p-value
	#	%	#	%	#	%	0.023
Minimal	47	59.5	48	60.8	71	89.8	<0.001
Mild	15	19	22	27.8	4	5.1	<0.001
Moderate	12	15.2	7	8.9	4	5.1	<0.001
Severe	5	6.3	2	2.5	0	0	<0.001

Table 1: BECK Depression Scale Results during study: p-value is for the change at depression percentage from baseline to the end of the study (24 months follow up).

Disclosure: **C. Verras:** None.

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The impact of depression, glycaemic control and in self-rated health in type 1 diabetes

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Background and aims: Depression has been associated with negative health outcomes related with diabetes such as poor glycemic control. To evaluate the frequency of depression in T1DM and its relationship to gender, habits and diabetic complications. To estimate the impact of depression in glycemic control and in Self-Rated Health.
Materials and methods: Patients over 18 years old with known T1DM attended by Argentineans doctors specialized in Diabetes were evaluated. The evaluation included: laboratory, habits and pharmacological treatment. Depression diagnosis: in patients with prior diagnosis and in those with the Beck Depression Inventory (BDI) >9 points (Severe depression BDI >30 point). Physical health were self-rated as excellent, very good, good, fair, or poor. A1C<7% was consider good glycemic control. Statistical analysis: Chi2, Student t, Spearman correlation, Multiple Logistic Regression (CSS / Statistica).
Results: 507 patients were included (age: 40.6± 14.6 years), T1DM duration: 17.4 ±12.0 years, Females: 55.1%, BMI 25.1±4.3 kg/m2; A1c 8.0 ± 1.5%, Fasting blood glucose 147.1 ±69.1 (mg/dl) . We found overweight in 46.3% and obesity in 11.9% . 37.7%.of them made Physical activity. Hypoglycemia was 67% in the last week and 13% had severe hypoglycemia in the last year, 75.1% of the population was under intensive diabetes therapy, external insulin pump in 8.6%, carbohydrate counting was made in 62.1%. Depression was found in 39.3% (prior diagnosis 4.7%, severe depression 15.9%). Diabetes management and outcomes in depressed group: Age: 41.8± 14.4 years (p=0.08), Females: 63.8% (p <0.001), obesity in 15.8% (p<0.02), A1c 8.2 ± 1.6% (p<0.03), Fasting blood glucose 155.3 ±69.3 (mg/dl) p <0.005), Intensive diabetes therapy 69.3% (p <0.01), external insulin pump 7.9% (p=0.67), hypoglycemia 68.3% (p=0.59), severe hypoglycemia 18.3% (p=0.006), carbohydrate counting 51.4% (p<0.005), Self-reported SMBG frequency 3.2±1.4 times per day (p=0.22). Physical health: in normal: excellent 16.2%, very good 64.5%, good, fair 18.4%, poor 0.9% and in depression excellent 6.1%, very good 35.8%, good, fair 44.2%, poor 13.9% (p<0.001). In multivariate analysis, depression were associated with: female gender (OR: 1.97, p<0.001) and coronary heart disease (OR:4.73, p<0.006)
Conclusion: Depression was a common occurrence in this sample of T1DM individuals. We have found association with female gender and coronary disease. Depression impact was found in poor or fair self-rated health, in patients with worst glycemic control, and increases severe hypoglycemia. It would be necessary to screen for depression in this population.

Factors associated with depression in T1DM			
Factors	Normal n=308	Depression n=199	p
Age (years)	39.7± 14.6	41.9± 14.5	p=0.08
Female gender	49.3%	63.8%	p <0.001
Duration of T1D (years)	16.6 ±11.9	18.5 ±12.1	p=0.06
BMI (kg/m2)	24.5±3.6	25.8±5.1	p=0.01
Insurance status (Private)	45.2%	49.1%	p=0.37
Education level (High school)	43.1%	36.0%	p=0.04
Working	76.6%	65.2%	p=0.008
Physical activity	44.0%	33.1%	p=0.01
Coronary disease	3.2%	12.7%	p=0.005
Retinopathy	18.6%	30.7%	p=0.002

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Disclosure: **E. Lopez Gonzalez:** None.

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What is the course of depression symptoms in type 2 diabetes? Risk factors and outcomes of 6-year depression trajectories using latent curve growth analysis

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Background and aims: Routine monitoring of depression in type 2 diabetes has been recommended, yet the longitudinal course of depression symptoms in this population remains unclear. This study aimed to describe trajectories of depression symptom severity over 6-years, and to identify predictors and outcomes of these trajectories.
Materials and methods: A community-dwelling cohort of 1,201 individuals with type 2 diabetes were recruited to the Fremantle Diabetes Study-Phase II (FDS2) from 2008-2011, and administered the PHQ-9 yearly for 6-years to assess depression symptoms. Glycemic control (HbA1c), body mass index (BMI), self-management behaviour and health-related quality of life (HRQoL) were measured at baseline and at subsequent 2-year intervals. Latent curve growth analysis was used to identify depression symptom trajectories and associated diabetes outcomes, and logistic regression models determined predictors of group membership.
Results: Three distinct trajectories of depression symptoms were identified: persistently low depression symptoms (No Depression Problem, 84.6%), a gradual worsening then slight improvement in symptoms (Recurrent Depression - Low Start, 7.8%), and a pattern of gradually improving symptoms which then relapsed (Recurrent Depression - High Start, 7.6%). Younger age, being unmarried, a lifetime history of depression, and poorer mental and physical HRQoL, significantly predicted membership of both depression groups. In turn, recurrent depression was associated with significantly higher BMI and HbA1c over time.
Conclusion: A sub-set of individuals with type 2 diabetes are at high risk of depression symptoms that remain recurrent over time. Regular screening for depression in primary care may better identify individuals at risk of a later relapse/progression in symptoms. Younger individuals with a history of depression and low HRQoL may benefit most from early and intensive depression treatment and ongoing follow-up to buffer the effect of depression on long-term health outcomes.
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Disclosure: **S.R. Whitworth:** None.

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Daytime napping and the risk of metabolic diseases: dose-response meta-analysis

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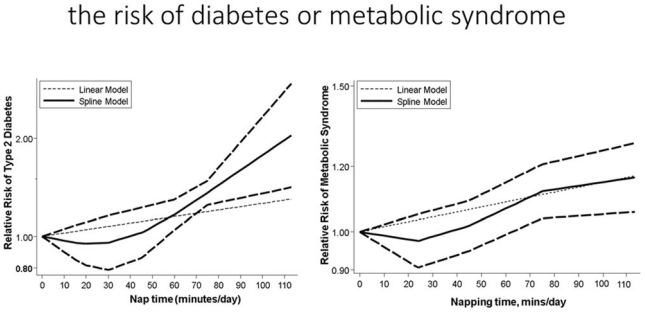
Background and aims: Sleep is an important component of a healthy life, along with a good diet and appropriate physical activity. Several recent studies have shown that a U-shaped curve describes the relation between the duration of sleep (hours of sleep/night) and metabolic diseases. However, some people cannot get enough sleep at night due to social life and work life related factors. Taking naps is widely prevalent around the world. Daytime naps are usually brief, but can range from a few minutes to a few hours. The frequency varies from taking an occasional nap to planned rest periods even several times daily for habitual nappers. Some individuals take a nap because they are excessively sleepy during the daytime as a result of a sleep disorder. We performed a meta-analysis to investigate the association between napping and the risk of metabolic diseases, and to quantify the potential dose-response relation.
Materials and methods: We searched electronic databases for articles published up to 2016. To ascertain the validity of the eligible studies, the quality of each report was appraised with reference to the STROBE statement. In addition, the Newcastle-Ottawa Scale for assessing the quality of observational studies in meta-analyses was used to quantify the validity of each study. The adjusted relative risk and 95% confidence interval were calculated with the random effect model. Dose-response relations were also evaluated by using restricted cubic spline models.
Results: 307,237 Asian and Western subjects stratified into 21 reports were selected. In each study, analyses were well adjusted for several confounders. Pooled analysis revealed that a longer nap (≥60 min/day) each significantly increased the risk of type 2 diabetes comparted with the absence of these factors (relative risk 1.45 (95% CI 1.25-1.69, p=0.03)). In contrast, a shorter nap (<60 min/day) did not (p=0.07). A dose-response meta-analysis showed a J-shaped relation between nap time and the risk of diabetes or metabolic syndrome, with no effect of napping up to about 40 minutes/day followed by a sharp increase in the risk at longer times. In contrast, nap time was not associated with an increased risk of obesity (1.13 (0.92-1.39, p=0.25) for a longer nap; 0.95 (0.88-1.02, p=0.15) for a shorter nap).
Conclusion: Longer nap was associated with increased risk of diabetes and metabolic syndrome. Further studies are needed to confirm the efficacy of a short nap.



Disclosure: **Y. Tomohide:** None.

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Diabetes and mental health: the INTERPRET-DD study

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Background and aims: Little is known about the relationship between mental disorders and clinical factors in people with type 2 diabetes in countries other than the USA or UK. The International Prevalence and Treatment Study (INTERPRET-DD) is following 3,000 patients in 15 other countries across the globe.
Materials and methods: At least 200 patients with type 2 diabetes were recruited in each country. Data (e.g. HbA1c, presence of complications) from medical records were collected and each participant completed the Patient Health Questionnaire (PHQ-9), the Problem Areas in Diabetes (PAID), and the WHO Well-being (WHO-5) scales. Demographic information was collected and patients underwent a psychiatric interview.
Results: Nearly half (44.8%) the participants were male, with mean duration of diabetes 8.8 years and mean age 54.1 years. Overall 10.2% received a diagnosis of Major Depressive Disorder (MDD) with comparable rates of past MDD (10.3%) and lower rates of recurrent MDD (5.1%). Diagnosis rates differed widely between countries, with Uganda, Kenya and India having the lowest proportion diagnosed at interview (1.0, 2.7 and 2.0% respectively) and Bangladesh having the highest proportion (29.9%). Nearly half (43.4%) of those with a current MDD diagnosis also reported a past episode, and about one-third (32.3%) reported recurrent episodes. Between 0 and 29% of those with MDD at interview had a diagnosis documented in their diabetes medical records. As expected, a higher proportion (16.3%) of people reported high levels of depressive symptoms (PHQ-9), poorer well being (WHO-5) and PAID scores. Analyses showed that those with MDD were more likely to be living in an urban rather than rural location ($p<0.001$) and were significantly more likely to be female (74 vs 26%; $p<0.001$). Age and duration did not significantly differ between those with and without MDD. Those with MDD were significantly more likely to have high levels of depressive symptoms, with mean PHQ-9 scores being consistently higher in those with MDD compared to those without ($p<0.01$). PAID scores were significantly correlated with depressive symptoms, both overall ($p<0.05$) and within each country ($p<0.01$). PAID scores were also significantly correlated with lower well-being (WHO-5) scores, older age, longer duration, higher HbA1c and history of diabetes complications. Multiple regression analysis demonstrated that being female, having a lower level of education ($p<0.001$), no regular income and urban living ($p<0.05$) were all independently associated with MDD, along with angina ($p<0.01$), higher PAID score ($p<0.0001$), family history of depression ($p<0.01$) and prior history of MDD ($p<0.0001$).
Conclusion: Our study (the first international study of clinically diagnosed diabetes and depression involving 15 countries) has found high rates of MDD and even higher rates of depressive symptoms with concomitant diabetes-related distress and overall poor well-being. Low levels of documentation of depression may lead to a lack of care for depression and poorer clinical outcomes.
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Disclosure: **C.E. Lloyd:** Grants; Association for the Improvement of Mental Programmes.

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Identifying depression among women and men with diabetes using three instruments: health insurance data, CES-D, PHQ-9 depression scales

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Background and aims: Several tools have been used to identify depression in people with diabetes, however, they have not been analysed in terms of accordance of identified persons. The aim of this study was to assess potential differences in identifying depression among women and men with diabetes when using three instruments.
Materials and methods: The study is based on a random sample (n=1860) of all insured persons of a statutory health insurance (covering 673.366 persons in Germany) with a documented diabetes diagnosis. A survey was performed, and survey data was linked on an individual level with health insurance data. Depression was identified using CES-D and PHQ-9 from the survey and health insurance data. The PHQ-9 focuses on signs and symptoms of depression based on DSM-IV. The CES-D is used to assess the occurrence of depressive symptoms over 7 days. Health insurance data on health care utilisation were available for the period covering 12 months before and after the baseline survey. Analysis were stratified for gender and divided into 7 groups for both sexes based on the results of screening-instruments and health insurance data (e.g. group 1: identified by all methods, group 7: identified by health insurance data only, in the following numbered 1) to 7)).
Results: We included 1580 participants, 597 women and 983 men. 42.5% of the women and 29.7% of the men were identified by at least one method as depressive. 1) 17.7% (95% confidence interval: 11.8%-24.0%) of females and 16.1% (10.6%-21.8%) of males with depression met the criteria for all three methods. 2) 9.1% (3.1%-15.4%) of women and 13.4% (7.9%-19.1%) of men with depression met the criteria for both screening tools but not for health insurance data. 3) 5.1% (0.0%-11.4%) of women and 6.5% (1.0%-12.2%) of men with depression met the criteria for PHQ-9 and health insurance data and 4) 8.2% of both women (2.0%-14.2%) and men (2.7%-13.9%) with depression met the criteria for CES-D and health insurance data. 5) 10.6% (4.7%-16.9%) of women and 11.3% (5.8%-17.0%) of men with depression met only the criteria for CES-D. 6) 5.9% (0.0%-12.2%) of women and 10.3% (4.8%-16.0%) of men with depression met only the criteria for PHQ-9 and 7) 43.7% (37.8%-50.0%) of women and 34.2% (28.8%-40.0%) of men with depression had a diagnosis in health insurance data only. The prevalence of depression for women ranged from 16.1% for PHQ-9, 19.3% for CES-D up to 31.7% in health insurance data. For men the prevalence ranged from 13.7% for PHQ-9, 14.5% for CES-D up to 19.3% in health insurance data.
Conclusion: In most groups, the prevalence of depression was higher among women compared to men in our sample. However, more men than women were identified as depressive when using PHQ-9 only in our sample. Either method does not cover all patients with depressive symptoms therefore whenever possible data linkage should be preferred over a single method to identify patients with depressive symptoms.
Supported by: BMBF - the Federal Ministry of Education and Research
Disclosure: **U. Linnenkamp:** None.

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Hospital based prevalence of major depression and its association with microangiopathy amongst adults with type 2 diabetes from India

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Background and aims: Depression is common but less addressed comorbidity in people with diabetes. This study aims at determining the prevalence of depression and its association with microvascular complications amongst adults with type 2 diabetes, attending a tertiary care diabetes centre from India.
Materials and methods: With prior approval from Institutional Ethics Committee, the study enrolled 775 established Type- 2 Diabetic subjects from March 2015 to January 2016, without prior history of antipsychotic treatment, attending a tertiary care diabetes centre at Nagpur, representing population from Central India. Of them, 72 patients refused to participate, thereby leaving 703 eligible cases with complete demographics, clinical and biochemical assessments. Nine- item Patient Health Questionnaire-9 (PHQ-9) was used for the evaluation for depression. The association of microvascular complications and other risk factors with depression was evaluated independently in terms of odds ratio. The adjusted odds ratios for the complications were obtained using multivariate logistic regression analysis after adjusting for covariates.
Results: Out of 703 eligible patients, 292 indicated depression with varying degree of severity (PHQ9 score > 5), thus providing a hospital based prevalence of 41.5%. Further, major depression was defined as PHQ9 score > 10, which resulted into a prevalence of 16.2%. Proportion of females showing major depression (20.7%) was significantly higher than that of males (14.1%) with p-value 0.024. Socio-economic strata, anthropological parameters, duration of diabetes, age and HbA1c did not show any association with major depression. Microvascular complications like neuropathy, retinopathy and nephropathy showed significant effect with corresponding ORs 2.06 [95% CI: 0.82-7.25; p-value: 0.006], 1.76 [95% CI: 1.02, 2.96; p-value: 0.033] and 2.39 [95% CI: 1.00, 3.91; p-value: 0.006] respectively. The effects of these complications were also studied in presence of covariates like age, gender, HbA1c, BMI and Haemoglobin using multivariate logistic regression analysis. Neuropathy and Nephropathy continued showing significant effect on major depression with ORs 1.81 [95% CI: 1.00, 3.27; p-value: 0.049] and 2.12 [95% CI: 0.98, 4.56; p-value: 0.048] respectively; however, retinopathy lost significance with corresponding OR of 1.29 [95% CI: 0.68, 2.42; p-value: 0.428]. Moreover, a higher risk of major depression was observed in patients with eGFR < 56 ml/min/1.73m2 with corresponding OR of 1.86 [95% CI: 0.89, 3.63; p-value: 0.075], although insignificant.
Conclusion: Study revealed a high prevalence of major depression in people with adult type2 diabetes and more so in female subjects. The risk increases significantly with associated nephropathy, neuropathy and retinopathy. eGFR<56ml/min/1.73m2 may correspond to higher risk of major depression. Clinicians need to focus on screening and early diagnosis of depression amongst people with type2 diabetes.
Disclosure: **S.S. Gupta:** None.

PS 077 Hypoglycaemia: clinical impact

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Assessment of the impact of fear of hypoglycaemia on glycaemic control in adults with type 1 diabetes

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Background and aims: Fear of hypoglycaemia (FoH) is quoted as a major barrier to the optimal control of type 1 diabetes (T1D) which reduces complications. To date evidence for a relationship between FoH and glycaemic control has been inconclusive. The aim of this study was to determine the association between FoH and glycaemic control in adults with T1D.
Materials and methods: We recruited in outpatient clinics at a secondary/tertiary diabetes centre. Patients completed the Hypoglycemia Fear Survey-II (HFS-II); the Gold score, a self-reported measure of hypoglycaemia awareness; a modified version of the Edinburgh Hypoglycaemia Scale, rating 17 individual symptoms of hypoglycaemia (4 autonomic, 3 neuroglycopenic) and a question on severe hypoglycaemia (SH) frequency in the preceding year. HbA1c and self-monitored plasma glucose (SMPG) data were obtained from medical records. Spearman's rank-order correlation was used to assess associations and the Mann-Whitney U test to investigate differences between two groups.
Results: The 150 patients had mean±SD age of 41.3±15.3 years; diabetes duration of 23.5±12.1 years; HbA1c of 69.4±17.9 mmol/mol. 44.0% were male, 49.7% on continuous subcutaneous insulin infusion therapy and 20.8% had impaired awareness of hypoglycaemia (Gold score ≥ 4). No significant association was found between HFS-II total or behaviour and worry subscale scores and HbA1c ($p = 0.305$, $p = 0.197$, $p = 0.562$ respectively). Those with SH in the past year had significantly higher HFS-II total scores than those without (median (interquartile range, IQR) 2.879 (2.242-3.546) vs 2.156 (1.727-2.546), $p < 0.001$), but there was no difference in HbA1c for SH history (63.0 (57.0-72.0) vs 67.0 (59.0-76.0) mmol/mol, $p = 0.121$). Higher HFS-II total scores associated positively with higher total or autonomic and neuroglycopenic Edinburgh scores (greater presence and intensity of hypoglycaemia symptoms) ($r = 0.445$, $p < 0.001$; $r = 0.258$, $p = 0.002$; $r = 0.378$, $p < 0.001$ respectively). Higher Gold scores (deteriorating hypoglycaemia awareness) associated with lower autonomic Edinburgh scores (less hypoglycaemia autonomic symptoms) but not with neuroglycopenic Edinburgh scores ($r = -0.216$, $p = 0.009$; $r = 0.115$, $p = 0.164$ respectively). There was no significant association between HFS-II total scores and number of SMPG tests done per day ($p = 0.782$).
Conclusion: Our data set finds no association between FoH, as measured by total score or behaviour or worry subscores, and HbA1c. Nor does FoH drive increased SMPG. In keeping with other data, previous SH associates with greater FoH, but not with HbA1c. FoH may lead to a range of different responses and behaviours, reflected by the different items in the HFS-II, which may explain the lack of association between summary scores and HbA1c and the discrepancies in the literature. The relationship between hypoglycaemia fear and experience and HbA1c is complex and requires further investigation.
Disclosure: **C. Halevy:** None.

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'Attitudes to awareness of hypoglycaemia' (A2A) questionnaire: an analysis of the HypoCOMPaSS data

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Background and aims: Risk of severe hypoglycaemia (SH) as a complication of diabetes therapy increases with age, longer diabetes duration, previous exposure to SH, and impaired awareness of hypoglycaemia (IAH). IAH has been associated with unhelpful health beliefs around hypoglycaemia. The 'Attitudes to Awareness of Hypoglycaemia' (A2A) questionnaire is designed to detect these. We investigated baseline A2A in adults with type 1 diabetes (T1D) and IAH, before and after participation in a successful research intervention to improve IAH (HypoCOMPaSS trial).
Materials and methods: 96 adults with T1D and IAH completed the A2A before and at 6, 12, 18 and 24 months after receiving hypoglycaemia avoidance education, educator support and random allocation use of insulin pump and/or real-time glucose sensing which improved hypoglycaemia awareness in 5 UK diabetes centres. Participants scored items in the A2A on Likert scales, including statements about degrees of belief, concern, and motivation to restore hypoglycaemia awareness (Q3-5); attitudes to hypoglycaemia (Q6-19) to identify unhelpful health beliefs, with subscores for 3 thinking styles (denial of serious consequences; determination to continue regardless of hypoglycaemia, and excessive fear of hyperglycaemia). Friedman test was used to compare percentiles of the same sample at the time points.
Results: Mean±SD age of participants, disease duration and HbA1c were 48.6±12.2, 29.1±12.4 years, and 8.3±1.2%, respectively; 63.5% were female, 43.3% had ≥1 SH in past 12 months, and 91.8% had IAH. The majority of the participants endorsed high baseline levels of concern and motivation but only moderate or lower baseline levels of beliefs of ability to restore hypoglycaemia awareness. The majority of the sample endorsed at least one of 14 attitudinal statements as 'Moderately True'; 70.4% rated at least one statement as 'Very True'; one third endorsed beliefs in ≥7 of the statements. Prevalence of IAH (Gold score ≥4) fell to 56.0% and 53.6% at 12 and 24 months, and of SH to 13.2% and 8.8%. In contrast, levels of belief, concern and motivation in ability to restore hypoglycaemia awareness did not change over time (p=0.773, p=0.178, p=0.756, respectively). There was a significant reduction in total scores for attitudinal statements at all follow-up time points (p<0.001), lost at 24 months (p=0.08), but no significant changes in any thinking styles subscores between baseline and all stages of follow-up (p=0.051, p=0.563, p=0.062, respectively).
Conclusion: Improvement in IAH and SH achieved by an educational and technological intervention was not accompanied by changes levels of belief in ability to, concern about or and motivation to restore hypoglycaemia awareness, or in the 3 different thinking styles associated with IAH at baseline, although there was an initial reduction in total scores for attitudinal statements. These findings suggest that while the intervention was able transiently to address some attitudinal beliefs believed to serve as barriers to hypoglycaemia avoidance, it did not reduce denial of serious consequences, underestimating need to treat hypoglycaemia nor overestimating the hyperglycaemia impact. Tackling these directly may be important for some people to regain awareness.
Supported by: EFSD Albert Renold Fellowship
Disclosure: **E. Sepúlveda:** None.

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Severity of hypoglycaemia and health related quality of life and work productivity in type 2 diabetes patients

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Background and aims: This study examined the association between severity of hypoglycemia and the health related quality of life (HRQoL) and work productivity of adults with type 2 diabetes mellitus (T2DM) in Europe (UK, Germany, France, Italy, Spain).
Materials and methods: Data from a nationally representative sample of adults (≥18 years) who participated in the EU National Health and Wellness Survey in 2013 were used in this study. The study population included patients who reported a diagnosis of T2DM and current treatment with an antihyperglycemic agent. Patients were categorized into 3 groups (severe, non-severe and no hypoglycemic event) based upon self-reported history of hypoglycemia in the past 3 months. Among those who reported hypoglycemia, severe event was defined as requiring third-party assistance. HRQoL and work productivity loss were measured using the validated questionnaires, Medical Outcomes Study Short-Form (SF-36) and the Work Productivity and Activity Impairment Questionnaire (WPAI), respectively. Multivariable regression models were used to adjust for baseline differences in patient characteristics.
Results: Of 1,269 patients who met the inclusion criteria, 617 (48.6%) reported experiencing at least one hypoglycemic event and 6.6% reported a severe hypoglycemic event in past 3 months. Patients who experienced a hypoglycemic event were slightly younger (mean age in years: 58.8, 60.7), more likely to be female (38%, 33%), more likely to report insulin use (56%, 37%) but similar use of SU (23%, 22%) than those without a hypoglycemic event. Compared to patients with no event or non-severe events, patients experiencing a severe event were younger (mean age in years: 60.7, 59.2, 56.2, respectively) and had a higher comorbidity burden (mean Charlson comorbidity score: 0.67, 0.71, 1.74, respectively). After controlling for covariates, increasing severity of hypoglycemia was inversely associated with HRQoL (measured as health utility scores) (means: no event = 0.64, non-severe = 0.62, severe = 0.58; p-trend < .001). This pattern was also observed for the mental health component scores (means: no event = 45.1, non-severe = 43.5, severe = 40.5; p-trend = .002) and the physical health component scores (means: no event = 42.2, non-severe = 41.3, severe = 38.7; p-trend = .008). Similarly, there was a significant increase in overall activity impairment (as measured by WPAI) with increasing severity of hypoglycemic events (means: no event = 41.7%, non-severe = 44.3%, severe = 59.2%; p-trend = .022). Work productivity loss followed the same pattern (means: no event = 27.1%, non-severe = 28.0%, severe = 38.9%) but the trend was not statistically significant (p-trend = .165).
Conclusion: Almost half of the patients treated for T2DM reported experiencing at least one hypoglycemia event in prior three months. Our study results suggest that increasing severity of hypoglycemia may be associated with a decrease in HRQoL and also an increase in activity impairment.
Disclosure: **M. Pawaskar:** Employment/Consultancy; Merck.

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Self-reported hypoglycaemia in insulin-treated patients with diabetes: results from an international survey of 7289 patients from 9 countries

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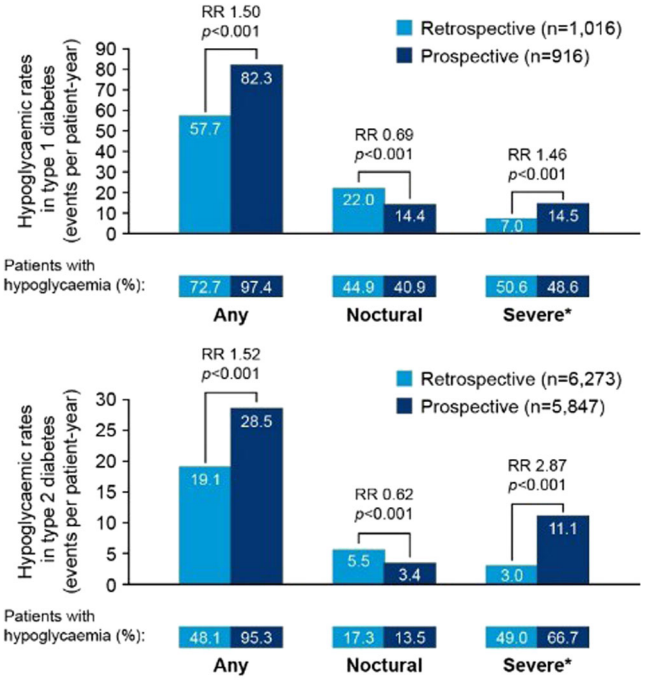
Background and aims: The non-interventional International Operations Hypoglycaemia Assessment Tool (IO HAT) study assessed the incidence of hypoglycaemia in patients with insulin-treated diabetes in Bangladesh, Colombia, Egypt, Indonesia, the Philippines, Singapore, South Africa, Turkey and the UAE.
Materials and methods: The incidence of hypoglycaemia was reported in self-assessment questionnaires completed at baseline and after the 28-day prospective period, and in patient diaries.
Results: Of 7289 patients (type 1 diabetes [T1D] n=1016, type 2 diabetes [T2D] n=6273), approximately 90% completed their diaries in the prospective period (28 days from baseline). At least 1 case of confirmed hypoglycaemia (capillary glucose <3.1 mmol/l) was recorded in patient diaries by 48.0% of patients with T1D and 12.6% of those with T2D. Based on patient recall, severe hypoglycaemia was reported for the prior 6 months, and any hypoglycaemia the 4 weeks before baseline (Fig. 1). Any hypoglycaemia was retrospectively reported by patients (TID 72.7%, T2D 48.1%). Nearly all patients reported events during the prospective period (T1D 97.4%, T2D 95.3%). Rates of 'any' and 'severe' hypoglycaemia were higher in the prospective period (p<0.001) compared to those in the retrospective period for T1D and T2D. In contrast, lower rates of nocturnal hypoglycaemia were reported prospectively vs. retrospectively (p<0.001).
Conclusion: These results are the first patient-reported dataset on hypoglycaemia in the participating countries and indicate that hypoglycaemia is under-reported and thus underestimated.

Figure 1. Retrospective and prospective rates of hypoglycaemia



RR, rate ratio. RR calculated from completers analysis set. Rates and prevalence calculated from full analysis set. 'Any' and 'Nocturnal' based on 4-week period for both retrospective and prospective analyses. *Retrospective data based on 6-month period and prospective data based on 4-week period.

Clinical Trial Registration Number: NCT02306681
Supported by: Novo Nordisk
Disclosure: **A. Jain:** Employment/Consultancy; Employed: Novo Nordisk Region International Operations AG.

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A population-based study on incidence and associated risk factors for hypoglycaemia in Canada: the INHYPO-DM study

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Background and aims: Hypoglycemia is a common and unfavourable side effect of secretagogues and insulin therapy that has a substantial negative impact on quality of life and clinical care. We aimed to determine the incidence of hypoglycemia in people with diabetes and identify factors associated with hypoglycemia.
Materials and methods: Self-reported data collected from a nationwide survey of people with diabetes treated with insulin and/or oral hypoglycemic agents (OHA), the largest of its kind in Canada, were used for this study. The survey was disseminated online between November 20 and December 2, 2015, to a nationally representative panel managed by Canada's largest research polling firm. Correlation analysis was conducted on all variables to explore factors associated with non-severe hypoglycemia (NSH), nocturnal hypoglycemia (NH), and severe hypoglycemia (SH). Using a multivariable logistic regression analyses, potential confounders were adjusted and factors significantly associated with the risk of daytime NSH, NH, and SH were identified.
Results: A total of 552 eligible people with diabetes, (17.1% type 1 (T1DM) and 82.9% type 2 (T2DM)), completed the survey across Canada. The average age of respondents was 52 and 56% of them were men. Among all survey respondents, recent (within last 30 days) NSH was reported by 55.4% (74.5% T1DM and 51.4% T2DM). NH was

reported by 33.9% (54.3% T1DM and 29.7% T2DM). Forty-one percent of the respondents (40.7% (54% T1DM and 37.9% T2DM), experienced SH in the last year. Incidence of hypoglycemia was positively associated with younger age (18-44 years old), those with no private drug coverage, those working full time, and those working shift work hours. Treatment with OHA in conjunction with insulin, especially for those who had 3 or more insulin injections a day, and who had poorly controlled blood glucose (A1C≥9%) were also at greatest risk. Based on the results of a logistic regression analysis, age (18-44 vs. ≥65) (OR: 3.08; 95% CI: 1.52-6.23), treatment type (OHA only vs. insulin plus OHA) (OR: 2.07; 95% CI: 1.15-3.77), and high A1C level (A1C≥9% vs. A1C≤7%) were significantly associated (OR: 1.87; 95% CI: 1.14-3.07) with NSH. High A1C level (OR: 2.17; 95% CI: 1.32-3.59) and shift work (OR: 2.26; 95% CI: 1.36-3.76) were associated with NH. Treatment type (OR: 2.64; 95% CI: 1.44-4.83), high A1C level (OR:1.7; 95% CI:1.05-2.78), full time work (OR:1.61; 95% CI:1.02-2.56), and shift work (OR: 1.97; CI:1.19-3.26) were associated with SH.

Conclusion: Despite the availability of safer glycemic lowering treatment, overall incidence of hypoglycemia remains remarkably high in both T1DM and T2DM as reported in this study. The odds of experiencing hypoglycemia were highest among younger adults, those with poor glycemic control, those who took multiple daily injections of insulin, and those who lead busy lives (working full time and/or shift work). These factors highlight an important diabetes population that may benefit from focused clinical attention and counselling in order to reduce hypoglycemia events.

Supported by: Sanofi Canada

Disclosure: **S.M. Reichert:** Grants; Sanofi Canada.

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Determinants of severe hypoglycaemia in a French population of adults with type 1 diabetes in 2015

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Background and aims: Since the DCCT study, it is commonly accepted that low HbA1c is a major risk factor of severe hypoglycemia (SH) in patients with type 1 diabetes. Using a large cohort, we have evaluated whether this is always the case 30 years after DCCT.

Materials and methods: Data from 1221 type 1 diabetics followed in consultation in a department of diabetology of a hospital during the last 2 years were collected using a database. SH for the last 6 months, age, gender, BMI, diabetes duration, HbA1c, number of self-monitoring of blood glucose by day (SMBG), number of mild hypoglycemia by weeks (MHW), and insulin treatment were analyzed. Logistic regression model was used to evaluate risk of SH. Data are expressed as mean +/- SD.

Results: Information about SH were available for 520 patients, age 40,7 years-old (+/-15.5), men 44%, diabetes duration 19,9 years (+/-12.5). 105 had at least one SH for the last 6 months (20%). Univariate analysis showed no difference in SH between the 4 HbA1c's clusters (<6.5, 6.5-7.4, 7.5-8.4, ≥8.5) neither by insulin treatment modality (multiple daily injection vs pump therapy) nor with the presence of diabetic retinopathy (OR=0,800 [0,385 - 1,661], p=0,55). Long diabetes duration and high occurrence of MHW were associated with an increased risk of SH. Multivariate analysis showed a significant increase in number of SH in patients with diabetes duration >10 years (<10 years: ref, 10-30 years: OR=2.80 [1.04-7.50] p=0,04; >30 years: OR = 5.55 [1.92-16.06] p=0,002), and in patients with a number of MHW ≥5 (MHW=0 : ref, MHW ≥5: OR= 6.76 [2.12-21.56], p=0,001).

Conclusion: Surprisingly, nowadays risk of SH remains high but is not related to low HbA1c neither treatment modality in adult subjects with type 1 diabetes. Diabetes duration and high occurrence of mild hypoglycemia are positively correlated to this acute complication. Further studies are necessary to explain this glycemic instability.

Disclosure: **J. Julla:** None.

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Comparison of two treatment approaches in older patients with type 2 diabetes (IMPERIUM)

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Background and aims: Older patients with type 2 diabetes mellitus (T2DM) often require complex care due to comorbidities, polypharmacy, and frailty. We report the final results of a pilot study evaluating the feasibility of the full-scale study comparing the efficacy and safety of two individualised treatment strategies in moderately ill and/or frail patients ≥65 years with T2DM whose individual HbA_{1c} targets were not met with diet/exercise and/or oral antihyperglycaemic medication (OAM).

Materials and methods: Patients were randomised to a non-glucose-dependent strategy (NGD, n=93) with a sulfonylurea (SU) as preferred OAM and insulin glargine as first injectable or a glucose-dependent strategy (GD, n=99) with a non-SU OAM and a GLP-1 receptor agonist as first injectable; 93 and 98 patients, respectively, were treated and included in the analyses. The primary endpoint was a composite of the proportion of patients achieving/maintaining individualised, preset HbA_{1c} targets (based primarily on life expectancy, hypoglycaemic burden/risk, comorbidities, and cognitive/functional status) without 'clinically significant' hypoglycaemia (any severe hypoglycaemia, or repeated hypoglycaemia causing interruption of patients' activities or sleep, or blood glucose <3 mmol/l). The study was stopped after an interim analysis demonstrated no difference between strategies in achieving the primary endpoint.

Results: Median treatment duration was 49.0 (0.1-78.6) weeks. Mean ± SD baseline characteristics were similar for GD vs NGD: age 71 ± 5 vs 71 ± 4 years, BMI 31.0 ± 5.7 vs 31.3 ± 4.8 kg/m^2, HbA_{1c} 8.4 ± 0.9% vs 8.2 ± 0.8%, fasting blood glucose 9.7 ± 2.5 vs 9.3 ± 2.4 mmol/l, and SU use 52% vs 41%. There was no significant difference between GD and NGD in achieving the primary endpoint (64.5% vs 54.9%; P=.190). HbA_{1c} improvement from baseline was similar for GD and NGD (LSM ± SE: -1.2 ± 0.10% vs -1.1 ± 0.10%; P=.390). Incidences of clinically significant hypoglycaemia for GD vs NGD were similar (0 vs 1.1%; P=.487); however, those for total, documented symptomatic (signs/symptoms and blood glucose ≤3.9 mmol/l), and asymptomatic (no signs/symptoms and blood glucose ≤3.9 mmol/l) hypoglycaemia were significantly lower for GD (10.2% vs 53.8%, 5.1% vs 36.6%, 8.2% vs 32.3%, respectively, P<.001 each). Incidences of treatment-emergent adverse events (TEAEs) for GD and NGD were similar (84.7% vs 79.6%, P=.355); whereas, those for TEAEs considered related or possibly related to treatment by the investigator were higher for GD (27.6% vs 8.6%, P<.001), with diarrhoea (6.1% vs 3.2%) reported most frequently.

Conclusion: Similar proportions of older, vulnerable patients with T2DM achieved/maintained glycaemic goals without clinically significant hypoglycaemia with GD and NGD. However, GD resulted in lower incidences of total, documented symptomatic, and asymptomatic hypoglycaemia. The potential benefits and clinical impact of reducing hypoglycaemic burden with GD in this vulnerable population with T2DM warrants further study.

Clinical Trial Registration Number: NCT02072096

Supported by: Eli Lilly and Company
Disclosure: **A. Sinclair:** Employment/Consultancy; Merck Sharp & Dohme, Takeda, Novartis, Eli Lilly and Company.

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Older people with type 2 diabetes are commonly overtreated with therapies associated with hypoglycaemia, even in the presence of chronic kidney disease

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Background and aims: The clinical consequences of severe hypoglycaemia are potentially serious, risking injury or harm and increasing mortality. Older people are particularly vulnerable due to advancing age, duration of diabetes, polypharmacy and multimorbidity. Chronic kidney disease (CKD) is the most commonly reported comorbidity predisposing to severe hypoglycaemia, particularly in association with sulfonylurea or insulin therapies. The International Diabetes Federation advises against intensive glycaemic management to HbA1c < 7.0% (53mmol/mol) in older people with CKD, a view supported by guidance from the International Society for Nephrology. Both advocate less stringent glycaemic targets aimed at reducing unnecessary overtreatment and avoidable risk of hypoglycaemia, promoting patient safety above all else. The aim of this project was to evaluate glycaemic management and prevalence of overtreatment with sulfonylurea and insulin therapies in older people with Type 2 diabetes, with and without CKD.
Materials and methods: Cross sectional observational study of people with Type 2 diabetes, aged ≥ 70 years, registered with 16 general practices, who had received a prescription for any sulphonylurea or insulin therapy within the previous 90 days. Data collected included age, sex, last recorded HbA1c and renal function. The Norfolk and Suffolk Primary and Community Care Research Office approved the project as service evaluation and audit.
Results: From a primary care population of 24,661 people aged ≥ 70 years, 3863 (15.7%) had a diagnostic code for type 2 diabetes. Of these, 1379 (35.7%) patients had received prescriptions for sulfonylurea or insulin therapies: 824 (59.8%) for SU therapies, 401 (29.1%) for insulin therapies and 154 (11.2%) for both insulin and SU therapies. Serum creatinine and HbA1c had been measured within the previous 18-months in 1347 (97.7%) and 1339 (97.1%) patients, respectively. Overall, 644 (47.8%) patients had CKD, with eGFR < 60ml/min/1.73m^2: 343 (25.5%) had CKD3A, 231 (17.1%) had CKD3B and 70 (5.2%) had CKD4-5. Patients with CKD were older, median age 80 (IQR 75-84) years vs. 76 (IQR 72-81) years in patients without CKD, p < 0.001. In spite of this, there was no significant difference in glycaemic attainment between patients with or without CKD. Amongst patients with CKD, median HbA1c was 7.5% (IQR 6.8-8.4%)(58, 51-68mmol/mol) compared to 7.5% (IQR 6.8-8.5%)(58, 51-69mmol/mol) in those without CKD, p = 0.535. Although the proportion of patients who had been prescribed SU therapies decreased progressively across worsening CKD staging, the proportion prescribed insulin, or both insulin and SU therapies increased, a difference that was highly significant, p ≤ 0.001. Overtreatment was common and as common in patients with CKD as in those without CKD. 193 (30.3%) patients with CKD had HbA1c < 7.0% (53mmol/mol), of whom 85 (13.3%) had HbA1c < 6.5% (48mmol/mol), compared to 205 (29.5%) and 76 (10.9%) respectively, amongst patients without CKD, p = 0.351.
Conclusion: Almost half of this cohort of older people with Type 2 diabetes prescribed sulfonylurea and insulin therapies had evidence of CKD, yet almost a third were potentially overtreated. Quality assurance programmes need to be implemented to reduce risk of hypoglycaemia in this vulnerable population.
Disclosure: **C.E. Hambling:** None.

PS 078 Psychological aspects: influences on life

828

Life chances of children diagnosed with type 1 diabetes in early childhood (<6 years): psychological well-being and social integration in the 3rd decade of life

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Background and aims: The concept of "life chances" (Lebenschancen) introduced by Max Weber, describes the likelihood, that an individual is able to satisfy one's needs. Onset of T1DM in early childhood is discussed as risk factor for impaired cognitive development, school performance und emotional well-being. In a cross-sectional study the socioeconomic, emotional and health status of young adults (19-30 yrs.) were compared between those with early (<6 yrs.) and later onset of T1DM.
Materials and methods: All patients with T1DM aged 19-30 yrs. (onset of T1DM < 18 years) visiting one of 26 specialized Diabetes out-Patient Units in Lower-Saxony (Germany) were invited to anonymously complete a comprehensive qustionnaire on their personal life Situation. The battery of questionnaires assessed demographic, educational, occupational, and Diabetes specific Status, emotional well-being (WHO-5; HAD-S) and Diabetes specific distress (PAID). patients diagnosed with T!DM < 6 years were compared to those diagnosed later in childhood.
Results: During the 3 months study period 306 (96%) of 318 eligible patients participated (47% female; age 24.1±3.5 yrs.; diabetes duration 11.7±5,8 yrs.; 43% CSII). Of them 51 (17%) were diagnosed ≤ 6 yrs. They reported of less diabetes distress (PAID) (21.3±14.7 vs. 28.0±20.5; p=.007) but higher HbA1c (8.7±1.7% vs. 8.2±1.6%; p=.03) than the ones with later onset. There were no group differences in well-being (WHO-5), rate of depressive / anxiety disorder (HAD-S), rate of critical life events. Overall severe life events and psychological disorders predicted HbA1c (each p 39 cut off score) mainly due to "worrying about future" and "feeling guilty". There were no differences between both groups according to highest levels of graduation from school (overall: 49% high school) and rate of unemployment (6%). These data point to a higher educational level and lower rate of unemployment compared to the regional background population.
Conclusion: Despite onset of T1DM in early childhood the majority of young adults solved the age specific life-tasks (education and occupation) successfully with good/acceptable metabolic control. About 25% were affected by anxiety of late complication, diabetes distress and reduced quality of life. Especially those facing demanding life events and emotional distress require specific psychosocial advice to better cope with T1DM and to improve their insufficient metabolic control.
Supported by: Deutsche Diabetes Stiftung
Disclosure: **K. Rölver:** None.

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Self-management obedience rate, metabolic control and cost of diabetes associated with medical insurance in China

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Background and aims: Though most of patients with type 2 diabetes can enjoy the medical insurance in China, some patients are still not covered by it. The association between insurance and metabolic control has not been yet studied in Chinese population. This study compares the self-

management obedience rate and metabolic control of type 2 diabetic patients with and without insurance in China.

Materials and methods: An observational cross-sectional study was performed on 5855 patients with type 2 diabetes mellitus(T2DM) across 50 representative centers in China. Demographics, medical history, diabetes related costs, insurance and self-management obedience were surveyed and patients underwent medical exams for metabolic parameters like HbA1c, FPG, TG, TC, etc. Analysis of One-way ANOVA and Chi-squared test were applied to test the differences of metabolic parameters, costs and the obedience rate of self-management between the two groups.

Results: In general, there were 4827 patients with medical insurance (MI) and 1028 patients without it (self-payment, SP). A total of 317 pairs of data were included in the analysis which were matched by sex, BMI, diabetes duration, smoking, anti-diabetic drugs and insulin use. Patients with MI had a lower level of HbA1c (8.092±2.058% vs 9.121±2.593%, p=3.76×10-5) and other metabolic parameters such as FPG, PBG, TG, TC and LDL(all p<0.05). Patients in SP had less 12-month DM costs (5641 vs 7840 RMB, p=0.0044) and lower obedience rate of self-management indicators including DM education, Exercise, Reexamination, Self-monitoring and Doctor visits (all p<0.05).

Conclusion: 17.56% T2DM patients are still not covered by medical insurance in China which may associated with a lower medical expenditure, obedience rate of self-management and consequently a poor metabolic control. Increasing the diabetes self-management support and popularizing the knowledge of medical insurance in the self-payment population may potentially help their metabolic control.

Table 1 Medical insurance and metabolic indicators

Group	HbA1c(%)	FPG(mmol/L)	PBG(mmol/L)	TG(mmol/L)	TC(mmol/L)	HDL(mmol/L)	LDL(mmol/L)
SP	9.121±2.593	8.461±3.493	12.240±5.008	2.278±1.996	5.167±1.559	1.325±0.584	2.986±1.179
MI	8.092±2.058	7.750±2.740	11.010±4.092	1.814±1.130	4.811±1.162	1.343±0.490	2.749±0.813
F	17.420	7.365	9.584	7.141	5.94	0.094	4.633
p value	<0.001	0.007	0.002	0.008	0.015	0.759	0.032

Values are represented by mean±sd. HbA1c = hemoglobin A1c; FPG=fasting plasma glucose; PBG= postprandial blood glucose; TG =triglycerides; TC=total cholesterol; HDL=high density lipoprotein; LDL=low density lipoprotein; SP=self-payment; MI=medical insurance.

Table 2 Medical insurance and obedience rate of self-management

Group	Education	Reexamination	Exercise	Drug use	Self-monitor	Consultant
SP	235(74.13%)	157(49.53%)	87(27.44%)	201(63.41%)	181(57.10%)	57(17.98%)
MI	259(81.70%)	196(61.83%)	114(35.96%)	249(78.55%)	211(66.56%)	83(26.18%)
χ2	4.849	9.229	4.924	16.914	5.620	5.730
p value	0.028	0.002	0.026	<0.001	0.018	0.017

SP=self-payment; MI=medical insurance.

Disclosure: **W. Li:** None.

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Adherence amongst type 2 diabetics: real-world physician and patients views and patient profiling

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Background and aims: Whilst the ultimate goal of all diabetes therapy is to achieve good glycaemic control, many patients are not achieving optimal control due to non-adherence factors. We explored the differing views of adherence amongst physicians and their patients, alongside characterising non-adherent versus adherent T2DM patients.

Materials and methods: Data were drawn from the 2015 Diabetes Disease Specific Programme in the US and EU. Diabetes specialists and primary care physicians (PCPs) completed forms for the next 10 consulting T2DM patients, including current therapy, HbA1c and physician-perceived assessment of patient compliance. The same patients were invited to complete a voluntary patient-reported form which included the validated Morisky Medication Adherence Scale (MMAS-8), patient demographics and characteristics, engagement with disease, lifestyle factors and need versus concern regarding injectable medicine. All results are statistically significant.

Results: A total of 352 specialists, 502 PCPs, and 8368 T2DM patients were included across US and 5EU. Of these, 4462 patients completed the validated adherence tool, MMAS-8. Physician-perceived patient adherence with T2DM medication differed greatly compared to patient-reported MMAS-8 adherence (low 6% vs 25%; high 61% vs 46%). Patient-reported low adherence profiles compared to high adherence were more likely to be current heavy smokers (21.5% vs 12.2%), working part-time (7.2% vs 4.7%), unemployed (10.6% vs 4.7%) or manual workers (40.0% vs 29.6%), clinically obese (36.9% vs 22.2%), made no changes to lifestyle (12.4% vs 5.8%), diet (11.7% vs 5.4%) and exercise (34.4% vs 20.3%). Low adherent patients also presented with higher HbA1c levels (7.7% vs 7.1%), poor glucose self-monitoring (23.1% vs 9.9%), a physician-perceived belief better glucose control can be achieved (46.2% vs 28.6%), a higher incidence of hypoglycaemic episodes (14.6% vs 7.2%) and were more likely to be prescribed insulin-containing regimens (21.6% vs 11.1). These patients were also receiving a higher number of products for all conditions, both prescribed and non-prescribed (6.8 vs 5.2). Low adherence patients currently not taking injectable medicine reported a low need but high concern about starting injectable therapy compared to high adherence patients (72.3% vs 57.4%). The same was observed amongst low adherence patients currently taking injectable medicine albeit to a lesser extent (9.6% vs 3.9%).

Conclusion: These real-world research findings suggest that low adherence T2DM patients, as measured by a patient-reported validated tool, is greatly under-recognised by physicians. Patients with low adherence are more likely to smoke heavily, be of a lower socio-economic demographic, have obesity and lifestyle concerns, present with poor glucose monitoring and control resulting in a higher number of hypoglycaemic episodes. Coupled with high product burdens and patient-perceived low needs for medicine, it is clear improved healthcare practitioner and patient awareness could result in better adherence to therapy which in turn can improve glucose control.

Disclosure: **V. Higgins:** None.

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Meta-analysis of improvement in glycaemic control by family intervention in patients with diabetes

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Background and aims: Observational data indicated that family support is associated with good glycemic control in patients with diabetes mellitus (DM). However, it has not been established that an intervention encouraging family members to support a patient's diabetes care contributes to the improvement of glycemic control. This meta-analysis aims to determine the effect of family interventions on glycemic control in patients with DM.

Materials and methods: Electronic literature search using two databases (MEDLINE and EMBASE, from 1950 to 2015) was conducted for randomized controlled trials (RCT) investigating the effect of study-specific diabetes care involving family members on a patient's glycemic control indicated by changes in hemoglobin A1C (A1C) level. The difference in A1C changes between groups with and without the family intervention was pooled with a random-effects model. Studies that compared glycemic control between two different types of family interventions were excluded.

Results: Twenty-five eligible studies were obtained from 570 RCT studies retrieved by the systematic literature search. Overall, the intervention group, which included instructions for family members, achieved a greater A1C reduction than the control group, which did not include such instructions (net A1C change (95% CI) -0.47% (-0.66 to -0.29). When

studies were stratified by DM types, family interventions for patients with type I DM (17 studies), type II DM (7 studies), and combining two types (1 study) elicited A1C changes (95% CI) by -0.41% (-0.63 to -0.19), -0.64% (-1.07 to -0.22), and -0.74% (-1.49 to 0.01), respectively. (Figure)
Conclusion: This meta-analysis confirmed the significance of family support in terms of improving glycemic control regardless of DM type. However, the extent of the reduction in A1C levels was modest.

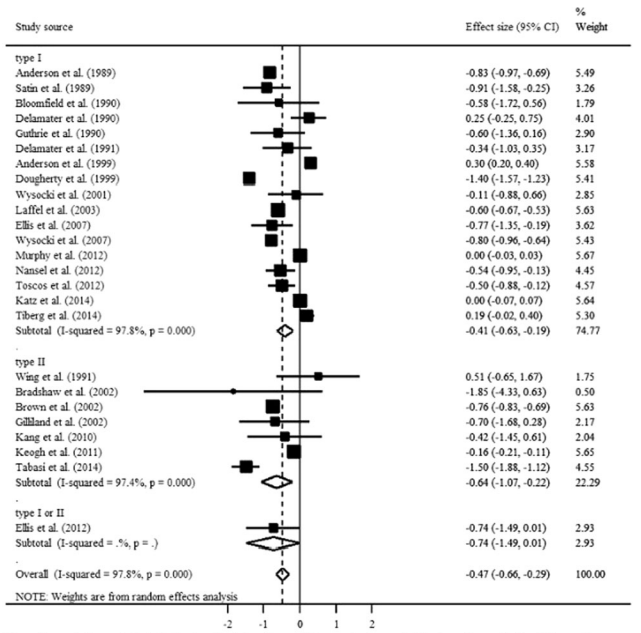

Figure legend: Forrest plots indicate effect size (i.e., difference in hemoglobin A_{1c} change in the intervention group compared with the control group.) The area of squares is proportional to the study weight (i.e., inverse of square of standard error of the effect size). Total effect size overall or by diabetes type is indicated in diamonds.

Disclosure: **M. Tsuruta:** None.

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Effects of patient, physician, and health care environment factors on insulin progression in patients with type 2 diabetes: the MOSAIc study

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Background and aims: Insulin progression or treatment intensification is the key to achieving and maintaining glycaemic goals in the management of type 2 diabetes. Factors associated with insulin progression and successful (or otherwise) outcomes are not fully understood. MOSAIc is a 2-year prospective cohort study of patients with type 2 diabetes across 18 countries in North and Latin America, Europe, Asia, and the Middle-East.
Materials and methods: We collected detailed data on patient, physician and health care environment factors at baseline and regular intervals within the prospective 2-year follow-up period of the study. Data was collected within the routine course of care in a variety of specialist and primary care clinics representative of care delivery in each country. In this analysis we examine the association between these factors and insulin progression. Insulin progression was defined as an increase in antidiabetic treatment complexity at Year 2 from the baseline (e.g., increasing the dose of basal insulin was not considered progression, whereas addition of a prandial dose of insulin to background basal therapy was considered progression). Multiple imputation via chained equations was used for missing data.
Results: In this analysis we included 2,706 patients with the mean (SD) age of 62.8 (10.8) years and 50% female. The overall mean (SD) baseline HbA1c was 65.0 (9) mmol/mol. Of these patients, 924 (34.1%) had a progression of their insulin therapy over the 2 year follow up of the study. Those who progressed were younger ($p<0.0001$) and more likely to be employed ($p=0.005$) and live in Middle East ($p<0.0001$) compared to those who did not progress (see Table 1). In the multivariable logistic regression model, age, marital status, employment, region, insurance and physicians' HbA1c goal at baseline were significantly associated with insulin progression.
Conclusion: Our analyses identified patient, physician and health care environmental factors that were significantly associated with insulin progression over time. It was notable that many patients did not undergo progression of therapy despite HbA1c values that would be considered sub-optimal in relation to current treatment guidelines.

At baseline	Progressed (n=924)	Did not progress (n=1,782)	Univariable P-value	Multivariable OR* (95% CI)
Age (years), mean ± SD	60.7±10.6	62.8±10.8	<0.0001	**0.99 (0.98-0.99)**
Female	50.6%	49.7%	0.6	1.01 (0.84-1.22)
Married or living together	74.8%	77.9%	0.07	**0.81 (0.65-1.00)**
Employed	41.7%	36.0%	0.005	**1.25 (1.01-1.56)**
Region				
- Europe	21.3%	20.4%	0.6	0.93 (0.69-1.24)
- Middle East	17.9%	11.2%	<0.0001	1.31 (0.96-1.80)
- North America	13.2%	16.7%	0.02	**0.68 (0.49-0.94)**
- Latin America	11.4%	11.1%	0.8	0.74 (0.52-1.04)
- Asia	36.3%	40.6%	0.03	Ref
Insurance				
- Public	52.7%	54.0%	0.5	**0.76 (0.59-0.98)**
- Private	25.1%	25.7%	0.7	0.94 (0.71-1.26)
- Uninsured	22.1%	20.3%	0.3	Ref
HbA_{1c} (mmol/mol), mean ± SD	66.1 ± 9	65.0 ± 9	0.3	1.01 (0.94-1.07)
Physician HbA_{1c} goal >53 mmol/mol	20.5%	16.0%	0.008	**1.37 (1.07-1.77)**
Hypoglycemia episode	20.9%	22.1%	0.5	0.91 (0.73-1.15)

Multivariable ORs are adjusted for age, sex, marital status, education, employment, insurance, region, HbA_{1c}, DM treatment type, hypoglycemia episode, BMI, and physician HbA_{1c} goal at baseline.

Clinical Trial Registration Number: NCT01400971
Supported by: Eli Lilly and Company
Disclosure: **S.C. Kim:** Grants; Eli Lilly and Company.

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The effect of the patient-physician relationship on insulin progression, diabetes distress, adherence, and HbA_{1c} control over time: insights from the MOSAIc study

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Background and aims: MOSAIc is a multinational prospective cohort study designed to understand the challenges associated with insulin progression among patients with type 2 diabetes. The study recruited patients

from 18 countries within North and Latin America, Asia, Europe, and the Middle-East. In this analysis we investigate how aspects of the patient-physician relationship were associated with insulin treatment progression, adherence to insulin, and HbA1c levels.

Materials and methods: Eligible patients were those with type 2 diabetes, ≥18 year old and using insulin for ≥3 months with/without other anti-diabetes medications. Patients were seen as part of their usual care in a variety of specialist and primary care clinics representative of care delivery in each country. We collected detailed data on patient, physician and health care environment factors at baseline and regular intervals within the prospective 2-year follow-up period of the study. Insulin progression was defined as an increase in antidiabetic treatment complexity at Year 2 from the baseline (e.g., increasing the dose of basal insulin was not considered progression, whereas addition of a prandial dose of insulin to background basal therapy was considered progression).

Multivariable linear or logistic regression models examined the effect of baseline Interpersonal Process of Care (IPC) sub-scale scores on a) the Diabetes Distress Scale (DDS), b) Diabetes Knowledge Test (DKT), c) self-monitoring blood glucose, d) adherence (number of missed injections in the last week), e) insulin treatment progression and f) HbA1c level at Year 2. We adjusted for demographic characteristics, socioeconomic status, and baseline scores of the DDS, DKT, self-monitored blood glucose, insulin adherence, and HbA1c levels. Multiple imputation via chained equations was used for missing data.

Results: Among 2706 patients included in this analysis, the mean age (SD) of patients was 62.1 (10.8) years, 50.0% were women and the mean (SD) HbA1c was 65.0 mmol/mol (9.0) at baseline. Among the domains of IPC survey, "Hurried communication" was significantly associated with higher (worse) scores on the DDS (β=0.12, 95% CI 0.04 to 0.20, p<0.01) at Year 2. Patient-rated scores of those physicians who were considered to be more proficient in clearly addressing the concerns of their patients ("elicited concerns" domain of the IPC Survey) (β=-0.18, 95% CI -0.28 to -0.07, p<0.01) or less judgmental towards their patients ("discriminated style") (β=-0.15, 95% CI -0.28 to -0.03, p=0.02) were significantly associated with lower HbA1c level at Year 2. IPC domains were not independently associated with diabetes knowledge, frequency of self-monitored blood glucose, adherence, or insulin progression over 2 years.

Conclusion: These findings suggest that key aspects of patient-physician communication may influence diabetes-related patient emotional distress and glycaemic control.

Clinical Trial Registration Number: NCT01400971

Supported by: Eli Lilly and Company

Disclosure: **B. Linetzky:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

PS 079 Insulin therapy

834

Faster-acting insulin aspart vs insulin aspart as part of basal-bolus therapy improves postprandial glycaemic control in uncontrolled type 2 diabetes: the onset® 2 trial

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Background and aims: This multicentre, double-blind, treat-to-target trial evaluated the efficacy and safety of faster-acting insulin aspart (faster aspart; an ultra-fast-acting mealtime insulin) vs insulin aspart (IAsp) in adults with uncontrolled type 2 diabetes (T2D) on basal insulin and oral antidiabetic agents.

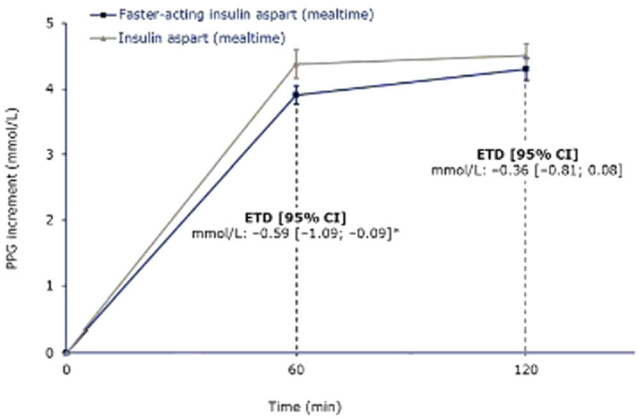
Materials and methods: After optimising basal insulin glargine during an 8-week run-in (mean HbA_{1c} 7.9% at baseline), subjects were randomised 1:1 to mealtime faster aspart (n=345) or IAsp (n=344), each with insulin glargine and metformin. Bolus insulin was titrated using a simple daily patient-driven algorithm.

Results: The change in HbA_{1c} from baseline to Week 26 (primary endpoint) was -1.38% for faster aspart and -1.36% for IAsp (estimated means); mean HbA_{1c} at end of treatment was 6.6% for both arms. Faster aspart demonstrated non-inferiority vs IAsp in reducing HbA_{1c} (estimated treatment difference [95% CI]: -0.02% [-0.15; 0.10]). Both basal-bolus (BB) regimens improved postprandial glucose (PPG) control. The estimated treatment difference for the 1-h PPG increment (meal test) was statistically significant in favour of faster aspart (Figure). Fasting plasma glucose levels remained the same within each group. Body weight change from baseline and rates of overall severe or confirmed hypoglycaemia (plasma glucose <3.1 mmol/L) were similar.

Conclusion: In T2D, mealtime faster aspart and IAsp in a BB regimen achieved excellent glycaemic control and reduced HbA_{1c} from baseline to 6.6%, confirming non-inferiority of faster aspart to IAsp, using a simple daily patient-driven titration algorithm. Faster aspart effectively improved 1-h PPG control vs IAsp without increasing overall hypoglycaemia rates.

Clinical Trial Registration Number: NCT01819129

Figure PPG increment (meal test) at Week 26



Full analysis set; observed data. Error bars: ± SEM. Estimated treatment difference (ETD; faster aspart – IAsp) for PPG increment changes from baseline. *Statistically significant in favour of faster aspart.

Supported by: Novo Nordisk A/S
Disclosure: **K. Bowering:** Employment/Consultancy; Novo Nordisk, Eli Lilly, AstraZeneca, Boehringer Ingelheim, Merck, Sanofi, Janssen. Honorarium; Merck, Eli Lilly, Boehringer Ingelheim, AstraZeneca, Novo Nordisk, Sanofi. Lecture/other fees; Novo Nordisk, AstraZeneca, Eli Lilly, Sanofi, Merck.

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Adding faster-acting insulin aspart to basal insulin significantly improved glycaemic control in adults with type 2 diabetes: the onset® 3 trial

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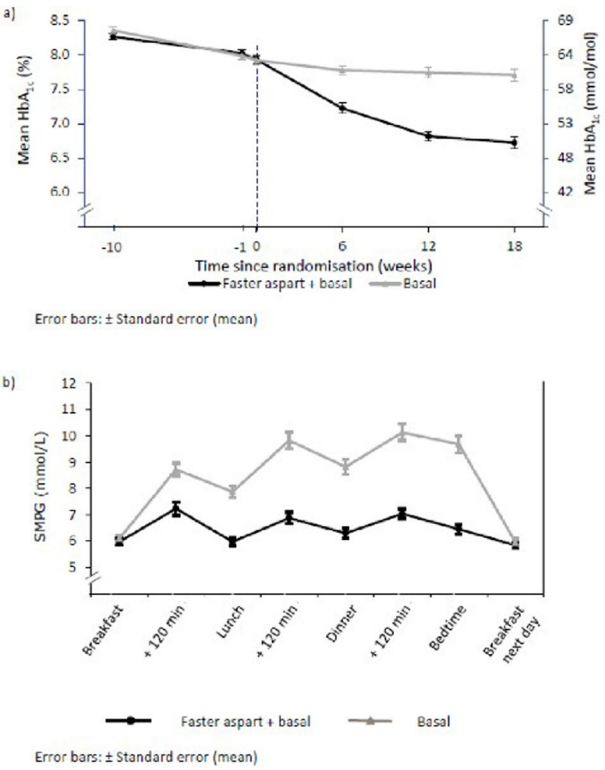
Background and aims: This multicentre, open-label, parallel group trial examined whether faster-acting insulin aspart (faster aspart; an ultra-fast-acting mealtime insulin) in a full basal-bolus (BB) regimen was superior to basal insulin therapy, both in combination with metformin, in terms of glycaemic control after 18 weeks of randomised treatment.
Materials and methods: Adults with type 2 diabetes (T2D) inadequately controlled with basal insulin and oral anti-hyperglycaemic medications underwent 8 weeks' optimisation of once-daily basal therapy (insulin detemir, glargine or NPH), followed by randomisation 1:1 to a BB regimen with mealtime faster aspart (n=116) or continuation of the same once-daily basal insulin (n=120), both in combination with metformin. Mean ± SD HbA_{1c} prior to randomisation was 7.9 ± 0.7%.
Results: Faster aspart in a BB regimen was superior to basal insulin for the primary endpoint, HbA_{1c} change from baseline to Week 18; HbA_{1c} was reduced from 7.9 to 6.8% in the BB arm, and from 7.9 to 7.7% in the basal arm: estimated treatment difference (ETD), (95% CI): -0.94% (-1.17; -0.72), P<0.0001 (Figure). HbA_{1c} ≤7% was achieved by 60.3% in the faster aspart BB arm vs 18.3% in the basal insulin arm. With a BB regimen, 2-hour postprandial plasma glucose (PPG; based on SMPG) was significantly lower, compared with basal insulin (Figure). There was no significant difference between groups, with respect to change in fasting plasma glucose from baseline (ETD, mmol/L: -0.12 [-0.66; 0.42]; NS). As expected, hypoglycaemia rate and weight gain were greater in the BB arm than in the basal arm.
Conclusion: In subjects with T2D, faster aspart in a BB regimen provided superior glycaemic control, achieving a final HbA_{1c} of 6.8% vs 7.7% in the basal-only arm. This effect appears primarily due to greater reduction in PPG (Figure).

Figure. Effect of mealtime faster-acting insulin aspart on glycaemic control: (a) mean HbA_{1c} vs time; (b) 8-point self-monitored plasma glucose (SMPG) profile at Week 18



Clinical Trial Registration Number: NCT01850615
Supported by: Novo Nordisk A/S
Disclosure: **M. Piletič:** Employment/Consultancy; Novo Nordisk, Eli Lilly (member of advisory board). Lecture/other fees; Novo Nordisk, Eli Lilly.

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BioChaperone Combo (BC Combo) reduces post-prandial glucose in type 1 diabetes compared to conventional lispro mix 75/25 (LMx)

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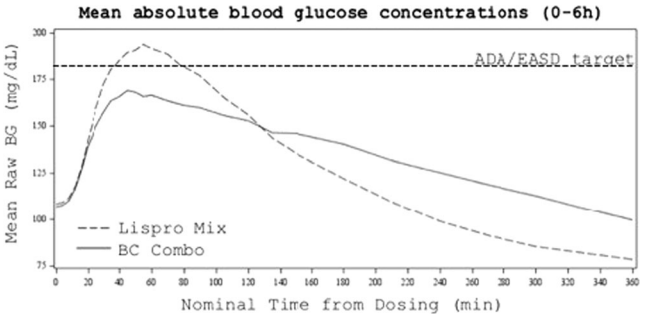
Background and aims: BC Combo is a co-formulation of prandial insulin lispro (25%) and basal insulin glargine (75%) with a rapid "prandial" insulin component and a prolonged flat "basal" component. BC Combo is designed to provide prandial glucose reduction for the largest meal of the day and to cover the daily basal insulin requirements in a single injection. The current trial was designed to evaluate the effect of BC Combo on mealtime glycemia compared to 75% insulin lispro protamine suspension (Lispro Mix 75/25 (LMx)).
Materials and methods: This was a double-blind, randomized, crossover mixed-meal study in T1DM subjects conducted at a single expert center. Subjects with T1DM received individualized doses of BC combo or LMx, immediately prior to a standardized mixed meal (508 kcal, 56% carbohydrates/17% protein/27% fat) on two occasions in a crossover sequence. Primary outcome measures included the incremental area under the blood glucose (BG) curve over the first 2 hours (incremental AUC-BG(0-2h)) after the meal and secondary glycemic endpoints including maximal and 1-hour (1h) post-prandial glucose excursions (PPG). Safety and hypoglycemic events were monitored.

Results: BC Combo was associated with a reduction in median incremental AUC-BG(0-2h) (min,max) vs. LMx (88 mg/dL*min (-31, 166) vs. 129 (20, 194), p=0.008). Starting from identical baseline controlled BG levels, BC Combo was associated with a reduction in BG-maximum (95%C.I.) of 23 mg/dL [(-34, -11), p=0.0004] and a decrease in PPG excursion at 1h of -24 mg/dL, [(-38, -9), p=0.0001] compared to LMx. LMx was associated with a late post-prandial BG drop below baseline, a lower BG-minimum relative to BC Combo (65 vs 94 mg/dL respectively; p=0.007) and numerically more hypoglycemic episodes (8 vs. 4, BG < 50mg/dL).

Conclusion: The time-action profile of BC Combo includes an early component suitable for mealtime glucose control. In comparison to Lispro Mix 75/25, BC Combo improved early postprandial glycemic excursions, with mean 2-hour PPG profiles below the ADA/EASD target threshold of 180 mg/dL, and reduced the risk of late postprandial hypoglycemia in a cohort with T1DM. Additional exploration of safety and efficacy of BC combo including use in T2DM is warranted.



Clinical Trial Registration Number: NCT02514954
Disclosure: **S. Bruce:** Employment/Consultancy; Adocia.

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IDegLira is efficacious across baseline HbA_{1c} categories in subjects with type 2 diabetes uncontrolled on SU, GLP-1RA or IGlar U100: analyses from completed phase 3b trials

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Background and aims: Previous analyses of phase 3a trials (DUAL I extension; DUAL II) showed insulin degludec/liraglutide (IDegLira) is efficacious irrespective of baseline HbA_{1c}. This post hoc analysis aimed to confirm this observation in additional populations from the DUAL III, DUAL IV and DUAL V treat-to-target trials.

Materials and methods: In the DUAL III trial , patients with type 2 diabetes uncontrolled on a glucagon-like peptide-1 receptor agonist (GLP-1RA) were randomised to IDegLira (n=292) or unchanged GLP-1RA (n=146) for 26 weeks. DUAL IV was a 26-week, randomised, double-blind trial comparing IDegLira (n=289) to placebo (n=146) in patients with type 2 diabetes previously uncontrolled on sulphonylurea (SU) ± metformin. DUAL V was a 26-week, randomised trial in patients with type 2 diabetes inadequately controlled on insulin glargine (100 units/ml [IGlar U100]; daily dose 20-50 U) comparing IDegLira (n=278) to continued IGlar U100 titration (n=279). IDegLira starting dose was 10 dose steps (1 dose step = 1 U IDeg + 0.036 mg Lira) in DUAL IV and 16 dose steps in DUAL III and V; maximum IDegLira dose: 50 dose steps. Subjects were grouped according to baseline HbA1c; ≤7.5, >7.5-≤8.5 and >8.5%.

Results: In all trials a higher baseline HbA_{1c} resulted in greater HbA_{1c} reductions (Table). The change in HbA_{1c} was significantly greater with IDegLira vs. comparator in all baseline HbA_{1c} groups with a similar treatment difference between baseline HbA_{1c} groups. In all trials for all baseline HbA_{1c} groups, IDegLira decreased mean HbA_{1c} to 9% (median 9.5%), HbA_{1c} was reduced to 6.9% with IDegLira vs. 7.8% with IGlar U100.

Conclusion: In conclusion, significant HbA_{1c} reductions occur with IDegLira regardless of baseline HbA_{1c} group or study population.

Table Change in HbA_{1c} by baseline HbA_{1c} category in DUAL III, IV and V

		Overall	Baseline HbA_{1c} ≤7.5%	Baseline HbA_{1c} >7.5%–≤8.5%	Baseline HbA_{1c} >8.5%
DUAL III	IDegLira, N	292	113	141	38
	Baseline HbA_{1c},% (±SD)	7.8 (±0.6)	7.2 (±0.2)	8.0 (±0.3)	8.8 (±0.2)
	ΔHbA_{1c},% (±SD)	-1.3 (±0.8)	-1.0 (±0.7)	-1.4 (±0.8)	-1.9 (±1.2)
	GLP-1RA, N	146	66	66	14
	Baseline HbA_{1c},% (±SD)	7.7 (±0.6)	7.2 (±0.2)	8.0 (±0.3)	8.9 (±0.3)
	ΔHbA_{1c},% (±SD)	-0.3 (±0.9)	-0.3 (±0.8)	-0.3 (±1.0)	-1.0 (±1.2)
	ETD [95% CI]*	-0.94 [-1.11;-0.78]	-0.74 [-0.95;-0.52]	-1.13 [-1.38;-0.88]	-1.18 [-1.95;-0.40]
	p value	p<0.001	p<0.001	p<0.001	p=0.004
DUAL IV	IDegLira, N	289	93	156	40
	Baseline HbA_{1c},% (±SD)	7.9 (±0.6)	7.2 (±0.3)	8.0 (±0.3)	8.8 (±0.2)
	ΔHbA_{1c},% (±SD)	-1.5 (±0.8)	-1.0 (±0.6)	-1.5 (±0.9)	-2.1 (±0.9)
	Placebo, N	146	48	80	18
	Baseline HbA_{1c},% (±SD)	7.9 (±0.6)	7.2 (±0.3)	8.1 (±0.3)	8.8 (±0.2)
	ΔHbA_{1c},% (±SD)	-0.5 (±0.8)	-0.2 (±0.7)	-0.6 (±0.9)	-0.7 (±0.9)
	ETD [95% CI]*	-1.02 [-1.18;-0.87]	-0.91 [-1.11;-0.72]	-1.00 [-1.23;-0.76]	-1.36 [-1.88;-0.84]
	p value	p<0.001	p<0.001	p<0.001	p<0.001
DUAL V	IDegLira, N	278	63	102	113
	Baseline HbA_{1c},% (±SD)	8.4 (±0.9)	7.2 (±0.2)	8.1 (±0.3)	9.3 (±0.6)
	ΔHbA_{1c},% (±SD)	-1.8 (±1.1)	-1.0 (±0.6)	-1.6 (±1.0)	-2.5 (±1.0)
	IGlar U100, N	279	64	118	97
	Baseline HbA_{1c},% (±SD)	8.2 (±0.9)	7.1 (±0.3)	8.1 (±0.3)	9.2 (±0.5)
	ΔHbA_{1c},% (±SD)	-1.1 (±1.0)	-0.5 (±0.8)	-1.0 (±0.9)	-1.7 (±0.9)
	ETD [95% CI]†	-0.59 [-0.74;-0.45]	-0.48 [-0.73;-0.23]	-0.55 [-0.80;-0.31]	-0.68 [-0.93;-0.42]
	p value	p<0.001	p<0.001	p<0.001	p<0.001

Based on the full analysis set. Missing data is imputed using last observation carried forward. *Analysed using ANCOVA with treatment, previous antidiabetic therapy and region as fixed factors and baseline HbA_{1c} as covariate. †Analysed using ANCOVA with treatment and region as fixed effects and baseline HbA_{1c} as covariate. ANCOVA, analysis of covariance; CI, confidence interval; ETD, estimated treatment difference; GLP-1RA, glucagon-like peptide-1 receptor agonist; HbA_{1c}, glycated haemoglobin; IDegLira, insulin degludec/liraglutide; IGlar, insulin glargine; N, number of subjects; SD, standard deviation

Clinical Trial Registration Number: DUAL III – NCT01676116; DUAL IV – NCT01618162; DUAL V – NCT01952145
Supported by: Novo Nordisk
Disclosure: **E. Jaeckel:** Employment/Consultancy; Novo Nordisk, Lilly, AstraZeneca, Boehringer, MSD, Janssen, Roche, Novartis. Grants; Novo Nordisk, Novartis, Gilead, Roche, Miltenyi Biotech, Biotest, Wacker Chemie, Fresenius DFG, BMBF, EU, JDRF, VW-Stiftung. Honorarium; NovoNordisk, Lilly, AstraZeneca, Boehringer, MSD, Janssen, Roche, Novartis. Lecture/other fees; Novo Nordisk, Lilly, AstraZeneca, Boehringer, MSD, Janssen, Roche, Novartis.

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Impact of BMI on HbA_{1c} reduction in response to IDegLira in subjects with type 2 diabetes uncontrolled on SU, GLP-1RA or IGlar U100: analyses from completed phase 3b trials

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Background and aims: Previous analyses of phase 3a trials (DUAL I extension; DUAL II) showed that insulin degludec/liraglutide (IDegLira) is efficacious irrespective of baseline body mass index (BMI) category in subjects with T2D who are insulin naïve or uncontrolled on basal insulin. This post hoc analysis aimed to confirm these findings with IDegLira in additional populations from the DUAL III, DUAL IV and DUAL V treat-to-target trials.

Materials and methods: In the DUAL III trial, patients with type 2 diabetes uncontrolled on a glucagon-like peptide-1 receptor agonist (GLP-1RA) were randomised to IDegLira (n=292) or unchanged GLP-1RA (n=146) for 26 weeks. DUAL IV was a 26-week,

randomised, double-blind trial comparing IDegLira (n=289) to placebo (n=146) in patients with type 2 diabetes previously uncontrolled on sulphonylurea (SU) ± metformin. DUAL V was a 26-week, randomised trial in patients with type 2 diabetes inadequately controlled on insulin glargine (100 units/ml [IGlar U100]; daily dose 20-50 U) comparing IDegLira (n=278) to continued IGlar U100 titration (n=279). Starting dose of IDegLira was 10 dose steps [1 dose step = 1 U IDeg + 0.036 mg Lira] in DUAL IV and 16 dose steps in DUAL III and V; maximum IDegLira dose: 50 dose steps for all trials. This analysis of three trials grouped subjects by BMI category; <30, ≥30-<35 and ≥35 kg/m^2.

Results: In all three trials, change in HbA_{1c} with IDegLira was similar between BMI groups (Table). Change in HbA_{1c} was significantly greater with IDegLira vs. comparator in all BMI groups ($p<0.001$ for all trials) with a similar treatment difference between BMI groups (p=NS for all trials).

Conclusion: This analysis confirmed IDegLira effectively reduced HbA_{1c} across all baseline BMI categories when used in subjects previously treated with SUs (as an add-on), GLP-1 RA or IGlar U100 (all in combination with other oral therapies).

Table: Change in HbA_{1c} by baseline BMI category in DUAL III, IV and V

		Overall	BMI <30 kg/m^2	BMI ≥30–<35 kg/m^2	BMI ≥35 kg/m^2
DUAL III	IDegLira, N	292	84	110	98
	Baseline HbA_{1c},% (±SD)	7.8 (±0.6)	7.8 (±0.5)	7.8 (±0.6)	7.7 (±0.6)
	ΔHbA_{1c},% (±SD)	-1.3 (±0.8)	-1.4 (±0.9)	-1.4 (±0.9)	-1.3 (±0.8)
	GLP-1RA, N	146	36	59	51
	Baseline HbA_{1c},% (±SD)	7.7 (±0.6)	7.6 (±0.6)	7.8 (±0.7)	7.7 (±0.5)
	ΔHbA_{1c},% (±SD)	-0.3 (±0.9)	-0.5 (±0.8)	-0.4 (±1.0)	-0.2 (±0.9)
	ETD [95% CI]*	**-0.94 [-1.11;-0.78]**	**-0.78 [-1.12;-0.44]**	**-0.90 [-1.18;-0.61]**	**-1.06 [-1.32;-0.79]**
	***p* value**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
DUAL IV	IDegLira, N	289	119	102	68
	Baseline HbA_{1c},% (±SD)	7.9 (±0.6)	7.9 (±0.6)	7.9 (±0.6)	7.9 (±0.6)
	ΔHbA_{1c},% (±SD)	-1.5 (±0.8)	-1.4 (±0.8)	-1.4 (±0.8)	-1.6 (±0.9)
	Placebo, N	146	53	53	40
	Baseline HbA_{1c},% (±SD)	7.9 (±0.6)	8.0 (±0.6)	7.8 (±0.6)	7.9 (±0.6)
	ΔHbA_{1c},% (±SD)	-0.5 (±0.8)	-0.5 (±0.9)	-0.4 (±0.8)	-0.5 (±0.8)
	ETD [95% CI]*	**-1.02 [-1.18;-0.87]**	**-0.95 [-1.21;-0.68]**	**-1.07 [-1.32; -0.82]**	**-1.11 [-1.42; -0.81]**
	***p* value**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**
DUAL V	IDegLira, N	278	99	110	69
	Baseline HbA_{1c},% (±SD)	8.4 (±0.9)	8.3 (±1.0)	8.3 (±0.8)	8.5 (±0.9)
	ΔHbA_{1c},% (±SD)	-1.8 (±1.1)	-1.8 (±1.1)	-1.8 (±1.2)	-1.9 (±0.9)
	IGlar U100, N	279	97	113	69
	Baseline HbA_{1c},% (±SD)	8.2 (±0.9)	8.3 (±1.0)	8.2 (±0.8)	8.3 (±0.8)
	ΔHbA_{1c},% (±SD)	-1.1 (±1.0)	-1.1 (±1.0)	-1.1 (±1.0)	-1.2 (±0.9)
	ETD [95% CI]†	**-0.59 [-0.74;-0.45]**	**-0.68 [-0.94;-0.42]**	**-0.52 [-0.76;-0.28]**	**-0.59 [-0.83;-0.34]**
	***p* value**	**p<0.001**	**p<0.001**	**p<0.001**	**p<0.001**

Based on the full analysis set. Missing data is imputed using last observation carried forward. *Analysed using ANCOVA with treatment, previous antidiabetic therapy and region as fixed factors and baseline HbA_{1c} as covariate. †Analysed using ANCOVA with treatment and region as fixed effects and baseline HbA_{1c} as covariate. ANCOVA, analysis of covariance; BMI, body mass index; CI, confidence interval; ETD, estimated treatment difference; GLP-1RA, glucagon-like peptide-1 receptor agonist; HbA_{1c}, glycated haemoglobin; IDegLira, insulin degludec/liraglutide; IGlar, insulin glargine; N, number of subjects; SD, standard deviation

Clinical Trial Registration Number: DUAL III – NCT01676116; DUAL IV – NCT01618162; DUAL V – NCT01952145
Supported by: Novo Nordisk
Disclosure: **E. Jódar:** Employment/Consultancy; Astra-Zeneca, Janssen, Lilly, NovoNordisk, FAES, FAES, MSD. Lecture/other fees; Lilly, Novonordisk, MSD.

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Safe and effective blood glucose lowering with IDegLira in elderly patients with type 2 diabetes uncontrolled on Oral Antidiabetic Drugs (OADs) and/or insulin glargine U100

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Background and aims: Elderly patients with type 2 diabetes (T2D) who are vulnerable to hypoglycaemia are a challenging population to treat. IDegLira is a once-daily, fixed-ratio combination of insulin degludec and liraglutide that has been investigated in the DUAL clinical trial programme; this analysis focused on elderly subpopulations uncontrolled on OADs (DUAL I) or uncontrolled on OADs and insulin glargine U100 (IGlar U100; DUAL V).

Materials and methods: This analysis investigated the safety and efficacy of IDegLira in elderly (≥65 years) subpopulations of DUAL I and DUAL V. These trials demonstrated the clinical benefits of IDegLira vs IDeg or Lira alone in patients uncontrolled on metformin ± pioglitazone (DUAL I) and vs continued IGlar U100 uptitration in patients uncontrolled on IGlar U100 ± metformin (DUAL V) (both 26 weeks), in the entire trial populations.

Results: In DUAL I and DUAL V respectively, 14% and 26% of patients were ≥65 years old (median: 69.6 and 69.8 years; body weight: 82.1 and 84.3 kg, respectively). HbA_{1c} reduction was significantly greater for IDegLira vs comparators with more patients achieving HbA_{1c} <7% (table). FPG reduction was similar for IDegLira vs insulin comparators of both trials but significantly greater for IDegLira vs Lira in DUAL I. IDegLira was associated with weight loss vs weight gain with both IDeg and IGlar U100, but less weight loss than Lira (statistically significant difference for all). Confirmed hypoglycaemia rates were similar for IDegLira vs IDeg in DUAL I and significantly lower vs IGlar U100 in DUAL V. IDegLira was insulin-sparing vs both insulin comparators. Safety profiles were consistent with the entire trial populations.

Conclusion: In elderly patients IDegLira led to better glycaemic control vs glucagon-like peptide-1 analogue liraglutide, and vs basal insulins, and had the advantage of weight loss compared with basal insulin alone, and low rates of hypoglycaemia.

		Change in HbA_{1c} from baseline, %	Change in FPG from baseline, mmol/L	Change in weight from baseline, kg	Confirmed hypoglycaemia	Daily insulin dose at end of trial, U	Patients with HbA_{1c} <7%, %
DUAL I	Observed data IDegLira (N=118) IDeg (N=61) Lira (N=57)	Mean (SD) -1.89 (1.06) -1.37 (0.89) -1.33 (0.95)	Mean (SD) -3.86 (2.71) -3.82 (2.77) -1.53 (2.19)	Mean (SD) -0.5 (3.1) 2.1 (3.5) -3.2 (3.6)	Eps/100 PYE 223.2 246.6 15.3	Mean (SD) 33 (13) 44 (27) NA	Proportion 90.7 75.4 66.7
	Estimate [95% CI], *p* IDegLira vs. IDeg	ETD: -0.54 [-0.80; -0.28], *p*<0.0001	ETD: -0.08 [-0.58; 0.42], NS	ETD: -2.59 [-3.65;-1.53], *p*<0.0001	ERR: 0.83 [0.48; 1.46], NS	ETD: -11 [-17; -5], *p*=0.0002	EOR: 3.29 [1.28; 8.46], *p*=0.0135
	Estimate [95% CI], *p* IDegLira vs. Lira	ETD: -0.66 [-0.93; -0.39], *p*<0.0001	ETD: -2.03 [-2.55; -1.51], *p*<0.0001	ETD: 2.54 [1.44; 3.65], *p*<0.0001	ERR: 18.10 [5.04; 65.02], *p*<0.0001	NA	EOR: 6.48 [2.53; 16.61], *p*<0.0001
DUAL V	Observed data IDegLira (N=68) IGlar U100 (N=77)	Mean (SD) -1.75 (1.03) -0.89 (0.82)	Mean (SD) -2.25 (2.65) -2.55 (2.43)	Mean (SD) -1.9 (2.7) 1.3 (3.1)	Eps/100 PYE 106.1 400.5	Mean (SD) 37 (11) 54 (24)	Proportion 70.6 37.7
	Estimate [95% CI], *p* IDegLira vs. IGlar U100	ETD: -0.71 [-0.95; -0.48], *p*<0.001	ETD: 0.12 [-0.43; 0.66], NS	ETD: -3.07 [-4.01; -2.13], *p*<0.001	ERR: 0.26 [0.14; 0.48], *p*<0.001	ETD: -17.05 [-23.12; -10.97] *p*<0.001	EOR: 5.52 [2.52; 12.10], *p*<0.001

Data based on the full analysis set, with the exception of the observed rates of confirmed hypoglycemia and insulin dose at end of trial, which are based on the safety analysis set. Missing data imputed using last observation carried forward. Confirmed hypoglycemia was defined as severe or plasma glucose <56 mg/dL. Change from baseline in HbA_{1c}, FPG, body weight and insulin dose are analyzed using an ANCOVA model, the number of confirmed hypoglycemic episodes are analyzed using a negative binomial regression model with a log link and the logarithm of the exposure time as offset, while the responder endpoints are analyzed using a logistic regression model with a logit link.
ANCOVA, analysis of covariance; EOR; estimated odds ratio; ETD, estimated treatment difference; ERR, estimated rate ratio; Eps, episodes; FPG, fasting plasma glucose; IDeg, insulin degludec; IGlar U100, insulin glargine U100; Lira, liraglutide; N, number of patients; NA, not applicable; NS, not significant; PYE, patient-years of exposure; SD, standard deviation.

Clinical Trial Registration Number: NCT01336023; NCT01952145
Supported by: Novo Nordisk
Disclosure: **A. Liebl:** Employment/Consultancy; Advisory panel: Boehringer-Ingelheim, Eli Lilly, Medtronic, MSD, Novo Nordisk, Roche. Lecture/other fees; Speakers bureau: AstraZeneca, Bayer, Becton Dickinson, Boehringer-Ingelheim, Eli Lilly, Medtronic, MSD, Novo Nordisk, Roche Consultant: Eli Lilly. Other; Research support: DexCom, Eli Lilly, Medtronic.

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Insulin glargine as an adjunct to oral antidiabetic drugs for Asians with type 2 diabetes: a pooled analysis to identify predictors of dose and treatment response

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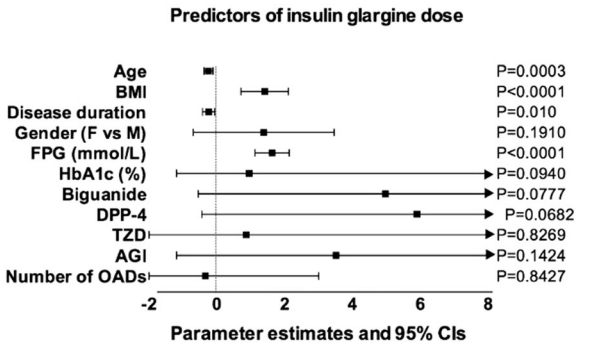
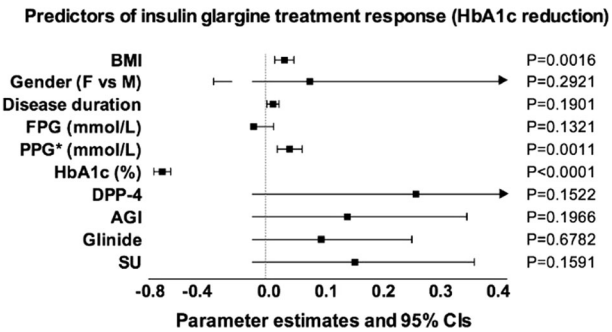
Background and aims: We performed a pooled analysis to identify factors that could predict insulin glargine dose and response to insulin

glargine therapy in Asian patients with type 2 diabetes mellitus (T2DM) who do not meet glycemic targets with oral antidiabetic drugs (OADs).
Materials and methods: We selected treat-to-target randomized controlled trials (RCTs) that enrolled, amongst others, Asian T2DM patients who did not meet a glycemic target of HbA1c ≤7% despite an established OAD regimen. Patients included in the pooled analysis received insulin glargine as an adjunct to OADs for 24 weeks. To identify predicting factors, multivariate analyses were performed.
Results: We selected 7 RCTs and extracted data from 724 Asian individuals. The median (min-max) age of patients was 55.9 years (33.6-77.3) and the group had a mean body mass index (BMI) of 26.4±4.02 kg/m2. The mean duration of T2DM for the group was 9.7 ±6.24 years. Mean (±SD) values for fasting plasma glucose (FPG), post-prandial glucose (PPG) and PPG excursion at baseline was 9.0±2.30 mmol/L, 12.8±3.67 mmol/L and 3.9±3.17 mmol/L, respectively. The group had a mean (±SD) baseline HbA1c of 8.7 ±1.03%. At the end of 24 weeks, the median (min-max) dose of insulin glargine was 25.7 ±17.31 U (0.4 ±0.23 U/kg), the mean HbA1c was 7.5% ±0.96, and 31.9% of patients (n=231) achieved target HbA1c levels of <7%. The Figure shows factors that would affect the insulin glargine dose and response to insulin glargine therapy, i.e. reduction in HbA1c.
Conclusion: Our pooled analysis identified predictors of insulin glargine dose and response to treatment in Asian patients who are inadequately controlled with an established OAD regimen. The results from this study provided a reference for dose adjustment of insulin glargine in clinical utility for Asian patients.

Panel A



Panel B



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Disclosure: **Y. Bi:** Other; Study sponsored by Sanofi.

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Basal insulin analogues for type 2 diabetes: systematic review and network meta-analysis

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Background and aims: Regulatory agencies in Europe and the United States recently approved several novel basal insulin analogues. However, their comparative effectiveness and safety compared with existing basal insulin analogues has not been clarified. We performed a systematic review and network meta-analysis to assess the efficacy and safety of available basal insulin analogues for type 2 diabetes.
Materials and methods: We systematically searched Medline, Embase, and the Cochrane Central Register of Controlled Trials up to September 2015 for randomised controlled trials with duration of more than 12 weeks that compared basal insulin analogues in patients with type 2 diabetes. Primary outcome was change in HbA_{1c}. Secondary outcomes included change in body weight and incidence of nocturnal and severe hypoglycaemia. We performed multivariate random-effects network meta-analyses and ranked the comparative effects of all treatments for all outcomes with surface under the cumulative ranking curves (SUCRA) probabilities.
Results: We included 29 studies comprising 14,268 adults with type 2 diabetes and assessing eight different basal insulin analogues. Glargine-300 and glargine were associated with marginally significant greater reductions in HbA_{1c} compared with detemir and degludec, however these differences were not clinically relevant (Table). Treatment with glargine-300 or glargine resulted in improved glycaemic control in comparison with degludec three-times-weekly [weighted mean difference (WMD) in HbA_{1c} change -0.29; 95% CI -0.49 to -0.10 and -0.28; -0.44 to -0.11, respectively]. Change in body weight was comparable among all insulin regimens, with the exception of detemir, which had a favourable weight profile versus all comparators (WMD ranging from -1.54 to -0.78). Incidence of severe hypoglycaemia did not differ among most insulin regimens, except for Neutral Protamine Lispro (NPL), which was associated with increased risk compared to glargine-300, glargine, detemir, and degludec. Glargine-300 and degludec were associated with lower nocturnal hypoglycaemic incidence against most comparators (Table).
Conclusion: Degludec and glargine-300 do not seem to confer significant improvements in glycaemic control or body weight compared to older regimens, apart from a decrease in the risk for nocturnal hypoglycaemia. Costs, patients' preferences and patient-reported outcomes should also be considered when deciding on an optimal insulin therapy for each individual.

Safety (incidence of nocturnal hypoglycaemia; OR 95% CI) Comparator Efficacy (change in $HbA1_c$; WMD 95% CI)

Glargine-300	**0.68 (0.58, 0.80)**	0.99 (0.64, 1.54)	**0.61 (0.44, 0.84)**	**0.42 (0.32, 0.56)**	**0.66 (0.53, 0.83)**	0.93 (0.72, 1.20)	**0.57 (0.34, 0.94)**
-0.02 (-0.14, 0.10)	**Glargine**	1.45 (0.96, 2.17)	0.89 (0.67, 1.18)	**0.62 (0.49, 0.78)**	0.97 (0.83, 1.13)	**1.37 (1.12, 1.64)**	0.83 (0.52, 1.35)
-0.05 (-0.27, 0.17)	-0.03 (-0.22, 0.16)	**Degludec-200**	0.61 (0.37, 1.01)	**0.43 (0.27, 0.68)**	0.67 (0.43, 1.03)	0.94 (0.63, 1.38)	0.57 (0.31, 1.07)
-0.07 (-0.34, 0.20)	-0.05 (-0.29, 0.19)	-0.02 (-0.33, 0.29)	**Ly 2963016**	0.70 (0.48, 1.01)	1.08 (0.79, 1.51)	**1.54 (1.09, 2.17)**	0.94 (0.54, 1.64)
-0.08 (-0.26, 0.10)	-0.06 (-0.20, 0.07)	-0.03 (-0.27, 0.20)	-0.01 (-0.29, 0.27)	**NPL**	**1.56 (1.22, 2.00)**	**2.22 (1.61, 2.94)**	1.35(0.79, 2.27)
-0.17 (-0.33, -0.01)	**-0.15 (-0.25, -0.04)**	-0.12 (-0.33, 0.10)	-0.10 (-0.36, 0.17)	-0.08 (-0.24, 0.07)	**Detemir**	**1.40 (1.01, 1.79)**	0.86 (0.52, 1.42)
-0.17 (-0.32, -0.01)	**-0.15 (-0.25, -0.04)**	-0.12 (-0.31, 0.07)	-0.10 (-0.36, 0.17)	-0.09 (-0.26, 0.09)	-0.00 (-0.15, 0.15)	**Degludec**	0.61 (0.37, 1.02)
-0.29 (-0.49, -0.10)	**-0.28 (-0.44, -0.11)**	-0.25 (-0.49, -0.00)	-0.23 (-0.52, 0.07)	-0.21 (-0.42, -0.00)	-0.13 (-0.32, 0.07)	-0.13 (-0.31, 0.06)	**Degludec 3/week**

Table: Network meta-analysis effect estimates of basal insulin analogues for efficacy (change in HbA1c) and safety (incidence of nocturnal hypoglycaemia): Drugs are reported in order of efficacy (change in $HbA1_c$) ranking. Data indicate column-to-row difference for change in HbA1c [i.e. a weighted mean difference (WMD) lower than 0.00 favours the column-defining treatment] and row-to-column ratio for incidence of nocturnal hypoglycaemia [i.e. an odds ratio (OR) lower than 1.00 favours the row-defining treatment]. Statistically significant differences are in bold.

Disclosure: **A. Tsapas:** None.

PS 080 Hypoglycaemia: the down side

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Glucagon response to insulin-induced hypoglycaemia in subjects with latent autoimmune diabetes in adults

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Background and aims: No studies, so far, have systematically examined counterregulatory (CR) responses to hypoglycemia (H) in subjects with latent autoimmune diabetes in adults (LADA). Whether they share with type 1 diabetes (T1DM) the loss of glucagon (G) secretion to H, is not known.
Materials and methods: To define and characterize α-cell responses to H in LADA we studied 9 GADab+ (mean±SD: age 46.1±7.3 yrs; BMI 23.3 ±2.4 kg/m^2; A_{1C} 7.1±0.6%, diabetes duration 3.6±3.1 yrs) and 9 GADab- subjects with type 2 diabetes matched for age, sex, BMI, diabetes duration, A_{1C}. Subjects were studied on 2 occasions at random, with i.v. insulin infusion in euglycemia (E) (plasma glucose, PG, 90 mg/dl) or during stepped H (PG 90, 78, 66, 54, and 42 mg/dl).
Results: PG was maintained at pre-selected plateaus, without any significant difference between groups (p>0.2). Glucose infusion rates (GIR) were lower in H than E studies in GADab+ (733±326 mg/kg vs 1101±398 mg/kg, p=0.039), as well as in GADab- (663±162 mg/kg vs 924±363 mg/kg, p=0.017), with no differences between groups (p>0.2). In E studies, there were no significant changes in plasma concentrations of any of the CR hormones in both groups, with the exception of G, whose plasma levels decreased significantly from baseline to the end of studies without differences between groups (from 62.1±28 to 50.3±30 pg/ml and from 87.5±39 to 75.4±34 pg/ml, GADab+and GADab-, respectively, both P<0.05). In H, all CR hormones increased significantly compared with the E studies, in both groups, however G increased in GADab- (incremental $AUC_{0\text{-}4h}$ 156 ±177 pg/ml.h, p=0.026) and to a lesser extent in GADab+ subjects (incremental $AUC_{0\text{-}4h}$ 48±100 pg/ml.h, p=0.186). Glycemic thresholds for G response were not different between GADab- and GAD ab+ subjects. Plasma adrenaline responses to H were superimposable in GADab- and GADab+ (p>0.2).
Conclusion: We conclude that similarly to T1DM, subjects with LADA exhibit severe impairment of the α-cell in terms of response to H. This finding might explain predisposition to H in people with LADA
Disclosure: **C.G. Fanelli:** None.

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Glucagon response in C-peptide positive vs C-peptide negative patients with type 1 diabetes under hypoglycaemic clamp vs real-life setting

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Background and aims: It has been suggested that in long-standing type 1 diabetes (T1D) counter-regulatory response to hypoglycaemia is blunted. Glucagon response to hypoglycaemia was tested in a hypoglycaemic clamp setting (clamp) versus hypoglycaemia under real-life conditions using a meal/insulin challenge (real-life) in C-peptide positive (C-pep+) vs. C-peptide negative (C-pep-) subjects with T1D.
Materials and methods: In the clamp study subjects underwent a hyperinsulinemic, hypoglycaemic clamp. In the real-life study subjects were investigated over 12 hours at the research center and received a standardized meal with 180% of their regular short-acting insulin dose. Glucagon response during hypoglycaemia was defined as the area under the glucagon curve during the first hypoglycaemic episode. Glucose was measured every 5min (Super GL glucose analyser). Glucagon samples were taken using for glucagon measurement validated BD P800 blood collection tubes and analysis was performed using a glucagon-specific sandwich ELISA (Mercodia, Sweden). C-pep+ was defined as C-peptide level ≥0.05nmol/l.
Results: Baseline characteristics were the following for the clamp group: 11 C-pep- (age 37.4±13.2 years, 6 female, BMI 25.0±2.3 kg/m2, HbA1c 58.2±8.5 mmol/l, diabetes duration 24.0±10.0 years) and 10 C-pep+ (age 39.6±12.9 years, 5 female, BMI 23.6±1.8 kg/m2, HbA1c 56.1±9.8 mmol/l, diabetes duration 2.5±2.2 years). Subjects in the real-life study were as follows: 11 C-pep- (age 31.5±7.7 years, 4 female, BMI 23.8±3.1 kg/m2, HbA1c 56.3±13.2 mmol/l, diabetes duration 18.7±8.3 years) and 5 C-pep+ (age 37.9±17.2 years, 2 female, BMI 23.0±1.3 kg/m2, HbA1c 60.9±10.1 mmol/l, diabetes duration 10.4±14.6 years). In the clamp study, C-pep+ had a significantly higher glucagon response during hypoglycaemia than C-pep- (Mann-Whitney-U: 4.7±3.23 vs. 1.5±1.70, respectively, p=0.0083). During the real-life experiment no statistically significant difference was seen (C-pep+ 0.1±0.08 vs. C-pep- 0.1±0.04, p=0.5569).
Conclusion: C-pep+ showed a higher glucagon response compared to C-pep- during hypoglycaemic clamp conditions, but in contrast to previous studies not during real-life conditions.
Clinical Trial Registration Number: NCT02028078, DRKS00009604, NCT02614768
Supported by: European Commission FP7-305343
Disclosure: **S. Zenz:** None.

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The association between hypoglycaemic events and ventricular arrhythmias in type 1 diabetes

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Background and aims: Significantly increased rates of ventricular arrhythmias were found in older type 2 diabetes patients during hypoglycaemic events. Little is known about this association in type 1 diabetes patients who have an increased risk of hypoglycaemia. We aimed to investigate the risk of ventricular arrhythmias in type 1 diabetes patient without macrovascular disease during hypoglycemic and control periods.
Materials and methods: In a prospective study, n=30 patients with type 1 diabetes under regular care participated. The patients wore continuous subcutaneous glucose (Medtronic iPro2) and ECG (EC-1-12H) monitors during their usual life for 132±51 hours. Hypoglycaemia (n=183, no severe event) was diagnosed if subcutaneous glucose ≤3.9 mmol/l. Linear mixed models were used to compare the difference in the rate of ventricular extrasystoles (VES) and heart rate between a 3 hour period after a hypoglycaemia and a control 3-hour period (the same time of another day) without a hypoglycaemia. Further, we separately analysed patients (n=5) with ≥10 VES over any investigated 3-hour period.
Results: Mean age of participants was 36.4±12.7 years, diabetes duration 19.2±12.1 years, HbA1c 8.4±1.2%, blood pressure 124±14/74±8 mmHg, BNP 20±16 ng/l volt; known hypertension n=7, neuropathy n=10. Altogether 18.3 million beats were analysed, 31,207 VES, 176 bigemin VES, 30 coupled VES, and 7 ventricular runs. The hourly VES rates were related to waist circumference (r=0.42), total, and LDL-cholesterol (r=0.56, r=0.60, all p<0.05). According to mixed models heart rate increased non-significantly during hypoglycaemia (difference daytime 0.5 SE 1.1, night-time 2.5 SE 1.6/min). The rate of VESs significantly increased during hypoglycaemia (daytime 0.88 SE 0.30, nigh-time 0.66 SE 030 VES/3 hours). In the subgroup with a higher rate of VESs, this increase was larger (daytime 4.33 SE 0.64, night-time 3.19 SE 0.64 VES/3 hours).

Conclusion: Ventricular arrhythmias were infrequent among younger type 1 diabetes patients with a long diabetes duration and remained markedly lower than the pathological cut-off of 100 VES/hour. Hypoglycaemic events statistically significantly but clinically insignificantly increased the rate of VESs. Our results suggest that non-severe hypoglycaemic events (an obligatory side effect of intensified treatment) do not increase the risk of ventricular arrhythmias in young people with type 1 diabetes.

Clinical Trial Registration Number: 507/2014
Supported by: Hungarian Diabetes Association
Disclosure: **V. Vass:** None.

845

Effects of antecedent hypoglycaemia on gastric and cardiac responses to subsequent hypoglycaemia in health

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Background and aims: In both health and patients with diabetes, acute hypoglycaemia triggers counter-regulatory responses causing an acceleration of gastric emptying (increasing carbohydrate absorption) and increased cardiac contractility. However, antecedent hypoglycaemia attenuates endogenous catecholamine and vagal responses to subsequent hypoglycaemia. Our objective was to evaluate the effects of antecedent hypoglycaemia on gastric and cardiac responses to subsequent hypoglycaemia in health.
Materials and methods: Ten healthy men (age 22.5 (1.0) years, BMI 23.8 (0.5) kg/m2) were studied on two occasions, each lasting 30 hours, separated by ≥6 weeks and randomised to either 'control' (Day 1 (C1) three euglycaemic clamps at a blood glucose of 6mmol/L followed day 2 (C2) one hypoglycaemic clamp at 2.8mmol/L), or 'antecedent hypoglycaemia' (Day 1 (AH1) three clamps at a blood glucose of 2.8mmol/L followed by day 2 (AH2) one clamp at 2.8mmol/L). Energy intake was controlled with the same meals given during both study periods. Gastric emptying (GE) was measured on day 1 and 2, for both control (GEC1 and GEC2) and antecedent hypoglycaemia (GEAH1 and GEAH2) periods, using scintigraphy, following ingestion of a meal consisting of 100g of minced beef labelled with 20 MBq 99mtechnetium-sulphur colloid. Radioisotopic data were acquired every minute for 180mins. Cardiac contractility was measured using fractional shortening (FS) via 2D echocardiography on days 1 and 2 of the control (FSC1 and FSC2) and antecedent hypoglycaemia (FSAH1 and FSAH2) periods. Data are mean (SE).
Results: Gastric emptying was faster (GEC2 AUC vs. GEC1 AUC, P=0.01) and fractional shortening greater (FSC2 vs. FSC1, P<0.01) during acute (single episode) hypoglycaemia compared to euglycaemia. When compared to a single episode of hypoglycaemia, gastric emptying was unaffected by antecedent hypoglycaemia (GEAH2 AUC vs. GEC2 AUC, P=0.74). In contrast, fractional shortening may be reduced (FSAH2 vs. FSC2, P=0.06), although this was not significant (Figure 1).
Conclusion: In health, the acceleration of gastric emptying during acute hypoglycaemia does not appear to be affected by antecedent hypoglycaemia whereas the increase in cardiac contractility may be. Accordingly, if similar gastric responses are observed in individuals with diabetes, treatment of hypoglycaemia with oral carbohydrate should remain effective in patients with recurrent hypoglycaemia.

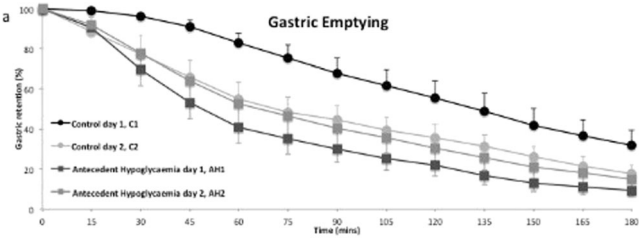

Figure 1a – Gastric emptying curves on day 1 and day 2 of Control and Antecedent Hypoglycaemia study periods
Figure 1b – Cardiac fractional shortening on day 1 and day 2 of Control and Antecedent Hypoglycaemia study periods

Clinical Trial Registration Number: ACTRN12614000986673
Supported by: PK is supported by a Royal Adelaide Hospital A.R. Clarkson Scholarship
Disclosure: **P. Kar:** Grants; PK is supported by a Royal Adelaide Hospital A.R. Clarkson Scholarship.

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High-intensity interval exercise reduces symptomatic responses to subsequent hypoglycaemia in patients with type 1 diabetes

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Background and aims: Antecedent moderate- and low-intensity exercise attenuates counterregulatory responses to subsequent hypoglycemia, thus increasing the risk of severe hypoglycemia. It is unknown whether high-intensity interval training (HIIT) produces similar effects. Since HIIT increases plasma lactate levels, and exogenous lactate suppresses counterregulatory and symptomatic responses to hypoglycemia, we hypothesized that elevated levels of endogenous lactate after HIIT mediate its potential suppressive effects. In patients with type 1 diabetes (T1DM) and impaired awareness of hypoglycemia (IAH), endogenous lactate might even further impair counterregulation. In contrast, high-intensity exercise has been suggested to restore defective counterregulation in a rat model of IAH.
Materials and methods: In a randomized cross-over study, 10 healthy controls (HC), 10 T1DM patients with normal awareness of hypoglycemia (NAH) and 10 T1DM patients with IAH underwent hyperinsulinemic (60 mU m2 m-1) euglycemic-hypoglycemic (2.6 mmol/L) clamps. Under euglycemic conditions, they performed either a high intensity interval training (HIIT) or rested for the same period of time (REST). HIIT consisted of a short warming up and three 30-s all-out sprints interspersed with 4 min active recovery. Plasma glucose, lactate, counterregulatory hormones and symptom scores were evaluated at regular intervals. A cognitive test battery was applied during the screening visit and during hypoglycemia.
Results: Plasma glucose levels were similar during the hypoglycemic phase after HIIT and REST for all groups. HIIT increased plasma lactate levels (from 0.9±0.2 to 13.2±3.0 mmol/L), and counterregulatory hormones, and decreased pH similarly in all groups (p<0.01 for all). Lactate remained elevated during subsequent hypoglycemia. Compared to REST, HIIT decreased total hypoglycemic symptom scores in T1DM-NAH (76 ±12 vs 53±10, p=0.02) and numerically, but not significantly in HC (51±10 vs. 42±7, p=0.19). HIIT did not affect hypoglycemic symptom scores in T1DM-IAH (22±3 vs 24±5, p=0.80). HIIT had no effect on

counterregulatory hormone responses to hypoglycemia (p=NS in all study groups). The deterioration in cognitive function in response to hypoglycemia tended to be less pronounced after HIIT than REST in T1DM-NAH.
Conclusion: The present findings show that short high-intensity interval training suppresses symptoms of subsequent hypoglycemia in T1DM-NAH. Exercise-induced lactate release may mediate this effect by providing an alternative fuel source for the brain, resulting in impaired detection of hypoglycemia and potentially preserved cognitive function. In addition, we found no evidence of restoration of defective counterregulation in response to HIIT in T1DM-IAH.
Clinical Trial Registration Number: NCT02308293
Supported by: EFSD, Dutch Diabetes Research Foundation
Disclosure: **H.M.M. Rooijackers:** None.

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Is the presence or absence of nocturnal hypoglycaemia unawareness related to changes in parameters for 24-hour glycaemic variability in type 1 diabetic patients?

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Background and aims: To investigate whether the presence or absence of nocturnal hypoglycemia unawareness (NHU) are related to changes in the standard deviation (SD) of glucose or the mean amplitude of glycemic excursions (MAGE) as parameters for 24-hour glycemic variability.
Materials and methods: This study included a total of 62 type 1 diabetic outpatients receiving intensive insulin therapy and undergoing continuous glucose monitoring (CGM) assessments at Jikei University Hospital. All patients were assessed for the SD of 24-hour glucose and the MAGE as parameters for glycemic variability. The patients were divided into two groups depending on whether or not they had NHU (<70 mg/dL) from 00:00AM to 06:00AM and were compared for the SD of glucose and the MAGE. Additionally, ROC analyses were performed on the SD of glucose and the MAGE to determine their cut-off values for predicting the presence of NHU.
Results: NHU was shown to be absent in 40 patients (64.5%) (HA group) and but to be present in 22 patients (35.4%) (NHU group). The HA and NHU groups were not significantly different in their background characteristics (mean age, 43 ± 14(SD) vs. 43 ± 14, P = 0.944; HbA1c, 8.9 ± 1.9% vs. 8.2 ± 1.7%; P = 0.192). The mean 24-hour glucose level was significantly lower in the NHU group at 144.8 ± 31.8 mg/dL vs. 171.3 ± 38.6 mg/dL in the HA group (P < 0.001). The SD of glucose was shown to be significantly higher in the NHU group at 66.5 ± 19.2 mg/dL vs. 50.5 ± 14.4 mg/dL in the HA group (P < 0.001). In contrast, the MAGE was not significantly different between the HA and NHU groups (95.2 ± 46.4 mg/dL and 108.4 ± 44.6 mg/dL, respectively; P = 0.272). ROC analysis identified the cut-off for the SD of glucose predicting the presence of NHU as ≥ 54.6 mg/dL (sensitivity, 0.78; specificity, 0.68; AUC, 0.76; P = 0.001).
Conclusion: While there was no difference in HbA1c between those with NHU and those without, the SD of glucose was shown to be significantly higher in those with NHU than in those without. Additionally, it was suggested that the SD of glucose ≥ 54.6 mg/dL had an 80% probability of predicting the presence of NHU.
Disclosure: **E. Sudo:** None.

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The association of hypoglycaemia severity and economic outcomes among patients with type 2 diabetes using basal insulin

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Background and aims: Hypoglycemia (HYPO) is a major limiting factor to insulin therapy, leading to non-optimal glycemic control and additional economic burden to patients with type 2 diabetes mellitus (T2DM). The aim of this study is to assess if economic outcomes differed by HYPO severity among patients with T2DM on basal insulin.
Materials and methods: Data were obtained from the 2011-2013 National Health and Wellness Survey, an annual Internet-based survey demographically representative of the US adult population. Eligible patients with T2DM were categorized as having no HYPO (n=938, 38.7%), non-severe HYPO (n=1,335, 55.1%), or severe HYPO (n=150, 6.2%) in the preceding 3 months. Among patients who experienced HYPO, one-third of all events were nocturnal, regardless of severity. Outcomes included healthcare utilization, work productivity and activity impairment, and annual direct and indirect costs estimated from the 2012 Medical Expenditure Panel Survey and the US Bureau of Labor Statistics, respectively.
Results: Multivariable models, adjusting for important covariates, suggested that patients with non-severe HYPO had more doctor's visits and higher indirect costs vs. patients without HYPO; patients with severe HYPO were generally associated with greater impairment in work productivity, more healthcare resource utilization and higher costs vs. patients with non-severe HYPO (Table 1).
Conclusion: Although the elevated economic burden seen in patients with HYPO may be multifactorial, findings highlight the opportunity for treatment options with better hypoglycemic profiles in T2DM patients utilizing basal insulin.

Table 1. Patient characteristics and economic outcomes by hypoglycemia severity among patients with T2DM using basal insulin.

Patient Characteristics/ Economic Outcomes		Hypoglycemia by Severity§			Non-severe vs. No	Severe vs. No	Severe vs. Non-severe
		No hypoglycemia in the past 3 months (n=938)	Non-severe hypoglycemia in the past 3 months (n=1,335)	Severe hypoglycemia in the past 3 months (n=150)			
Patient Characteristics					**Crude p-value**		
Age (Mean±SD)		60.79±10.99	61.03±10.52	55.32±13.00	0.602	**<0.001**	**<0.001**
Female (N, %)		352, 37.5%	518, 38.8%	58, 38.7%	0.538	0.326	0.974
Charlson Comorbidity Index (Mean±SD)		0.88±1.38	1.05±1.56	1.51±1.80	**0.008**	**<0.001**	**0.001**
Basal + rapid/pre-mix (N, %)		447, 47.7%	830, 62.2%	84, 56.0%	**<0.001**	**<0.001**	0.141
Economic Outcomes					**Adjusted p-value‡**		
WPAI * (LS Mean±SE)	Absenteeism %*	3.98±0.99	4.2±0.87	14.02±9.34	0.877	0.074	0.089
	Presenteeism %*	15.45±1.38	18.55±1.38	33.67±8.47	0.119	**0.004**	**0.024**
	Overall work impairment %*	18.01±1.6	21.3±1.57	36.6±9.11	0.165	**0.007**	**0.035**
	Activity impairment %	38.81±1.01	41.13±0.89	54.38±3.58	0.090	**<0.001**	**<0.001**
HRU[b] in the past 6 months (LS Mean±SE)	Number of Dr.'s visits	6.97±0.22	7.73±0.21	10.28±0.81	**0.014**	**<0.001**	**0.001**
	Number of ER visits	0.3±0.09	0.31±0.07	0.93±0.17	0.745	**<0.001**	**<0.001**
	Number of Hospitalizations	0.23±0.02	0.19±0.02	0.56±0.11	0.105	**<0.001**	**<0.001**
Costs (LS Mean±SE)	Indirect* $ (USD)	6,422±667	8,537±748	11,856±3,408	**0.039**	**0.044**	0.278
	Direct $ (USD)	30,273±1,228	30,728±1,039	58,887±6,291	0.760	**<0.001**	**<0.001**

Note:
§ Among eligible patients, 75% of patients responded to the question of "any hypoglycemia episodes in the past 3 months"; Non-severe hypoglycemia refers to self-managed events; severe hypoglycemia refers to events that required assistance.
‡All generalized linear models adjusted for the following covariates: age, gender (females vs. males), ethnicity (non-Hispanic black, Hispanic, other vs. non-Hispanic white), income (<$50K, declined to answer vs. >$50K), insurance (don't have vs. have), presence of diabetes complications (have any vs. none), HbA1c (don't know, uncontrolled vs. controlled), years diagnosed with diabetes, number of oral antidiabetic drugs, Charlson comorbidity index (CCI; excluded diabetes and diabetes-related complications), body mass index (overweight, obese, unknown vs. underweight/normal weight), and exercise (1+times vs. 0 times in past month).
a. WPAI=Work productivity and activity impairment; absenteeism, presenteeism, overall work impairment and activity impairment scores represent impairment percentages, with higher scores indicating greater impairment. *Calculated only for employed respondents.
b. HRU=healthcare resource use; SD=standard deviation; SE=standard error; N/A=not applicable; LS=least square.

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Disclosure: **L.K. Lee:** Employment/Consultancy; I am an employee of Kantar Health, which received funding from Sanofi to conduct this study.

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Dangerous antidiabetic overtreatment of patients with type 2 diabetes having experienced severe hypoglycaemia

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Background and aims: Severe hypoglycaemia (SH) is clearly associated with a critical prognosis. In addition to its acute life-threatening potential, SH may increase cardiovascular and all-cause mortality in patients with long-standing and complicated diabetes. Thus, we investigated whether in patients with T2DM who had experienced SH individual morbidity and treatment targets had sufficiently been respected.
Materials and methods: Prospective population-based observational trial capturing all episodes of SH between 2007 and 2014 in the Lippe-Detmold area. SH was defined as a symptomatic event requiring treatment with intravenous glucose or administration of glucagon and being confirmed by a blood glucose measurement of <50 mg/dl. Extensive clinical patient characteristics and circumstances of SH were registered.
Results: A total of 1,080 episodes of SH in 747 patients were registered. 51.9% (561/1080) of all cases were related to T2DM with an initial blood glucose of 33.9±11.2 mg/dl. The hypoglycaemic patients were characterized as follows: age 77.3±10.2 years, diabetes duration 15.2±8.7 years, HbA1c 6.6±1.4% (reference 6.0%), creatinine clearance <60 ml/min 59.3% and <30 ml/min 22.6%. 29.0% of patients suffered from dementia and 18.5% from cancer. The Charlson comorbidity index was 8.8±2.7 with a mean of 5.1±2 severe comorbidities. 36% were living in institutional homes or were cared by home nursing services.
Conclusion: A major proportion of our cohort of individuals with T2DM and SH showed a geriatric multimorbidity with a short life expectancy and was clearly overtreated with antidiabetic drugs or insulin. Severe comorbidities as well as contraindications to oral agents were disregarded resulting in a high risk for SH. In patients with T2DM, SH could effectively be prevented if individual morbidity and therapeutic targets would adequately be respected.
Disclosure: **T. Wohland:** None.

PS 081 Devices: glucose monitoring

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Effects of an automated carbohydrate-assessment tool (GoCARB) on glycaemic profile in individuals with type 1 diabetes: a clinical pilot study

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Background and aims: Quantification of carbohydrates (CHO) is a cornerstone of type 1 diabetes (T1D) management, in order to achieve satisfactory glucose control. However, accurate CHO estimation remains a challenging task in daily life. GoCARB is a novel computer vision-based system for smartphones designed to support individuals with T1D in CHO decision making. The aim of this study was to investigate the clinical applicability of GoCARB in individuals with T1D under free-living conditions compared to their usual CHO estimation methods.
Materials and methods: This was a randomized prospective single-center two period cross-over trial involving 20 CHO-trained individuals with T1D on sensor-augmented pump therapy (mean±SEM: age 35±14 years, HbA1C 7.5±0.6%, duration of diabetes 17±10 years). Following a two week familiarization phase using a GoCARB training version without automated CHO output patients were assigned to one week use of GoCARB and one week of usual CHO estimation methods with cross-over in between, in random order. The use of the app and transfer into insulin dosing were at the patients' own discretion. Glycemic profile was assessed using continuous glucose monitoring. Patient satisfaction was evaluated by feedback questionnaire. Statistical analysis was performed on an intention-to-treat approach using paired comparison and general linear models with fixed and random effects. Results are presented as mean±SEM.
Results: The average reported daily CHO-containing meal/snack frequency was 4.4±0.6, 47±1% of which were assessed using the GoCARB system. Daily basal and bolus insulin for GoCARB and control was 19.9±1.5 vs. 20.1±1.5 U and 27.5±2.3 vs. 30.0±2.3 U (p=0.67 and 0.11). Average daily bolus frequency was 6.8±0.4 for GoCARB and 7.3 ±0.5 for control (p=0.12). Average glucose levels were 8.67±0.26 and 8.89±0.27 mmol/l in GoCARB and control, respectively (p=0.15). Time spent in hyperglycemia (>12 mmol/l) was significantly lower in GoCARB compared to control (15.0±2.0% vs. 18.2±2.1%, p= 0.048). Postprandial incremental area under the glucose curve (iAUC) over 180 min tended to be lower in GoCARB when compared to control (incremental glucose 205.2±28.8 vs. 270±39.6 mmol/l/min, p=0.13). Time in target (3.9-10 mmol/l) was 65.9±2.7% for GoCARB and 63.2 ±2.8% for control (p=0.21). Patients spent 2.3±0.8 and 2.6±0.7% in hypoglycemia (<3.5 mmol/l) in GoCARB and control (p=0.58), respectively. Standard deviation and J-index were significantly lower in GoCARB when compared to control (3.0±0.1 vs. 3.2±0.2 mmol/l, p=0.01; 0.14 ±0.01 vs. 0.15±0.01, p=0.03). MAGE tended to be lower in GoCARB when compared to control (5.77±0.29 vs. 6.21±0.29, p=0.09). Eighty percent of the patients would be ready to use GoCARB in their daily life and 100% would recommend the app to others.
Conclusion: In this first clinical study, use of GoCARB in patients with T1D under daily life conditions reduced time spent in hyperglycemia as well as glycemic variability. A larger, long-term study will be required to further explore the efficacy and usability of the GoCARB system in T1D self-management.
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Supported by: FP7
Disclosure: **L. Bally:** None.

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The clinical application of learning-type closed-loop blood glucose (BG) control algorithm in patients with type 1 diabetes

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Background and aims: "Artificial pancreas" consists of continuous glucose monitoring equipment (CGM), insulin infusion equipment (insulin pump) and the automatic control algorithm. Automatic control algorithm can automatically control the amount of insulin infusion, allowing the patient to maintain BG at normal levels. There is repetitiveness in glucose-meal-insulin dynamics, but no clinical trial considers it and the possibility of learning from one day to another. To clinically evaluate the capability of "learning", a learning-type closed-loop BG control algorithm, termed as L-MPC, will be tested its clinical feasibility, and to observe its application results in the patients with T1DM.
Materials and methods: With insulin therapy optimization and model identification in advance, the closed-loop clinical trials last six days for each subject. In each day, the trail starts at 8am and ends at noon with 50g CHO diet at 9am. To study the influences of alcohol and exercise, subjects drink 50mL beer and/or ride 15-min bike on the fourth and/or sixth day. We enrolled 30 patients with T1DM in the endocrinology department of our hospital, according to the different learning gain in L-MPC (L=0.5;L=0.2;L=0.8), The patients were divided into three groups.Each group contains 10 patients for comparing the effect of glycemic control in different learning gain.
Results: The results show that in different learning gain, which is L = 0.5, L = 0.2, L = 0.8, the gender, age, weight and course duration were M / F = 6/4, (35.8 ± 13.2) years, (68.7±14 .4) kg, (4.23 ± 4.51) years; M / F = 4/6, (48.5 ± 14.0) years, (61.2 ± 9.0) kg, (11.0 ± 9.7) years; M / F = 6/4, (39.2 ± 12.4) years, (64.7 ± 11.6) kg, (11.9 ± 9.7) years Respectively. Compared with the first day, the blood glucose index(BGI) in the third day has decreased trend among all three groups (P = 0.563; 0.184; 0.380), and L = 0.2 group learns best.The percentage of time when BG within [3.9, 10]mmol/L in the third day has increased trend compared to that in the first day (P = 0.260; 0.074; 0.334), and L = 0.2 group still learning the best, with glycemic control relative to be the best. In different learning gain groups,the percentage of time with low blood glucose (BG<3.9 mmol/L) in the third day compared with the first day were 0.54% vs 2.43%; 1.62% vs 3.51%; 0% vs 0% (P = 0.415; 0.524; free).Duration of hypoglycemia is very short, and for L = 0.8 group ,there was no incidence of hypoglycemia on either the first day or the third day.
Conclusion: This is the first clinical study verifying the capability of learning algorithm in controlling BG level in T1DM patients. L-MPC is effective for BG control in T1DM and has excellent robustness to alcohol and exercise disturbances. It can help achieve better control of BG in T1DM patients, with promising results in different learning gain groups, and the duration of hypoglycemia is very short. Especially for L=0.2 group, the BGI and the glycemic stabilizing effect are the best, but the hypoglycemia is inevitable in order to achieve the desired BG quickly. Thus, the duration of hypoglycemia has increased in group L = 0.2 compared with the other two groups. Therefore, the L-MPC in T1DM still have good promising results. However there are some limitations of this study, for example, the small size of patients enrolled. The sample size needs to be expanded for further verification, before it is applied in clinic.
Clinical Trial Registration Number: OPC-14005355
Supported by: EFSD Clinical Research Programme
Disclosure: **Z. Jinping:** None.

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Better postprandial glucose control with a new closed-loop system as compared with open-loop treatment in patients with type 1 diabetes

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Background and aims: Postprandial period (PP) control is still a challenge for closed-loop (CL) control algorithms. Although recent at-home studies have demonstrated better daytime glucose control with CL systems as compared to pump therapy, few studies with inconsistent results have investigated systematically the PP. This randomised study compares, in subjects with type 1 diabetes (T1D), a new developed CL algorithm implementing sliding mode reference conditioning (SMRC) with current open-loop (OL) during the PP.
Materials and methods: 20 T1D subjects (F/M 13/7, disease duration 22.6±9.9y, A1c 7.7±0.7%) underwent an 8-hour standardized mixed meal test (60g carbohydrate, CH) on 4 occasions, after normalization of plasma glucose (PG) to euglycaemia using an iv feedback insulin infusion. They wore Paradigm Veo® devices and two continuous glucose monitors (CGM, Enlite-2®). In addition to CGM, PG was measured every 15 min. On 2 occasions (CL1/CL2), after meal-announcement a bolus was given followed by 15-min based-on-CGM adjustments of basal rate. Alternatively, in OL1/ OL2 usual pump therapy was used and boluses were based on individual insulin/CH ratios. In case of hypoglycaemia (PG<70mg/dl), oral glucose (OG, 15g/15min) was given until recovery.
Results: CL improved PG control in the early and late PP (CL1=CL2<OL1<OL2; mean±SD, p<0.01, all): $PG_{0\text{-}8h}$ 123±47 and 125 ±44 vs 152±53 and 159±54 mg/dl; PG_{max} 180±48 and 186±42 vs 212±48 and 222±47 mg/dl. Time-in-range (70-180mg/dL) was greater with CL 381±97 vs OL 307±120 min (p=0.001). Neither the time-below 70mg/dl (CL 30±42 vs OL 18±37 min), nor the need for OG were significantly different (CL 40.0% vs OL 22.5% of tests, p=0.017). Furthermore, the mean needed rescues/test (range) was similarly low in both: CL 0.825 (0-4); OL 0.475 (0-6).
Conclusion: Our CL algorithm effectively and consistently controls PP excursions achieving euglycaemia in the postabsorptive state without an increased risk of hypoglycaemia.
Clinical Trial Registration Number: NCT02100488
Disclosure: **C. Quirós:** None.

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A prospective study of the prevalence of self glucose measurement of blood glucose according to guidelines in persons with type 1 diabetes in Sweden

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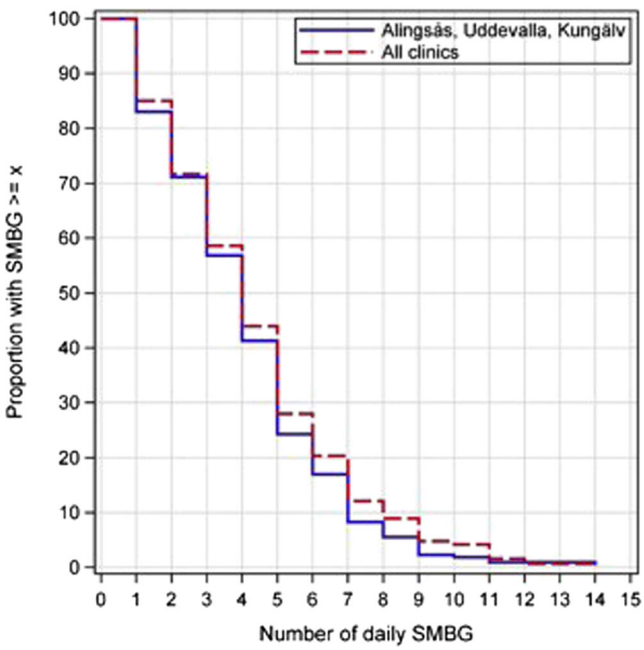
Background and aims: Regular monitoring of blood glucose levels among persons with type 1 diabetes is crucial to obtain good glycaemic control. This is generally performed by self-measurement of blood glucose (SMBG), but patients may need other tools to control glucose levels. The primary aim of this study was to evaluate the extent to which persons with type 1 diabetes perform regular SMBG according to guidelines. Secondary objectives were to investigate possible predictors for good SMBG compliance as well as studying the association between SMBG frequency and HbA1c.

Materials and methods: A random sample of 100-125 persons with type 1 diabetes at each of 5 outpatient diabetes clinics in Sweden was recruited for participation. Inclusion criteria were type 1 diabetes and age > 18 years. Exclusion criteria were usage of continuous glucose monitoring (CGM) or Flash Glucose Monitoring (FGM). The primary endpoint was the proportion of patients measuring 4 or more glucose values per day. Glucose meters were checked of the number of SMBG measurements. Patients completed a questionnaire of psychosocial factors and SMBG habits. Diabetes risk factors, complications and treatments were retrieved from local patient record databases. Predictors analyzed for SMBG compliance were sex, age, diabetes duration, type of insulin delivery (pump vs. injections) smoking, level of education, occupation, residential status, stress level, physical activity level, BMI and diabetic complications.
Results: In the cohort (n=329), 43,9% (95% CI 38,4%-49,5%) performed SMBG >=4 times per day. For the 3 clinics where more than 70% of patients participated the frequency of SMBG >=4 times per day was similar to the whole sample proportion (41.0%, 95% CI 34,7%-47,9%). The proportion of various numbers of SMBG performed is shown in the figure. Predictors for performing more frequent SMBG were: older age (p=0.0331) and female sex (p=0.0097), using multiple regression (R2=0.04). More frequent SMBG was associated with lower HbA1c (p<0.0001, R2=0.07), analyzed with quadratic regression adjusting for potential confounders. One SMBG per day compared to 0 was associated with a 2.4 mmol/mol or 0,2% lower HbA1c level, which increased monotonically to a 7.9 mmol/mol or 0,8% reduction in Hba1c at 4 measurements. Median patient belief in recommended number of daily SMBG was 4. 18.3% rated that that 3 daily SMBG should be made, but there were 11.4% rating that 2 or fewer should be made.
Conclusion: Despite glucose meters and strips being free in Sweden, less than 50% of patients measure capillary blood glucose levels 4 times per day or more. This indicates the need of further support in performing SMBG and increased availability of other tools for glucose monitoring such as CGM and FGM.



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Disclosure: **E. Ahlén:** None.

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Real-time tracking of risk for hypoglycaemia in diabetes using multiple data sources

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Background and aims: Hypoglycaemia in diabetes can be triggered by many factors, including intense exercise, inadequate carbohydrate intake, or excessive insulin/glucose-lowering medications. It is therefore logical to assume that information from disparate sources may contribute to assessment of hypoglycaemia risk. While self-monitoring of blood glucose (SMBG) is the primary information source driving treatment decisions for a majority of patients, additional data (exercise, food intake, insulin injection) become increasingly available to diabetes decision support systems.
Materials and methods: We introduce a new method for tracking hypoglycaemia risk and predicting hypoglycaemic episodes based on a probability aggregation procedure used to combine multisource data. Data may include, but are not limited to, SMBG readings and information about recent exercise, carbohydrate intake, or insulin delivery. Besides daily fasting SMBG readings, other data may be beneficial but are not strictly necessary—the procedure would work regardless of their availability. The method was first developed using training data from 169 diabetes patients with type 1 diabetes (28,215 SMBGs and 12,915 records of recent exercise, food, and insulin collected over 1 month and coded on a 0-6 less-usual-more scale). Then all method parameters were fixed, and the procedure was run on an independent test data set of 114 patients (188,390 SMBGs and 28,224 records of recent exercise, food, and insulin collected over 10 months).
Results: Table 1A presents the odds for hypoglycaemia when the method issued a hypoglycaemia warning. Odds ratios increased significantly when predicting progressively deeper hypoglycaemia, reaching 3.2-fold increase in the odds for significant hypoglycaemia (<2.8 mmol/l) with all available data. Additional data enhanced prediction, most prominently for hypoglycaemia < 2.8 mmol/l. Table 1B compares risk metrics for hypoglycaemia computed over 24 hours following hypoglycaemia warning versus no warning. All metrics indicate elevated risk and significantly more pronounced low blood glucose excursions following hypoglycaemia warning (all $P < 0.001$).
Conclusion: Real-time tracking of hypoglycaemia risk is possible, even with sparse (eg, daily fasting) SMBG data. Even imprecise additional information such as patients' recollection of exercise, food, or insulin further improves prediction of impending hypoglycaemia. Key defining characteristics of this method are robustness and ability to handle missing data—the probability aggregation procedure permits various data sources to be available or unavailable to the overall risk estimation at any time. Clinically, several metrics indicated heightened risk for hypoglycaemia following a hypoglycaemia warning; the odds for significant hypoglycaemia (<2.8 mmol/l) exceeded 3-fold with all information present. Thus, clinically meaningful messages about upcoming significant hypoglycaemic events can be presented to the patient.

Table 1. Odds and Risks for Impending Hypoglycaemia Tracked From Progressively Richer Data Sources

A: Odds	Odds Ratios for Hypoglycaemia Depending on Data Availability		
	< 3.9 mmol/l	< 3.3 mmol/l	< 2.8 mmol/l
SMBG alone	1.7	1.9	2.6
SMBG + recollection of exercise	1.8	2.0	2.9
SMBG + recollection of exercise, food, and insulin	1.8	2.1	3.2
B: Risks	**Hypoglycaemia in the 24 h Following a Hypoglycaemia Warning**		
	Following Warning for Hypoglycaemia	No Warning for Hypoglycaemia	*P* Value
Nadir of blood glucose	4.3 mmol/l	5.4 mmol/l	< 0.001
Low blood glucose index*	3.5	1.9	< 0.001
Percent readings < 3.9mmol/l	15.9	9.1	< 0.001
Percent readings < 3.3mmol/l	9.9	5.0	< 0.001
Percent readings < 2.8mmol/l	4.6	1.7	< 0.001

*The low blood glucose index is a metric of the risk for hypoglycaemia that increases with progressively lower and more frequent hypoglycaemic readings. An index of > 2.5 indicates moderate/high risk; ≤ 2.5 indicates low risk

Supported by: Sanofi
Disclosure: **C. Fabris:** None.

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Clinical outcomes of the United4Health (U4H) project in patients with diabetes: a European large-scale telehealth deployment project

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Background and aims: Evidence is required for the effectiveness of telehealth (TH) for patients with diabetes mellitus (DM) in a routine service. The aim of this study was to evaluate the impact of large scale deployment of TH for patients with DM on the reduction in HbA1c and healthcare resources used in 9 regions of 8 European countries
Materials and methods: The project was designed as a prospective observational study. 6,497 diabetic patients received TH services from 1 January 2014 - 30 September 2015 and, in accordance with the study protocol, 2,541 were included in the evaluation cohort with a minimum follow-up of 6 months. 1,016 patients were allocated to the intervention group (IG) and 1,525 to the comparator group (CG). Nominated health professionals accessed data uploaded to web-based portals to monitor patients' health status and provide counselling on lifestyle and medication adjustments by phone, secure online messaging or text messages, when required. Ethical approval for the study was obtained in regions where this was necessary.
Results: Most patients had type 2 DM with 10.4% having type 1. The two groups were different in age (67.6 vs 65.6 years old, p=0.006), current smokers (12.0% vs 15.3%, p<0.001), assisted at home (72.5% vs 63.2%, p=0.001), type 1 diabetics (8.5% vs 11.6%, p=0.028) and number of years with the disease (13.4 vs 12.7 years, p=0.040) for IG and CG respectively. Almost half were male with an age-adjusted comorbidity index of 4.87 ±1.87 vs 4.74±2.01 (p=0.112). The mean length of follow-up was 334.1 ±77.1 vs 369.1±58.5 days (p<0.001). Fewer patients were lost-to-follow-up in the IG (8.2% vs 22.1%, p<0.001). Regression analysis showed HbA1c had been reduced significantly more in the IG than in the CG (-0.224, p < 0.001). The reduction was higher in patients with higher baseline HbA1c values (-0.791, p < 0.001), but not for patients receiving insulin treatment (0.246, p < 0.001). Annual face-to-face contacts with GP or diabetologist after adjustments for all possible confounders have been reduced in the IG but not statistically significant (-0.182, p = 0.445). Moreover, logistic regression analysis showed that patients in the CG were more likely to be hospitalised (5.25, 95% CI 1.73 - 15.95, p=0.003).
Conclusion: The U4H project showed that large scale deployment of telehealth services for diabetic patients is feasible. Although, it showed that there may be some beneficial effects in terms of reduction in HbA1c levels and health care resources used, these findings have to be further explored, mainly because of the differences between the two groups.
Supported by: The project U4H was partially funded under the EC CIP ICT-PSP
Disclosure: **G.E. Dafoulas:** None.

PS 082 Devices: accuracy

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A clinical trial of the accuracy and treatment experience of the flash glucose monitor FreeStyle Libre in persons with type 1 diabetes

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Background and aims: Traditionally, frequent capillary self measurements of blood glucose (SMBG) has been the only and most effective way to follow blood glucose levels for persons with type 1 diabetes. In the last decades continuous glucose monitoring (CGM) has become available to many patients as a komplement to SMBG. In Sweden a flash glucose monitoring system (FGM) came onto the market in 2014, FreeStyle Libre. Few independent studies have evaluated the accuracy and treatment experience of the system. The aim of this study was therefore, to evaluate the accuracy and treatment experience of the FreeStyle Libre.
Materials and methods: We studied 50 patients in 2 outpatient clinics in Sweden from Jun.- Dec. 2015. Patients carried a FreeStyle Libre for 10-14 days and measured capillary blood glucose levels with a hospital blood glucose system at least six times a day during that period. All endpoints reported were predefined and the study registered on clinicaltrials.gov. A questionnaire earlier used for evaluating treatment experience of CGM was used to rate patients experience of the system. The primary endpoint was the mean absolute relative difference (MARD) for FreeStyle Libre. This study was performed independently from FreeStyle Libres manufacturers and no salaries, FGM systems, sensors or costs were hence covered by the manufacturer but by independent funders.
Results: The MARD for the whole study period was 13.2% (95% CI 12.0-14.4%). During the first week MARD was 13.6% (95% CI 12.1-15.4%) and the second week 12.7% (95% CI 11.5-13.9%). The mean absolute difference (MAD) for the whole study period was 1.10 mmol/l, the first week 1.14 mmol/l and second week 1.05 mmol/l. The Correlation coefficient for the whole study period was 0.96. For glucose values <4; 4-10; >10mml/l the MARD was 20.3%(95% CI 17.7-23.1%); 14.7%(95% CI 13.4-16%) ;9.6%(95% CI 8.5-10.8%) respectively and the MAD 0.69mml/l; 0.99mml/l;1.31mml/l respectively. Patients rated their experience of the FreeStyle Libre with 10 statements on a visual analogue scale (VAS) (0-10) as positive, mean from 8.22 - 9.8 (Table 1).
Conclusion: The FreeStyle Libre had a similar overall MARD as CGM systems in earlier studies when studied in similar at home conditions. Most patients wished to use the FreeStyle Libre system after the study was completed and the overall satisfaction was very high.

Tabel 1

Questions	Mean score Visual Analogue Scale (0-10)
Q1 My experience of FreeStyle Libre was very positive	9,04
Q2 The insertion of FreeStyle Libre was easy and problem free	9,08
Q3 I felt safe and free while using the FreeStyle Libre	8,92
Q4 It was easy to use the FreeStyle Libre	9,80
Q5 It was easy to interpret the information on the FreesStyle Libre screen	9,64
Q6 I did not experience discomfort while using the FreeStyle Libre	9,06
Q7 I had no connection problems with scanning while using the FreeStyle Libre	9,7
Q8 The FreeStyle Libre lost connection how many times	0,48
Q9 The FreeStyle Libre sensor was comfortable to wear in my daily life	8,32
Q10 The FreeStyle Libre did not disturb in my daily life	8,22
Q11 I would like to use the FreeStyle Libre in my daily life	9,4

Clinical Trial Registration Number: NCT02677454
Supported by: Novonordisk foundation & R&D Fyrbodal sweden & Region of Västra Götaland
Disclosure: **A.F. Ólafsdóttir:** Grants; Novonordisk foundation, Region of Västra Götaland, Regional research and development foundation of Fyrbodal.

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Standardised evaluation of three continuous glucose monitoring systems mimicking real-life conditions

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Background and aims: Continuous glucose monitoring (CGM) has become an essential tool in diabetes management. In order to use CGM for treatment decisions, CGM systems have to be reliable over a wide range of glycaemia as well as in situations with rapidly changing glucose levels. The aim of this study was to evaluate the performance of three CGM systems under real-life conditions.
Materials and methods: In this monocentric study we evaluated the performance of 3 commercially available CGM systems (Abbott Libre, Dexcom G4 Platinum, Medtronic Enlite) in 12 type 1 diabetic subjects (age 33 ± 11 years, 42% women, BMI 22.5 ± 2.4 kg/m2, diabetes duration 17 ± 12 years, HbA1c 59.2 ± 12.4 mmol/mol) over a period of 12 hours. Routine clinical conditions were mimicked by meal and exercise tests. The sensors were inserted 24 hours prior to the test in parallel and were calibrated according to manufacturers' instructions. Reference plasma glucose samples were taken every 5 minutes throughout the study and measured with Super GL analyser.
Results: Glucose measurement accuracy was determined for each CGM system according to ISO 15197: 2013 guideline (±15%; ± 0.83 mmol/L for glucose <5.55 mmol/L). The systems fulfilled these criteria as follows: 73.1% (Abbott), 56.1% (Dexcom) and 52.0% (Medtronic). Additional accuracy metrics are indicated in Table 1.
Conclusion: In summary, the Abbott sensor showed superior performance based on all accuracy metrics applied in this study. All sensors

were less accurate during hypoglycaemia. Future CGM generations need to be improved regarding accuracy in the low glucose range.

Table 1 – Performance Characteristics of the Three CGM Systems

	Abbott	Dexcom	Medtronic
Total MARD	13.22 ± 10.87	16.75 ± 12.31	21.36 ± 17.60
Data points, total (n)	462	540	502
MARD <3.9 mmol/L	14.61 ± 10.34	23.77 ± 15.66	26.86 ± 20.02
Data points <3.9 mmol/L (n)	81	88	87
MARD 3.9-≤10 mmol/L	13.68 ± 11.53	16.34 ± 11.64	20.94 ± 15.34
Data points 3.9-≤10 mmol/L (n)	301	362	334
MARD >10 mmol/L	10.09 ± 7.93	11.56 ± 7.24	17.15 ± 21.85
Data points >10 mmol/L (n)	80	90	81
Parkes Error Grid Zone A (%)	85.71	83.52	71.12
Parkes Error Grid Zone B (%)	14.29	15.74	27.49
Parkes Error Grid Zone C (%)	0	0.74	1.39

*MARD – Mean Absolute Relative Difference

Clinical Trial Registration Number: NCT02614768
Supported by: European Commission FP7-305343
Disclosure: **F. Aberer:** None.

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Decision support via dynamic tracking of HbA_{1c} using sparse SMBG measurements: effect of calibration

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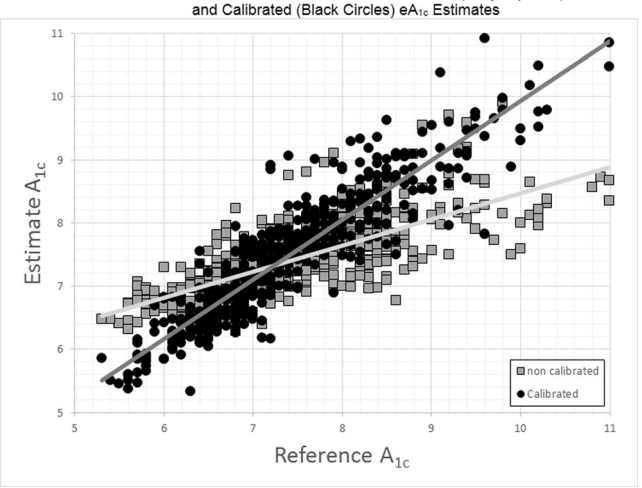
Background and aims: We previously introduced the eA_{1c}, a real-time tracker of average glycaemia and estimate of HbA_{1c} from infrequent self-monitoring blood glucose (SMBG) data. Tested in type 1 and 2 diabetes mellitus (T1DM and T2DM, respectively), the eA_{1c} yielded mean average relative deviation (MARDs) between 5% and 8%. We now confirm the accuracy of our method on independent data sets and explore the accuracy immediately and up to 6 months after calibration by laboratory HbA_{1c}.
Materials and methods: Data from 2 studies were used in the analysis. Study 1 was a 12-week, prospective, national, multicentre, uncontrolled, single-arm, open label study in patients with T1DM and T2DM. SMBG data were collected using an iBGStar meter, and HbA_{1c} was measured 7 times. Study 2 was a 12-month, prospective, national, single-centre, controlled, open-label study in T1DM. SMBG data were collected using a OneTouch UltraSmart meter, and HbA_{1c} was measured at baseline and every 3 months thereafter. Study 1: A total of 51 patients were enrolled and included in the study; 50 completed the study. Mean age (± SD) was 54.1 ± 12.6 years, 56.9% were male, and baseline HbA_{1c} was 7.1%. Study 2: A total of 120 patients were enrolled and included in the study; 97 completed the study. Mean age (± SD) was 39.2 ± 14.4 years, 42.5% were male, and baseline HbA_{1c} was 8.0%. The latest eA_{1c} algorithm was applied a posteriori to the SMBG data, using the first and second laboratory HbA_{1c} values as calibrations and the others as reference. We contrasted the accuracy of calibrated eA_{1c} versus non-calibrated.
Results: 219,970 SMBG records with mean (± SD) blood glucose of 167 ± 89 mg/dl [range, 40-600], were used in the analysis, corresponding to 986 lab HbA_{1c} values: 7.2 ± 0.9% [5.6-9.9]. Standard eA_{1c} performed as expected: MARD = 7.5 ± 5.4%, MAD = 0.55 ± 0.42, and correlation ρ= 0.8. Calibrated eA_{1c} improved MARD to 4.4 ± 3.8% ($P < 0.001$), with MAD = 0.32 ± 0.28 and ρ = 0.9 (Figure). Focusing on Study 2, we analysed the performances of calibrated eA_{1c} over the 9 months following the last calibration. Accuracy remained improved (compared with standard eA_{1c}) at 3 months (MARD = 3.6%, ρ= 0.93), 6 months (MARD = 4.0%, ρ= 0.89), and 9 months (MARD = 4.7%, ρ= 0.88).
Conclusion: The standard algorithm performed as expected with MARD, MAD, and correlation within the bounds of previously published results. Calibration using reference HbA_{1c} significantly improved performance, reducing MARD up to 2 fold in the months following the calibration. With 75% of estimates falling within 6% of lab values (NGSP standard: 92.5%) and estimate within 1% A1c of reference 96% of the time (old NGSP: 95%), the calibrated, eA_{1c} algorithm showed very robust performance for an SMBG-based system. These performances were confirmed up to 9 months after the last calibration.

Figure:
Estimated Versus Reference HbA_{1c} for Standard (Grey Squares) and Calibrated (Black Circles) eA_{1c} Estimates



Supported by: Sanofi
Disclosure: **M.D. Breton:** Grants; BD, Roche Diagnostics, Sanofi U.S., Tandem Diabetes. Stock/Shareholding; TypeZero Technologies.

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Accuracy of a subcutaneous continuous glucose monitoring system in critically ill patients

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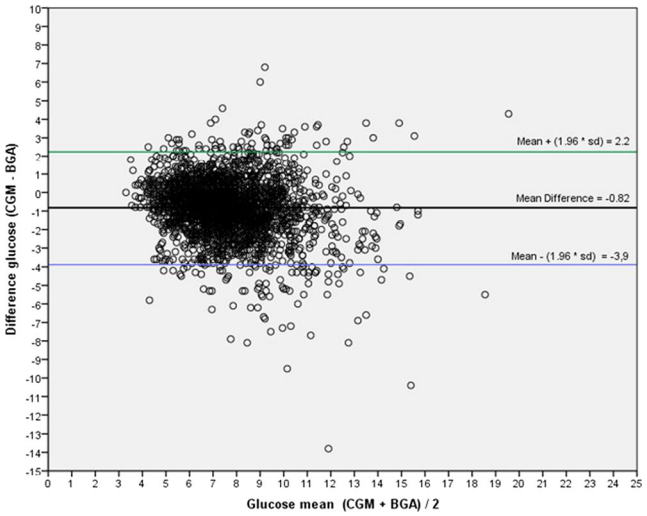
Background and aims: Achieving adequate glucose control is important but challenging in critically ill patients. Continuous glucose monitoring (CGM) systems should be able to improve glycaemic management since they are able to provide real-time glucose information. However, these devices are still not implemented in clinical practice, in part because of questions concerning its accuracy in patients admitted to intensive care units. With this study, we investigated the level of accuracy of the FreeStyle Navigator I in critically ill patients.
Materials and methods: For this retrospective study we used data from a randomized controlled trial that compared the efficacy of the CGM system with point-of care measurements to guide insulin treatment during three consecutive days. We evaluated accuracy of CGM measurements relative to reference arterial blood gas analysis (BGA) measurements in these patients. Results are presented as correlation coefficients, and presented in Bland-Altman plots and Clarke error grids.
Results: A total of 70,527 CGM measurements in 156 patients were obtained. After time-matching CGM and reference BGA measurements in a 1:1 ratio, the accuracy assessment was performed with 2837 paired points, with a median of 27 measurements per patient. Mean CGM glucose was 7.2 mmol/L (SD = 1.9) compared to a mean BGA glucose of 8.0 mmol/L (SD = 2.1). The median absolute difference was 1.0 mmol/L [IQR 0.5-1.8]. Median absolute relative difference was 9.7% [IQR -19.0-0]. Glucose correlation coefficient between CGM and BGA was 0.70 ($P < 0.001$). The intraclass correlation coefficient was 0.64 (95% CI 0.45-0.75, $P < 0.001$). Bland-Altman plots showed that bias was -0.82 mmol/L with limit of agreement of -3.9 mmol/L to 2.2 mmol/L (figure). The percentage of values falling in Clark error grid zone A and B (clinically acceptable treatment zone) was 98.4%, with 1.1% of data in

zone D and 0.1% in zone E. In 13 of the 30 points in zone D the CGM system failed to detect a potentially dangerous low reference glucose (<4 mmol/L), with a mean absolute difference of 1.8 mmol/L between BGA and CGM (range 1.1-3.0 mmol/L).
Conclusion: The FreeStyle Navigator I CGM system showed an acceptable accuracy in a cohort of critically ill patients.



Disclosure: **S. Rijkenberg:** None.

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Impact of xylose on glucose-dehydrogenase-based blood glucose meters for patient self-testing

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Background and aims: The term "functional food" defines nutrition with additional supplements, which are supposedly beneficial for the health of the consumers. The food industry is increasingly using artificial sweeteners and is enriching food with fibers. One example for such an added ingredient is xylose, which is enriched in certain edible algae and mushrooms. However, xylose is known to interfere with glucose-dehydrogenase-based (GDH) blood glucose measurement systems for patients self-testing. The aim of our study was to investigate the extent of xylose interference on commercially available blood glucose meters (AccuChek Aviva, AccuChek Connect, Contour Next, FreeStyle Freedom Lite, FreeStyle Insulinx, MyStar Extra, OneTouch Verio IQ, and Wellion Calla).
Materials and methods: Heparinized whole blood was drawn from a healthy subject and manipulated to contain three different glucose concentrations (50-80 mg/dL, 130-160 mg/dL, and 250-300 mg/dL) and four different xylose concentrations (0 mg/dL, 25 mg/dL, 50 mg/dL, and 100 mg/dL). Each sample was measured in parallel 3 times with 2 different strip lots and the test meters. The YSI GlucoStat analyzer served as the reference method, and samples were additionally tested before and after the test series.
Results: The mean results were used to determine the capture rate of xylose in addition to glucose in the tested samples, i.e. the xylose amount present in the sample wrongly displayed as glucose. No xylose interference was seen with four meters: AccuChek Aviva (mean capture rate 0%), AccuChek Connect (-2%), MyStar Extra (10%), and Wellion Calla (8%). In contrast, substantial interference was observed with Contour Next (100%), FreeStyle Freedom Lite (104%), FreeStyle Insulinx (120%), and OneTouch Verio IQ (162%).
Conclusion: We observed pronounced xylose interference in several GDH-based blood glucose meters. Our findings may become important with a more prevalent use of xylose in dietary and functional food products, in particular in products designed for weight loss. Our findings may be considered when selecting meters for patients who are consuming such products as part of their lifestyle treatment regimen.
Supported by: Sanofi
Disclosure: **A. Pfützner:** Employment/Consultancy; Sanofi, Lifecare, NovoNordisk, Insulin-NG. Grants; Sanofi.

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Effects of hydroquinone-containing skin-lightening creams on capillary glucose measurements before and after serial hand washings

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Background and aims: Self-monitoring of blood glucose using point-of-care glucometers is a pivotal part of diabetes care. We previously showed that hydroquinone-containing skin lightening creams caused false increase in capillary glucose measurement. As a means to prevent false capillary glucose readings, it is a common practice to swab patients' finger with water soaked gauze, or to apply hand sanitizers before the glucose measurement is performed. The magnitude and clinical impact of these creams on capillary glucose measurement is less well known. Also, it is not known if routine finger swabbing, application of hand sanitizer or one hand washing is sufficient to avoid the effect of these contaminants. We sought to determine the effect of hydroquinone-containing creams on capillary glucose measurement in terms of technical and clinical impact and the number of hand washing needed to reverse these effects.
Materials and methods: We included 91 consenting diabetic and non-diabetic participants in an experimental study in Buea, Cameroon. To assess the effects of the hydroquinone-containing cream, two glucometers with different enzymatic principles (Accu-Chek active® and OneTouch ultra2®) were used to measure fasting capillary blood glucose after initial hand washing (reference) and after application of a calibrated amount of cream. To determine the means to reverse the effects of the cream, capillary glucose was measured after finger swabbing, sanitizer application, and a series of three hand washings, following cream application. The technical impact was evaluated by comparing glycaemia values obtained after the various interventions to the reference. Parke's error grid and total allowable error analyses were used to assess the clinical impact of the differences in measurement. Data were analyzed using R statistical software version 3.2.2.
Results: Accu-Chek active® measured glycaemia in mg/dl changed from 134±85 (reference) to 162±98 ($p<0.0001$), 161±101 ($p<0.0001$), 172 ±104 ($p<0.0001$), 150±94 ($p<0.0001$), 138±86 ($p=0.0003$), and 132±83 ($p=0.009$) after cream application, finger swabbing, sanitizer application and first, second and third hand washings, respectively. Corresponding values for OneTouch ultra2® were 138±79 at baseline, then 180±100 ($p<0.0001$), 182±103 ($p<0.0001$), 202±117 ($p<0.0001$), 160±98 ($p<0.0001$), 143±90 ($p=0.057$), and 133±79 ($p<0.0001$) respectively. The mean cream-attributed glucose increment (95% CI) was 28 (18-37) mg/dl for Accu-check® and 41 (30-53) mg/dl for OneTouch ultra®. These differences were clinically significant and reversed only with two or more hand washings. After cream application, Accu-Check® had 9.9% of values in zones C-E of the Parke's error grid while OneTouch Ultra2® had 18.7%.
Conclusion: Hydroquinone-containing creams cause significant false increase in capillary glycaemia irrespective of the enzymatic principle of the glucometer used, leading to potentially wrong clinical decisions. Finger swabbing with wet gauze or application of hand sanitizer are insufficient to prevent the effect of these creams. A minimum of two hand washings is required before capillary glucose measurement.
Disclosure: **S. Choukem:** None.

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The C-peptide correlations and influence on the glycaemic variability parameters recorded using continuous glucose monitoring system in patients with type 2 diabetes

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Background and aims: Glycemic variability is an important aspect of glycemic control, being recognized as an independent risk factor for the development of chronic diabetes complications. The endogenous secretion of insulin varies widely among patients with type 2 diabetes and might be an important factor involved in the control of glycemic variations in this category of patients.
Materials and methods: This is a retrospective study in which we included data from patients with type 2 diabetes who had a complete continuous glucose monitoring (CGM) recording (first 24 hours of recording starting from the midnight after insetion with no pause in recorded values) and peptide C levels measurement available. We collected from patients' medical charts data on age, gender, anthropometric paramateres - weight, heigh, body mass index (BMI), visceral fat area (VFA) and percent of body fat (PBF) meaured by bioimpedance, diabetes duration and treatment, HbA1c and C-peptide levels. The following glycemic variability parameters were calculated with GlyCulator (www.pediatria.umed.pl/team/glyculator): mean level of 24h interstitial glucose value (MG), median glucose value and standard deviation (SD); percentage coefficient of variation (%CV); weighted average of glucose values around 100 mg/dl (M100); J index; percentage of glucose values above 180 mg/dl (%above180) or below 70 mg/dl and 54 mg/dl (%below70 and %below54); mean amplitude of glycemic excursion (MAGE); fractal dimension (FD); continuous overall net glycemic action (CONGA) at 1, 2, 4 and 6 hours.
Results: We collected data from 53 patients with type 2 diabetes aged 55.6±8.96 years and with a median duration of diabetes of 4 years (0; 9.75 years). Of these 12 (22.6%) were insulin treated. The median value of C-peptide was 2.71 ng/ml (1.91; 3.89) and the mean HbA1c was 8.29 ±1.90%. The C-peptide concentration was significant correlated with body weight (r=0.518, p<0.001), BMI (r=0.535, p<0.001), PBF (r=0.343, p=0.012) and VFA (r=0.409, p=0.012), M100 (r=-0.259; p=0.041), CONGA-1 (r=-0.337, p=0.014) and CONGA-2 (r=-0.234, p=0.028). The peptide-C quartile correlated significantly with body weight (r=0.520, p<0.001), BMI (r=0.481, p<0.001), VFA (r=0.338, p=0.013), M100 (r=-0.294; p=0.032), J index (r=-0.271; p=0.050), %above126 (r=-0.275; p=0.046), CONGA-1 (r=-0.332, p=0.015) and CONGA-2 (r=-0.298, p=0.030). Compared to the patients with C-peptide levels in the lowest quartile, the ones with levels in the highest quartile had significantly higher values of mean glucose levels (180.69 mg/dl vs. 141.37 mg/dl, p=0.017), median glucose levels (180.5 mg/dl vs. 135.00 mg/dl, p=0.033), J index (50.50 vs. 30.77, p=0.020), %above126 (93.73% vs. 59.03%, p=0.013) and %above180 (49.65% vs.15.97%, p=0.026) and significantly lower weight (79.2 kg vs.101.8 kg, p=0.004), BMI (28.2 kg/m2 vs. 34.8 kg/m2, p=0.002) and VFA (133.9 cm2 vs. 155.00 cm2, p=0.048).
Conclusion: C-peptide concentration was correlated with glycemic variability parameters evaluated by CGMs.
Disclosure: **A.E. Craciun:** None.

PS 083 Insulin usage

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Similar glucose control, post-prandial glucose excursions and safety in people with type 1 diabetes on MDI using SAR342434 or insulin lispro and insulin glargine (U100): SORELLA 1 study

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Background and aims: SAR342434 insulin lispro (SAR) was developed as rapid-acting follow-on to insulin lispro U100 (LIS). In a phase I clamp study, PK and PD between SAR and LIS were similar with the 2 rapid acting insulin analogues (RAIA). To the best of our knowledge, SORELLA 1 is the first long-term randomized study of a follow-on RAIA intended for submission to the FDA and EMA for approval.
Materials and methods: The randomized, controlled, 6 month open-label period of this phase 3 study compared the efficacy and safety of SAR and LIS in people with T1DM using MDI on once daily insulin glargine (GLA100) as basal insulin. 507 people [mean age 43 (SD 14.2) yr, duration of T1DM 19.1 (12.3) yr, BMI 26.0 (4.1) kg/m^2 were randomized (1:1) to SAR (n=253) or LIS (N=254). The baseline characteristics were similar in both groups including insulin doses: rapid-acting insulin 0.36 (0.17) U/kg, basal insulin 0.34 (0.17) U/kg, total insulin 0.70 (0.28) U/kg]. The dose of SAR or LIS was adjusted to achieve a 2-hr postprandial plasma glucose of 6.7 - 8.9 mmol/L (120-160 mg/dL), while avoiding hypoglycemia. The recommended target range for fasting, pre-prandial plasma glucose was 4.4 - 7.2 mmol/L (80-130 mg/dL). Primary endpoint was HbA1C change from baseline to week 26 (tested for non-inferiority of SAR versus LIS at the 0.3% non-inferiority margin) with secondary endpoints including 7-point self-monitored plasma glucose profiles.
Results: Mean baseline HbA1c was similar in both groups (SAR: 8.08% [SD 0.78] and LIS: 7.99% [SD 0.64]). SAR was non-inferior to LIS for change in HbA1C (%) [LS Mean difference (SE) vs LIS 0.06 (0.07); (95% CI (-0.08 to 0.20) Table)]. Postprandial glucose excursions and changes in insulin dose were also similar (Table) with the two RAIA. There was no meaningful difference in the % people reporting hypoglycemia across the categories defined by ADA (severe hypoglycemia: SAR 7.9%; LIS 7.5%). Percentages of patients reporting adverse events, hypersensitivity events and injection site reactions were comparable in both treatment groups. Anti-insulin lispro antibody incidence and prevalence were not different in the 2 treated groups.
Conclusion: We conclude, SAR342434 was as effective, safe and well-tolerated as insulin lispro in people with T1DM.

SORELLA 1

	SAR342434	LIS
Randomized and treated	**252 (99.6%)**	**254 (100%)**
Efficacy results		
HbA1c; Baseline (%; mean, SD)	8.08 (0.78)	7.99 (0.64)
LS mean change at Week 26 95% CI	-0.42 (0.05) (-0.52 to -0.32)	-0.47 (0.05) (-0.57 to -0.38)
LS mean difference vs LIS 95% CI	0.06 (0.07) (-0.08 to 0.20)	
Insulin dose at Week 26 (U/kg; SD) Basal insulin Prandial insulin	 0.36 (0.32) 0.37 (0.16)	 0.35 (0.16) 0.35 (0.17)
Postprandial glucose excursions (mmol/L; mean; SD) MMRM		
Breakfast Baseline	0.79 (4.66)	0.34 (4.25)
LS mean (SE) change at Week 26	-0.46 (0.30)	0.19 (0.30)
LS mean difference (SE) vs LIS 95% CI	-0.64 (0.42) (-1.45 to 0.18)	
Lunch Baseline	0.94 (4.49)	0.91 (4.05)
LS mean (SE) change at Week 26	0.14 (0.30)	-0.26 (0.31)
LS mean difference (SE) vs LIS 95% CI	0.40 (0.43) (-0.44 to 1.25)	
Dinner Baseline	0.40 (4.48)	0.06 (4.01)
LS mean (SE) change at Week 26	0.48 (0.31)	0.56 (0.32)
LS mean difference (SE) vs LIS 95% CI	-0.07 (0.45) (-0.95 to 0.80)	
Safety		
Severe hypoglycemia n/N; %	20/252 (7.9%)	19/254 (7.5%)
Treatment emergent AEs (TEAEs) n/N; %	108/252 (42.9%)	106254 (41.7%)
Treatment emergent SAEs	8/252 (3.2%)	14/254 (5.5%)
TEAEs leading to death	1/252 (0.4%)	0
TEAEs leading to permanent IMP discontinuation	1/252 (0.4%)	1/254 (0.4%)
Hypersensitivity reactions	13/252 (5.2%)	10/254 (3.9%)
Injection site reactions	3/252 (1.2%)	2/254 (0.8%)

LS (least square) mean: MMRM mixed-effect model for repeated measures adjusted on randomization strata, treatment, visit (Week 12, Week 26), treatment-by-visit interaction, baseline value and baseline value-by-visit interaction

Clinical Trial Registration Number: NCT02273180
Supported by: Study funding and editorial support provided by Sanofi.
Disclosure: **S.K. Garg:** Other; Advisory Boards Consulting fees: Medtronic, Roche, Merck, Lexicon, Novo-Nordisk, Sanofi, Eli Lilly. Research Grants: Eli Lilly, Novo Nordisk, Merck, Lexicon, Medtronic, Dario, NCI, T1D Exchange, NIDDK.

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Nocturnal glycaemic control with glargine titration based on bedtime in addition to fasting plasma glucose in type 1 diabetes

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Background and aims: The study compared the usual (evening) glargine titration based on fasting plasma glucose (FPG) (mean of 6 days, algorithm #1, titration 1/week) with an algorithm #2 based on increments/decrements of nocturnal PG (difference between bedtime PG, BT_PG, and next morning FPG on days with post-dinner PG at the target 100-130 mg/dl with optimized evening prandial insulin) (Δ [BT_PG-FPG] of 6 days, titration every 2 weeks) (Alg #2). Both algorithms aimed at target FPG 100-130 mg/dl.
Materials and methods: 58 T1DM subjects on basal-bolus (all glargine) and FPG >130 mg/dl (24 M, age 35±7 yrs, diabetes duration 12±5 yrs, BMI 24.1±0.7 kg/m^2, A1C 7.4±0.4%) were randomized to Alg #1 or Alg #2 (Table 1) and studied (parallel group design)
Results: After 3 months, FPG decreased with Alg #1 and Alg #2 vs baseline (from 154±17 to 140±16, and from 151±18 to 128±8, $p<0.05$), but more on Alg #2 ($p<0.05$). A1C decreased more on Alg #2 (from 7.5 ±0.4 to 7.1±0.3%) vs Alg #1 (from 7.4±0.4 to 7.3±0.4%) ($p<0.05$). Intra-subject variability of FPG was lower on Alg #2 vs Alg #1 (CV 9±1.4 vs 15±2.7%, $p<0.05$). Incidence of nocturnal confirmed hypoglycemia (PG <70 mg/dl) and rates (5.4±1.4 vs 7.6±2.7 events/pt-year) were lower on Alg #2 vs Alg #1 ($p<0.05$).
Conclusion: We conclude that algorithm #2, which includes bedtime PG, more specifically allows titration of glargine than algorithm #1 based only on FPG.

Alg #1	Change in Gla U	Alg #2	Change in Gla U
FPG mg/dl		Δ BT_PG-FPG mg/dl	
131-150	+1	≥+21 - +30	+1/+2
100-130	0	+11 - +20	0/+1
		-10 - +10	0
99-90	-1	-11 - -20	-1
<80	-2	≤-21	-2

Disclosure: **F. Porcellati:** None.

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Demographic and clinical factors may predict insulin progression and glycaemic control among patients with type 2 diabetes using insulin: learnings from the MOSAIc study

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Background and aims: MOSAIc is a multinational prospective cohort study designed to understand the challenges associated with insulin progression among patients with type 2 diabetes. The study recruited patients from 18 countries within North and Latin America, Asia, Europe, and the Middle-East. This analysis describes characteristics of patients who experienced progression of their insulin therapy during the 2-year prospective follow-up period stratified according to their glycaemic control status.
Materials and methods: Eligible patients were those with type 2 diabetes, ≥18 year old and using insulin for ≥3 months with/without other anti-diabetes medications. Patients were seen as part of their usual care in a variety of specialist and primary care clinics representative of care delivery in each country. We collected detailed data on patient, physician and health care environment factors at baseline and regular intervals within the prospective 2-year follow-up period of the study. Insulin progression was defined as an increase in antidiabetic treatment complexity at Year 2 from the baseline (e.g., increasing the dose of basal insulin was not considered progression, whereas addition of a prandial dose of insulin to background basal therapy was considered progression). Adequate glycaemic control goal at Year 2 was defined as HbA1c ≤ 53.0mmol/mol. Multiple imputation via chained equations was used for missing data.
Results: During the course of the study period, a total of 924 patients (34.1%) experienced progression of their insulin therapy. Of those, only 28% achieved glycaemic control at Year 2. Those who achieved glycaemic control were older, with lower baseline HbA1c; the majority were male, non-smokers, and with family history of diabetes. Additionally, 30% of this group still had their HbA1c level above their physician's stated treatment goal.
Conclusion: In conclusion, there are important demographic and clinical differences at baseline among T2D patients with insulin progression by glycaemic control status. Of note, the majority of patients progressing insulin therapy were not able to achieve optimal glycaemic outcomes according to current treatment guidelines, nor in relation to their physician's current stated goal.

Table 1: Characteristics of those patients who progressed insulin therapy during the study

Baseline Characteristic*	End of Study HbA_{1c} ≤53mmol/mol (n=255)	End of Study HbA_{1c} >53mmol/mol (n=669)	P value**
Age, years	62.7±10.6	59.9±10.5	0.01
Male	135(53)	333(50)	0.45
HbA_{1c}, mmol/mol, (SD)	57.4± (8.5)	69.4±(8.5)	<0.01
Family history of diabetes	144(56)	408(61)	<0.01
Other Diabetes Medication			
Basal insulin	122(48)	330(49)	0.76
Short-acting insulin	27(10)	64(10)	0.75
Mixed insulin	65(25)	179(27)	0.72
Metformin	114(45)	374(56)	<0.01
Sulfonylurea	62(24)	199(30)	0.14
Dipeptidylpeptidase-4 inhibitor	28(11)	77(11)	0.79
Glucagon-like peptide-1 agonist	11(4)	25(4)	0.66
Thiazolidinedione	9(4)	40(6)	0.20
Region			
Asia	87(34)	248(37)	0.45
Europe	72(28)	124(19)	<0.01
Middle East	42(17)	123(18)	0.61
North America	31(12)	91(14)	0.64
Latin America	23(9)	83(12)	0.30

* Mean ± SD for continuous and Number (%) for categorical variables
** Univariate linear or logistic regression

Clinical Trial Registration Number: NCT01400971
Supported by: Eli Lilly and Company
Disclosure: **A.K. Ali:** Employment/Consultancy; Eli Lilly and Company. Stock/Shareholding; Eli Lilly and Company.

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A cloud-based electronic health records study of treatment intensification patterns in type 2 diabetes patients uncontrolled on ≥2 oral anti-diabetes drugs

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Background and aims: Clinical inertia is an ongoing barrier in diabetes care. To further understand the extent of clinical inertia, this study assessed treatment intensification patterns and their associated demographic and clinical characteristics in patients (pts) with uncontrolled type 2 diabetes (T2D) using data from a US cloud-based electronic health records (EHR) platform.
Materials and methods: Insulin-naive adult pts with T2D prescribed ≥ 2 different types of oral antidiabetes drug (OAD), with the most recent prescription in the 6 months prior to an uncontrolled HbA_{1c} level (i.e., HbA_{1c} > 7.0%), were identified from Jan. 2011 to Dec. 2015 in the Practice Fusion EHR database with > 30 million pts across the US. The most recent uncontrolled HbA_{1c} date following the prescription of a 2nd OAD marked the index date. The baseline period was defined as the 6 months prior to the index date; the observation period was defined as the 6 months post index date. Treatment intensification patterns during the observation period were assessed and used to classify pts into 4 cohorts: a) no intensification, and intensification with b) an additional OAD, c) a basal insulin, and d) a glucagon-like peptide-1 receptor agonist (GLP-1 RA). Baseline demographics and clinical characteristics including age, gender, mean HbA_{1c}, and mean BMI of pts across the cohorts were compared using chi-square tests or ANOVA.
Results: Of the 25,365 eligible pts, the majority did not intensify their treatment regimens (71.7%; n = 18,197); 19.9% (n = 5,047) of pts received an additional OAD; 6.7% (n = 1,690) added a basal insulin; and 1.7% (n = 431) added a GLP-1 RA. Baseline age, gender, mean HbA_{1c}, and mean BMI were significantly different across the 4 cohorts (all P values ≤ 0.001). In particular, and compared to pts who intensified with an additional OAD, basal insulin, or GLP-1 RA, pts with no intensifications were older and had a lower mean HbA_{1c}. Compared to pts who intensified with a basal insulin or GLP-1 RA, pts with no intensifications were also less likely to be female. While all pts had obesity (mean BMI > 30 kg/m2), pts who intensified with a GLP-1 RA had the highest BMI (mean: 37.0 kg/m2; standard deviation: 7.5). Table 1 presents the distribution of the demographic and clinical characteristics across the cohorts.
Conclusion: Clinical inertia is common among adult pts with uncontrolled T2D. In this EHR database covering a broad range of US practices, the majority of pts on ≥ 2 OADs with uncontrolled HbA_{1c} levels had no change in therapy. This was especially true in older pts and those with lower levels of uncontrolled HbA_{1c}. When pts intensified therapy, most added an additional OAD. Intensification to injectable forms of therapy was infrequent, occurring less than 10% of the patients.

Table 1. Distribution of demographic and clinical characteristics across treatment intensification

Characteristic	No intensification (n = 18,197)	Additional OAD (n = 5,047)	Basal insulin (n = 1,690)	GLP-1 RA (n = 431)	*P* value
Age, years, mean (SD)	62.4 (12.3)	61.5 (12.0)	60.8 (12.5)	57.6 (11.1)	< 0.001
Female, n (%)	8,759 (48.2)	2,375 (47.1)	873 (51.7)	232 (53.8)	0.001
HbA_{1c}, %, mean (SD)	8.2 (1.3)	8.6 (1.4)	9.5 (1.8)	8.7 (1.4)	< 0.001
BMI, kg/m^2, mean (SD)	32.3 (6.8)	32.4 (6.9)	32.9 (7.1)	37.0 (7.5)	< 0.001

Supported by: Sanofi US, Inc.
Disclosure: **L. Kallenbach:** Employment/Consultancy; Sanofi US.

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What are the HbA_{1c} thresholds for initiating insulin therapy in people with type 2 diabetes in UK primary care?

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Background and aims: For people with type 2 diabetes mellitus (T2DM), insulin therapy is often eventually required to maintain optimal glycaemic control. Concerns of both physicians and patients surrounding the use of insulin create barriers to initiating insulin therapy, increasing the likelihood that effective treatment is delayed and increasing the risk of developing complications. Despite consensus guidelines to the contrary, data from epidemiological and observational studies highlight that initiation is delayed in many cases until HbA1c has exceeded values of 75mmol/mol. We aimed to characterise the level of glycaemic control at which insulin was initiated in a large primary care cohort of people with T2DM in the UK.
Materials and methods: We performed a retrospective cohort analysis using a primary care sentinel network (Royal College of General Practitioners Research and Surveillance Centre). We identified the first insulin prescription in a cohort of people with T2DM between 1st January 2005 and 31st July 2015. We excluded people who had their first prescription within 12 months of joining their registered practice to ensure only people receiving their first insulin prescriptions were captured. We compared the HbA1c value at which insulin was initiated against a number of potential influencing factors, using linear regression. Factors included patient age, gender, ethnicity, socioeconomic status, smoking status, alcohol use, duration of diabetes, body mass index (BMI), comorbidities, and number of concomitant and previous diabetes medications. Socioeconomic status was measured using index of multiple deprivation (IMD) score, with higher scores in people with higher levels of deprivation. The analysis was performed using R version 3.2.3.
Results: From 58,717 people with T2DM we identified 4,527 (7.7%) people with a first insulin prescription and an HbA1c measurement preceding the initiation of treatment. The mean insulin initiation threshold was at HbA1c of 83.4 (SD 22.7) mmol/mol. There was no association between the threshold for insulin initiation and age, gender, alcohol consumption, BMI, or number of concurrent therapies. A lower glycaemic threshold (HbA1c in mmol/mol) for insulin initiation was associated with

Asian ethnicity (estimate -2.99; 95% CI -5.78 to -0.19; p=0.036), absence of retinopathy (-2.11; -3.63 to -0.59; p=0.007), coronary artery disease (-3.28; -5.23 to -1.33; p<0.001), and hypertension (-1.59; -3.15 to -0.03; p=0.046). A higher threshold was associated with higher IMD score (estimate 95% CI 0.09; 0.05 to 0.14; p<0.001), current smoking (3.79; 1.30 to 6.28; p=0.003), and a higher number of previous diabetes medications. Overall model performance was limited.

Conclusion: The threshold for insulin initiation in the UK is high and is likely to be contributing to poor glycaemic control. The high HbA1c threshold for insulin initiation equates to a mean capillary glucose of 13.0mmol/L, which is just above the renal threshold and therefore likely to lead to symptoms. Clinicians only moderately tailor insulin initiation thresholds by patient factors. Good glycaemic control is vital for prevention of complications and therefore approaches to improve this situation are urgently needed.

Supported by: Eli Lilly and Company

Disclosure: **W. Hinton:** Grants; Eli Lilly.

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Defining insulin responders with a composite measure in an integrated real-world health system database compared to a clinical trials database

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Background and aims: Most insulin-treated patients with type 2 diabetes (T2D) do not meet the hemoglobin A_{1c} (HbA_{1c}) goal of <7% suggested by treatment guidelines. Previous analyses in an integrated insulin lispro clinical trial (CT) database described a composite HbA_{1c} measure which identified more patients with clinically relevant HbA_{1c} reductions than an HbA_{1c} target alone. The present analysis evaluated this composite HbA_{1c} measure in a real-world (RW) database and compared it with the results from a CT database.

Materials and methods: The US-based MedMining electronic medical records database has de-identified data on 30,040 individuals in the Geisinger integrated health system from 2004 to 2015. This analysis included 1134 patients with T2D ≥18 years of age who initiated any insulin regimen (with first use as the index date), and had HbA_{1c} values available at the baseline (BL) index date and the 6-month post-index endpoint, both within 90-day windows. Responders were defined as patients with an endpoint HbA_{1c} <7% and/or a ≥1% absolute decrease from BL in HbA_{1c}. BL demographics, percentage and characteristics of responders in this RW database were compared to those in a CT database.

Results: Patients in this RW cohort (N=1134) were 57.1 ± 30.5 years of age (mean ± SD), with BL HbA_{1c} of 9.1% ± 1.8%, similar to the CT cohort (N=4908; 57.9 ± 9.7 years and 8.8% ± 1.2%, respectively). The RW cohort had more female and Caucasian patients (53.5% and 96.7%, respectively) than in the CT cohort (48.4% and 66.0%, respectively). The body mass index (mean ± SD) in the US-based RW database (37.3 ± 8.8) was higher than in the international CT database (31.0 ± 5.7). Overall, the proportions of patients identified as responders were similar between the RW and CT cohorts (Table), with the composite measure identifying more responders than the HbA_{1c} <7% definition. In both the RW and CT cohorts, the composite measure identified increasingly greater proportions of responders across higher BL HbA_{1c} categories ≥9%, while the proportions of responders reaching HbA_{1c} <7% remained consistently low. Irrespective of the starting insulin regimen, the composite measure identified greater proportions of responders in both cohorts than the HbA_{1c} <7% definition.

Conclusion: In both the RW and CT cohorts, a composite HbA_{1c} measure (≥1% absolute decrease in HbA_{1c} from BL and/or HbA_{1c} <7%), identified more patients with clinically meaningful responses to insulin therapy than an HbA_{1c} target alone, particularly in patients with high baseline HbA_{1c}. This composite model of defining insulin therapy response may be useful in population management and quality measures.

Baseline Variable and Subgroups	Responder Defined as HbA_{1c} <7% [n(%)]		Responder Defined as HbA_{1c} <7% and/or ≥1% Absolute Decrease in HbA_{1c} from BL [n(%)]	
	CT Cohort	RW Cohort	CT Cohort	RW Cohort
Overall	1991/4908 (40.6%)	420/1134 (37.0%)	3561/4908 (72.6%)	707/1134 (62.3%)
HbA1c ≥7% to <8%	677/1134 (59.7%)	187/374 (50.0%)	677/1134 (59.7%)	187/374 (50.0%)
HbA1c ≥8% to <9%	664/1638 (40.5%)	92/268 (34.3%)	1067/1638 (65.1%)	128/268 (47.8%)
HbA1c ≥9% to <10%	326/1135 (28.7%)	49/182 (26.9%)	915/1135 (80.6%)	129/182 (70.9%)
HbA1c ≥10% to <11%	153/572 (26.7%)	29/117 (24.8%)	525/572 (91.8%)	90/117 (76.9%)
HbA1c ≥11%	72/294 (24.5%)	63/193 (32.6%)	278/294 (94.6%)	173/193 (89.6%)
Basal Insulin Only	317/774 (41.0%)	191/568 (33.6%)	605/774 (78.2%)	352/568 (62.0%)
Bolus Insulin Only	123/348 (35.3%)	161/391 (41.2%)	227/348 (65.2%)	239/391 (61.1%)
Basal + Bolus Insulin	792/1806 (43.9%)	59/139 (42.4%)	1259/1806 (69.7%)	97/139 (69.8%)

Disclosure: **I. Conget:** None.

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Exploring ideal time points for predicting glucose fluctuations in type 1 diabetics receiving insulin degludec: a continuous glucose monitoring study

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Background and aims: Insulin degludec (IDeg) is a novel ultra long acting basal insulin that allows once a daily dosage and a significantly lower the risk of hypoglycemia especially in Type 1 diabetes mellitus (T1DM) patients. Meanwhile, continuous glucose monitoring (CGM) alongside insulin use has become an ultimate tool to measure glycemic variability and prevent unwanted hypoglycemia. Unfortunately, the use of this device comes at a cost making it less practical for long-term use. In this study, we aimed to identify the optimal time-point for Plasma glucose (PG) measurement that best represents the treatment effect, especially glucose fluctuations, of IDeg users in T1DM by using CGM data.

Materials and methods: A total of 32 T1DM patients who were treated with IDeg at our university hospital were evaluated. Each patient had a CGM device placed on day 1 and was given 4 meals of standardized diabetic diet (1 meal on day 1 and 3 meals on day 2) in outpatient setting. The 24-hour CGM data acquired on day 2 was used for the current analysis. The 24-hour mean glucose value and daily glycemic variability [standard deviation (SD) of blood glucose and mean amplitude of glycemic excursions (MAGE)] was evaluated. The correlation between the parameters acquired from CGM and pre-prandial PG, 1 hour post-prandial PG, and 2 hour post-prandial PG was studied. Furthermore, regression models were constructed to best predict glucose fluctuations acquired by the CGM parameters.

Results: The patient characteristics were as follows [all values expressed as median (interquartile range)]: age, 47 (40-53); HbA1c, 7.9 (7.4-8.2) %; 24-hour mean glucose values, 151 (124-168) mg/dL; standard deviation (SD) of glucose, 67.1 (49.5-78.2); and MAGE, 115.3 (80.3-151.5). The 24-hour mean PG was significantly correlated with pre-breakfast PG (r = 0.41; p= 0.001), pre-lunch PG (r = 0.35; p=0.007), and pre-dinner PG (r = 0.41; p=0.001). The SD of glucose was correlated with the 1 hour post-breakfast PG (r = 0.23; p= 0.01), 1 hour post-lunch PG (r = 0.25; p= 0.05) and 1 hour post-dinner PG (r = 0.31; P = 0.001). MAGE was significantly correlated with 2 hour post-dinner PG (r = 0.28; P = 0.03) but not with 2 hour post-lunch or post-breakfast PG. Additionally, regression analysis suggested that the 24-hour mean PG, SD of glucose and MAGE could be predicted with the equation, (pre-dinner PG) x 0.29 + 115, (1 hour post-dinner PG) x 0.11 + 43.8, and (2 hour post-dinner PG) x 0.23 + 75.6, respectively.

Conclusion: Our current study found that 24-hour mean PG, SD of 24-hour glucose and MAGE in T1DM patients receiving IDeg can be

predicted by measuring pre-dinner PG, post-dinner 1 hour PG or post-dinner 2 hour PG. Further studies are needed to accommodate for the needs of patients on multiple glucose lowering agents.
Clinical Trial Registration Number: UMIN000013817
Disclosure: **S. Sawano:** None.

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Changes in blood pressure before and after initiation of continuous subcutaneous insulin infusion therapy in a large cohort of adults with type 1 diabetes

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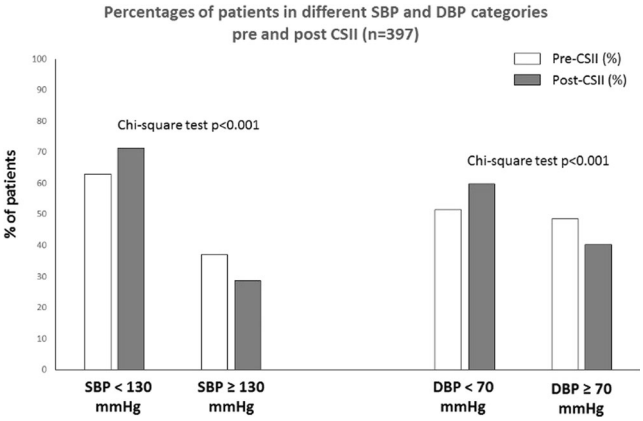
Background and aims: Among people with type 1 diabetes, use of continuous subcutaneous insulin infusion (CSII) therapy is associated with lower cardiovascular mortality compared to treatment with multiple daily insulin injections but mechanisms are unclear. Limited data exist about the impact of CSII therapy on blood pressure (BP). The objective of the present study was to describe the changes in BP before and after initiation of CSII therapy in a large cohort of adults with type 1 diabetes (T1DM).
Materials and methods: In adults with T1DM with at least 12 months of CSII therapy, we obtained demographic and biomedical data from electronic and paper records. Mean BP and HbA1c levels were calculated from values obtained up to 15 months before and up to 15 months after CSII initiation. Data shown are mean ± SD or mean (95%CI).
Results: We analyzed 1793 BP readings (pre-CSII 2.1±0.8, post-CSII 2.5 ±0.7 readings/patient) from 397 adults (antihypertensive treatment-naïve: n=286, on stable antihypertensive treatment: n=111); %female: 56; age: 39±13 years; diabetes duration: 20±12 years. CSII therapy was associated with a significant reduction in systolic blood pressure (SBP): -1.8 (95% CI: -0.8, -2.7) mmHg and diastolic blood pressure (DBP): -1.4 (95% CI -0.8, -2.0) mmHg; both: p 0.001. The proportions of individuals with SBP≥130 mmHg was reduced from 37% to 29%, p 0.001, and DBP≥70 mmHg reduced from 49% to 40%, p 0.001 (Figure). Mean HbA1c fell from 73±19 to 67±15 mmol/mol, paired difference -6.1 (95% CI -4.7, -7.4) mmol/mol, p 0.001. There was no change in body weight (pre vs. post: 74.8±15.2 vs. 75.0±15.4kg, p=0.44). In multiple regression analysis reduction in SBP was only related to pre-CSII SBP (beta 0.46, p<0.005). Reduction in DBP was related to pre-DBP (beta 0.47, p<0.005) and weight loss (beta 0.12, p=0.007). The variance explained by models predicting change in SBP and DBP was 22% for both. No significant correlation was found between change in blood pressure and sex, age, duration of diabetes, changes in HbA1c or pre-CSII weight. When comparing patients taking stable antihypertensive treatment and antihypertensive treatment-naïve individuals, SBP reductions (F=.06, p=0.80) and DBP (F=.78, p=0.37) reductions were similar after adjusting for the pre-CSII SBP and DBP.
Conclusion: We found significant reductions in systolic and diastolic blood pressure after starting CSII therapy. Pre-CSII SBP and DBP was related to reduction in SBP and DBP. Once adjusted for the pre-CSII SBP and DBP there was no difference between those on anti-hypertensive drugs and drug naive individuals. Mechanistic studies evaluating the impact of CSII on BP and other cardiovascular risk factors are warranted.



Disclosure: **K. Markakis:** None.

PS 084 Devices: continuous glucose monitoring

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Flash glucose monitoring improves metabolic control and treatment satisfaction in people with type 1 diabetes

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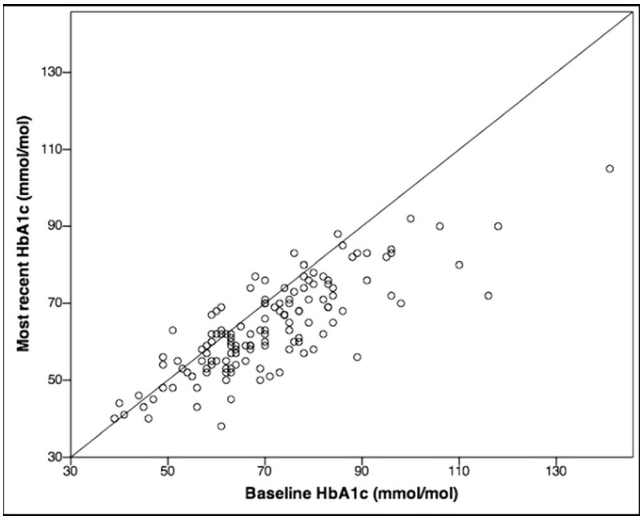
Background and aims: Hypoglycaemic events and fluctuations in blood glucose levels are limitations for good metabolic control in individuals with type 1 diabetes (T1D). Flash glucose monitoring (FGM) is a new method for glucose and glucose trend monitoring which requires an active initiative from the patient. The aim of this study was to evaluate the effects on HbA1c and diabetes treatment satisfaction after introduction of FGM in people with T1D.
Materials and methods: Patients with T1D in a university hospital setting, treated according to routine clinical management, were given FGM if their individual metabolic goal was not met or if they had problems with blood glucose fluctuations or repetead hypoglycaemic events. Their HbA1c was measured before initiating of FGM and every third month thereafter. A DTSQs questionnaire was completed before FGM initiation and a DTSQs follow-up questionnaire three months later. Data are given as mean(SD).
Results: 142 patients with a follow-up time between 3 and 12 months and a baseline HbA1c level of 70.5(15.9) mmol/mol were included in this prospective clinical follow-up study. 8 patients discontinued FGM. HbA1c was 65.1(12.2) mmol/mol at the 3 months follow up and 63.8(12.1) at the latest measurement. The mean decrease was 7.1 (9.4) mmol/mol. 68% improved their HbA1c more than 2 mmol/mol, 32% more than 10 and 8% detoriated more than 2 mmol/mol. The mean HbA1c change was 10.9 (9.8) (min:max -44:+7) mmol/mol, in those with baseline HbA1c 70 mmol/mol, 5.39 (-23:+9) in those with baseline HbA1c between 60 and 69 and 0.5 (5.0) (-13:+12) in those with baseline HbA1c < 60 mmol/mol. Individual changes in HbA1c are given in figure 1. Patients experience of FGM were positive with self estimated treatment satisfaction of 2.6 (+3 much more pleased/0 no change/-3 much less pleased) and rating for continued FGM use 2.8. Unacceptable high blood glucose was less often present (-0.3) as were unacceptable low blood glucose levels (-0.4). These improvements were as common in those with baseline HbA1c <60 mmol/mol (-0.4 and -0.6, respectively).
Conclusion: FGM adds clinical significant advantage to individuals with 1 diabetes in terms of HbA1c reduction and improved self estimated treatment satisfaction. HbA1c reductions were larger in those with higher baseline levels, but occurred also in individuals with HbA1c below 55 mmol/mol. Improvements in treatment satisfaction occurred over the whole HbA1c range, and were considerable also in those with lower baseline HbA1c levels.



Disclosure: **M. Löndahl:** None.

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Using novel flash glucose-sensing technology for 12 months reduces hypoglycaemia in individuals with type 2 diabetes on intensive insulin therapy

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Background and aims: Published data evaluating the use of sensor glucose monitoring by individuals with type 2 diabetes (T2DM) are limited, particularly in those treated with intensive insulin therapy for a wear period of 6 months or more. This study assesses the impact of using new technology to manage glycaemic control as an alternative to self-monitoring of blood glucose (SMBG) levels over 12 months in subjects with T2DM on intensive insulin therapy.
Materials and methods: 224 subjects with T2DM were randomised into a 6 month European study (26 sites). Individuals were randomised (1:2 respectively) to a control group (n=75), using capillary glucose-testing (FreeStyle Lite™), or an intervention group (n=149), using sensor glucose data (FreeStyle Libre™ Flash Glucose Monitoring System), for self-management. Subjects in the intervention group were invited to continue to use FreeStyle Libre for an additional 6 months in an open access phase. 139 subjects completed 6 months and elected to continue. This population was aged 59.3±9.6 years (mean±SD) with a screening HbA1c of 8.72 ±0.96% (71.8±10.5 mmol/mol), average duration of insulin use of 9±6 years, average SMBG tests per day of 3.9±0.67 reported at baseline and comprised 63.3% males.
Results: Within subject analysis of time in hypoglycaemia (sensor glucose time below 3.9 mmol/L [70 mg/dL] for >15 minutes) was significantly reduced by 50% at 12 months compared to baseline (-0.27±0.67 hrs/24hrs mean±SD]); p<0.0001. Nocturnal hypoglycaemia (11pm to 6am, <3.9 mmol/L [70 mg/dL]) reduced by 52%; p=0.0002. Reductions in overall hypoglycaemia were also evident in the pre-specified age group analysis (<65 years, 42% and aged >65yrs, 57%). There was no change in time in range (sensor glucose 3.9-10.0 mmol/L [70-180 mg/dL]) or glucose variability (SD). SMBG testing fell from a mean of 3.9 (median 3.9) at baseline to 0.3 times per day (median 0.0) at 3 months and remained at 0.2 times (median 0.0) per day during the 6-12 month open access phase. During the open access phase (month 6-12), average frequency of sensor scanning was 7.3 times/day. Throughout this period, subjects wore a

sensor and obtained FreeStyle Libre glucose results for 83.6±13.8% [88.3] (mean±SD [median]) of the time. During the 6 month open access phase the intervention group experienced; 134 occurrences of anticipated skin symptoms/sensor-insertion events expected with equivalent device use (e.g. erythema, itching and rash) from 28 (20.1%) subjects. There were 16 instances of device-related adverse events (e.g. infection, allergy) from 9 subjects. No device-related serious adverse events were reported.
Conclusion: Use of FreeStyle Libre by subjects with T2DM on intensive insulin therapy over 12 months in a home environment demonstrated a high frequency of interstitial glucose monitoring as a safe and effective replacement of blood glucose monitoring with continued benefits of reduced time in day and nocturnal hypoglycaemia.
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Supported by: ADC
Disclosure: **T. Haak:** None.

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Using novel flash glucose-sensing technology for 6 months results in a high rate of concordance by young adults with type 1 diabetes

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Background and aims: Sustained frequent use of continuous glucose monitoring devices (CGM) has been shown to be less likely in younger adults. We assessed the concordance rate to new sensor technology in 18-24 year olds as part of a study evaluating the impact of new sensor technology on hypoglycaemia compared to conventional self-monitoring of blood glucose (SMBG).
Materials and methods: 241 individuals with well controlled (HbA1c 6.74±0.56% [50.1±6.1mmol/mol]) type 1 diabetes (T1DM), age 43.7 ±13.9 years and average duration of diabetes 22±12 years (mean±SD), were recruited into a 6 month European study (23 sites). The control group (n=121) used capillary glucose-testing (FreeStyle Lite™) and the intervention group (n=120) used sensor glucose data (FreeStyle Libre™ Flash Glucose Monitoring System) to support self-management. Of the 241 subjects, 19 were aged 18-24.
Results: After 6 months of device use there was no significant difference between age groups in the number of times the sensor was scanned daily. Intervention group patients aged 18-24 years (n=10) scanned the sensor on average 11.3±4.6 (mean±SD) times daily, whilst subjects aged 25 years or over (n=101) scanned the sensor 14.9±10.2 times daily. The amount of time a sensor was worn and sensor derived glucose results were obtained via a scan was also not significantly different between the age groups. The rate of sensor wear and glucose results collection was 91.4% for young adults and 92.9% for subjects aged 25 years or over. Frequency of SMBG testing for the intervention group young adults dropped from 4.7±1.1 (mean±SD) times daily during baseline (2 weeks of masked sensor wear), to 0.2±0.4 at 6 months when sensor results were visible. In the control group, SMBG remained unchanged at 4.9 tests per day during baseline and 4.7 at 6 months. At 6 months, there was no significant interaction of age with treatment group for the primary endpoint of time in hypoglycaemia <3.9 mmol/L (70 mg/dL). The overall reduction in time in hypoglycaemia was significantly reduced by 38.0% (mean difference -1.24±0.239 hours per day [mean±SE]; p<0.0001). Further analysis of sensor glucose data at 6 months demonstrates that young adults significantly increased time in range (TIR) (sensor glucose 3.9-10.0 mmol/L [70-180 mg/dL]) by 2.9±0.89 hours per day (mean±SE); p=0.0055. Adults aged 25 or over also significantly increased TIR by 0.9 ±0.31 hours per day (mean±SE); p=0.0073. Time in hyperglycaemia was also significantly improved in young adults, hours/day >10.0 mmol/L (180 mg/dL) reduced by 2.40±0.834 hours per day (mean±SE); p=0.0113. Similarly, hours per day >13.3 mmol/L (240 mg/dL) reduced by 2.34±0.486 hours per day (mean±SE); p=0.0002.
Conclusion: This study demonstrated a high level of concordance to using novel flash glucose monitoring technology by young adults with well controlled type 1 diabetes resulting in significant improvement in time in range with significantly reduced time in both hypoglycaemia and hyperglycaemia for this group.
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Supported by: ADC
Disclosure: **J. Bolinder:** Honorarium; Abbott Diabetes Care, Integrity Appl., Sanofi. Lecture/other fees; AstraZeneca, Lilly, Sanofi.

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Performance of a fourth-generation glucose sensor during a pivotal Hybrid Closed-Loop (HCL) trial

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Background and aims: A fourth-generation sensor was assessed during the pivotal trial of the Medtronic hybrid closed-loop (HCL) system to assess sensor performance as a critical component of this system.
Materials and methods: Following a 2-week run-in phase to establish baseline parameters, 124 subjects (55 male, 30 age ≤21 yr) with type 1 diabetes entered a 3-month study phase with the Medtronic MiniMed 670G HCL system, which includes an insulin pump, a fourth-generation glucose sensor, and a proportional-integral-differential control algorithm. Subjects' mean (±SD) age was 37.8 ±16.5 yr, duration of diabetes 21.7±13.7 yr, BMI 26.2±5.3 kg/m2. Subjects calibrated the sensor periodically with a home glucose meter, administered mealtime and correction boluses as needed, and could temporarily adjust the target glucose value from 120 to 150 mg/dL. The study phase included a 6-day, 5-night hotel stay for frequent sample testing (FST) of venous blood glucose using i-STAT values for reference. The FST was conducted during one day and one night of the hotel stay. Sensor and reference blood glucose values were compared. The percentage of glucose values in various ranges was calculated for blood and sensor glucose measurements.
Results: The mean ±SD (median) blood glucose value was 153.9 ±29.7 (149.0) mg/dL; the mean ±SD (median) sensor glucose value was 148.4±13.1 (148.2) mg/dL (p=0.016). The overall mean (±SD) absolute relative difference (MARD) between 3710 paired sensor and i-STAT reference glucose values was 10.3±9.0% (median, 8.2%). There were 870 paired i-STAT-sensor points in the >180 mg/dL reference range, 2763 in the 71-180 mg/dL range, and 77 in the ≤70 mg/dL range. Accuracy parameters were similar for daytime and nighttime hours (overall daytime MARD, 10.4±9.66% [median, 8.1%]; overall nighttime MARD 10.2±8.30% [median, 8.3%]). The distribution of the percentage of values and sensor accuracy in various glucose concentration ranges are shown in the Table.
Conclusion: Data from the fourth-generation sensor were in good agreement with frequently-sampled blood glucose values. The percentage distribution of glucose values in ≤70, 71-180, and >180 mg/dL ranges determined by i-STAT blood samples was similar to that determined by the fourth-generation sensor. These parameters support the sensor's use for automated control of insulin delivery in the MiniMed 670G system.

Table. Percentage distribution of i-STAT blood and sensor glucose values and sensor accuracy in various ranges during frequent sample testing. Values are mean ± SD (median).

Reference glucose range (mg/dL	MARD (%)	Percentage of points in range	
		i-STAT (%)	Sensor (%)
>180	11.0±8.2	25.1±21.2 (19.5)	21.8±9.7 (20.6)
71 to 180	9.8±8.7	72.5±20.5 (76.5)	75.7±9.3 (76.2)
≤70	12.3*	2.4±4.8 (0.0)	2.5±2.0 (2.0)
*For reference values ≤70 mg/dL, accuracy given as mean absolute difference in mg/dL.			

Clinical Trial Registration Number: NCT02463097
Disclosure: **S.W. Lee:** Employment/Consultancy; Medtronic, Inc.

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Can FreeStyle Libre™ sensor-based glucose data support decisions for safe driving?

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Background and aims: The Driver and Vehicle Licensing Agency (DVLA) issues driving licences in the UK to comply with both European Union and UK legislation. In the UK there are additional standards for many individuals with diabetes to meet, in order to obtain or retain their licence including; awareness of hypoglycaemia, no more than one severe hypoglycaemic event per year and blood glucose (BG) testing relevant to driving. Modern glucose monitoring technology is a significant component of diabetes care; however, to date the DVLA does not recognise systems that use interstitial glucose measurements. The aim was to demonstrate whether continuous interstitial based sensor results could provide additional information for safe driving.
Materials and methods: We analysed data from 2 European studies of FreeStyle Libre sensor-based glucose monitoring technology used by adults on intensive insulin therapy with either type 1 diabetes (T1DM) or type 2 diabetes (T2DM), 241 and 224 subjects, respectively. The analysis was confined to sensor readings taken up to 2 hrs after day time BG fingerstick test results of ≥5 mmol/L (90 mg/dL); theoretically a safe driving level for those with insulin-treated diabetes if measured a minimum of 2 hours before and every 2 hours whilst driving.
Results: Analysis of masked sensor data over a 14-day period for individuals with well controlled T1DM (HbA1c<7.5% [58 mmol/mol]), showed; sensor glucose results <3.9 mmol/L (70 mg/dL) occurring up to 2 hours after a BG result ≥5 mmol/L (90 mg/dL) on 13.8% (1610/11628) of occasions. The same analysis for intensive insulin therapy treated T2DM showed sensor glucose results <3.9 mmol/L occurring up to 2 hours after a BG test result ≥5 mmol/L on 4.4% (365/8203) of occasions. Results up to 1.5 hours after a BG test were 10.0% (1160/11601) and 3.1% (254/8152) for the type 1 and type 2 populations respectively. Diabetes specialists recommend glucose levels of ≥5 mmol/L as safe to drive. FreeStyle Libre displays the current glucose level accompanied by a glucose trend arrow indicating the direction and rate of change of glucose. Analysis of sensor results between 5 and 7 mmol/L demonstrated the trend arrow descending on 14.7% (1163/7894) and 9.4% (305/3233) of occasions for the type 1 and 2 populations respectively. Availability of such information before driving may prompt additional action and so reduce the frequency of subsequent low results. Prior to randomisation, T1DM patients completed a hypoglycaemia status questionnaire which included asking if they checked their BG before driving. At study end, the likelihood of checking sensor glucose before driving were significantly increased for intervention patients compared to control using BG testing (p=0.0262). Those reporting they did not test their glucose before driving reduced from 24% to 16% (intervention vs. control). Frequency of sensor scanning per day was 15.1 for those with T1DM and 8.3 for T2DM.
Conclusion: Sensor-based glucose information with directional arrows has the potential to actively and opportunistically support licence holders' assessment of safe glucose levels both prior to driving and retrospectively. These facilities are not available with standard BG testing, thus, FreeStyle Libre offers distinct advantages over standard BG testing to support concordance with driving safety standards for individuals with type1 and type 2 diabetes.
Clinical Trail Registration Number: NCT02082184
Supported by: ADC
Disclosure: **G. Rayman:** None.

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Fifth generation glucose sensor system with 10-day wear and fewer calibrations

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Background and aims: The fifth generation sensor is a novel, subcutaneous glucose oxidase-based sensor. In addition to sensor design improvements, this sensor incorporates redundant electrochemical sensing elements suitable for a sensor life of 10 days. The system also utilizes diagnostics, fault detection, and a custom algorithm to allow for improvements in accuracy, reliability, and sensor longevity with 1 calibration per day. Data from an ongoing human feasibility clinical trial were analyzed to demonstrate these advances. To evaluate sensor performance stability beyond its calibration schedule, data were also evaluated with 1 calibration every 36 hours.
Materials and methods: Subjects with Type 1 or Type 2 diabetes wore up to 4 sensors in the arm and abdomen for a period of 10 days. Participants took daily reference blood glucose values using the Bayer® CONTOUR® NEXT LINK RF Blood Glucose Meter and participated in 3 in-clinic sessions where meal challenges were administered and blood glucose values were recorded every 15 minutes for 3-4 hours. Data were retrospectively processed to simulate calibration frequencies of 1 calibration every 24 hours and 1 calibration every 36 hours.
Results: Sensor design, diagnostics, and algorithm improvements show strong performance throughout wear, as captured in Table 1. Results suggest little degradation in performance when extending the calibration interval from 24 to 36 hours, indicative of the stability of the sensor and reliability of the system. A subset of 73 sensors (6942 evaluation points) that were placed using final taping recommendations were used to evaluate sensor lifetime and results indicate a lifetime of 9.7 days with 90.4% of sensors performing into day 10.
Conclusion: Feasibility clinical data for the fifth generation glucose sensor suggest strong and reliable performance with minimal calibrations and for a wear duration period of 10 days. Evaluation is ongoing.

Table 1: Arm and Abdomen Performance

Performance Metrics versus SMBG	1 calibration every 24 hours	1 calibration every 36 hours
Overall Mean-ARD	11.2%	11.6%
Mean-ARD Days 1-4	11.8%	11.9%
Mean-ARD Days 5-7	10.3%	10.8%
Mean-ARD Days 8-10	11.4%	11.8%
Overall Hypo-MAD	10.9 mg/dl 0.605 mmol/L	11.3 mg/dl 0.627mmol/L
20/20 Agreement	85.2%	83.8%
40/40 Agreement	98.1%	98.0%
Reliability	98.8%	98.7%
Sensor Startup Time	90 minutes	
Number for Sensors	142	
Evaluation Points	13428	

Disclosure: **J. Ulloa:** None.

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Implantable CGM with daily transmitter adhesive demonstrates high use compliance in a 180 day pivotal trial

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Background and aims: CGM use compliance can be linked to improvements in glycemic control. Poor compliance could be due to issues related to skin reactions or irritations linked to adhesives needed for CGM 7+ day system transmitters. The Eversense® CGM System (Senseonics Inc. MD, USA) consists of an implanted fluorescence-based glucose sensor that lasts up to 180 days, a wearable smart transmitter with a daily silicone-based adhesive patch, and a mobile app to display real-time glucose readings. This system was investigated in the prospective, single-arm, 180 day PRECISE study.
Materials and methods: Adult subjects (n=71) with T1DM and T2DM were enrolled at 7 clinical sites. Subjects used the CGM system at home and in-clinic. CGM wear time and adhesive patch related adverse events (AEs) were assessed. Subjects filled in an study specific questionnaire discussing the device features.
Results: Compliance with CGM wear was high with a median wear time of 23.5 hours/day and 25th percentile greater than 23 hours/day. The high wear compliance is accompanied with a very low incidence of adhesive patch related AEs. Only 3 adhesive patch related AEs were reported in 3 subjects over a total wear time of 8894 days, on average 1 AE every 2965 days. All three AEs were scored as mild or moderate in severity and resolved between 2 to 18 days. Patients scored the ability to remove and replace the transmitter on a daily basis as one of the highest likability factors (8.6 out of 10 points, SD 4.4) of the system.
Conclusion: Clinical data from the 180-day PRECISE study has demonstrated a high level of compliance of the Eversense® CGM system over the full study duration. Use and wear of the daily adhesive patch was shown to be safe, well tolerated and a highly liked feature, which possibly promoted the high level of wear compliance observed.
Clinical Trial Registration Number: NCT02154126
Disclosure: **M. Link:** None.

PS 085 Health care: technology and algorithms

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Evaluation of the use of new communication technologies in patients with type 2 diabetes

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Background and aims: The increased use of the internet is changing the way health care providers and the general population can interact. Type 2 Diabetes Mellitus is a chronic disease with special needs related to achieve good metabolic control in order to avoid the development of chronic complications. To achieve this goal, telematics systems (TS) may be useful. The aim of this study was to assess the efficacy and satisfaction of patients with a telematics system based on an online platform.
Materials and methods: This is a pilot, multicentre, randomized, monitored non-PAS study including 49 Type 2 diabetic patients (18 to 65 years old) receiving insulin treatment. The study consisted of two cohorts: Standard Management Arm (SM): 25 patients following routine clinical practice and Comprehensive Management Arm (CM): 24 patients monitored through comprehensive clinical care between Primary Healthcare and Specialized Healthcare, supported by telemedicine tools. Patients on CM sent information about glycaemia and insulin dose monthly through the TS and doctors made adjustment of treatment according to glycaemia values. Efficacy was measured in terms of HbA1c, number of episodes of hyper and hypoglycaemia. A satisfaction survey was completed by the patients before and at the end of the study. Costs were also analysed.
Results: HbA1c improved in 57,9% of patients on CM vs 54,55% on SM (p value >0,05) over a median of 6 months follow-up duration. Symptomatic hypoglycaemic and especially nocturnal episodes showed better evolution in CM group although we did not found statistically significant differences related to the small size of the sample. Patients on CM showed an improvement in satisfaction with the use of TS according to the comfort in the use of this technology (7, 6 vs 8, 7 p value =0, 04) at the end of the study. At the end of the 6-month Study, there were 68 on-site consultations less in the CM arm versus SM arm, representing savings of €3,672.
Conclusion: Results found in this study suggest that CM intervention can lead to improvement in glycaemic control and self-management in diabetes care. Telematics systems can help to diminish health care resources use and this can contribute to a reduction in economic costs although we have to consider the short time of the follow up period. Further studies are warranted to evaluate the economic impact of these strategies on Diabetes Care.
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Disclosure: **A. Oleaga:** Grants; SANOFI.

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The results of extended study of smart phone based the S-Diabetes Care programme in policyholders with type 2 diabetes

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Background and aims: The fact that complications of diabetes result in greater expenditure and reduced productivity is a socioeconomic concern.

However, less than 10% of patients with diabetes meet the recommended targets for glycemia, lipid, and blood pressure. The aim of this study is to evaluate whether adding mobile application coaching to community primary care for diabetes management would improve the glycemic control in patients with type 2 diabetes.

Materials and methods: This is a randomized, controlled, open-label study conducted in our hospital. The study subjects were 136 Korean adult policyholders with type 2 diabetes. All subjects attended usual dietary education program after engagement. And then, they were divided into two groups: intervention group, n=72; control group, n=64. Intervention group received 'S-Diabetes Care Program (S-Care)' which allowed subjects to enter data (blood pressure, weight, blood glucose values, diet, medications, and activity) on a mobile phone and receive educational, behavioral, and motivational messages according to their data. Subjects could communicate with providers by mobile application. All subjects kept their previous management for diabetes and S-Care did not get involved with prescription throughout the study. Biochemical variables including serum hemoglobin A1c (HbA1c) were measured at baseline, after 3, and 6 months. Primary outcome was the change in HbA1c. After 6 months, crossover-extension study followed in subjects who approved. Initial control group received S-Diabetes Care Program, and initial intervention group just were managed with conventional treatment for diabetes. After 6 months, biochemical variables were also measured. We used Wilcoxon's signed rank test to analyze the difference within each group, and t-test to compare between two groups.

Results: There was no difference in baseline characteristics between control and intervention group. In intervention group, HbA1c was significantly decreased from 8.1 ± 1.5% at baseline to 7.5 ± 1.0% at 3 months, and 7.5 ± 1.1% at 6 months (p value < 0.001). In control group, HbA1c was also significantly decreased from 8.1 ± 1.3% at baseline to 7.7 ± 1.3% at 3 months (p value = 0.012). However, HbA1c was rather increased to 7.9 ± 1.5% and there was no difference in HbA1c compared with baseline value (p value = 0.199). Extension study was conducted in 105 subjects: initial intervention group, n=54; initial control group, n=51. In previous control group, HbA1c significantly decreased to 7.3 ± 1.0 after applying S-Care. Compared with HbA1c at 6 months, p value was < 0.001. In previous intervention group, HbA1c after extension study was 7.5 ± 1.1, and the significant reduction in HbA1c was maintained even after stopping S-Care (p value compared with HbA1c at 6 months was 0.456).

Conclusion: In this study conducted in policyholders with type 2 diabetes, addition of a smart phone-based behavioral coaching intervention to conventional treatment for diabetes improved glycemic control, suggesting the educational role of mobile communication in behavior related to blood glucose. And its effect was maintained not only over study period but also 6 months after stopping the intervention.

Supported by: Samsung Fire & Marine Insurance Co., LTD

Disclosure: **E.S. Lee:** None.

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Reduced utilisation of health care provider resources with automated basal insulin titration in patients with type 2 diabetes

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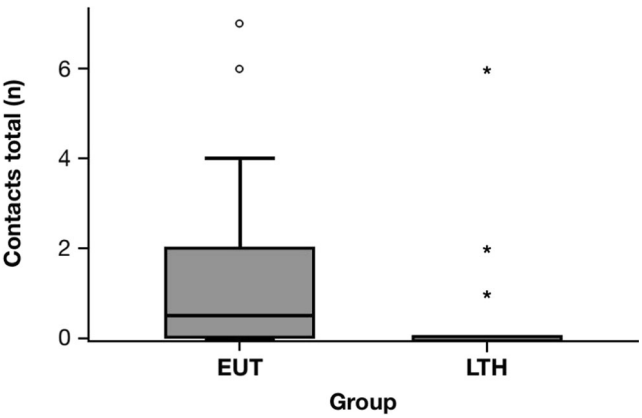
Background and aims: Basal insulin titration in the real world is mostly guided by health care providers (HCP). Use of health information technology for insulin titration may offer similar glycaemic effectiveness and lead to reduced utilisation of HCP resources. LTHome (LTH) is a web tool that applies a rules engine-based algorithm for insulin titration.

Materials and methods: Patients with type 2 diabetes, age 18-75 years, with HbA_{1c} >7.0% and computer literacy were randomized to receive basal insulin glargine that was titrated by LTH or by HCP-driven titration (enhanced usual therapy [EUT]). Titration instructions were provided throughout the 12-week trial. HbA_{1c}, hypoglycaemia incidence, Hypoglycemia Fear Survey (HFS), and Diabetes Distress Scale (DDS) were assessed at 12 weeks. HCP visits were recorded throughout.

Results: 139 patients with comparable baseline characteristics were randomised to LTH or EUT. At the end of the trial period, HbA_{1c} reduction (-1.0% and -1.1%; P = 0.66) and hypoglycaemia incidence were similar between groups. A significant difference in change of satisfaction scores in favour of LTH was observed for the HFS score (P = 0.04) and DDS (P = 0.04). Outside of the scheduled study visits at 4, 8, and 12 weeks, fewer patients required additional HCP visits with LTH vs EUT (by Week 4: 5 vs 30, P <0.001; by Week 8: 1 vs 9, P = 0.0434; by Week 12: 2 vs 7, P = 0.28). 22 patients with EUT had 1 to 2 additional visits vs 4 with LTH, 6 with EUT had 3 to 4 additional visits vs 0 with LTH, 4 with EUT had 6 to 7 additional visits vs 1 with LTH. Total number of visits for all patients was significantly less with LTH compared with EUT (78 vs 11, P < 0.001). Mean number of visits/patient is shown in figure.

Conclusion: Automated basal insulin titration led to significantly fewer visits and improved patient satisfaction, while obtaining similar glycaemic safety and effectiveness.



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Disclosure: **J. Sieber:** Employment/Consultancy; Sanofi.

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Use of a glucose alert pathway and connectivity blood glucose meters reduces inattention to hospital diabetes management

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Background and aims: In hospitalised patients with diabetes, hypoglycaemia and hyperglycaemia are associated with infections, mortality and increased costs. Clinical inertia and inattention to diabetes are major barriers to achieving optimal glycaemic control. We investigated the effect of a novel Glucose Alert Pathway (GAP) and Connectivity Blood Glucose Meter (CBGM) technology on nursing and medical staff action in response to suboptimal glycaemia.

Materials and methods: The study was a prospective, pre- and post-implementation audit on two wards (medical and surgical) in a tertiary referral hospital over 3 months. The intervention consisted of two components: GAP (a paper-based glucose management and clinical escalation guideline) coupled with CBGM with visual alerts for out-of-range capillary blood glucose levels (BGL). Consecutive inpatients with diabetes admitted with length of stay (LOS) ≥ 1 day were assessed for capillary BGL measures, diabetes treatment and hospital outcomes. BGL data was analysed per patient-day. The primary outcome was appropriate staff action on patient-days with reportable low or high BGLs (defined as BGL

15.0 mmol/L, or two consecutive BGL > 10.0 mmol/L at least 4 hours apart). Appropriate nursing staff action was defined as documented evidence of escalating (ie notifying) to medical staff. Appropriate medical staff action was defined as documented evidence of adjusting treatment (ie revision of BGL measuring frequency, prescription of correctional insulin, diabetes therapy adjustment or escalation to the endocrinology service). Secondary outcomes included adverse glycaemic days (patient-days with BGL 15.0 mmol/L), hospital complications (infections, acute kidney injury, myocardial infarct and intensive care admission) and LOS.
Results: Over the study period, 157 patients with diabetes (age: 69±14 years, HbA1c: 7.4±1.5%, 32% insulin-requiring) were recruited. In the 6-weeks baseline and 8-weeks intervention periods, there were 72 patients (359 patient-days) and 85 patients (311 patient-days) respectively. Reportable BGLs occurred in 148 (42%) and 114 (37%) of patient-days in the baseline and intervention periods respectively (p=0.12). Of patient-days with reportable BGLs, appropriate nursing staff action increased from 34% at baseline to 58% at intervention period (p<0.001). Similarly, appropriate medical staff action increased from 33% to 50% (p=0.004). However, there was no increase in diabetes medication adjustment (baseline 24%, intervention 29%, p=0.3) or endocrinology consults (baseline 14%, intervention 17%, p=0.6). There was a significant 24% decrease in adverse glycaemic days as a proportion of all patient-days (baseline: 29%, intervention: 22%, p=0.03). There was no difference in hospital complications or LOS.
Conclusion: The GAP coupled with CBGM technology, increased nursing and medical staff attention to diabetes in hospital, however, there was evidence of ongoing clinical inertia with little action to adjust diabetes medications or seek endocrinologist assistance. Overall, the intervention resulted in a quarter reduction of adverse glycaemic days in hospital.
Disclosure: **M. Kyi:** None.

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Randomised trial of a diabetes clinical decision support system: use rates, provider satisfaction, and impact on clinical outcomes

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Background and aims: CV Wizard is a web-based EHR-integrated point-of-care clinical decision support (CDS) system that presents personalized cardiovascular (CV) risk information to primary care providers (PCP) and patients in both a low numeracy and high numeracy format. Here we report use rates, PCP satisfaction, and impact of the CDS system on clinical outcomes of eligible diabetes patients.
Materials and methods: Nineteen primary care clinics with 7035 eligible diabetes patients with high CV risk were randomized to either usual care (UC) or use of the CDS system. This CDS system identifies target high risk patients and provides prioritized and personalized EHR-linked web-based CDS for management of glucose, BP, lipids, tobacco, weight, and aspirin to both the PCP and the patient at the point of care. Consented providers (n=102) were surveyed at baseline and 18 months after implementation, with survey response rates of 92% at baseline and 80% at follow-up. Electronic health record (EHR) data and multilevel regression models tested for differential trends in CV risk among patients at UC and CDS clinics.
Results: The CDS system was used at 70-80% of targeted visits made by diabetes patients. Compared to UC, PCPs in the CDS group reported increased use of CV risk calculation while seeing patients (73% vs. 28%, p=.006), being more prepared to discuss CV risk reduction priorities with patients (98% vs. 78%, p=.03), being more able to provide accurate advice on aspirin use for primary prevention (75% vs. 48%, p=.02), and more frequent discussion of CV risk reduction with patients (60% vs. 30%, p=.06). PCPs at CDS clinics reported that the CDS system improved CV risk factor control (98%), saved time talking to patients about CV risk reduction (93%), efficiently elicited patient treatment preferences (90%), was useful for shared decision making (95%), influenced treatment recommendations (89%), and helped initiate CV risk discussions (94%); 85% of PCPs reported that their patients liked CV Wizard. During the 14-month period when the final CDS intervention was in place, CV risk declined 0.2% per visit for diabetes patients at UC clinics, and 0.6% per visit for diabetes patients at CDS intervention clinics.
Conclusion: In a randomized trial, the CV Wizard CDS system was successfully integrated into the workflow of primary care visits with sustained use rates of 70-80% at targeted clinic visits, high PCP satisfaction, perceived positive impact on shared decision making and patient-centered care, and a favorable effect on CV risk factor control.
Clinical Trial Registration Number: NCT01420016
Supported by: NIH R01HL102144, P30DK092928
Disclosure: **P.J. O'Connor:** None.

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Hyperglycaemia guidance (protocol) improves glycaemic control during nil-by-mouth periods in patients with type 2 diabetes in hospital

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Background and aims: Hyperglycaemia is associated with poorer post-operative outcomes of type 2 Diabetes Mellitus patients such as wound infection, higher readmission rate and potentially longer length of stay in the hospital. The Endocrine Society guideline recommend the use of glucose management protocols optimise glycaemic control during perioperative and nil-by-mouth periods. The aim of this study was to evaluate the efficacy of Nil-by-Mouth (NBM) Guidance which we have developed for our institution. The NBM Guidance provides concise recommendations of dose and timing of insulin administration for type 2 DM patients who are kept nil-by-mouth while awaiting procedures such as surgery, radiological investigations and endoscopy. This guidance highlights the role of basal insulin for DM patients when they are nil-by-mouth.
Materials and methods: NBM Guidance was implemented at 6 wards in 2015. There was an extensive teaching program focusing on the use of the guidance to the doctors and allied healthcare professionals in the 6 wards. Baseline demographic data, diabetes medications usage and laboratory parameters were extracted from electronic medical records. The post procedures capillary blood glucose (CBG) readings of the patients who were on the NBM guidance were compared with the nil-by-mouth patients in the hospital prior to the implementation of the NBM guidance. Post procedure CBG was defined as CBG within the same day as the procedure. Proportion of capillary glucose readings which were above and within target range of the 2 patient groups were compared using chi-square tests.
Results: There were 281 patients with 418 CBG readings in the pre-NBM Guidance implementation group and 264 patients with 704 CBG readings in the post-NBM Guidance implementation group. There were no significant difference for mean HbA1c (7.3%±2.06 vs 7.5%±1.65 , p=0.34) and mean creatinine (103.4±111.56 umol/L vs 109.4±110.84 umol/L, p=0.56) . There was a significant decrease in proportion of CBG readings above 10mmol/L and 14mmol/L post procedures between the pre-NBM and post-NBM guidance implementation groups as evidenced by 39.7% vs 23.9%, p<0.001 and 10.8% vs 5.8%, p=0.003 respectively. Crucially, there was a significant increase in the proportion of glucose readings within the target range of 4-10mmol/l post procedures between the pre-NBM and post-NBM guidance implementation groups; 60.0% vs 74.7%, p<0.001. There was no statistically significant increase in hypoglycaemia peri-procedure (0.7% vs 1.2%, p= 0.28).
Conclusion: Implementation of the Nil-by-mouth Guidance has led to an improvement in glycaemic control in the post-procedure periods for the patients with DM as evidenced by decrease in proportion of

hyperglycaemic CBG and an increase in proportion of CBG in the target range post-procedures . Improvement in glycaemic control post procedures can potentially have positive impact on clinical outcomes and length of stay.
Disclosure: **W. Loh:** None.

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Efficacy of an education and treatment programme for people with type 2 diabetes on a non-intensive insulin regimen (MEDIAS2 BOT+SIT+CT): results of a RCT

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Background and aims: MEDIAS2 BOT+SIT+CT (More Diabetes Selfmanagement for People with type 2 diabetes on a Basal insulin supported Oral Therapy or on Supplementary Insulin Treatment with prandial insulin or Conventional Insulin Treatment with biphasic insulin) is a newly developed diabetes education and treatment programme for people with type 2 diabetes(PWD-T2) on a non-intensive insulin treatment regimen. The development of MEDIAS2 BOT+SIT+CT was based on self-management and empowerment theory. The efficacy of the newly developed intervention MEDIAS 2 BOT+SIT+CT (IG) was evaluated in a randomized trial with a six month follow-up period. The control group (CG) participated in an established education programme (Treatment- and education programme for type 2 diabetic patients with injection of prandial and/or basal insulin). The primary outcome of this study was the reduction of HbA1c.
Materials and methods: Both interventions in IG and CG consisted of 6 lessons. At baseline and follow-up the HbA1c was measured in a central laboratory. Participants also completed questionnaires to measure diabetes distress, empowerment, diabetes knowledge and hypoglycaemia awareness.
Results: 186 PWD-T2 participated in the study (IG vs. CG: age 63.0 ±8.4 vs. 63.2 ±7.6 yrs.; diabetes duration 11.5 ±5.7 vs. 11.4 ±7.0 yrs.; duration of insulin treatment 3.6 ±4.0 vs. 3.6 ±4.5 yrs.; HbA1c 8.0 ±1.3 vs. 7.9 ±1.2%; respectively 63.9 ± 14.2 mmol/mol vs. 62.8 ± 13.1 mmol/mol; hypoglycaemia-unawareness score 2.0 ±1.0 vs. 2.1 ±0.9). There were no significant differences at baseline between IG and CG. HbA1c was significantly more reduced in IG compared to CG (-0.7 ±0.1 vs. -0.3 ±0.1 percentage Points, respectively -7.7 ±1.1 mmol/mol vs. 4.4 ±1.1 mmol/mol; p=.02). Hypoglycaemia awareness did not deteriorate in both groups (1.9 ±0.1 vs. 2.1 ±0.1 p=.276). Diabetes distress and empowerment improved in both groups, but there were no significant between group differences.
Conclusion: Participation in the newly developed MEDIAS2 BOT+SIT+CT treatment and education programme led to a significant improvement of glycaemic control without a measurable increase in risk for hypoglycaemia. An important component of the newly developed MEDIAS2 BOT+SIT+CT was self-titration of insulin doses; this might have contributed to the positive impact of this programme on glycaemic control. In summary, MEDIAS2 BOT+SIT+CT has been proven as an effective treatment and education tool.
Clinical Trial Registration Number: NCT00901992
Disclosure: **N. Hermanns:** Grants; BERLIN CHEMIE, ABBOTTt, YPSOMED. Honorarium; YPSOMED, NOVO NORDISK, ABBOT DIABETES CARE. Lecture/other fees; BERLIN CHEMIE,

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CVD risk reduction in patients with type 2 diabetes going through a multifactorial treatment programme

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Background and aims: Patients with dysregulated type 2 diabetes (T2D) are often referred from primary to secondary care for treatment, with increased focus on improving hyperglycaemia, hypertension and dyslipidaemia. We aimed to evaluate the effects of a multifactorial treatment programme on metabolic outcomes and risk of cardiovascular disease (CVD).
Materials and methods: The study is a retrospective observational cohort study, based on data from all patients with T2D referred to the type 2 clinic between Jan 1st 2001 and Nov 1st 2014. Data were extracted from the electronic medical records as part of quality of care assurance. Logistic regression was used to assess change in proportions reaching treatment goals and linear mixed model analysis for repeated measurements to asses change in estimated risk of CVD using commonly available risk engines.
Results: We included a total of 3985 patients (male 59%). 630 (16%) patients went through more than one treatment programme (mean duration 9.5±5.6 months). There was a decrease in HbA1c, systolic BP, diastolic BP and LDL cholesterol of 12.7±0.4 mmol/mol, 6.3±0.4 mmHg, 2.6 ±0.2 mmHg and 0.40±0.02 mmol/l resp. (p<.0001)(Table 1). The effect on HbA1c was greater in males (p=0.0091). The proportion of patients who met the treatment goal for HbA1c (<53 mmol/mol) increased from 27% to 56%; for blood pressure (<140/<85 mm Hg) from 41% to 55%, and for LDL-cholesterol (<2.6 mmol/l) from 55% to 72% (p<.0001 for all comparisons). According to the UKPDS risk engine this led to an absolute 5-year CHD risk reduction of 1.6% with a higher risk reduction in males (2.2%) than in females (0.8%) (p=0.009) Similar results were seen using the Swedish NDR model which predicts the 5-year risk of a new CVD in a diabetic population, again with higher relative risk reduction for males than females (p=0.021). Looking at 10-year CVD risk, we used the Framingham risk score, which only included 10% with diabetes, and found an absolute risk reduction of 3.6%, but with no relative gender difference.
Conclusion: We conclude that multifactorial treatment of dysregulated T2D in a specialized clinic results in significant improvement in their metabolic outcomes and thereby in reductions of CVD risk. However, common risk engines illustrate this risk differently.

	Baseline	Follow-up	*P* value
Treatment programmes (*n*)	4748		NA
Age (years)	60.3 (51.5-68.0)	61.1 (52.4-68.8)	NA
Gender F/M (%)	41/59		NA
Diabetes duration (years)	6.3 (2.0-11.1)	7.1 (2.8-11.9)	<.0001
HbA1c (mmol/mol)	65 (53-81)	53 (46-61)	<.0001
BMI (kg/m^2)	30.0 (26.5-34.4)	29.8 (26.5-34.0)	0.1830
Systolic BP (mmHg)	139 (127-154)	133 (123-146)	<.0001
Total cholesterol (mmol/l)	4.7 (4.0-5.5)	4.2 (3.6-4.9)	<.0001
Estimated CHD 5-year risk: UKPDS Risk Engine (%):			
• All	4.6 (2.4-8.6)*	3.0 (1.7-5.4)*	<.0001
• F	2.8 (1.6-5.5)	2.0 (1.1-3.5)	<.0001
• M	6.1 (3.6-10.5)	3.9 (2.4-6.5)	<.0001
Estimated CVD 5-year risk: NDR Risk Engine (%):			
• All	27.2 (17.6-41.0)*	21.7 (14.0-33.0)*	<.0001
• F	23.1 (14.3-34.6)	18.3 (11.5-28.7)	<.0001
• M	32.0 (20.9-47.2)	25.0 (16.4-38.9)	<.0001
Estimated CVD 10-year risk: Framingham Risk Engine (%):			
• All	30.4 (17.6-46.5)*	26.8 (15.6-41.8)*	<.0001
• F	21.9 (13.0-34.3)	18.4 (11.5-29.5)	<.0001
• M	37.5 (23.6-53.3)	33.9 (21.5-48.5)	<.0001

Table 1: Summary statistics for risk factors used in risk models and estimated risk of coronary heart disease (CHD) and cardiovascular disease (CVD) in male (M) and female (F). Data are median (IQR) unless otherwise stated. * p<.0001 for gender differences.

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PS 086 Hypoglycaemia: influence of insulin type

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Effect of hypoglycaemia frequency during insulin titration on outcomes in people with type 2 diabetes adding insulin glargine 100 U/mL to OADs: a subject-level pooled analysis

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Background and aims: Hypoglycaemia occurring during uptitration of basal insulin introduced to patients with type 2 diabetes (T2D) may prevent optimal doses and glycaemic targets being achieved. The influence of hypoglycaemia frequency during uptitration of Gla-100 on glycaemic parameters at 6 months was examined.

Materials and methods: A post-hoc subject-level analysis using standardized data from 16 Gla-100 RCTs (FPG target ≤ 5.6mmol/L; duration ≥ 24 weeks) was performed. Insulin-naive T2D patients, uncontrolled on OADs, were grouped in hypoglycaemia (plasma glucose < 3.9 mmol/L or assistance required) quartiles (0, 1-3, 4-6, > 6 events) according to their frequency during uptitration (Weeks 0-8) after commencing Gla-100 as add-on therapy to OADs. Clinical outcomes at baseline, 3, and 6 months were examined for all participants and also by concomitant OAD use (metformin [MET] only, sulfonylurea [SU] only or MET + SU). 3,549 patients who received Gla-100 treatment for 24 weeks were included, of which 623 (18%), 906 (26%), and 1,624 (46%) received MET, SU, or MET + SU therapy, respectively.

Results: Mean age was similar (58 years) across all groups. Those with ≥ 4 hypoglycaemic events during titration had the lowest baseline body weight, FPG, and HbA_{1c}, and longer known duration of T2D (Table). Those experiencing ≤ 3 events had higher BMI, FPG, and HbA_{1c} at baseline, and greater change in insulin dose from baseline to Week 24. Analysis of concomitant OAD use showed differential effects on HbA_{1c} reduction. MET-treated subjects had the shortest T2D duration and highest baseline BMI; the SU-treated group had the longest duration of T2D and lowest BMI (Table). In all OAD subgroups, most patients experienced ≤ 3 hypoglycaemic events during titration. The frequency of hypoglycaemia was inversely related to baseline BMI in all but the MET + SU-treated group with > 6 events. Gla-100 dose titration from baseline was lowest in those experiencing most hypoglycaemic events, regardless of OAD therapy.

Conclusion: In people with T2D, a lower frequency of hypoglycaemia during Gla-100 uptitration was associated with a shorter T2D duration and higher BMI requiring greater insulin dose titration. They attained a smaller HbA_{1c} decrement at 6 months compared to those who experienced more hypoglycaemia during titration, but achieved greater HbA_{1c} reduction and had a lower BMI and longer T2D duration. Differences in prevailing insulin resistance and residual beta-cell function may underlie these findings. Concomitant OAD therapy affected efficacy outcomes and overall hypoglycaemia risk during the initial insulin uptitration period.

	Gla-100 + OAD	Frequency of hypoglycaemic events during titration (Week 0–8)			
		0	1–3	4–6	>6
Number of subjects	Overall (n = 3,549)	2573	732	152	92
	MET (n = 623)	535	75	6	7
	MET+SU (n = 1,624)	1047	421	100	56
	SU (n = 906)	683	168	35	20
Baseline C-peptide, nmol/L	Overall (n = 2,165)	1.2 (0.6)	1.0 (0.6)	1.0 (0.6)	0.8 (0.5)
	MET (n = 250)	1.1 (0.5)	0.7 (0.3)	0.5 (0.3)	0.3 (–)
	MET+SU (n = 918)	1.2 (0.6)	1.1 (0.5)	1.1 (0.6)	0.8 (0.4)
	SU (n = 739)	1.3 (0.8)	1.0 (0.6)	1.0 (0.6)	0.7 (0.6)
Duration of T2D, years	Overall (n = 3,546)	8.6 (6.0)	9.5 (6.4)	10.2 (6.5)	9.9 (7.4)
	MET (n = 623)	6.9 (5.3)	9.1 (6.9)	11.4 (8.8)	11.5 (6.9)
	MET+SU (n = 1,622)	9.2 (6.0)	9.8 (6.3)	10.0 (6.4)	10.6 (8.4)
	SU (n = 905)	9.5 (6.6)	9.6 (6.8)	10.2 (6.6)	8.0 (5.2)
Baseline BMI, kg/m^2	Overall (n = 3,548)	31.0 (5.3)	29.6 (4.9)	28.3 (4.5)	28.4 (4.5)
	MET (n = 623)	31.8 (5.7)	29.3 (4.9)	27.6 (5.0)	26.0 (4.4)
	MET+SU (n = 1,623)	31.4 (5.0)	30.4 (4.8)	28.7 (4.4)	29.7 (4.5)
	SU (n = 906)	29.6 (5.2)	27.7 (4.6)	27.0 (3.8)	25.9 (4.1)
Baseline FPG, mmol/L	Overall (n = 3,515)	10.8 (3.0)	10.4 (2.9)	10.3 (3.6)	10.2 (3.1)
	MET (n = 618)	10.4 (3.0)	9.8 (2.9)	8.4 (1.6)	9.8 (2.7)
	MET+SU (n = 1,600)	10.6 (2.8)	10.0 (2.7)	9.8 (3.5)	9.0 (2.3)
	SU (n = 906)	11.2 (3.2)	11.4 (3.2)	12.3 (3.7)	13.4 (3.3)
Baseline HbA_{1c}, %	Overall (n = 3,546)	8.8 (1.1)	8.6 (1.0)	8.5 (1.0)	8.5 (0.9)
	MET (n = 622)	8.8 (1.1)	8.4 (1.0)	8.5 (0.8)	9.0 (1.0)
	MET+SU (n = 1,623)	8.7 (1.0)	8.4 (0.9)	8.4 (0.9)	8.2 (0.8)
	SU (n = 906)	9.0 (1.1)	9.0 (1.0)	9.1 (1.0)	9.3 (0.7)
HbA_{1c} at week 12* → 24, %	Overall (n = 3,447)	7.5 (1.0) → 7.2 (1.0)	7.1 (0.9) → 7.0 (1.0)	7.1 (0.9) → 7.1 (0.9)	7.0 (0.9) → 7.0 (0.9)
	MET (n = 603)	7.3 (1.0) → 7.0 (1.0)	6.9 (0.9) → 6.8 (1.0)	7.5 (0.4) → 7.4 (0.7)	7.4 (1.2) → 7.5 (1.3)
	MET+SU (n = 1,589)	7.4 (0.9) → 7.1 (0.9)	7.1 (0.8) → 6.9 (0.8)	7.0 (0.7) → 7.0 (0.7)	6.9 (0.6) → 6.9 (0.7)
	SU (n = 881)	7.8 (1.2) → 7.6 (1.2)	7.5 (1.1) → 7.4 (1.2)	7.5 (1.1) → 7.5 (1.2)	7.2 (1.4) → 7.2 (1.3)
HbA_{1c} change at week 12* → 24, %	Overall (n = 3,447)	-1.3 (1.0) → -1.5 (1.2)	-1.4 (1.0) → -1.5 (1.1)	-1.4 (1.0) → -1.4 (1.0)	-1.5 (0.9) → -1.5 (1.0)
	MET (n = 603)	-1.4 (1.1) → -1.7 (1.2)	-1.5 (1.1) → -1.6 (1.3)	-1.1 (0.9)→ -1.1 (1.0)	-1.6 (1.5) → -1.4 (1.5)
	MET+SU (n = 1,589)	-1.3 (0.9) → -1.5 (1.1)	-1.4 (0.9) → -1.5 (1.0)	-1.4 (0.9) → -1.4 (1.0)	-1.3 (0.7) → -1.4 (0.8)
	SU (n = 881)	-1.2 (1.1) → -1.4 (1.2)	-1.4 (1.2) → -1.5 (1.3)	-1.6 (1.1) → -1.6 (1.2)	-2.1 (1.2) → -2.1 (1.1)

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Disclosure: **B.M. Frier:** Other; Served on advisory panels for Johnson & Johnson, MSD, Eli Lilly, Novo Nordisk, Consultant for Locemia Solutions, Speakers bureaus for Eli Lilly, Novo Nordisk, MSD, Sanofi, Boehringer Ingelheim, Takeda.

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Reaching individualised Fasting Plasma Glucose (FPG) targets without nocturnal hypoglycaemia with IDegAsp BID vs BIAsp 30: a meta-analysis

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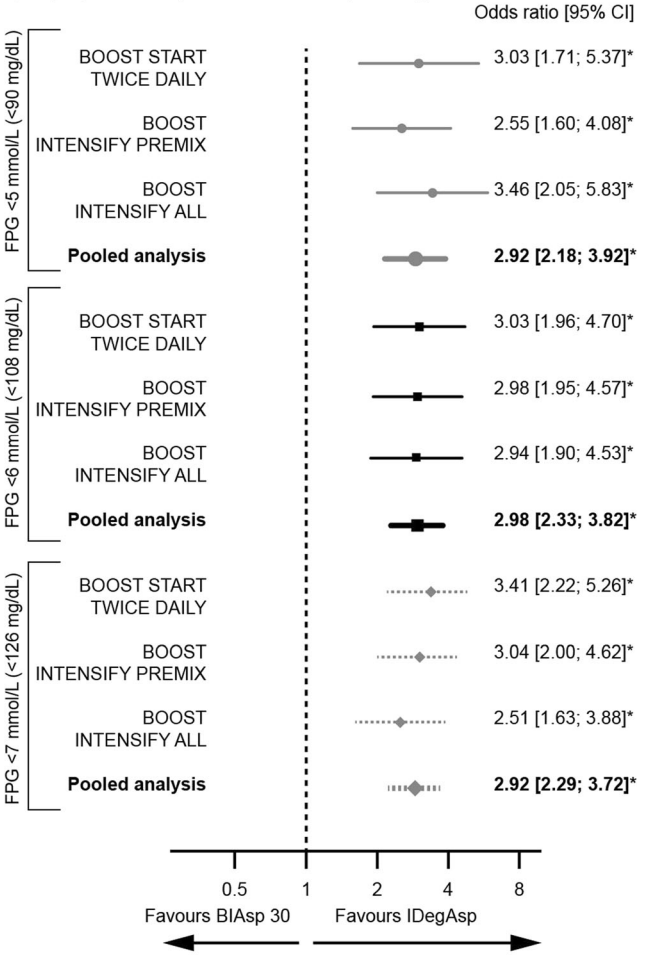
Background and aims: ADA/EASD 2015 guidelines recommend personalised glycaemic targets to balance the benefits and risks (e.g. hypoglycaemia) in individual patients. Nocturnal hypoglycaemia can be a frightening side-effect that may limit treatment adherence, consequently jeopardising glycaemic control. Assessing the likelihood of reaching glycaemic targets without nocturnal hypoglycaemia with different therapies may aid achievement of individualised targets.

Materials and methods: The proportion of patients reaching FPG targets (<5 mmol/L, <6 mmol/L or <7 mmol/L) without nocturnal hypoglycaemia (any severe or confirmed [blood glucose <3.1 mmol/L] self-monitored event between 00:01 and 05:59, inclusive) during the maintenance period (last 12 weeks of treatment) was assessed using data pooled from three 26-week, treat-to-target phase 3a/b trials of IDegAsp (a novel co-formulation of 70% insulin degludec [IDeg] and 30% insulin aspart [IAsp]) twice daily (BID) vs. biphasic IAsp 30/70 (BIAsp 30) BID in the IDegAsp clinical development program (BOOST). Patients were insulin naïve (BOOST START TWICE DAILY) or switched from basal or pre-mix insulin (BOOST INTENSIFY PREMIX I or INTENSIFY ALL).

Results: End-of-trial HbA_{1c} did not differ between IDegAsp and BIAsp 30 in the three treat-to-target trials. Patients were significantly more likely to reach all FPG targets without nocturnal hypoglycaemia with IDegAsp vs. BIAsp 30: the odds ratio (OR) ranged from 2.92 to 2.98 for all three FPG targets ($p<0.0001$ for all analyses) (Fig 1).

Conclusion: Treatment with IDegAsp BID vs. BIAsp 30 BID resulted in a significantly greater number of patients achieving FPG targets without nocturnal hypoglycaemia (OR 2.92-2.98, $p<0.0001$). IDegAsp BID may help achieve personalised treatment targets, e.g. reduced FPG without nocturnal hypoglycaemia, for a wide range of patients with T2D.

Fig 1. Treatment odds ratios for patients achieving FPG targets without nocturnal hypoglycaemia
(IDegAsp BID/BIAsp 30 BID; estimate [95% CI])



*$p<0.0001$. BIAsp 30, biphasic insulin aspart 30; BID, twice daily; CI, confidence interval; FPG, fasting plasma glucose; IDegAsp, insulin degludec/insulin aspart.

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Meta-analysis comparing hypoglycaemia rates of insulin degludec with insulin glargine U100 across clinical trials with up to 2 years' duration

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Background and aims: Insulin degludec (IDeg) is a basal insulin with a long and stable glucose-lowering effect with low day-to-day variability. A pre-specified meta-analysis of hypoglycaemia across the IDeg core phase 3a trials has previously been conducted and published. The aim of this post hoc meta-analysis was to compare the rate of hypoglycaemia with IDeg vs. insulin glargine U100 (IGlar) across the phase 3a trials including all available trial extensions (n=4) plus one new trial and to confirm the results from the previous pre-specified meta-analysis.
Materials and methods: The post hoc meta-analysis included two trials in patients with type 1 diabetes (T1D) and six trials in patients with type 2 diabetes (T2D); IDeg: n=3454; IGlar U100: n=1709. Hypoglycaemia was defined as rates of self-reported confirmed hypoglycaemia (BG <3.1 mmol/L [56 mg/dL] or severe hypoglycaemia requiring assistance) and nocturnal (00:01-05:59 both incl.) confirmed hypoglycaemia. Rates were analysed with a negative binomial regression model on patient level data.
Results: IDeg resulted in statistically significantly lower rates of confirmed and nocturnal confirmed hypoglycaemia vs. IGlar U100 in T2D, and for nocturnal confirmed hypoglycaemia in T1D (Table). Analyses of the maintenance period (from 16 weeks onwards), demonstrated more pronounced benefits with IDeg vs. IGlar U100 in both T1D and T2D.
Conclusion: This post hoc meta-analysis confirms and extends the outcomes of the previously published pre-specified meta-analysis. Even with the inclusion of additional trial data for up to two years' duration, the lower rates of both overall (T2D) and nocturnal confirmed (T1D and T2D, respectively) hypoglycaemia with IDeg vs. IGlar U100 are maintained.

	Confirmed hypoglycaemia (Rate ratio (IDeg/IGlar U100) [95% CI])		Nocturnal confirmed hypoglycaemia (Rate ratio (IDeg/IGlar U100) [95% CI])	
	Total period	Maintenance period†	Total period	Maintenance period†
T1D (n=958)	1.08 [0.94; 1.23]	1.01 [0.87; 1.18]	0.80 [0.67; 0.96]*	0.73 [0.60; 0.90]*
T2D (n=4205)	0.85 [0.76; 0.94]*	0.80 [0.71; 0.91]*	0.68 [0.58; 0.81]*	0.65 [0.53; 0.80]*
T2D insulin-naïve (n=2755)	0.82 [0.71; 0.94]*	0.76 [0.64; 0.90]*	0.61 [0.48; 0.78]*	0.54 [0.40; 0.72]*

CI, confidence interval; IDeg, insulin degludec; IGlar U100, insulin glargine; T1D, type 1 diabetes; T2D, type 2 diabetes. n refers to the full analysis set
*$p \leq 0.05$; †From week 16 onwards to trial end

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Disclosure: **A. Philis-Tsimikas:** Employment/Consultancy; Lilly, DexCom, Voluntis, Novo Nordisk, Sanofi. Grants; Merck, Novo, Sanofi, Lilly, Amylin, AstraZeneca, Pfizer, Janssen, Genentech.

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Achieving FPG target without hypoglycaemia: a meta-analysis of insulin degludec vs insulin glargine U100

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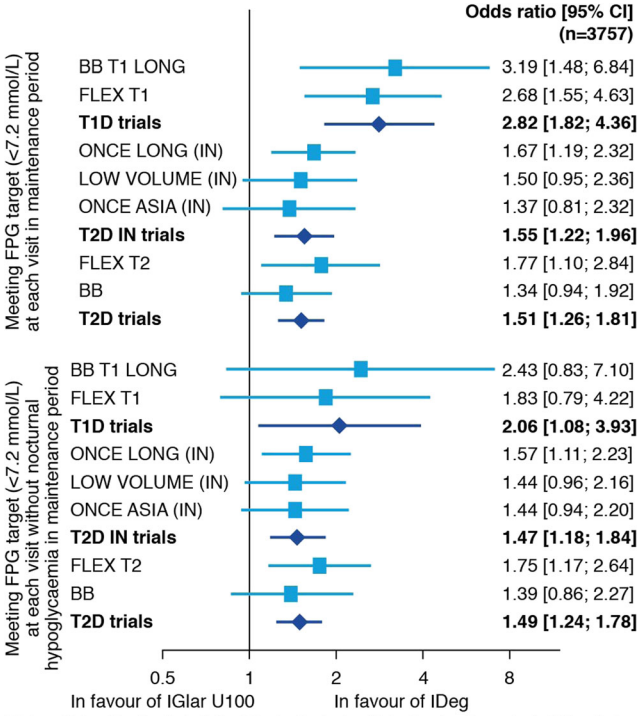
Background and aims: Insulin degludec (IDeg) is a basal insulin with a long and stable glucose-lowering effect and low day-to-day intra-patient variability compared with insulin glargine U100 (IGlar). This meta-analysis investigated the proportion of patients meeting the laboratory-measured FPG target of <7.2 mmol/L (130 mg/dL), which is the recommended pre-meal plasma glucose (PG) goal according to the 2015 ADA Standards of Medical Care in Diabetes, as well as doing so without experiencing nocturnal confirmed hypoglycaemia.
Materials and methods: The patients who reached the FPG target were defined as those with a FPG level <7.2 mmol/L (130 mg/dL) at each visit during the maintenance period. Nocturnal confirmed hypoglycaemia was defined as any severe or confirmed (blood glucose (BG) <3.1 mmol/L [56 mg/dL]) self-monitored event occurring between 00:01 and 05:59, inclusive. The maintenance period is defined as all visits from week 16 onwards. Patients (type 1 diabetes [T1D] or type 2 diabetes [T2D]) from seven open-label, randomised, treat-to-target trials treated with either IDeg (n=2501) or IGlar U100 (n=1256) were included.
Results: Use of IDeg resulted in significantly more patients reaching the FPG target at each visit throughout the maintenance period, as well as doing so without experiencing nocturnal confirmed hypoglycaemia, compared with IGlar U100 (Figure). These results were similar across the three patient populations: T1D, T2D previously insulin treated and T2D insulin naïve.
Conclusion: In conclusion, more patients treated with IDeg can achieve target FPG without experiencing nocturnal confirmed hypoglycaemia compared with IGlar U100.



BB, basal–bolus; IDeg, insulin degludec; IGlar, insulin glargine; IN, insulin naïve; Responder for FPG: patient meeting the FPG target of <7.2 mmol/L for all visits (≥2) from week 16 onwards. For FLEX T2 and FLEX T1 trials, IDeg Flexible arm is excluded from statistical analyses. For LOW VOLUME trial, IDeg U200 is considered as IDeg arm. For BB T1 LONG, BB and ONCE LONG trials patients without at least three valid FPG measurements (at week 16 or later) are not considered. For ONCE ASIA, FLEX T2, LOW VOLUME and FLEX T1 trials patients without at least two valid FPG measurements (at week 16 or later) are not considered.

Supported by: Novo Nordisk
Disclosure: **L.F. Meneghini:** Employment/Consultancy; Novo Nordisk, Sanofi Aventis.

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Patients with type 2 diabetes treated with IDegLira have a greater chance of reaching glycaemic targets without hypoglycaemia and weight gain than with insulin glargine U100 (IGlar U100)

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Background and aims: This post hoc analysis of DUAL V explored whether patients achieving glycaemic targets (Hb_{A1c} <7% or a fasting plasma glucose [FPG] target of <7.2 mmol/L) also achieved composite endpoints relevant to diabetes management. The FPG target of 7.2 mmol/L was selected to better reflect the targets aimed for in clinical practice versus the ambitious target used in DUAL V. When considering the DUAL V trial target of 4.0-5.0 mmol/L, IDegLira was superior to IGlar U100 in HbA_{1c} reduction, rate of confirmed hypoglycaemia and weight change, all p<0.001.
Materials and methods: DUAL V was a 26 week open-label, treat-to-target trial that randomised patients (n=557) with type 2 diabetes (T2D) uncontrolled (HbA_{1c} 7-10%) on IGlar U100 (20-50U) to either once-daily insulin degludec/liraglutide (IDegLira; 16 dose steps initially) or continued IGlar U100 titration, both + metformin. The proportion of DUAL V patients achieving glycaemic targets without confirmed hypoglycaemia (<3.1 mmol/L or requiring assistance) in the last 12 weeks of treatment and/or weight gain from baseline to week 26 were analysed using a logistic regression model based on the full analysis set and last observation carried forward-imputed data. The proportion of patients achieving HbA_{1c} targets were analysed across a range of baseline HbA_{1c} categories.
Results: The odds of reaching a FPG target of <7.2 mmol/L or HbA_{1C} <7% without hypoglycaemia and/or weight gain are statistically significantly higher for IDegLira vs IGlar U100 treated patients (Table). Across baseline HbA_{1c} groups (≤7.5, >7.5-≤8.5 and >8.5%) more patients achieved HbA_{1c} <7% (87% vs 66%; 76% vs 50%; 59% vs 31%), HbA1c <7% with no hypoglycaemia (67% vs 45%; 55% vs 30%; 47% vs 19%) and HbA_{1c} <7% with no hypoglycaemia and no weight gain (51% vs 25%; 39% vs 11%; 32% vs 5%) with IDegLira vs IGlar U100 (p<0.005 for all). Importantly, FPG and HbA_{1c} were significantly reduced at weeks 4, 8 and 12 with IDegLira vs IGlar U100 demonstrating better glycaemic control with IDegLira shortly after transferring from IGlar U100 to IDegLira.
Conclusion: This analysis suggests that the clinical advantages of IDegLira over IGlar U100 in DUAL V would also be observed in clinical practice, at titration targets closer to a real world setting, allowing patients to experience improvements in glycemic control without the detrimental effects of hypoglycaemia or weight gain. A greater proportion of patients achieved HbA_{1c} <7% with IDegLira vs IGlar U100 regardless of baseline HbA_{1c}.

Proportion of patients reaching HbA_{1c} target or FPG target by end of trial without confirmed hypoglycaemia* and/or weight gain†.

	Proportion of patients achieving treatment targets (%)		OR	95% CI	*p* value
	IDegLira	IGlar U100			
FPG <7.2 mmol/L	77.7	73.8	1.19	0.80; 1.79	0.3864
FPG <7.2 mmol/L without confirmed hypoglycemia	57.9	40.9	1.95	1.38; 2.76	<0.0001
FPG <7.2 mmol/L without weight gain	54.3	24.0	4.09	2.80; 5.98	<0.0001
FPG <7.2 mmol/L without confirmed hypoglycaemia and weight gain	41.4	14.3	4.55	2.96; 6.98	<0.0001
HbA_{1c} <7.0%	71.6	47.0	3.45	2.36; 5.05	<0.001

Data based on full analysis set and last observation carried forward. Estimated odds ratio and p-values are from a logistic regression with treatment and region as fixed effects and baseline FPG/HbA_{1c} (and body weight, where weight gain was included in the composite) as covariates. *Confirmed hypoglycaemia was defined as requiring assistance or <3.1 mmol/L in the last 12 weeks of treatment; † from baseline to week 26; OR, odds ratio.

Clinical Trial Registration Number: NCT01952145
Supported by: Novo Nordisk
Disclosure: **I. Lingvay:** Employment/Consultancy; Advisory panel: NovoNordisk – paid to UTSW, Board member: Janssen (Data Monitoring Board), Research support: NovoNordisk, Merck/Pfizer, GI Dynamics.

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Lower rate of hypoglycaemia with IDegLira vs insulin glargine regardless of dosing time or hypoglycaemia definition in patients with type 2 diabetes

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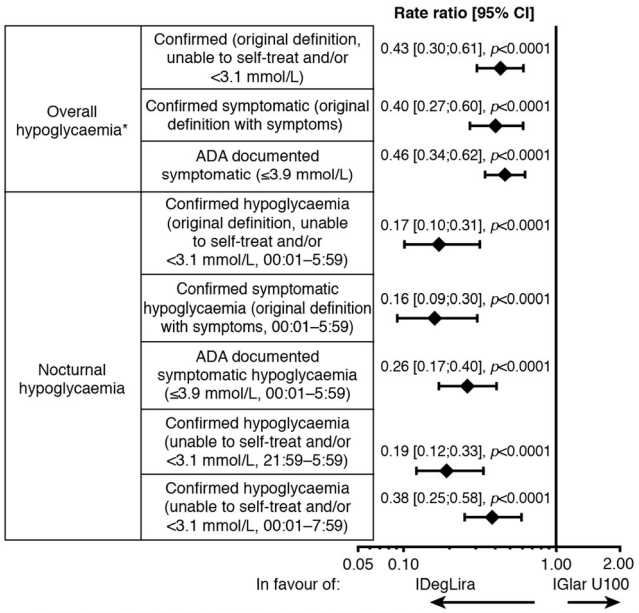
Background and aims: In the DUAL V trial, patients assigned to insulin degludec/liraglutide (IDegLira) had greater HbA_{1c} reduction (end of trial [EOT] HbA_{1c} 6.6 vs 7.1%) vs insulin glargine (100 units/mL [IGlar U100]) with a significantly lower confirmed hypoglycaemia rate (original definition: plasma glucose <3.1 mmol/L or unable to self-treat).
Materials and methods: This *post hoc* analysis investigated whether the lower hypoglycaemia rate with IDegLira vs IGlar U100 was consistent irrespective of dosing time (AM [00:00-11:59] or PM [12:00-23:59]) or definition of hypoglycaemia used (overall/nocturnal confirmed, with/without symptoms, ADA documented symptomatic).
Results: Hypoglycaemia rates (episodes per patient-year of exposure [PYE], based on the original definition) were significantly lower with IDegLira vs IGlar U100, whether both were dosed at AM or PM, for confirmed hypoglycaemia (AM: 2.18 vs 6.86 PYE, rate ratio 0.34 [95% CI 0.15; 0.74], *p*=0.006; PM: 2.26 vs 4.59 PYE, rate ratio 0.46 [95% CI 0.31; 0.68], *p*<0.001) and for nocturnal confirmed hypoglycaemia (AM: 0.22 vs 1.67 PYE, rate ratio 0.15 [95% CI 0.05; 0.45], *p*<0.001; PM: 0.23 vs 1.12 PYE, rate ratio 0.18 [95% CI 0.09; 0.37], *p*<0.001). The rate of hypoglycaemia was significantly lower for IDegLira vs IGlar U100 for confirmed (2.23 vs 5.05 PYE), confirmed symptomatic (1.56 vs 3.75 PYE) and ADA documented symptomatic (8.03 vs 15.63 PYE) (Figure). Nocturnal hypoglycaemia rates were significantly lower (62-84%) for IDegLira vs IGlar U100 across the different definitions used.
Conclusion: In conclusion, despite a lower EOT HbA_{1c}, the hypoglycaemia rate is lower with IDegLira vs IGlar U100, regardless of dosing time or hypoglycaemia definition.

Figure. Estimated rate ratio of hypoglycaemia for IDegLira vs IGlar U100 according to hypoglycaemia definition.



Number of hypoglycaemic events are analysed based on the full analysis set using a negative binomial regression model with a log link and the logarithm of the exposure time as offset. The model includes treatment and region as fixed effects. *One episode of severe hypoglycaemia was reported in the trial, which was in the IGlar U100 arm. IDegLira, insulin degludec/liraglutide; IGlar, insulin glargine.

Clinical Trial Registration Number: NCT01952145
Supported by: Novo Nordisk
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Cost of severe hypoglycaemia among patients with type 2 diabetes in UK, by treatment regimens

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Background and aims: Hypoglycemia (hypo) in patients with diabetes can be induced by many factors including certain medications, notably insulins and sulfonylureas. We assess the real world impact of specific treatment combinations on severe hypo events (requiring hospitalization) and related secondary care cost to UK healthcare system.
Materials and methods: A retrospective cohort design was used with Clinical Practice Research Datalink with linked hospital admission data during 2008-2013. Patients were identified based on type 2 diabetes (T2D) clinical diagnosis and/or therapeutic history and assigned to mutually exclusive subgroups based on treatment regimen at index date (first prescription for an antihyperglycemic agent). Outcomes were count and cost of hospital admissions with a hypo-related diagnosis code.
Results: We identified 110,206 T2D patients, mean age 64.9, mean time since diagnosis 5.4 years, mean HbA1c at index 7.4%; 439 hypo admissions were observed over a 128,060 person-years of treatment. The table shows crude event rates of hospitalizations / 1,000 person-years. Mean cost / admission was £2,045; mean length of stay 7.3 days.
Conclusion: The rates of severe hypo events vary substantially between alternative regimens, with the highest rates and costs in SU or insulin-based regimens. This highlights a potential unmet need in avoidable hypo events and costs.

Treatment regimen	n	Event rates (admissions per 1,000 person-years	Mean cost per admission (£)	Treatment regimen	n	Event rates (admissions per 1,000 person-years	Mean cost per admission (£)
All Drug Combinations (mono & combo)	110,206	3.4	2,045				
Insulin + SU	1,782	7.7	1,585	**Monotherapy**			
Insulin alone or insulin + non-SU	14,971	12.1	1,952	Metformin	51,739	0.2	2,221
SU alone or SU + non-insulin	34,324	5.0	2,183	SU	11,355	7.8	2,045
Metformin + SU (dual therapy)	16,340	4.0	2,116	DPP-4i (sitagliptin)	113 (101)	0	n/a
Non-insulin/ Non-SU	59,129	0.2	1,964	Insulin	7,803	16.8	2,138
Metformin + DPP-4i (dual therapy; met + sitagliptin)	237 (204)	0	n/a	Other	1,152	2.0	547

Supported by: Grant support from Merck & Co., Inc.
Disclosure: **Y. Tang:** Employment/Consultancy; Merck.

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Development of a protocol to allow commercial pilots with insulin-treated diabetes to fly

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Background and aims: In 2012, the UK Civil Aviation Authority (CAA) decided to issue Class 1 medical certificates to commercial pilots with insulin-treated diabetes, subject to strict criteria. The development and implementation of the protocol is described.
Materials and methods: An expert CAA committee that included diabetes specialists with clinical expertise of hypoglycaemia and experience of developing guidelines for safe driving, reviewed scientific publications and recommended relevant practical assessments e.g. measuring blood glucose on the flight deck, that were undertaken. A protocol for assessment, oversight and pre- and in-flight glucose monitoring was devised.
Results: Pilots with insulin-treated diabetes who apply for medical certification under the protocol are assessed individually. Essential information includes a detailed medical report from their specialist, biochemical profiles and complete cardiological and ophthalmological assessment. Applicants are examined to identify diabetes complications. Evaluation of hypoglycaemia awareness is undertaken, including glycaemic threshold for symptoms, Gold score measurement and detailed assessment of glucose monitoring records including frequency of out of range results. If all is acceptable and the applicant has practised the operational aspects, a flight test is performed with an flight examiner to assess ability to carry out the pre-flight briefing, in-flight testing, and knowledge of the actions to be taken. A commercial pilot has to fly as part of a multi-pilot crew. The Blood glucose monitoring protocol comprises: (a) before flight/duty - testing at least 1 hour before reporting for duty or at least 2 hours before flying (this allows satisfactory glycaemic control to be confirmed or notification of unfitness); - less than 30 minutes before take-off. (b) in-flight blood glucose testing - at least every hour while flying; - within 30 minutes of anticipated landing time; - If any hypoglycaemia symptoms are experienced. Blood testing must employ an ISO 9000 certified device and a spare must be carried. All commercial pilots must brief their co-pilot, and ensure the co-pilot cross-checks their blood glucose result and that it is recorded on the flight voice recorder. Pilots who have to correct a high or low reading should document the action taken. The 'acceptable' range is defined as 5.0 to 15.0mmol/l. If testing reveals a value in the caution ranges of 4.0-5.0 and 15.0-20.0 mmol/l then corrective action is required. If the level is <4.0 or >20mmol/l then 'pilot-flying' duties should be passed to the co-pilot while corrective action is taken. Any incapacitating symptoms as a result of low/high blood glucose should be managed as for any other incapacitating event. Commercial pilots are re-assessed by an independent specialist appointed by the CAA every 6 months. All home monitoring records are reviewed with verification of all pre- and in-flight glucose levels.
Conclusion: The programme, protocol and assessment are practical and their introduction represents a means of allowing insulin-treated pilots to perform complex occupational duties with very low risk. The longer term safety of this policy is being monitored prospectively by the UK and Republic of Ireland to evaluate its suitability for extension to other European countries.
Disclosure: **S. Mitchell:** Employment/Consultancy; I am employed by the UK civil Aviation Authority.

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Living with one's spouse reduces the risks of overweight status and metabolic syndrome among patients with type 2 diabetes: a cross-sectional study

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Background and aims: Among patients with type 2 diabetes (T2D), overweight status increases insulin resistance and exacerbates glycemic control, and metabolic syndrome increases the risk of cardiovascular disease. Unfortunately, it is quite difficult to manage body weight in patients with T2D. Several studies have revealed that living with one's spouse improves glycemic control, although the effects of living with one's spouse on overweight status and metabolic syndrome are unclear. Therefore, this study aimed to assess the effects of living with one's spouse on overweight status and metabolic syndrome among patients with T2D.
Materials and methods: We performed a cross-sectional study of patients with T2D (June 2010 to March 2016), and assessed marital status and metabolic syndrome-related information using a medical chart review. The participants' body mass indexes (BMI) and body fat masses were assessed using a bioelectrical impedance body composition analyzer (InBody720, InBody, Korea). Univariate and multivariate logistic regression analyses were performed to determine the association of marital status with overweight status (BMI $\geq$ 25.0 kg/m^2) and metabolic syndrome. Metabolic syndrome was diagnosed according to International Diabetes Federation Worldwide Definition of Metabolic Syndrome.
Results: After the screening, we evaluated 270 consecutive patients with T2D, who included 180 married patients who were living with their spouse (67%) and 90 single patients (33%). Compared to the single group, the married group exhibited a significantly lower BMI (24.5 ± 4.7 kg/m^2 vs. 26.5 ± 5.5 kg/m^2, p = 0.003), lower HbA1c levels (7.0 ± 1.3% vs. 7.3 ± 1.2%, p = 0.04), a lower body fat mass (18.9 ± 9.1 kg vs. 23.5 ± 12.1 kg, p = 0.002), and a lower rate of metabolic syndrome (54% vs. 68%, p = 0.03). In the univariate logistic regression analysis, the married group exhibited a risk reduction of 56% for being overweight (unadjusted odds ratio [OR]: 0.44, 95% confidence interval [CI]: 0.26 to 0.75, p = 0.003). In the multivariate logistic regression analysis, the married group exhibited a risk reduction of 50% for being overweight, after adjusting for age, sex, diabetes duration, beta-cell function (as assessed using the C-peptide index), insulin use, and exercise habits (adjusted OR: 0.50, 95% CI: 0.28 to 0.87, p = 0.01). Among men, living with one's spouse reduced the risk of metabolic syndrome by 58% (adjusted OR: 0.42, 95% CI: 0.18 to 0.95, p = 0.04), although no significant risk reduction was observed among women who lived with their spouse (adjusted OR: 1.32, 95% CI: 0.51 to 3.39, p = 0.56).
Conclusion: In this cross-sectional study, being married and living with one's spouse reduced the risk of being overweight by approximately 50% among patients with T2D. Men who were married and lived with their spouse also exhibited a risk reduction of 58% for metabolic syndrome. In contrast, being single was a risk factor for overweight status and metabolic syndrome, especially among male patients. These findings suggest that social supportive care is needed to help single patients with T2D manage their body weight.
Disclosure: **Y. Kondo:** None.

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Personalised diabetes care: from diabetes monitoring to putting diabetes in a patient's context

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Background and aims: Treatment of diabetes is shifting from 'disease management' to 'personalised care'. Guidelines advise to integrate

patient's preferences, needs and possibilities of self-management into daily diabetes care. However, such a policy requires a new approach of patients. In close collaboration with patients and diabetes care providers the Dutch Diabetes Federation developed a conversation model for general practitioners (GPs) and endocrinologists. The model contains systematically discussing not only health related factors such as HbA1c, but also personal factors such as quality of life, diabetes knowledge and skills for self-management, illness perceptions, social context etc. It consists of four steps; discussing these factors, setting goals, assessing treatment options and finally making a shared decision about treatment and care. We intend to implement it nation-wide. Here we present the results of the pilot study about the usefulness and applicability of the conversation model.

Materials and methods: Twenty-five GPs were trained during 2x2 hours to use the conversation model in practice. They applied it during a patient's annual diabetes check-up of 20 minutes. Patients received information about the new conversation and were recommended to prepare the following four questions before visiting the GP; do you have health problems?, do you want to solve your health problems?, how do you want to do that? what kind of support do you need? GPs and patients filled out a (online) questionnaire after the conversation. GPs about shared decision making, and usefulness and applicability of the model. Patients about shared decision making and satisfaction about the conversation.

Results: From November 2015 till February 2016, 157 patients with diabetes type 2 were included. GPs reported that 66% of the conversations took no more than 25 minutes. The majority of the patients spoke more than 50% of the consultation time (66%). In 60%, the conversation model was largely / completely applicable. In 82% the model supported GPs to gain insight into the patient's personal factors. In eight out of ten patients, the GP and patient made shared decisions about goals and treatment. Most of the patients prepared the conversation with their GP (77%). Patients reported that they set goals together with their GP (95%), and were sufficiently informed about the treatment options (92%) and the treatment related pros and cons (88%). Half of the patients feel more involved in making treatment decisions than before (53%). Almost all patients reported that they made a good to excellent treatment choice (96%). Patients gave a 8.8 (mean, range 7-10) for the conversation with their GP and 57% experienced the conversation with their GP more pleasant than before.

Conclusion: Personalised approach according to our conversation model seems feasible in daily diabetes care. Where the usual annual check-up has a medical focus, the annual conversation has a more integrated approach. Such an integrated approach seems promising.

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Disclosure: **H.A. van Vugt:** None.

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Delays in treatment intensification with oral anti-diabetics impact incidence of complications of type 2 diabetes: an individual patient simulation study

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Background and aims: Despite guideline recommendations, many patients whose HbA1c remains above target while on oral antidiabetes drugs (OADs) fail to have their treatment intensified. This study uses the Archimedes Model® to estimate the consequences of a delay in treatment intensification with OADs on glycemic control and long term outcomes in patients with type 2 diabetes. The simulated cohort and projections for times to intensification were derived from real world management observed in a recent US retrospective claims study.

Materials and methods: The Archimedes Model® is a detailed computer model of human physiology, disease progression, and healthcare delivery. This model was used to simulate outcomes over 20 years for alternative treatment intensification scenarios. A cohort of 600,000 individual hypothetical patients with HbA1c ≥ 8% on metformin (monotherapy or combination therapy) and no history of insulin or glucagon-like peptide-1 receptor agonist use was created based on real world data observed in a recent US retrospective claims study. The cohort included three strata based on the number of OADs taken at baseline (1, 2, or 3+), and the intensification sequence was assumed to be add on sulfonylurea, dipeptidyl peptidase-4 inhibitor, and thiazolidinedione. The intensification scenarios included either no delay (per guidelines), or delay based on the time to intensification observed for 1 year in the retrospective claims study, and a gamma distribution fit to these data to extrapolate over subsequent years. The delay scenario was composed of delaying <6 months, 6 to 12 months, and >12 months; results for these subgroups were pooled using a weighted average based on the size of each subgroup.

Results: At baseline, the cohort mean HbA1c was 9.1%, mean age 57 years, and 60% were male. At 1 year, HbA1c was 6.8% for patients intensifying without delay, and 8.2% for patients delaying intensification. Projected cumulative 5 year risk and % change with no delay vs. delayed intensification are summarized in Table 1. At 5 years, the risk of major adverse cardiac event (MACE) (defined as stroke, myocardial infarction, or CHD death) for no delay vs. delay was reduced by 18.0%. For no delay vs. delays of <6 months, 6 to 12 months, or >12 months, 5 year risk of MACE was reduced by 3.2%, 12.7%, and 26.8%, respectively. Severe hypoglycemia 5 year risks were, however, increased from 12.9% for delay to 19.0% for no delay (47.8% change). At 20 years, the results had similar trends as at 5 years.

Conclusion: Intensifying OAD therapy at guideline-recommended time intervals led to greater reductions in HbA1c, a lower risk of complications, but a higher risk of hypoglycemia than delaying intensification. These results highlight the potential impact of avoiding delays in prescribing combination therapy and timely treatment intensification on long term outcomes.

Table 1. Cumulative 5-Year Risk of Complications

	No Delay	Pooled Delay	% Change
MACE	4.9%	5.9%	-18.0%
MI	2.9%	3.9%	-25.1%
Stroke	1.8%	1.9%	-6.8%
HF	3.1%	3.6%	-13.8%
Blindness	3.8%	3.9%	-1.7%
Amputation	4.0%	5.0%	-20.4%
MacroAlb	3.2%	3.5%	-8.6%
Death	7.9%	8.2%	-3.1%

MI = Myocardial Infarction; HF = Heart Failure; MacroAlb = Macroalbuminuria; MACE = MI, Stroke, and CHD Death; Death = All-cause mortality

Disclosure: **J. Mukherjee:** Employment/Consultancy; Bristol-Myers Squibb.

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Suboptimal glycaemic control in patients with type 2 diabetes: retrospective data from 22,272 individuals

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Background and aims: Despite clinical guideline recommendations, glycaemic control in patients with type 2 diabetes remains suboptimal. Understanding treatment patterns and associated outcomes is crucial to address this issue.

Materials and methods: As part of the DISCOVER programme, a retrospective cohort study was conducted using data from electronic medical records in Canada, France, Germany and the UK. Patients with type 2 diabetes initiating second-line antidiabetic therapy (baseline) and with ≥ 6 months of follow-up were assessed for their characteristics and response to treatment.

Results: We identified 22 272 patients who initiated a second-line therapy (add-on therapy or switch from one therapy to another) from February 2011 to April 2014. Table 1 shows median HbA_{1c} levels for patients who remained on second-line treatment for ≥ 6 months; overall 44.6% achieved HbA_{1c} < 7.0% at 6 months. The most frequently used second-line treatments were dipeptidyl peptidase-4 (DPP4) inhibitors or sulphonylureas, with metformin. The proportion of patients achieving HbA_{1c} < 7.0% was slightly higher with the former (45.4%) than with the latter (41.0%).

Conclusion: Although all assessed therapies were associated with a decrease in median HbA_{1c} at 6 months, more than 50% of patients still had HbA_{1c} levels ≥ 7.0%. The 3-year follow-up period of the DISCOVER study will provide valuable data on longer-term glycaemic control.

Table 1. Glycaemic control in patients receiving second-line therapy for ≥ 6 months

Second-line therapy[a]	n (%)[d]	Median HbA_{1c} (IQR), %[e] At baseline[f]	At 6 months	Proportion with HbA_{1c} < 7% at 6 months, %[e]
Metformin monotherapy	443 (89.9)	7.6 (6.8–8.9)	6.8 (6.3–7.4)	57.1
DPP-4i monotherapy	745 (83.5)	7.4 (6.6–8.1)	6.9 (6.3–7.5)	53.8
SU monotherapy	1486 (90.1)	7.9 (7.2–8.9)	6.9 (6.4–7.6)	50.2
Metformin and DPP-4i[b]	5422 (79.1)	7.9 (7.4–9.0)	7.1 (6.5–7.7)	45.5
Metformin and SU[c]	7304 (83.3)	8.6 (7.8–10.0)	7.2 (6.6–8.0)	41.0
All other therapies	3086 (85.4)	8.4 (7.3–10.3)	7.0 (6.4–8.0)	46.5
Overall	18 486 (83.0)	8.2 (7.5–9.6)	7.1 (6.5–7.8)	44.6

[a]Add-on therapy or switch from one therapy to another.
[b]Patients for whom a DPP-4i was added to metformin or patients who switched to this combination therapy from other first-line treatments.
[c]Patients for whom a SU was added to metformin and patients who switched to this combination therapy from other first-line treatments.
[d]Numbers and proportions of patients who remained on second-line therapy for ≥ 6 months.
[e]For patients who remained on second-line therapy for ≥ 6 months.
[f]Initiation of second-line therapy (add-on therapy or switching from one therapy to another).
DPP-4i: dipeptyl-peptidase 4 inhibitor; IQR: interquartile range; SU: sulphonylurea.

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Differences in persistence by class of oral therapy for the treatment of type 2 diabetes

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Background and aims: Higher rates of adherence and persistence to antihyperglycaemic medications among patients with type 2 diabetes (T2DM) are associated with improved glycaemic control, reduced cardiovascular events and mortality, and reduced costs. Sub-optimal adherence is a significant problem for those managing and living with T2DM, with rates routinely reported as ranging between 60-80% depending on the medication class, patient population, and environment. While adherence is acknowledged as a multi-factorial predicament, medication factors are an important dimension. We compared medication persistence across all commonly used non-insulin medication classes used for treatment of type 2 diabetes.

Materials and methods: We performed a retrospective cohort analysis using a primary care based population (Royal College of General Practitioners Research and Surveillance Centre cohort). We identified prescriptions for all medication classes utilised by people with T2DM between 1st January 2004 and 31st July 2015. We compared crude median, first time, persistence across each class with non-persistence defined as prescription gap of ≥ 90 days. We also compared non-persistence between classes, adjusted for known confounders of adherence, using Cox regression. Confounders included: age, gender, ethnicity, socioeconomic status, alcohol use, smoking status, glycaemic control, duration of diabetes, diabetes complications, comorbidities, number of previous and concurrent diabetes medications. The analysis was performed using R version 3.2.3.

Results: From 58,717 people with T2DM and a median duration of follow up, since diagnosis of T2DM, of 6.75 (IQR 7.58) years we identified 78,524 prescriptions for new medications. Metformin and sulphonylureas were the most commonly initiated and had the longest crude median persistence of 2.78 (95% CI 2.72-2.85) years and 2.16 (2.09-2.24) years, respectively. Early data for sodium glucose co-transporter-2 (SGLT2) inhibitors show good persistence however median rates had not yet been reached. In regression models shorter persistence was associated with younger age, non-white ethnicity, extremes of HbA1c, peripheral neuropathy, renal disease, dementia, depression, heart failure, and a higher number previous diabetes medications. Longer persistence was associated with hypertension, and a higher number of concurrent diabetes medications. After adjusting for confounders, non-persistence with SGLT2 inhibitors was similar to metformin (HR 1.06; 95% CI 0.93-1.20). All other classes had higher non-persistence rates: sulphonylureas (HR 1.23; 1.20-1.25), dipeptidyl peptidase-4 inhibitors (HR 1.50; 1.45-1.55), thiazolidinediones (HR 1.61; 1.55-1.67), meglitinides (HR 1.93; 1.75-2.13), alpha-glucosidase inhibitors (HR 1.83; 1.60-2.10).

Conclusion: There is considerable variability in persistence between medication classes after adjusting for important confounders. These data will help facilitate proficient medication selection from the vast array of therapies currently available. We plan further work to understand the factors underlying these differences including a qualitative analysis of behavioural and attitudinal factors not available from electronic records.

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Clinical practice and outcomes versus clinical guidelines: a real-world perspective on the updated NICE guidelines

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Background and aims: Clinical guidelines, such as those set by The National Institute for Health and Care Excellence (NICE) in the UK, set out a structure for the management of type 2 diabetes mellitus (T2DM) at the European level, particularly from the health economics perspective. However, guidelines developed using indirect treatment estimates from randomised clinical trials may provide misleading perspectives on the relationship between baseline haemoglobin A1c (HbA1c) and expected therapy-related changes in HbA1c in patients with T2DM.
Materials and methods: Using patient-level data (PLD) from the EDGE study (Effectiveness of Diabetes with vildaGliptin and vildagliptin/mEtformin), a prospective, 1-year, worldwide, 'real-life', and the recently published NICE clinical guidelines for the management of T2DM, we contrasted guideline vs. real-world (EDGE) derived estimates of HbA1c change at 1-year for metformin+sulphonylurea (Met+SU) compared with metformin+vildagliptin (Met+Vilda) in patients inadequately controlled on metformin. Multivariate analysis of EDGE PLD provided baseline HbA1c adjusted 1-year changes in HbA1c; these were contrasted with the guideline-developed equations for adjusted HbA1c treatment changes. The two approaches were assessed with respect to expected lifetime incidence of T2DM-related vascular complications, expressed as changes in patient life expectancy (LE) and quality-adjusted life expectancy (QALE) using the CORE diabetes model.
Results: Based on the EDGE data, the estimated change in HbA1c at 1-year for Met+Vilda and Met+SU was −1.33% and −1.05%, respectively (delta: −0.28%). Applying the guideline equations, estimates for Met+Vilda and Met+SU were −0.95% and −0.99%, respectively (delta: 0.04%). These alternative predictions of change in HbA1c translated to a difference in the predicted change in QALE of 0.10 years between arms (Met+Vilda vs. Met+SU: 0.41 vs. 0.31 years) based on the EDGE data, compared with 0.01 years (Met+Vilda vs. Met+SU: 0.31 vs. 0.30 years) for the guidelines-based analysis; a similar relationship was observed for LE. Gains in LE and QALE favouring Met+Vilda were driven by lower cumulative incidence of major microvascular and cardiovascular complications.
Conclusion: Analysis of the real-world data suggests that current NICE guidelines for the management of T2DM might underestimate the HbA1c lowering potential and health gains of Met+Vilda compared with Met+SU in routine clinical practice.

Table: Summarising cost, QALE and LE for Met+SU vs. Met+Vilda

	Met+SU		Met+Vilda	
	EDGE	Guidelines	EDGE	Guidelines
Baseline profiles (mean, SD)^				
Age (years)	56.9 (11.5)		59.2 (11.3)	
Diabetes duration (years)	5.8 (4.8)		7.2 (6.5)	
Sex (% male)	55		51	
Baseline HbA1c (%)^^	8.2 (1.3)		8.1 (1.4)	
Baseline BMI (kg/m^2)	28.7 (4.8)		30.0 (4.9)	
Predicted outcomes[a]				
Change in A1c	−1.05 (1.16)	−0.99[b] (0.58)	−1.33 (1.24)	−0.95[b] (0.61)
Change in QALE	0.31	0.30	0.41	0.31
Change in LE	0.26	0.24	0.32	0.24
Incremental outcomes: Met+Vilda vs. Met+SU			**EDGE**	**Guidelines**
Δ A1c (%)			−0.28	0.04
Δ Change in QALE			0.10	0.01
Δ Change in LE			0.07	−0.01

[a]**Change in QALE/LE**$_\infty = \beta_0 + \beta_1(Age_0 - \overline{Age_0}) + \beta_2(Duration\ diabetes_0 - \overline{Duration\ diabetes_0}) + \beta_3 Male + \beta_4(A1c_0 - \overline{A1c_0}) + \beta_5(BMI_0 - \overline{BMI_0}) + \beta 6_6 A1c_1$
[b]$\boldsymbol{A1c_{\Delta 1}} = c_{1,1} + \beta(A1c_0 - 7.5) + d_{1,1i}$
$c_{1,1}$: amount HbA1c is expected to change after 1-year on the reference treatment, $d_{1,1i}$: extent to which treatment is better or worse than the reference treatment, $A1c_0$: baseline HbA1c, Age_0: baseline age, Male: proportion male, $A1c_0$: baseline HbA1c, $A1c_1$: HbA1c change at 1-year, BMI_0: baseline BMI, SD: standard deviation.
^Baseline profiles, treatment efficacy, and modelling assumptions derived from previously published analysis of the EDGE data using a published and validated disease model of T2DM.
^^EDGE baseline HbA1c used in both EDGE and guidelines-based analysis of HbA1c change; change refers to the difference between baseline and 1-year estimates in each arm.

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Type 2 diabetes-related chronic complications` development prediction

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Background and aims: It has been considered that individuals with type 2 Diabetes mellitus (T2DM) should be stratified by their risk of developing T2DM-related chronic complications. We aimed at proposing a clinical prediction rule (CPR) to predict the occurrence of these complications and improve the identification of subjects that should be followed in primary care institutions or hospitals.
Materials and methods: A prospective cohort of 600 subjects with T2DM [51% male, 35% using insulin, mean age of 65 (±11) years, diabetes duration of 13 (±10) years, T2DM onset on average at 52 (±13) years and body mass index (BMI) of 29 (±5)] undergoing diabetic foot screening in a public Hospital and a Family Health Unit were consecutively included. Several clinical variables were collected at the first appointment and analysed to predict the presence of each, none and of ≥ 4 out of the 6 most common T2DM-related chronic complications' history [namely, retinopathy, nephropathy, cerebrovascular, myocardial infarction, peripheral neuropathy (DPN) and vascular disease (PVD)]. Data concerning autonomic neuropathy and metabolic complications were not collected. The predictive models were created using logistic regression with a backward-stepwise approach. We have transformed our model into a more easy to apply rule by multiplying each logistic regression

coefficient by 28 and rounding to the nearest integer. CPRs accuracy were assessed through the area under the receiver operating characteristic curve (AUC).

Results: At 10 years of T2DM duration less than 1% of the subjects had >3 complications, being DPN the most common (33%). The remaining had a prevalence below 7%. Subjects followed in the Hospital had a higher number of complications ($p<0.001$). Variables associated with retinopathy were age, living alone, physical autonomy, T2DM onset age, insulin use and previous diabetic foot ulcer (DFU); with nephropathy were physical autonomy and T2DM for more than 10 years; with stroke were physical autonomy, T2DM for more than 10 years, glycated haemoglobin, hypertension and BMI; with myocardial infarction were male gender, physical autonomy and insulin use; with DPN were age, physical autonomy, T2DM onset age and previous DFU; and with PVD were T2DM for more than 10 years and previous DFU. We propose, to be applied in the primary care setting, the following CPR for the estimation of the probability of not having any complication: 105 - 3*(age in years) - 27*(limited physical autonomy) - 13*(insulin use) + 2 (T2DM onset age in years) - 60*(DFU history). This CPR presented an AUC of 0.81 [95% confidence interval (CI) 0.78-0.85]. For the tertiary setting, we propose the following CPR for the estimation of the probability of having ≥ 4 T2DM-related chronic complications: -41 + 62*(limited physical autonomy) - 2*(T2DM onset age in years). This CPR presented an AUC of 0.83 (95% CI 0.76-0.91).

Conclusion: Several CPR have been proposed for the prediction of a specific diabetes-related complication (most commonly cardiovascular disease, coronary heart disease or retinopathy). We proposed, for the first time, CPRs to identify subjects that should be followed more thoroughly in an Hospital (CPR for ≥ 4 complications) and those that can be less frequently followed in the primary care setting (CPR for no complications). Biochemical and genotypic characteristics should be studied in order to improve the proposed CPR discrimination and external validation should be conducted.

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PS 088 Socio economics

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Quantifying the health economic value associated with levels of glycaemic control, weight change and hypoglycaemia in type 1 diabetes

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Background and aims: Therapeutic guidelines advocate the use of patient-optimised management strategies and individualised targets for the management of type 1 diabetes mellitus (T1DM). Therapy-related consequences of treatment, such as weight gain and hypoglycaemia are known to act as a potential barrier to attaining optimal glycaemic control. We therefore sought to ascertain the respective contribution of hypoglycaemia, weight change and improved blood glucose control on predicted life expectancy and quality-adjusted life years (QALYs) in T1DM subjects.

Materials and methods: This study used the Cardiff T1DM model with microvascular disease progression rates derived from DCCT and EDIC and cardiovascular event rates from the Swedish National Diabetes Registry to predict outcomes associated with various ages, levels of glucose control, weight and rates of hypoglycaemia.

Results: Mean life expectancy predicted was 63.6 years. Achieving and maintaining a 1% reduction in HbA1c was associated with an estimated gain of 1.37 QALYs per patient. Changes in weight (±3kg) and hypoglycaemia frequency (±30%) produced a combined QALY gain of ±0.59 (70% attributable to weight change). HbA1c reductions resulted in larger QALY benefits among younger patients with high baseline HbA1c; for a 30-year old patient, 1.48 QALYs were gained for 9% versus 10% HbA1c, and 0.99 QALYs were gained for 7% versus 6% HbA1c. For a 50-year old 1.01 QALYs were gained for 9% versus 10% HbA1c, and 0.61 QALYs were gained for 7% versus 6% HbA1c.

Conclusion: This analysis quantifies the QALY improvements associated improved glycaemia control in subjects with T1DM, and highlights that the beneficial effects of improved glycaemic control on QALYs may be partially offset by characteristic treatment-specific adverse effects, such as weight gain and hypoglycaemia.

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Health economic impact of hypoglycaemia among 7,289 insulin-treated patients with diabetes: results from an international survey in 9 countries

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Background and aims: Hypoglycaemia is a key consideration in the individualisation of treatment in patients with diabetes. However, because

observational studies are predominately conducted in Western countries and are limited in number and consistency of design, actual hypoglycaemia rates in clinical practice, and their socio-economic impact, remain unclear for many countries.

Materials and methods: The International Operations (IO) Hypoglycaemia Assessment Tool (HAT) study is a non-interventional, real-world, observational study of self-reported (using self-assessment questionnaires) hypoglycaemic events in Bangladesh, Colombia, Egypt, Indonesia, the Philippines, Singapore, South Africa, Turkey and the UAE among 7,289 patients with insulin-treated type 1 (T1D) and type 2 diabetes (T2D). This abstract reports the health economic (HE) implications, including direct and indirect costs, of hypoglycaemic episodes occurring in the 6-month retrospective and 4-week prospective periods of IO HAT.

Results: Baseline characteristics are shown in Figure 1. Rates of any hypoglycaemia (per patient, per month) were 4.8 and 6.9 in patients with T1D and 1.6 and 2.4 in those with T2D during the retro- and prospective periods, respectively. For both patients with T1D or T2D, reporting of any and severe hypoglycaemic events were significantly higher ($p<0.001$) in the prospective period, whereas that of nocturnal hypoglycaemic events was significantly higher ($p<0.001$) in the retrospective period. The most common direct impact of hypoglycaemia was increased blood glucose monitoring which occurred in 43.8% (T1D) and 20.0% (T2D) of patients in the 4-week prospective period. Other impacts (in patients with T1D and T2D) included telephone contacts with a health care team member (6.4 and 5.9%, respectively), additional clinic appointments (5.8 and 4.3%) and post-hypoglycaemic event hospital admissions (3.0 and 1.7%). Indirect impacts of hypoglycaemia included reduced work/study punctuality (arriving late or leaving early) in patients with T1D (11.5 and 9.4%) and T2D (3.5 and 3.7%). In addition, some reported absence from their workplace or studies (T1D 6.3%; T2D 3.5%).

Conclusion: Hypoglycaemia is a major concern in diabetes treatment and is not just a barrier to reaching appropriate glycaemic targets, but also increases HE costs. This study details both direct and indirect HE impacts (to healthcare, employers or patients) of hypoglycaemic episodes in patients with T1D or T2D across non-Western countries.

Table 1. Baseline characteristics

	T1D (N=1,016)	T2D (N=6,273)
Age (years)	35.0 (13.0)	57.7 (10.9)
Male/female (%)	42.6/56.6	43.9/55.2
Duration of diabetes (years)	14.5 (9.8)	13.2 (7.7)
Duration of insulin use (years)	13.5 (9.8)	6.1 (5.1)
BMI (kg/m^2)	25.0 (4.8)	29.2 (5.9)
HbA_{1c} (mmol/mol)	66.6 (18.1)	70.7 (20.1)
HbA_{1c} (%)	8.2 (1.7)	8.6 (1.8)
FBG (mmol/l)	8.7 (4.2)	8.9 (3.6)
PPG (mmol/l)	10.3 (4.7)	11.6 (4.4)

Data are presented as mean (SD) unless otherwise stated. SD, standard deviation; N, total number of patients participating; HbA_{1c}, haemoglobin A_{1c}; FBG, fasting blood glucose; PPG, postprandial glucose; T1D, type 1 diabetes; T2D, type 2 diabetes.

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Validating prescribing choice in older patients with type 2 diabetes: an economic analysis of patient outcomes based on real world data

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Background and aims: Prescribing for type 2 diabetes (T2D) should take into account estimates of economic value associated with alternative approaches to glycaemic control.

Materials and methods: Using the CORE Diabetes Model, a cost-effectiveness analysis (UK perspective) evaluated intra-group changes in patient risk factor profiles associated with escalation to second-line treatment based on retrospective data from the UK Clinical Practice Research Datalink (CPRD) in patients with T2D ≥ 65 years. Lifetime costs and quality-adjusted life years (QALYs) were estimated for: metformin (M) [control] and M + sulfonylurea (SU), dipeptidyl peptidase-4 inhibitor (DPP4) or thiazolidinediones (TZD) [treatment arms]. Costs and utilities (discounted at 3.5% annually) were sourced from the literature.

Results: At baseline, patients (n = 6,619) were approximately 72 years, with diabetes duration 6-7 years, weight of 86-90kg and HbA1c of 8%. At year 1, vs baseline, weight increased with M+SU and M+TZD (0.60 and 1.28kg, respectively); weight decreased for M+DPP4 (-1.21kg). M+SU, M+DPP4 and M+TZD were associated with HbA1c changes of -1.02%, -0.76% and -0.57%, respectively. M+DPP4 was associated with the largest QALY gain (control vs treatment: 5.48 vs 5.61; delta: 0.13) and a cost/QALY estimate of £21,318. M+SU was associated with a relatively small incremental cost (control vs treatment: £19,228 vs £19,507; delta: £279) and QALY gain (control vs treatment: 5.34 vs 5.36, delta: 0.02) and a cost/QALY of £17,640. M+TZD was cost-saving and associated with QALY gains, as small incremental treatment costs were offset by savings and benefits from complications avoided. The probability that M+DPP4, M+SU and M+TZD following M was cost-effective, at a willingness to pay threshed of £30,000, was 61%, 54% and 74%, respectively.

Conclusion: Patients prescribed a DPP4, SU or TZD following M were associated with health gains and economic analysis confirmed the cost-effectiveness of these prescribing decisions.

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Correlates of absenteeism and productivity at work among adults in the UK who are overweight/obese

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Background and aims: Excess weight has been associated with a myriad of health consequences, such as hypertension, altered glucose metabolism, osteoarthritis, and many others. The objective of this analysis was to evaluate the association between body weight, comorbid conditions, health-related quality of life, medical resource utilisation, and degree of health problem-related work impairment in an overweight and obese population in the UK.

Materials and methods: Employed adults (full-time, part-time, or self-employed) in the UK with body mass index (BMI) ≥30 kg/m^2 or BMI ≥27-29.9 kg/m^2 with comorbidities (hypertension, dyslipidaemia, type 2 diabetes mellitus [T2DM], or prediabetes) were identified from a national, internet-based study (N = 2,004). The Work Productivity and Activity Impairment (WPAI) questionnaire was used to calculate overall health problem-related work impairment scores, which capture both time spent and productivity at work. This score was used to dichotomize respondents based on the sample median of 10: those with more impairment (>10; n = 808) or less impairment (≤10; n = 1,196). Characteristics examined included BMI, common comorbid cardiovascular and metabolic disorders,

physical and mental health as measured by the SF-36, self-reported health behaviours, and healthcare resource use in the 6 months prior to the survey.

Results: Respondents with greater impairment reported a higher BMI (34.8 [standard deviation (SD): 8.11] vs 32.8 [SD: 4.48] kg/m^2, p <0.05). Those with greater impairment had higher rates of self-reported hypertension, T2DM, and hypercholesterolaemia, although rates were not statistically different. Interestingly, the rate of self-reported prediabetes was higher among those with more impairment (17.6% vs 5.7%, p <0.05). Those with more impairment had worse physical and mental composite summary scores (44.7 vs 52.9 and 40.6 vs 50.2, respectively, p <0.05). Interestingly, those with worse impairment were more likely to report taking steps to lose weight (74.0% vs 68.4%, p <0.05), but less likely to report exercising at least 15 days in the past month (14.2% vs 18.1%, p <0.05) compared to those with less impairment. Health care resource utilisation in the 6 months prior to the survey was higher among those with more impairment compared to those with less impairment: % visited general practitioner (70.2 vs 56.8, p <0.05); % visited any health care provider (85.0 vs 74.7, p <0.05); mean number of any healthcare provider visits (5.3 vs 2.6, p <0.05); % visit to emergency room (19.4 vs 6.4, p <0.05); % hospitalised (17.1 vs 4.2, p <0.05).

Conclusion: These data suggest that improvements in cardiometabolic health status may be associated with improvements in absenteeism and productivity at work among adults in the UK who are obese or overweight with comorbidities. Future research should explore the relationships between changes in cardiometabolic health and work productivity over time.

Supported by: Janssen Global Services, LLC

Disclosure: **E.S. O'Brien:** Employment/Consultancy; Janssen Global Services, LLC.

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Willingness To Pay (WTP) for flexibility at the workplace for people with diabetes

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Background and aims: Work life with type 2 diabetes presents challenges to the person with diabetes and people they work with. Workplace accommodations can potentially improve and prolong the working lives of people with diabetes, but workplace accommodations come at a cost and require employers and employees to consider the perceived benefits in lieu of anticipated costs. In this study we investigate the relative value attached to workplace accommodations for diabetes and what people with diabetes and their colleagues are prepared to pay for them.

Materials and methods: The study is based on data obtained in a survey where subjects were recruited from online panels in three samples: people with type 2 diabetes (N=693), general population (N=600), and type 2 diabetes age and gender adjusted general population sample (N=539). The survey included a Discrete Choice Experiment which elicited preferences for workplace accommodations relevant for diabetes. The choice of accommodations presented in the survey was determined by the author group. Participants were presented with 6 discrete choice questions about workplace accommodations.The method creates a value hierarchy, where subjects express preferences in terms of willingness to pay (WTP). Answers from respondents who reported that they did not understand the questions were excluded from further analysis.

Results: People with type 2 diabetes had the lowest average WTP for workplace accommodations. This result may be explained by the fact that our subjects are still in work and trust their own resilience. It may also reflect the view that dispensation for diabetes contributes to stigmatization. Here it is important to recognise that the type 2 diabetes population differed in important respects to the general population. The type 2 diabetes population had a higher share of people who worked part-time (17% vs 4%), a lower share with more than three years of further education (42% vs 55%) and more people with diabetes worked in the public sector (45% vs 40%). These differences were reduced somewhat in the adjusted general population. Interestingly, the adjusted general population had a significantly higher WTP. This population was older and had a higher proportion of men and factors such as levels of disposable income may account for higher WTP in this group, as may anticipation of future health risks, though why this generates higher WTP than an actual diagnosis is uncertain.

Conclusion: More research is needed into the factors influencing these results. This is especially true if fear of stigmatization or concern about the consequences of preferential treatment contributes to people with diabetes concealing the challenges they experience in their working lives.

Attribute	General Population WTP € [CI]	Adjusted General Population WTP € [CI]	T2DM WTP € [CI]	Difference Gen. Pop. - T2DM P-value	Difference Adjusted General Population - T2DM P-value
Possibility of part-time	€43 [36-51]	€76 [62-97]	€36 [31-42]	0.122	<0.001*
Customizing job description	€36 [30-44]	€61 [49-76]	€28 [23-33]	0.042*	<0.001*
Additional break with pay	€8 [3-13]	€18 [10-26]	€11 [7-15]	0.392	0.132
Time off with pay**	€47[39-57]	€68 [53-85]	€57 [50-64]	0.096	0.228
Time off without pay**	€38 [30-46]	€53 [41-69]	€27 [21-34]	0.046*	<0.001*
Average	€34 [28-42]	€55 [43-71]	€32 [26-38]	0.404	<0.001*

Disclosure: **B. Cleal:** None.

906

Socioeconomic status is associated with glycaemic control, health behaviors and diabetic complications in Korean men with type 2 diabetes

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Background and aims: Lower socioeconomic status has been associated with increased risk of type 2 diabetes. But the association between socioeconomic status and glycemic control, metabolic parameters and complications in type 2 diabetes has not been well studied. This study aimed to investigate the association between socioeconomic status (SES) and glycemic control, metabolic parameters, health behaviors, and diabetic complications in Korean men with type 2 diabetes.
Materials and methods: A total of 2580 male type 2 diabetes patients enrolled in Korean National Diabetes Program from multiple medical centers in Korea, from Mar 2006 to Dec 2012. Baseline metabolic parameters, levels of glycemic control, health behaviors from individual questionnaires, and complication status were collected from the participants. The factors of SES included the level of education (less than high school, high school graduate, college graduate and more) and monthly house income (<2 million won, ≥2 and <4 million won, ≥4 million won). Microvascular complications included micro/macro-albuminuria, proliferative/non-proliferative diabetic retinopathy (PDR/NPDR), and macular edema. And macrovascular complications included carotid artery disease (CAD), peripheral arterial disease (PAD), cardiovascular disease, and cerebrovascular disease.
Results: The age ranges from 14 to 86, mean age of 52.3±10.4 years old. Lower educational level was associated with older age ($p<0.001$), lower body mass index (BMI, $p=0.0377$), higher postprandial glucose ($p=0.0018$), higher HbA1c ($p=0.0073$), and higher prevalence of microalbuminuria ($p=0.0453$), CAD and PAD ($p=0.0433$). Less daily alcohol drinking (glass, $p=0.0437$), more smoking amount (pack-year, $p=0.0002$), less physical activity ($p<0.001$), less fat intake ($p=0.0067$), and more carbohydrate intake ($p=0.0264$) were also associated with lower education. Lower income was associated with older age ($p<0.001$), lower BMI ($p<0.001$), higher HbA1c ($p=0.0139$), and higher prevalence of NPDR ($p=0.0021$). And less daily alcohol drinking (gram, $p=0.0404$), less total energy ($p=0.0068$) and fat intake ($p=0.0399$), and more carbohydrate intake ($p=0.0004$) were presented.
Conclusion: There were significant associations between SES and health behavior and nutrition in men with type 2 diabetes. Lower socioeconomic status was associated with poorer glycemic control, higher prevalence of macrovascular and microvascular complications. These findings will have implication for public health policies.
Supported by: Korea HT R&D project, ministry of health and welfare, Korea
Disclosure: **S. Kim:** None.

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Cost effectiveness of insulin sparing treatment regimes in type 2 diabetes: data from a real world clinical database

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Background and aims: The majority of the cost of diabetes is spent managing the complications of diabetes. Several models have suggested drugs with higher acquisition cost, but lower risk of hypoglycaemia or weight gain may be cost beneficial, but these models are limited by the cost utility estimates. Eclipse (Electronic Care Leading to Improved Patient Safety & Empowerment) software is currently utilized in more than 6000 surgeries in 800 Clinical Commissioning Groups in England for the optimization of medicines management. It monitors individual patient records with prescribing safety alerts, cost and availability of medications, cost of referrals to secondary care specialists, hospital admissions and out-patient visits.
Materials and methods: We identified a single practice in the UK that underwent a change of management and practitioners at the end of 2007. There was an accompanying change in treatment strategy from a UKPDS based strategy of metformin, suphonylurea and NPH insulin to an insulin sparing strategy of metformin, DPP-4 inhibitors (predominantly vildagliptin), thiazolidinediones (pioglitazone), GLP-1 analogues (liraglutide) and analogue insulins. The Eclipse system monitored average cost per patient with diabetes over a 7-year period. All figures were compared to the local average accounting for geographic and socio-economic factors.
Results: Prior to the change in management, the practice had low prescribing costs compared to the national average, but higher secondary care activity such that the total cost per 1000 patients with type 2 diabetes was 2-fold higher than the local primary care trust average (£4230 vs. £2100 per 1000 patients per annum). Over subsequent years, prescribing costs initially rose as there was increased expenditure on branded drugs. Five years after the instigation of the new treatment protocols, prescribing costs for diabetes was higher than the local (or national) average, however, due to reductions in unplanned admissions and referral costs, total costs reached the point of neutrality (Table 1). Subsequent years demonstrated further reductions in total patient cost, and due to reductions in co-morbidities and thus co-prescriptions, by the end of year 7 all costs including drug acquisition costs, showed substantial savings compared to local or national average.
Conclusion: Eclipse presents a good way to monitor cost effectiveness of treatment strategies using real-life data thus eliminating the selection biases that naturally accompany recruitment to and retention in randomized controlled trials. These data suggest, in a population with type 2 diabetes the higher acquisition cost of insulin sparing drugs may be offset by reduced unplanned admissions and referral to specialists such that cost neutrality is achieved in 5 years with subsequent years demonstrating cost benefit.

Table 1
Breakdown of the costs per 1000 patients in practice using insulin sparing treatment regime compared to local average. Where +ve figure presented that represents an increase in expenditure compared to local average, a –ve figure represents a cost saving)

Year ending	2012	2013	2014
Prescription costs*	+11.2%	+5.7%	-16.6%
Unplanned admission costs	-52%	-88%	-57.7%
Referral costs	-86.1%	-72%	N/A **
Total cost per 1000 patients	-0.7%	-5.2%	-19.7%

* includes all prescriptions for co-morbidities in addition to diabetes related treatment costs
** No referrals to specialists were recorded in this year.

Disclousre: **W.D. Strain:** None.

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Safety and efficacy of IDegLira titrated once weekly (1W) vs twice weekly (2W) in patients with type 2 diabetes uncontrolled on oral antidiabetic drugs: DUAL VI study

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Background and aims: IDegLira, a once-daily combination of insulin degludec and liraglutide, provided the advantages and mitigated the main side effects of each of its components, in a previous 52-week trial of insulin-naïve patients with type 2 diabetes (T2D) using twice-weekly titration (DUAL I). This trial compared the safety and efficacy of a simpler titration algorithm to that used in previous DUAL trials in insulin-naïve patients.
Materials and methods: In this 32-week, open-label, non-inferiority trial, insulin-naïve patients uncontrolled on metformin ± pioglitazone were randomised 1:1 to receive IDegLira, titrated either 1W based on the mean of two pre-breakfast plasma glucose (PG) readings (n=210) or 2W based on the mean of three pre-breakfast PG readings (i.e. six readings/week, as for DUAL I-V trials; n=210).
Results: Mean HbA_{1c} decreased from baseline (8.2%/8.1%) to 6.1% with IDegLira titrated 1W and 6.0% titrated 2W, and non-inferiority by <0.3% was confirmed (estimated treatment difference: 0.12 [-0.04; 0.28] (Table)). Similar proportions of patients achieved HbA_{1c} targets and composite endpoints in each arm. Mean fasting PG was similar after 32 weeks. Weight change was -1.0 kg for 1W vs -2.0 kg for 2W titration. Rates of severe or blood glucose-confirmed symptomatic hypoglycaemia were low in both arms: 0.16 events/patient-year of exposure (PYE) for 1W and 0.76 events/PYE for 2W titration. Mean IDegLira dose at 32 weeks was 41 dose steps (41 U IDeg/1.48 mg Lira) for both arms. The safety profile of IDegLira was consistent with previous findings; both titration algorithms were well tolerated.
Conclusion: In conclusion, a pragmatic titration algorithm with 1W dose adjustments based on two PG readings resulted in a similar safety and glycaemic efficacy profile to that with 2W adjustments based on three preceding PG values in insulin-naïve patients.

	1W titration of IDegLira	2W titration of IDegLira	
	Observed changes		Estimated treatment difference [95% CI], MMRM analysis
HbA_{1c} (%), mean change from baseline (SD)	−2.01 (1.09)	−2.02 (0.98)	0.12 [−0.04; 0.28]†
Fasting PG (mmol/L), mean change from baseline (SD)	−4.33 (2.81)	−4.55 (2.57)	0.22 [−0.11; 0.55]
Weight (kg), mean change from baseline (SD)	−1.0 (4.2)	−2.0 (4.9)	1.09 [0.22; 1.96]
	Proportion of patients (%) achieving composite endpoints		Estimated odds ratio [95% CI], logistic regression
HbA_{1c} <7%	89.9	89.5	0.95 [0.51; 1.78]
HbA_{1c} <7% with no hypoglycaemia	85.7	83.5	1.14 [0.66; 1.96]
HbA_{1c} ≤6.5%	83.6	85.0	0.88 [0.52; 1.49]
HbA_{1c} ≤6.5% with no hypoglycaemia	79.4	79.0	1.02 [0.63; 1.66]

Change in HbA_{1c}, fasting PG and weight were based on FAS and analysed by a mixed model for repeated measurement (MMRM) with treatment, visit, region and previous treatment as factors, and baseline value as covariate. Interactions between visit and all factors and the covariate were included. Responder endpoints were based on FAS and analysed by a logistic regression model with treatment, region and previous treatment as fixed factors and baseline HbA_{1c} as covariate.
†p=0.012, test for non-inferiority by 0.3%; CI, confidence interval; FAS, full analysis set; PG, plasma glucose; SD, standard deviation; 1W, once weekly; 2W, twice weekly.

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Disclosure: **S.B. Harris:** Employment/Consultancy; Novo Nordisk, Sanofi, Merck, Bristol-Myers Squibb/Astra Zeneca, Eli Lilly/ Boehringer Ingelheim, Janssen. Grants; Sanofi, Abbott, Astra Zeneca, Novo Nordisk. Honorarium; Sanofi, Novo Nordisk, Eli Lilly, Astra Zeneca, Merck, Medtronic. Lecture/other fees; Sanofi, Novo Nordisk, Eli Lilly, Astra Zeneca, Merck, Medtronic.

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Impact of delaying treatment intensification in type 2 diabetes uncontrolled on basal insulin: a longitudinal study of US Administrative Claims database

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Background and aims: Given the progressive nature of Type 2 diabetes, a patient's treatment regimen often needs to be intensified, in order to achieve and maintain glycemic control. However, delays occur despite high HbA1c values. The aim of the study is to evaluate the effect of delaying treatment intensification with glucagon-like peptide-1 (GLP-1) receptor agonists (RAs) (GLP-1 RA) in longitudinal change of clinical outcomes and economic burden in patients with type 2 diabetes (T2DM) uncontrolled on basal insulin (BI).
Materials and methods: Adult (>18 years) patients with T2DM who initiated basal insulin (BI) between 1/1/2005 and 12/31/2012 were identified in the IMPACT™ health insurance claims database. Patients were categorized into 3 cohorts based on time starting GLP-1 RA post BI initiation: ≤6m (Early intensification, EI), >6m (Delayed intensification, DI), and not receiving any anti-diabetic injectable 24 months post BI initiation (No intensification, NI). The starting date of GLP-1 RA served as index date for EI and DI groups, and 24 months post BI initiation served as index date for NI group. Patients having HbA1c value ≥7% and not using other bolus insulin prior to index date, with continuous enrollment during 6-month baseline and one-year follow-up were included in the

analysis. Multivariate generalized linear mixed models were used to assess the effect of time to intensification on changes in clinical and economic outcomes from baseline to follow up adjusting for important covariates.

Results: A total of 1552 patients met the study criteria, of which 44.3% were female with mean age of 54 years. The proportion of patients receiving EI, DI, and NI were 9.0%, 37.9%, and 53.2%, respectively. The median time to treatment intensification was 111 days for EI group, and 540 days for DI group. At baseline the HbA1c values for EI, DI, and NI groups were 9.16%, 9.07%, and 9.34%, respectively. At the end of one-year follow-up, the HbA1c reduction was significantly larger in EI group (mean reduction=1.01%; 95%CI: 0.67% to 1.35%) than in DI (0.68%, 0.48% to 0.88%, DI vs. EI: p<0.0001) and NI (0.11%; 0.01% to 0.42%, NI vs. EI: p<0.0001) groups; During 6-month baseline, the mean semi-annual total cost for the three groups were comparable (EI: $10,851, DI: $8,038, NI: $8460; p=0.095). At the end of one-year follow-up, the mean semi-annual total cost decreased by 6% for EI group (95%CI: -14% to 31%), but increased significantly by 20% for DI group (10% to 29%; DI vs. EI: p=0.0557) and 43% for NI group (35% to 50%; NI vs. EI: p<0.0001).

Conclusion: This study found that patients with T2DM who delayed intensification after failure of glycemic control with basal insulin were associated with poorer glycemic control and higher total cost. Strategies to improve timely intensifications are needed to help patients achieve and maintain recommended glycemic target and potentially limit financial consequences.

Disclosure: **L. Tong:** None.

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Drivers of and barriers to optimal basal insulin (BI) titration: results of a quantitative survey

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Background and aims: To evaluate the drivers of and barriers to optimal BI titration in the USA, France and Germany.

Materials and methods: Online survey of 386 healthcare professionals (HCPs) and 318 patients with type 2 diabetes (T2DM) on long-acting BI for 6-36 months, including 243 current BI users (currently self-titrating n=95), and 75 users who had discontinued BI within the past 12 months.

Results: Fasting self-monitored plasma glucose (SMPG) targets as outlined in country-specific guidelines were not used by all HCPs; instead, HCPs preferred higher fasting SMPG targets than recommended by these guidelines (Table). For current BI users, mean start dose was 15 U and mean current dose was 25 U. A dose increase of <10 U was seen by 27 months for 62% of current BI users despite 49% of them reporting not reaching HbA_{1c} target. Main barriers to optimal titration for current BI users not reaching HbA_{1c} target were concerns over weight gain (52%), frustration over time to reach goal (43%), perception that dose increase meant worsening of disease (38%) and fear of hypoglycaemia (37%) (Table). HCPs perceived the main barriers to target attainment in self-titrating patients to be fear of hypoglycaemia (74%) and low patient involvement/motivation (63%) (Table). Overall, 26% of current BI users indicated that they preferred self-titrating their BI; the percentage of current BI users self-titrating was 39%.

Conclusion: Generally, HCPs prefer a slow, safe approach to titration to higher glucose targets than recommended to avoid hypoglycaemia. However, patients not at target are frustrated about time taken to reach target and are less concerned about hypoglycaemia than HCPs. Preference for self-titration needs improving and patients need encouraging to self-titrate.

Table. Survey results

HCP responses		Overall	USA			France		Germany	
HCP specialty		**Overall (N=347)**	**PCP (N=104)**	**NP (N=27)**	**CDE (N=25)**	**Endo / Diabeto (N=67)**	**Nurse / CDE (N=27)**	**PCP (N=68)**	**Endo / Diabeto (N=29)**
Mean recommended fasting SMPG target (mg/dl)	General	N/A	135	124	N/A	111	N/A	145	142
	Elderly	N/A	141	130	N/A	135	N/A	153	159
Responses for current BI users not at HbA_{1c} target and HCPs		**Overall**		**USA**		**France**		**Germany**	
		Patients (N=118)	**HCPs (N=376)**	**Patients (N=59)**	**HCPs (N=171)**	**Patients (N=30)**	**HCPs (N=104)**	**Patients (N=29)**	**HCPs (N=101)**
Barriers to titration for patients[a], and HCPs' perception of patients' barriers to reaching target[b] in patients who self-titrate (%)[c]	Concern about weight gain	52	57	49	50	63	71	45	55
	Frustration that time to reach goal is too long	43	52	37	63	57	38	41	49
	Dose increase means disease is getting worse	38	55	31	58	53	70	38	34
	Fear of hypoglycaemia	37	74	27	67	63	87	31	74
	Low motivation and involvement	27	63	22	65	33	56	31	67

[a]Data are percentage of patients who agreed completely/somewhat that each of the reasons listed are reasons they have not successfully reached their HbA_{1c} target; [b]Data are percentage of HCPs who agreed completely/somewhat that each of the barriers listed are reasons that patients who self-titrate do not successfully reach their HbA_{1c} target; [c]Multiple answers could be selected. BI, basal insulin; CDE, certified diabetes educator; Diabeto, diabetologist; Endo, endocrinologist; HCP, healthcare professional; N/A, not applicable; NP, nurse practitioner; PCP, primary care physician; SMPG, self-monitored plasma glucose.

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Disclosure: **L.D. Berard:** Employment/Consultancy; Abbott, AstraZeneca, Bayer, BD, Boehringer Ingelheim, Eli Lilly, Janssen, Lifescan, Merck, Novo Nordisk, Sanofi.

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Safety and efficacy of a pragmatic self-titration 1 unit/day (INSIGHT) algorithm for insulin glargine 300 U/mL (Gla-300)

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Background and aims: New basal insulin GLA-300 provides a flat and prolonged PK/PD profile with a comparable glycemic control and less hypoglycemia vs glargine 100 U/mL (GLA-100).

Materials and methods: In the phase III EDITION trials, insulin was titrated by the HCP based on the median of the last 3 fasting prebreakfast SMPGs. Titration was scheduled once weekly, no more often than every 3 days. However, with GLA-100, the INSIGHT pragmatic 1U/day self-titration protocol is widely used in Canada. The objective of this 12-week, randomized, descriptive pilot study was to compare safety and efficacy of two titration algorithms, INSIGHT and EDITION, for GLA-300 in T2DM patients (insulin-naïve or on basal insulin ± OAD) mainly in a primary care setting.

Results: Baseline characteristics of the 212 patients randomized in the study were similar: age 62.3 years, BMI 34.2 kg/m2, A1c 8.4%, insulin naïve 37.0%, prior basal insulin dose 57.2 U. Comparable number of patients in each titration group reached primary endpoint of a fasting SMPG ≤ 5.6 mmol/L without nocturnal (0:00-6:00 h) hypoglycemia (confirmed: SMPG ≤3.9 mmol/L or symptomatic or severe) at 12 weeks (INSIGHT algorithm 22.8%; EDITION 20.6%). No between treatment differences in number of severe hypoglycemia were noted (1 INSIGHT algorithm vs 3 EDITION). The percentages of patients achieving A1c ≤7% was 28.7% (INSIGHT algorithm) vs 28.4% (EDITION) with a similar mean A1c (SD) of 7.6% (0.9) at week 12. Similar number of patients experienced nocturnal hypoglycemia (INSIGHT algorithm 28.7%; EDITION 27.5%). Mean (SD) insulin dose at week 12 was comparable in both titration algorithms (INSIGHT algorithm 67.0 U (37.8); EDITION 70.0 U (43.1)). Mean change (SD) in weight from baseline was 0.41 kg with INSIGHT algorithm and 0.15 kg (2.4) with EDITION. No between groups differences in adverse events were noted.

Conclusion: In conclusion, application of a self-titration of 1 U/day algorithm with GLA-300 resulted in a good safety profile, was effective and comparable to the previously tested EDITION algorithm.

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Supported by: Sanofi Canada

Disclosure: **J. Yale:** Other; Sanofi Canada.

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Biphasic vs basal bolus insulin in type 2 diabetes: real world clinical outcomes at one year post insulin initiation in UK general practice

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Background and aims: Treatment intensification using insulin therapy is often necessary in people with type 2 diabetes (T2D) but there is limited evidence from real-world practice to inform the specific choice of insulin regimen when added to oral therapy. We aimed to evaluate the 'real world' effects of biphasic compared with basal-bolus insulin regimens on glycaemic control and body weight in a large cohort of UK patients starting insulin in primary care.
Materials and methods: Data were sourced from the The Health Improvement Network (THIN) database. Insulin naïve patients were propensity-score matched based on their baseline characteristics and grouped by insulin regimen. Changes in HbA1c and body weight at 1 year post-insulin initiation were determined. A linear regression model was fitted to determine the mean differences between these groups, while adjusting for confounders.
Results: In a cohort of 18,227 patients (mean age 52.8±14.1yrs, HbA1c 8.64±1.83%; weight 90.8±18.7kg, 53.2% male) at 1-year post insulin-initiation, the mean adjusted difference in change in HbA1c was 0.05% lower in the basal-bolus regimen group compared to the biphasic group (β = -0.05%; 95%CI:-0.09 to 0.001; p = 0.05), independent of age, duration of diabetes, BMI, baseline HbA1c, e-GFR, lipid profile and occurrence of hypoglycaemia. However, for body weight, the mean adjusted difference in change was 0.16kg less (β = -0.16; 95%CI:-0.42 to -0.10, p-value = 0.235) in the basal-bolus insulin regimen group compared to the biphasic regimen, independent of other glucose-lowering therapies combined with the insulin regimen, age, BMI, BP, antihypertensive medication, height, baseline HbA1c, e-GFR and lipid profile.
Conclusion: Among patients starting insulin in routine practice in the UK, those receiving a basal-bolus regimen showed only a marginally greater reduction in HbA1c level compared with those receiving a biphasic insulin regimen. Weight change was not significantly different between the two insulin regimen groups.
Disclosure: **U.C. Anyanwagu:** None.

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Impact of time to basal insulin initiation on glycaemic control in type 2 diabetes patients: an electronic medical record database analysis

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Background and aims: Many patients who would benefit from insulin therapy do not receive it or do not receive it in a timely manner. As such, clinical inertia is highly prevalent despite a key goal of T2DM treatment being achieving HbA1c control earlier and maintaining control longer. This study assessed treatment patterns and HbA1c control in a real world setting by evaluating patients with uncontrolled T2DM initiating treatment with basal insulin using a retrospective electronic medical record database.
Materials and methods: Patients with a T2DM diagnosis (ICD-9-CM codes 250.x0 or 250.x2) between 1/1/2007 and 12/31/2014, were identified in the GE Centricity database. Patients initiating basal insulin (date of first observed basal insulin record termed index date) and having an HbA1c test >7% in the 6 months pre-index date were identified. Patients were required to have 24 months pre- and 12 months post-index date physician history. Patients were stratified by duration of time with uncontrolled HbA1c (>7%) before basal insulin initiation (i.e, <6, 6-12, 12-18, 18-24 months). Study measures included pre- and post-index date HbA1c and patient demographic and treatment characteristics.
Results: A total of 37,053 patients met the inclusion criteria. Mean (SD) patient age and Charlson comorbidity index score was 60.4 (12.0) years and 1.0 (1.5), respectively, and 65.6% of patients received an oral antidiabetic agent in the 24 months before basal insulin initiation. Before initiating basal insulin, mean (SD) HbA1c was 9.5% (1.9), with 40.7% of patients uncontrolled <6 months, 15.3% uncontrolled 6-12 months, 16.0% uncontrolled 12-18 months, and 28.0% uncontrolled 18-24 months. There was little variation in baseline HbA1c by duration of time uncontrolled (range: 9.2% [1.7] for patients uncontrolled 6-12 months to 9.6% [1.8] for patients uncontrolled 12-18 months). Patients with uncontrolled HbA1c <6 months had the largest change in HbA1c during follow-up (mean [SD] change in HbA1c of 1.2% [2.2], 62.3% had HbA1c < 8%), while patients with uncontrolled HbA1c 18-24 months had the smallest change in HbA1c during follow-up (mean [SD] change in HbA1c of 0.8% [1.8], 43.5% had HbA1c < 8%). Despite improvements in HbA1c control during follow-up, only 20.3% of patients achieved HbA1c ≤ 7% and only 53.4% of patients achieved HbA1c ≤ 8%.
Conclusion: In a large clinical practice database, despite improvements in HbA1c following basal insulin initiation, over 46% of patients still had HbA1c >8% during follow-up, with patients with the longest period of uncontrolled Hba1c during baseline the least likely to achieve HbA1c <8%. This study highlights a large unmet need in diabetes treatment and suggests there would be benefit from earlier introduction of basal insulin or alternative therapeutic options to assist patients in achieving HbA1c targets.
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Clinical perspectives from the BEGIN and EDITION programmes: trial-level meta-analyses outcomes with either degludec or glargine 300 U/mL vs glargine 100 U/mL in type 2 diabetes

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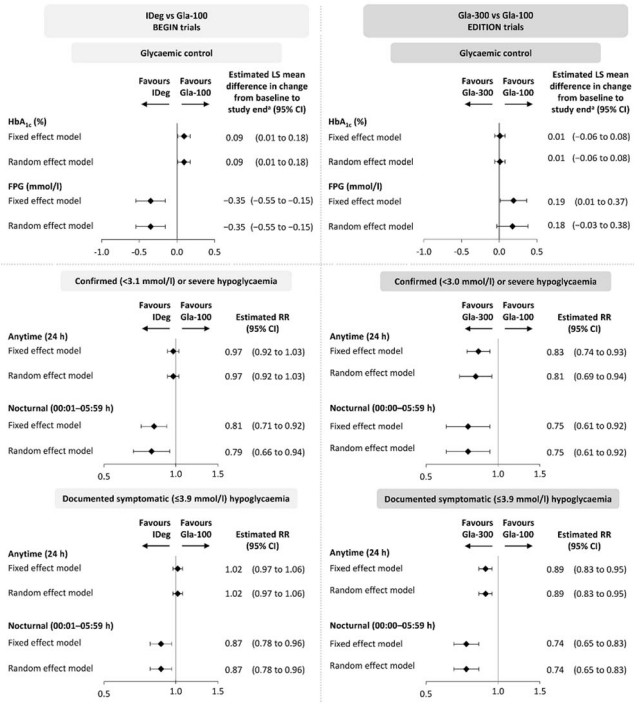
Background and aims: The BEGIN and EDITION programmes included a broad population of adults with T2DM on basal-bolus or basal-oral therapy as well as those who were insulin naïve. Efficacy and safety of insulin degludec (IDeg) and insulin glargine 300 U/ml (Gla-300) were compared with insulin glargine 100 U/ml (Gla-100) in the BEGIN and EDITION programmes, respectively.
Materials and methods: HbA_{1c}, fasting plasma glucose (FPG) and hypoglycaemia incidence with IDeg or Gla-300 vs Gla-100 were explored in 2 trial-level meta-analyses of clinical trials in T2DM (Figure).
Results: HbA_{1c} reduction was significantly greater for Gla-100 vs IDeg despite FPG reduction being significantly more pronounced with IDeg (Figure). HbA_{1c} reduction was comparable with Gla-300 and Gla-100, whereas FPG reduction was significantly greater with Gla-100 in the fixed but not random effect model. Risk of ≥1 confirmed (<3.1 mmol/l) or severe hypoglycaemic event was lower with IDeg vs Gla-100 at night (00:01-05:59 h) but comparable at any time (24 h) (Figure). Risk of ≥1 confirmed (<3.0 mmol/l) or severe hypoglycaemic event was lower with Gla-300 vs Gla-100 at night (00:00-05:59 h) and also at any time (24 h). Furthermore, the risk of ≥1 documented symptomatic (≤3.9 mmol/l) hypoglycaemic event with IDeg or Gla-300 vs Gla-100, both at night and at any time, closely reflected that of confirmed (<3.1 or <3.0 mmol/l)

or severe events (Figure). Risk of ≥1 severe hypoglycaemic event was comparable with IDeg or Gla-300 vs Gla-100.

Conclusion: In trial-level meta-analyses in T2DM, Gla-100 reduced HbA_{1c} more than IDeg despite IDeg having a greater FPG-lowering effect. Hypoglycaemia risk was lower with IDeg vs Gla-100 for nocturnal but not anytime events. Gla-300 provided comparable glycaemic control to Gla-100 with lower risk of anytime and nocturnal hypoglycaemia. Head-to-head trials of IDeg vs Gla-300 are warranted.

Figure. Estimated LS mean differences in HbA_{1c} and FPG, and relative risk of ≥1 hypoglycaemic event, in T2DM†: two trial-level meta-analyses of data from IDeg vs Gla-100 and Gla-300 vs Gla-100 clinical trials



†T2DM analysis pool includes studies: Five BEGIN studies (IDeg vs Gla-100): BEGIN Basal-Bolus type 2 (52 weeks, basal-bolus), BEGIN Once Long (52 weeks, insulin-naïve), BEGIN Low Volume (26 weeks, insulin-naïve), BEGIN Flex (26 weeks, insulin-naïve or basal ± OADs), BEGIN Once Asia (26 weeks, insulin-naïve); Four EDITION studies (Gla-300 vs Gla-100): EDITION 1 (26 weeks, basal + mealtime insulin), EDITION 2 (26 weeks, basal insulin + OADs), EDITION 3 (26 weeks, insulin-naïve), EDITION JP 2 (26 weeks, basal insulin + OADs). Study durations selected based on time of primary endpoint. No significant heterogeneity of treatment effect across studies was observed (p>0.05). [a]LOCF analysis was used for the BEGIN studies and MMRM analysis was used for the EDITION studies except for FPG data in EDITION JP 2, for which LOCF analysis was used.

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Improvement in quality of life after initiation of basal insulin therapy, results from the ORBIT study

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Background and aims: The effect of insulin therapy on quality of life in real world is uncertain. Till now, there is no large scale study regarding whether the introduction of basal insulins (BI, including insulin glargine, detemir and NPH) therapy can improve the health-related quality of life (HRQoL) in real world clinical practice. To determine the effects on HRQoL after initiating BI in insulin naïve patients with type 2 diabetes mellitus (T2DM) uncontrolled by oral antihyperglycemic drugs (OADs), we investigated the change of HRQoL and its predictors based on an Observational Registry of Basal Insulin Treatment (ORBIT) study.

Materials and methods: Totally 18,995 patients inadequately controlled (HbA1c≥7%) with OADs and willing to accept BI treatment were registered at 209 secondary or tertiary hospitals at eight geographic regions of China. Type of BI to initiate was at physician's discretion and patient's willingness. HRQoL was assessed at baseline and at the end of follow-up (6 months) using the EuroQol-5 dimensions 3 levels (EQ-5D-3L) questionnaire. A cohort population who kept using BI during follow-up was used for analysis. Chinese time trade-off values were used for calculation of EQ-5D utility index. Descriptive statistics and paired t-test were used to describe and compare the EQ-5D utility index and visual analogue scale (VAS) score, while the Chi-square test was conducted to compare the proportions of levels of each dimension between baseline and 6 months. Linear ordinary least squares regression model was used to explore the predictors of changes in EQ-5D VAS score.

Results: Excluding patients who stopped any insulin or switched BI therapy to premixed insulin during follow-up, 12358 patients were included in this study. HbA1c decreased from 9.5% to 7.4% after 6 months. The HRQoL measured by the EQ-5D VAS score increased by 5.6 ($p < 0.001$) from 76.6 to 82.3, and EQ-5D utility index score increased by 0.02 ($p < 0.001$) from 0.95 to 0.97 during 6 months. Statistically significant improvement was found in all five dimensions. The percentage of patients reporting no problems in the mobility, pain/discomfort, and anxiety/depression dimensions of EQ-5D increased significantly ($p < 0.001$) from 95.7% to 97.2%, 82.5% to 91.8%, and 85.3% to 95.4%, respectively. After controlling for age, gender, education degree, rural/urban area, BMI, duration of diabetes, HbA1c level, frequency of SMBG, hypoglycemia in past one month and times of insulin injection at baseline and 6 months as well as changes of diet consumption, physical activity, BI types, cost and total insulin dose during follow-up, we found that times of dose titration ($p = 0.0006$), dose increase ($p < 0.0001$), physical activity ($p < 0.0001$), consumption of fruit ($p = 0.0004$), HbA1c reduction ($p < 0.0001$) and use of long-acting BI analogues ($p = 0.0099$) were positively associated with the increase of VAS score, while the baseline HbA1c level ($p < 0.0001$) and consumption of staple food ($p < 0.0001$) were negatively associated with the increase of VAS score.

Conclusion: BI therapy in real world can improve the quality of life in patients with T2DM uncontrolled by OADs, especially for patients initiated with long-acting BI and with positive insulin dose titration, fruit intake, physical activity and better glycemic control.

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Disclosure: **P. Zhang:** None.

PS 090 Features of pregnancy in type 1 diabetes

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Glycaemic control and complication rate eleven-years post-partum in women with type 1 diabetes

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Background and aims: The aim of the present work was assess the degree of glycemic control and the presence of complications 11 years post-partum in women with type 1 diabetes (T1DM).
Materials and methods: In total 237 patients with T1DM were enrolled in the study; they were separated into 2 groups (Gr.): Gr.1 - 87 women with prepregnancy care(PC); based on albuminuria (NormA, MicrA) levels women were separated into 2 subgroups: SGr.1a - 46 women with NormA, SGr.1b - 41 women with MicrA. Gr.2 - 59 women without PC. Repeated examinations were performed 11 years post-partum.
Results: At entry HbA1c(%) levels for SGr.1a, 1b and Gr.2 were: 6.09 (0.08), 6.7 (0.8), 8.87 (1.6), respectively; by the end of the pregnancies they statistically decreased in all the groups (P=0.024, P=0.000, P=0.000, respectively). At entry percent of preproliferative retinopathy for SGr.1a, 1b and Gr.2 were 6,5; 24,3 and 13,5%,respectively; by term the percent has not increased. In SGr.1a patients percent of pre-eclampsia and preterm deliveries before 37 weeks of gestation were lower, than in SGr.1b and Gr.2 (pre-eclampsia - P1a-1b =0.0003; P1a-2 = 0.0001; preterm deliveries P1a-1b =0.0001; P1a-2 = 0.0001). Perinatal mortality was observed in SGr.1b (3.07%) and in Gr.2 (3.1%) of women. Repeated examinations 11 years post-partum showed that HbA1c levels were statistically higher, than at term: SGr.1a - 7.1 (0.11); SGr.1b - 7.4 (0.9) and Gr.2 - 8.4(1.68) (P=0.000). Kidney function indices worsened 11 years post-partum: in SGr.1a 8.6% of patients had MicrA; in SGr.1b - 70.7% - MicrA, and 21.9% - MacrA; in Gr.2 - 27.1% - MicrA, and 5.08% -MacrA. Five patients from SGr.1b are on regular hemodialysis. Percent of reproliferative retinopathy in SGr.1b and Gr.2 were 56.09% and 25.4%; percent of proliferative retinopathy in SGr.1a, 1b and Gr.2 were: 2.17, 19.5 and 10.1% (respectively). Patients from SGr.1b had statistically higher complications rates, than from SGr.1a (preproliferative retinopathy - P=0.0009, proliferative retinopathy - P=0.042, MicrA - P=0.0003, MacroA -P=0.03) and SGr.1b - Gr2 (preproliferative retinopathy - P=0.042, MicrA - P=0.0009, MacroA - P=0.035). While, women from Gr2 had statistically higher complication rates, than SGr1a (preproliferative retinopathy -P=0.041, MicrA -P=0.04).
Conclusion: If MicrA is present in the 1st trimester, pregnancy in T1DM patients significantly increases the risk of pre-eclampsia, preterm deliveries and perinatal mortality. Eleven years post-partum in all observation groups glycemia control deteriorated; the highest indices were registered in women without PC. The higher complication rate (retinopathy, micro- and macroalbuminuria) was found if pregnancy proceeded with MicroA. Even 11 yrs postpartum the lowest rate of complications was observed in women who received PC.
Disclosure: **N. Asatiani:** None.

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Changes in pre-conception treatment and glycaemic control in women with type 1 diabetes: 15 years one centre observation

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Background and aims: Pregnancy complicated by type 1 diabetes (T1DM) is associated with a high risk of obstetric and neonatal complications. Pregnancy planning and excellent glycemic control prior to conception help limit the number of outcomes. New therapeutic tools and methods of care have become available for women with type 1 diabetes (T1DM). The aim of the study was to assess the clinical characteristics of T1DM women, their pre-conceptional medical care and glycemic control during the first pregnancy visit over a period of 15 years.
Materials and methods: We analyzed the medical records of 524 pregnant T1DM women. The women received diabetes care during pregnancy between 1998 and 2012. This period was analyzed as three 5-year intervals. We assessed details of the patients' characteristics, use of various therapeutic tools, and glycemic control as assessed by HbA1c level at the 1st pregnancy visit. Appropriate statistical methods were used.
Results: We did not observe differences in the women s age (mean 28.4 ±5.2 for the entire study group), T1DM duration (mean 11.8±7.5 years), pre-pregnancy BMI (mean 23.9±4.4 kg/m2), and pregnancy week at the 1st visit (8.7). The number of women planning pregnancy did not change and reached 32.1% in the 1st period (1998-2002), 44.4% in the 2nd period (2003-2007) and 40.4% in last period (2008-2012) (p=0.11). The number of women treated with modern technologies increased rapidly: the use of rapid-acting analogues of insulin went up from 2.6% to 46.5% and 95.6%; (p<0.001). Similarly, personal pumps before pregnancy were available only to 4.6% of T1DM women in the first period, which increased to 23.5% and 33.3% in the 2nd and 3rd period, respectively; (p<0.001). We observed a small decrease of HbA1c level at the 1st pregnancy visit over the study period from 7.4±1.6% (57mmol/mol) to 7.1±1.5% (54mmol/mol) and 7.0±1.4% (53mmol/mol); (p<0.05). Additionally we found a statistically lower level of HbA1c at the 1st visit in female T1DM patients who entered pregnancy planning program within all three study periods. Specifically, in the 1st time period in planned pregnancies the HbA1c level reached 6.8% (51mmol/mol) and was better than in unplanned pregnancies - 7.6% (60mmol/mol); (p=0.003). For the 2nd and 3rd period, the following values were recorded: 6.6% (49mmol/mol) vs 7.1% (54mmol/lmol); (p=0.009) and 6.2% (44mmol/mol) vs 7.5% (58mmol/mol); (p=0.01), respectively.
Conclusion: We observed a rise in the use of modern therapeutic tools (insulin analogs, personal pumps) in T1DM women before their pregnancies. This was accompanied by a some improvement in glycemic control at the 1st pregnancy visit. However, the proportion of women planning their pregnancies remained stable.
Disclosure: **K. Cyganek:** None.

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Insulin and glucose in the three trimesters of pregnancy are independently associated with neonatal growth parameters in women with type 1 diabetes

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Background and aims: In addition to maternal glucose promoting fetal growth in the 2nd and 3rd trimesters (T), 1st T conditions such as fasting plasma glucose and embryo culture media are associated with neonatal growth parameters. Overgrown foetuses can also exert siphoning on maternal glucose. We aimed to analyze the association of maternal insulin doses and glycemic regulation in the three trimesters with neonatal and placental growth parameters at birth.
Materials and methods: We studied a cohort of 472 women with Type 1 DM and singleton pregnancies attended in the Diabetes and Pregnancy Clinic of the center. The dependent variables analyzed were: birth weight (BW), BW standard deviation score (BWSDS), neonatal length, ponderal index (PI) and placental weight (PW). PI was calculated as 100*BW/

length3. Maternal mean self-monitored blood glucose (MBG, mg/dl), HbA1c (%) and insulin doses (IU/kg/day) in 1st, 2nd and 3rd T were addressed as potential independent variables. Analyses were adjusted by gestational age (GA) at booking, maternal age, height, prepregnancy BMI, smoking habit, prior pregnancies, prior macrosomia, nephropathy, weight gain, GA at delivery and fetal sex. Statistics: results are expressed as %, mean + SD or P50 (P25,P75) according to distribution; multiple linear regression analyses (forward method); significance was set at a bilateral p<0.05.

Results: Average BW was 3350 g (2970, 3700), BWSDS 0.95 ± 1.22, length 49 cm (47.5, 50.0), PI 2.90 g/cm3 (2.70, 3.06), PW 620 g (330, 770); 50.3% of newborns were male and GA at delivery was 38.0 weeks (37.0, 38.0). B coefficients and significant p values are displayed in the table only for insulin and glucose-related variables. 1st T insulin dose, 2nd T MBG and/or 3rd T HbA1c were positively associated with neonatal growth parameters while 3rd T insulin dose displayed a negative relationship. No glucose or insulin variable was associated with PW.

Conclusion: In this cohort insulin and glycemic parameters in the three trimesters were independently associated with neonatal growth parameters. The positive association of 1st T insulin dose can be attributed to the growth promoting effects of maternal insulin and the negative association of 3rd T insulin dose to fetal glucose siphoning.

Maternal characteristic	Birthweight (g)		BWSDS		PI (g/cm3)		Length (cm)	
	B	p	B	p	B	p	B	p
1st T MBG (mg/dl)								
2nd T MBG (mg/dl)	6.878	0.000	0.016	0.000			0.026	0.001
3rd T MBG (mg/dl)								
1st T insulin (IU/kg/day)			0.722	0.042				
2nd T insulin (IU/kg/day)								
3rd T insulin (IU/kg/day)	-259.69	0.002	-0.951	0.000			-0.838	0.028
1st T HbA_{1c} (%)								
2nd T HbA_{1c} (%)								
3rd T HbA_{1c} (%)					0.049	0.002		

Disclosure: **R. Corcoy:** None.

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Association of haemoglobin glycation index with macrosomia in pregnancies complicated by preexisting type 1 diabetes

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Background and aims: Haemoglobin glycation index (HGI) is defined as a difference between measured glycated haemoglobin A_{1c} (HbA_{1c}) and HbA_{1c} level predicted from patient's average glucose in self-monitoring record. It is considered a marker of individual's propensity for glycation for a given mean blood glucose level. It has been postulated that patients more prone to glycation may be at higher risk of diabetes complications. However, the use of HGI and its interpretation in clinical practice remain controversial. It is unknown if in pregnancies complicated by diabetes, HGI can be useful as an independent risk predictor of foetal and maternal adverse outcomes, in addition to HbA_{1c}. Our aim was to assess whether HGI predicts adverse pregnancy outcomes, and whether this association is independent from the patient's mean glucose.

Materials and methods: We analysed 442 singleton pregnancies in women with type 1 diabetes (T1D) that was diagnosed at least 1 year before conception. Cases of multiple pregnancy or miscarriage (till 20th week of pregnancy) were excluded. HbA_{1c} was assayed in every trimester in 375 term pregnancies and in the first two trimesters in 67 women with preterm delivery. Mean glucose in each trimester was calculated as an average of 7 to 9 daily glucometric self-measurements over a period of one week preceding the corresponding HbA_{1c} measurement. To predict HbA_{1c} from the mean glucose, linear regression model was used. Then HGI was calculated as the difference between measured and predicted HbA_{1c}.

Results: In logistic regression models adjusted for same-trimester HbA_{1c} we found that HGI in the second trimester was a statistically independent from HbA_{1c} predictor of macrosomia. If measured HbA_{1c} was higher than glucose-predicted HbA_{1c}, a protective association with macrosomia was observed (p=0.016, odds ratio for 1% HGI increase 0.33). This association was attenuated in the third trimester (p=0.086, odds ratio 0.52). There was no significant association of HGI with preterm deliveries, low birth weight, stillbirths of congenital malformations, when adjusted for HbA_{1c}. In other words, higher glycation (measured as HGI) for a given blood glucose level was associated with less risk of macrosomia and lower mean birth weight, but not higher incidence of low birth weight.

Conclusion: HGI is associated with the risk of macrosomia, but not with other adverse pregnancy outcomes in women with type 1 diabetes. Observed counterintuitive direction of association with macrosomia suggests that HGI is just a function of patient's average glycaemia. HGI captures changes in glycaemic control not reflected by HbA_{1c}. Higher HGI does not seem to correspond with increased individual's propensity for glycation of proteins and elevated risk of adverse outcomes. Our data do not support hypothesis that HGI is associated with adverse pregnancy outcomes in T1D.

Disclosure: **I. Lason:** None.

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Insulin resistance in the early pregnancy and subsequent preeclampsia in women with type 1 diabetes

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Background and aims: Despite improvement in diabetes care over the years, incidence of preeclampsia is still very high even in a well controlled type 1 diabetic mothers. Aside from the already studied effects of hyperglycemia, it could be suspected that insulin resistance (IR) may also play an important role in preeclampsia. However, data on the association between preeclampsia and IR in women with T1DM is scarce. Therefore, the aim of our study was to determine whether insulin resistance in the early pregnancy might contribute to higher rate of preeclampsia in this population.

Materials and methods: Prospective single-center study on a population of 165 women with T1DM admitted to the perinatal center for women with diabetes between June 2012 and December 2014. According to pregnancy outcome women were divided into 3 subgroups: normotensive (N=141), developing pregnancy induced hypertension (PIH) (N=8), developing preeclampsia (PE) (N=16). Anthropometric, clinical and laboratory data were collected in the first trimester (<12th week). Insulin resistance was quantified using estimated glucose disposal rate formula (eGDR, milligrams/kilogram/minute). Decreasing eGDR correlates with increasing IR.

Results: Women from subgroups did not differ according to the age, BMI and waist-to-hip ratio. Women from PE subgroup were diagnosed with diabetes at the younger age (10±4 y) as compared to normotensive (17±8 y) and PIH women (21±8 y), P=0.001. Significantly higher proportion of women developing PE had some form of diabetic vasculopathy (PE- 12/16=75% vs PIH- 1/8= 12.5% vs normotensive- 28/141=19.9%). The eGDR [median, inerquartile range] in PE women was significantly lower (10.1, 6.6-11.0) than in normotensive (10.8, 9.6-11.5) and PIH women (11.3, 10.8-11.8), P=0.018. There was no difference between eGDR in normotensive and PIH women. PE women had significantly higher HbA1c levels (7.8%, 6.6-8.9%) than normotensive women (6.6%, 5.9-7.5%), P=0.047, with no difference observed in PE vs PIH (6.6%, 6.3-6.9) and normotensive vs PIH. No difference was observed in total cholesterol, LDL, HDL and triglycerides between subgroups.

Conclusion: Increased insulin resistance in the early pregnancy and younger age at diagnosis of diabetes are observed in women with type 1 diabetes with subsequent preeclampsia but not pregnancy induced hypertension. Further studies are needed to investigate the role of eGDR assessment in the prediction of preeclampsia in pregnant women with type 1 diabetes.

Supported by: PUMS, PTD

Disclosure: **P. Gutaj:** None.

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The alteration of placental vascular permeability and AGE-RAGE system in rats with gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) is defined as a glucose intolerance of any degree with onset or first recognition during pregnancy. Accelerated formation of advanced glycation end products (AGEs) is associated with hyperglycemia. AGE-RAGE system is an important mechanism of diabetes-related vascular complications. Low molecular weight heparin (LMWH) can competitively bind to receptor for advanced glycation end products (RAGE) and the affinity of this binding is higher than AGEs. This study aimed to investigate the alteration of placental vascular permeability in GDM rats and whether the alteration is related to the AGE-RAGE system.

Materials and methods: Adult pregnant rats were randomly divided into three groups: control group, GDM group and LMWH group. Streptozotocin (STZ) was administered intraperitoneally in rats of GDM group and LMWH group to induce GDM rats model. Rats in LMWH group received LMWH subcutaneously since pregnant day 4. All rats were sacrificed at pregnant day 16. The vascular permeability was measured by Miles assay presented by leakage of Evans Blue. Endothelial cell tight junction protein Occludin was measured by western blot. Maternal serum levels of AGEs were measured by ELISA. RT-qPCR was adopted to measure the expression of VEGF mRNA and RAGE mRNA.

Results: GDM rats exhibited a significant increase in leakage of Evans Blue, serum levels of AGEs and expression of VEGF mRNA and RAGE mRNA when compared with the control rats (leakage of Evans Blue, 0.53 ±0.05ng/mg vs 0.35±0.06ng/mg, P<0.01; serum levels of AGEs, 121.26 ±28.22ng/ml vs 67.43±22.84ng/ml, P<0.01; expression of VEGF mRNA, P<0.01; expression of RAGE mRNA, P<0.01). The expression of Occludin was reduced in GDM rats compared tocontrol rats(P<0.05). The intervention of LMWH resulted in a significant decrease in leakage of Evans Blue and expression of VEGF mRNA when compared with GDM rats (leakage of Evans Blue, 0.44±0.02ng/mg vs 0.53±0.05ng/mg, P<0.05; expression of VEGF mRNA, P<0.01). The expression of Occludin was higher in rats treated with LMWH than that in GDM group (P<0.05).There were no differences between LMWH group and GDM group in serum levels of AGEs and expression of RAGE mRNA.

Conclusion: The placental vascular permeability in GDM rats increases, which may be associated with the alteration of AGE-RAGE system. The intervention of LMWH exhibits a protective effect against GDM in alteration of vascular permeability.

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Disclosure: **Y. Shi:** None.

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ATLANTIC DIP: type 1 and 2 diabetes: prepregnancy, pregnancy and beyond

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Background and aims: Many patients with type 1 and 2 diabetes struggle to meet therapeutic targets. However, during pregnancy women are highly motivated to achieve tight glycaemic goals and typically receive intensive education and support from a specialist service. Those who attend prepregnancy care (PPC) benefit from additional input for approximately 6 months prior to pregnancy. This study sought to assess the impact of pregnancy and PPC on long term treatment goals in women with diabetes.

Materials and methods: We included women with type 1 and 2 diabetes who attended the Atlantic DIP programme for PPC and antenatal care between January 2006 and December 2014. Women were evaluated at six months prepregnancy (or at first PPC visit), and again at twelve months post-partum.

Results: In total 269 women were included, 177 (66%) with type 1 diabetes and 92 (34%) with type 2 diabetes. One hundred and seventeen (44%) attended prepregnancy care. At 12 months post-partum, 70 (26%) were again attending PPC, 26 (9.7%) were pregnant again, 40 (14.9%) were lost to follow up and the remaining 133 (49.5%) were attending routine diabetes clinics. For all women despite achieving tight glycaemic control in the first trimester of pregnancy (mean HbA1c 7.2 ±1.6%), there was no significant difference in HbA1c before and twelve months after pregnancy (before: 7.8±1.9%, after: 7.6±1.7%, p=0.26). Furthermore, there was no difference in mean systolic/diastolic blood pressure (SBP/DBP), lipid profile, albumin-creatinine ratio (ACR) or weight in women before and after pregnancy. However, at 12 months post partum, those who attended PPC in preparation for pregnancy had a lower SBP (126.3±14.6mmHg vs 119.8±17.0mmHg, p=0.001), DBP (77.6 ±9.9mmHg vs 74.8±8.0mmHg, p=0.04) and weight (75.0±14.4kg vs 81.0±20kg, p=0.04) than those who did not attend PPC. In addition, women who achieved a first trimester HbA1c of <7.0% after PPC continued to demonstrate superior glycaemic control at 12 months post-partum (6.8±1.3% vs 8.4±1.8%, p<0.001).

Conclusion: Despite intensive education and personal motivation during pregnancy, women with diabetes do not have a sustained improvement in measures of diabetes control and weight at 12 months post-partum. However the benefits of PPC continue beyond pregnancy with women who have attended PPC continuing to have better glycaemic control, lower BP and lower weight at 12 months post-partum. Our challenge now is to engage all women in PPC so that these long term benefits can be gained by all women with established diabetes.

Supported by: Health Research Board of Ireland

Disclosure: **A.M. Egan:** None.

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Parity and future risk of diabetes in patients with breast cancer

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Background and aims: Diabetes increase risk of breast cancer. Multiparity increase risk of diabetes while it decrease risk of breast cancer. Little is known about the impact of parity on diabetes in women with breast cancer.The aim of this study was to examine the association between parity and the risk of diabetes among breast cancer patients.

Materials and methods: This retrospective cohort study enrolled breast cancer patients without diabetes from 2002/1/1 to 2014/12/31 in our hospital. Incident diabetes after breast cancer being diagnosed was defined by medical chart and clinical physicians. Information of Parity was obtained by history and was defined as the number of live births (no live births [nulliparity], one, two, three and four or more live births [grandmultiparity]).

Parity and risk of diabetes was estimated with Cox proportional hazard regression models, adjusting for age at breast cancer diagnosis, hypertension, body mass index (BMI) and treatment with chemotherapy.
Results: Overall, 3508 breast cancer patients were included. Over an average of 5.5 person-years of follow-up, 397 incident cases of diabetes were noted after breast cancer being diagnosed. The BMI (kgs/m2) were 22.7, 23.7, 23.6, 24.3 and 25.5 for nulliparity, parity one, two, three and grandmultiparity, respectively (p for trend <0.001). After adjusting competing risk of death, in multivariate analysis, nulliparity, parity 1, 3 and grandmultiparity were associated with an increased risk of being diagnosed with diabetes compared with parity 2 [relative risks: 1.74 (95% confidence interval 1.12-2.67), 1.28 (0.77-2.09), 1.32 (0.92-1.90) and 2.12 (1.47-3.10), respectively.
Conclusion: Higher parity is associated with increasing BMI. However, only nulliparity and grandmultiparity increase future risk of diabetes for patients with breast cancer.
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Disclosure: **C. Lee:** None.

PS 091 Pregnancy: factors that impact outcome

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Glucose alteration in pregnancy and risk of adverse neonatal outcomes

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Background and aims: Both gestational diabetes (GDM) and pregestational diabetes in pregnancy (DM) are associated with worse neonatal outcomes compared with normal pregnancy. Others clinical conditions, primarily represented by hypertensive and thyroid disorders, could complicate pregnancy outcome. Aim of our study was to estimate the risks of adverse neonatal outcomes of pregnancies complicated by GDM and DM considering also the role of other important concomitant diseases.
Materials and methods: Administrative data of the Italian Puglia region from 2002 until 2012 were analyzed. Singleton pregnancies complicated by GDM and DM and their neonatal outcomes were selected. Risks of adverse neonatal outcomes for GDM and DM compared with normal pregnancy were estimated by multivariate analyses after adjusting for age, hypertensive and thyroid disorders and drugs use, the latter being a proxy of pregnancy complexity.
Results: From a total of 135,163 pregnancies 1,357 complicated by GDM and 234 by DM were selected. Both GDM and DM were associated with higher risks of neonatal hypoglycemia (OR=10.1; 95%CI 8.5-11.9 and OR=36.0; 95%CI 27.1-47.8, respectively), SGA (OR=1.7; 95%CI 1.4-2.0 and OR=5.8; 95%CI 4.4-7.7, respectively), LGA (OR=1.7 95%CI 1.3-2.1 and OR=7.9 95%CI 5.7-10.9, respectively), jaundice (OR=1.7; 95%CI 1.5-2.0 and OR=2.6; 95%CI 1.8-3.6, respectively), fetal malformations (OR=2.2; 95%CI 1.7-2.8 and OR=3.5; 95%CI 2.2-5.7, respectively), hypocalcemia and hypomagnesemia of newborn (OR=1.8; 95%CI 1.2-3.0 and OR=9.2; 95%CI 5.5-15.5, respectively) and cesarean section delivery (OR=1.9; 95%CI 1.7-2.2 and OR=8.5; 95%CI 5.6-12.9, respectively) compared with pregnancies with normal glucose tolerance. Pre-gestational diabetes but not GDM was associated with high risk of respiratory distress (OR=2.7; 95%CI 1.7-4.3 and OR=1.1; 95%CI 0.9-1.5, respectively) and polidramnios (OR=46.5; 95%CI 19.52-110.57 and OR=2.32; 95%CI 0.57-9.52, respectively).
Conclusion: GDM and DM are associated with adverse neonatal outcomes independently of the presence of other clinical conditions complicating the pregnancy. Greater attention should be placed to the care of pregnant women with GDM and DM.
Disclosure: **B. Pintaudi:** None.

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The excessive weight gain during pregnancy is associated with adverse maternal and fetal outcomes

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Background and aims: The incidence of obesity and gestational diabetes (GDM) is rising worldwide along with the number of overweight (OW)/obese (OB) women who get pregnant. Both obesity and GDM are risk factors for adverse maternal and neonatal outcomes. Nonetheless, the relative impact of the two conditions on the overall risk is still undefined. The aim of our study is to ascertain the independent role of pre-pregnancy

BMI (pgBMI), gestational weight gain (GWG), and GDM on maternal and fetal outcomes.
Materials and methods: We analyzed the data of 1198 pregnant women (age 34.5±4.6 years; pgBMI 23.9±4.7 kg/m^2; Caucasian 94.7%; primiparous 42%; twin pregnancy 2.2%) screened for GDM between January 2010 and March 2015. Data on maternal and fetal outcomes were based on hospital discharges and medical records of both mother and newborn.
Results: At conception 22.9% of women were OW and 11.7% OB; during pregnancy 33.2% had excessive GWG. Mean gestational age at delivery was 39.7±1.7 weeks and 37% of women had Cesarean section (CS). Prevalence of GDM was 39.7%. Women with GDM were more frequently OB (18.7% *vs.* 6.7%; p<0.0001) than those with normal glucose tolerance (NGT), with no difference in GWG. CS frequency was superimposable in GDM and NGT women. Prevalence of fetal macrosomia was 7.2%, with no difference between GDM and NGT women, while SGA (7.6% *vs.* 3.5%) and LGA (6.4% *vs.* 1.9%) were more frequent in newborns of GDM women (p<0.01). Offspring of women with GDM were born slightly earlier (39.6±1.6 vs. 39.8±1.8 wks; p<0.05). There was no difference in neonatal hypoglycemia. GWG correlated with neonatal body weight. This correlation persisted after adjustment for gestational age at delivery and pg-BMI. Prevalence of excessive GWG increased with increasing pg-BMI. Offspring of women with GWG lower than recommended were born earlier (39.4±2 *vs.* 39.8±1.6 weeks; p<0.0001) and weighed less (3100±511 g *vs.* 3287±524; p<0.001) than those born to mothers with regular GWG. On the contrary, offspring of women with excessive GWG weighed more (3405±510 g *vs.* 3287±524; p<0.01). On a logistic regression analysis, excessive GWG was an independent risk factor for macrosomia (OR 2.95, 95% CI 1.14-7.70; p<0.05) and gestational age at delivery over 40 weeks (OR 1.91, 95% CI 1.19-3.07; p<0.01). Factors associated with CS were twin pregnancy (OR 16.48, 95% CI 2.10-129.58; p <0.01), parity (OR 1.48, 95% CI 1.03-2.12; p<0.05), gestational age at delivery <37 weeks (OR 4.17, 95% CI 1.73-10.06; p<0.002), prior CS (OR 62.52, 95% CI 14.78-264.49; p<0.0001) and, of borderline statistical significance, also excessive GWG (OR 1.63, 95% CI 0.99-2.67; p=0.05). GDM was not an independent risk factor for adverse outcomes in this cohort.
Conclusion: Excessive GWG rather than GDM is associated with adverse maternal and fetal outcomes. These findings call for early education and implementation of healthy lifestyle in women planning a pregnancy and careful monitoring of GWG during the pregnancy.
Disclosure: **C. Bianchi:** None.

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Sleep quality in early pregnancy is associated with early-onset gestational diabetes and adverse perinatal outcome in obese pregnant women

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Background and aims: Sleep-Disordered Breathing (SDB) is characterized by impaired ventilation during sleep resulting in intermittent hypoxia. Evidence concerning association between SDB and maternal and neonatal outcome remains conflicting. We evaluated self-reported parameters of sleep quality in early pregnancy: daily amount of sleep (AOS), number of days with snoring per week (SD) number of days with insufficient amount of sleep per month (IAS) as predictors of hyperglycemia and perinatal complications in obese pregnant women.
Materials and methods: 905 participants of DALI study that aimed at prevention of gestational diabetes mellitus (GDM) diagnosed following IADPSG/WHO 2013 criteria. Inclusion criteria involved singleton viable pregnancy, gestational age (GA) below 20 weeks, BMI >=29.0 kg/m2 and no history of diabetes. Statistical analysis: logistic regression and ROC analysis with sleep quality parameters as predictors and GDM and perinatal complications as dependent variables.
Results: Characteristic of the study group at entry: maternal age: 31.5±5.4 years; GA: 15.3±2.3 weeks; BMI: 34.5±4.0 kg/m2; AOS: 7.8±1.4 hours; IAS: 10.6±9.6 days/month; SD: 1.7±2.5 days/week; NC: 36.5±2.2 cm; multiparity: 62.7%, shift work: 20.9%, nonsmokers: 52.9%. Perinatal outcomes: chronic hypertension:12.6%, preeclampsia: 2.9%, gestational hypertension (PIH): 11.1%, GDM at entry (early-onset GDM/e-GDM): 25.0%, GDM in late pregnancy: 26.7%, premature delivery: 5.7%, congenital malformations: 4.4%. Independent predictors for snoring were NC: OR 1.17 (1.11-1.25) and BMI: OR 1.06 (1.03-1.09). Snoring was associated with a risk of e-GDM: OR 1.52 (1.12-2.1). SD>3 days was independently associated with a risk of PIH: OR 1.13 (1.02-1.25). In women without e-GDM, shift work predicted fetal malformations: OR 2.3 (1.04-5.21).
Conclusion: Self-reported information concerning snoring in early pregnancy can be useful in assessment of fetomaternal risk in obese pregnant women.
Clinical Trial Registration Number: ISRCTN70595832
Supported by: HEALTH-F2—2009-242187
Disclosure: **A. Zawiejska:** None.

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Ethnic variations in glucose and the interaction with fetal growth in a multi-ethnic inner city antenatal cohort

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Background and aims: Non-white ethnicity is a known risk factor for developing gestational diabetes mellitus (GDM). The prevalence of adverse materno-fetal outcomes in women diagnosed with GDM additionally varies according to ethnicity. However few studies have explored the impact of ethnicity on the interaction between glycaemia and fetal birthweight. We aimed to determine whether there was an independent effect of ethnicity on the interaction between maternal glycaemia, early pregnancy BMI and fetal birthweight.
Materials and methods: A retrospective analysis of pregnant women at risk of developing GDM in an inner-city healthcare centre was conducted (n=4562). This cohort underwent a diagnostic 75g oral glucose tolerance test at 24-28 weeks gestation. Baseline maternal demographics, glycaemia and fetal birthweight were compared across five ethnic groups: White Caucasian (Group 1, n=1379), Black African-Caribbean (Group 2, n=591), South

Asian (Group 3, n=392), Mixed ethnicity/ any other Asian ethnicity (Group 4, n=392) and Other/ Ethnicity unknown (Group 5, n=701). Regression analyses were used to determine if an interaction between glucose, early pregnancy BMI and fetal birthweight existed that was dependent on ethnicity: gestational age and diagnosis of GDM were adjusted for.

Results: Significant differences were observed in maternal age, early pregnancy BMI, fasting plasma glucose (FPG), 120-minute value glucose and fetal birthweight across the five ethnic groups (Table 1). Following adjustment for gestational age and GDM diagnosis, a 1mmol/L increase in FPG increased birthweight by 157g ($p<0.001$): this interaction was ethnic group dependent (effect size significantly smaller in ethnic groups 4 and 5). Interactions between 120-minute glucose value and fetal birthweight were also dependent on ethnicity with black African-Caribbean women exhibiting the largest effect size (27.6g increase in birthweight for every 1mmol/L increase in glucose). These interactions persisted following adjustment for early pregnancy BMI. The interaction between early pregnancy BMI and fetal birthweight was not dependent on ethnicity.

Conclusion: This study has demonstrated an ethnic group dependent effect on the interaction between glucose and fetal birthweight. However ethnicity did not impact on the interaction with early pregnancy BMI and fetal birthweight. These data suggest that ethnicity specific glucose thresholds for the diagnosis of GDM may be warranted.

Table 1: Baseline maternal demographics, assessments of glycaemia and fetal birthweight across the five ethnic groups

	Group 1: White Caucasian	Group 2: Black African-Caribbean	Group 3: South Asian	Group 4: Mixed/ Any Other Asian	Group 5: Other/ Not Known	P value
N	1379	591	392	1499	701	
Mean (SD) age (years)	34.1 (±5.3)	30.8 (±6.0)	31.9 (±4.2)	32.0 (±5.5)	32.2 (±5.6)	<0.001
Mean (SD) BMI (kg/m^2)	25.5 (±6.1)	28.1 (±6.0)	25.4 (±4.9)	25.4 (±5.8)	25.5 (±5.3)	<0.001
Mean (SD) FPG (mmol/L) *	4.33 (±0.46)	4.34 (±0.66)	4.47 (±0.69)	4.40 (±0.68)	4.31 (±0.70)	<0.001
Mean (SD) 120 min glucose (mmol/L)	5.42 (±1.36)	5.54 (±1.52)	6.09 (±1.74)	5.69 (±1.58)	5.60 (±1.55)	<0.001
Mean (SD) Birthweight (g)	3388 (±571)	3261 (±566)	3065 (±582)	3269 (±546)	3294 (±524)	<0.001

Abbreviations and footnotes:
SD, standard deviation; BMI, body mass index; FPG, Fasting plasma glucose.
Birthweight centile: Fetal birthweight adjusted for gestational age

Supported by: Novo Nordisk UK Research Foundation
Disclosure: **R. Agha-Jaffar:** None.

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Does the use of ultrasound (u/s) monitoring during GDM pregnancy help to prevent neonatal macrosomia?

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Background and aims: The aim of the study is to examine whether the intensive management of women with gestational diabetes mellitus (GDM), which takes into account not only glucose control but also the findings of serial u/s fetal measurements may help to reduce neonatal macrosomia.

Materials and methods: In this prospective study 60 pregnant women with GDM were included. Of them 35 women (58.3%) were treated with diet (GDM-D) following the Institute of Medicine recommendations and the other 25 (41.7%) were treated with insulin as well (GDM-I). For insulin initiation the following were taken into consideration: 30% of fasting glucose values >95mg/dl or of 1-hour glucose values >130mg/dl, despite complying with the diet instructions, and/or findings of the fetal u/s, namely abdominal circumference (FAC)>70percentile (pctl) or amniotic fluid index (AFI)>20. The patients were followed-up every two weeks at the outpatient diabetic clinic. The fetal u/s was performed by the same examiner at the time of GDM diagnosis (U/S-diag) and then monthly up to delivery (U/S-final).

Results: Regarding the pre-pregnancy BMI in the whole group of GDM women, 48% were of normal weight, 29% overweight and 23% obese. The GDM-I presented a greater percentage of obese women compared to the GDM-D subgroup (38.1% vs 12.1% respectively, p=0.048). The weight gain for the whole group was 10.5±3.4kg. There were no differences between the two subgroups according to weight gain, family history of diabetes, previous history of GDM and smoking. Concerning the u/s findings: (i) in the total GDM group FAC>70pctl at the initial U/S-diag was 16/60 (26.7%), compared to the U/S-final 8/60 (13.3%), p=0.022. Specifically in the GDM-D subgroup the percentages were as follows: FAC>70pctl in the U/S-diag was 7/35 (20%), while in the U/S-final it was 1/35 (2.9%), p=0.022, whereas in the GDM-I subgroup these were 9/25 (36%) and 7/25 (28%) respectively, p=ns. There was no correlation found between FAC and pre-pregnancy BMI or weight gain. (ii) AFI>20 was detected in the initial U/S-diag at 10/60 (16.7%), while in the U/S-final it was 4/60 (6.7%), p=0.058. Particularly in the GDM-D subgroup the percentages were as follows: AFI>20 in the U/S-diag was 5/35 (14.3%), while in the U/S-final it was 2/35 (5.7%), whereas in the GDM-I subgroup they were 5/25 (36%) and 2/25 (8%) respectively. Gestational age at delivery was 38.2 ±1.4 weeks (GDM-D= 38.4±1.4 vs GDM-I= 37.8 ±1.2, p=ns). The percentage of caesarean delivery was 31.7% (19/60), specifically in the GDM-D subgroup 10/35(28.6%) while in the GDM-I it was 9/25(36%), p=ns. Birth weight was 3238±477g (GDM-D=3194 ±462g and GDM-I= 3299±498g, p=ns). Of the neonates 2/60 were less than 10pctl (GDM-D=1/35 and GDM-I=1/25), while over the 90pctl was 1/60 in the GDM-I subgroup. The classification at the same percentile range (≤10, 11-89, ≥90) of the U/S-final compared to the actual birth weight was almost identical in 58/60 cases (accuracy = 96.7%).

Conclusion: The intensive management of GDM women taking into account the u/s fetal measurements contributed to the reduction both of u/s macrosomia by 50% and of amniotic fluid by over 60%. The actual result of this approach was that 57/60 neonates presented with normal birth weight where as only 1/60 was large for gestational age and 2/60 small for gestational age.

Disclosure: **E. Anastasiou:** None.

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Are there differences in the outcome of in vitro fertilisation vs spontaneous conception in pregnancies complicated by gestational diabetes?

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Background and aims: In Vitro Fertilization (IVF) is a popular method of assisted reproduction. IVF is associated with an increased risk of Gestational Diabetes Mellitus (GDM). In literature, there is limited evidence regarding the effect of IVF on the outcome in pregnancies complicated by GDM. The aim of our study was to investigate the clinical characteristics of IVF vs spontaneous conception pregnancies complicated by GDM and their affect on the maternal and fetal outcomes.

Materials and methods: A cross-section study was conducted in 102 singleton IVF vs 102 spontaneous conception pregnancies diagnosed with GDM. The demographic and clinical characteristics of the study groups are as follows: [(Mean±SD) age: 38.23±4.9 vs 34.1±3.2 years, p<0.001; BMI: 25.8±5.3 vs 23±4.1 kg/m2, p<0.001; HbA1c: 5.2±0.5 vs 5.2±0.7%, p=NS; Fasting Blood Glucose (FBG): 84.1±8.4 vs 84.2±7.1 mg/dl, p=NS; 1-hour postprandial BG: 103.6±11 vs 106.5±10mg/dl, p=NS;

week of diagnosis GDM: 21.8±5.2 vs 23.8±6.2, p=0.03; percentage of insulin users: 79.2 vs 77.3%, p=NS; week of starting insulin: 22.8±5 vs 24.8±5, p=0.03; insulin dose: 34.7±10 vs 29.5±16 iu/day, p=0.03; miscarriage history: 32.5 vs 30.8%, p=NS; smoking history: 30.5 vs 31.2%, p=NS].

Results: The summary of obstetric and neonatal history between the two groups is as follows: [Maternal weight gain: 10.4±4.4 vs 11.9±3.7 kg, p=0.053; week of delivery: 36.9±2 vs 37.4±0.7, p=0.04; neonatal birth weight: 2857±517 vs 2891±341 g, p=NS; women experienced hypoglycemia episodes: 24.5 vs 26.5%, p=NS; pre-eclampsia rate: 4.9 vs 3.9%, p=NS; Respiratory Distress Syndrome: 14.7 vs 12%, p=NS; Neonatal hypoglycemia: 17.6 vs 14.7%, p=NS; Jaundice: 20.6 vs 18.6%, p=NS; Neonatal Intensive Care Unit admittance: 15.6 vs 14.7%, p=NS; Caesarean Section (CS): 86.3 vs 56.9%, p<0.001]. There were 2 cases of perinatal mortality in the IVF group. Associations between clinical characteristics and the adverse outcomes were tested among the IVF group. CS as a complication was not included in the analysis. However, the data showed a significant higher incidence of CS in the IVF group, which could be partially attributed to physicians and/or patient's preference. Regarding the glycemic control, 1-hour Postprandial BG was associated with maternal-fetal complications (r=0.504, p<0.001). In addition, it was noted that there was no correlation between the FBG and HbA1c with maternal-fetal complications. Insulin dosage was associated with higher rate of maternal hypoglycemia (r=0.513, p=0.001), but it did not affect fetal outcome. History of preceding miscarriage was associated with smoking (r=0.299, p=0.007). BMI was not correlated with the week of GDM diagnosis. The statistical difference of the age and the BMI for the IVF group vs the spontaneous conception group may indicate that both age and increased BMI may have a negative affect on fertility.

Conclusion: There was no significant difference in the adverse pregnancy outcome of the IVF vs the spontaneous conception pregnancies complicated by GDM. It is apparent that strict postprandial metabolic control, which can be reached with intense early insulin therapy, can limit the incidence of the adverse pregnancy outcome. Our results indicated a need for earlier GDM screening in the IVF vs spontaneous conception pregnancies.

Disclosure: **P. Thomakos:** None.

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DALI lifestyle intervention in pregnant women with BMI ≥29 kg/m^2. Continuous glucose monitoring substudy

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Background and aims: The DALI study compared the impact of three interventions (healthy eating (HE), physical activity (PA), and HE + PA) vs a control group on the risk of GDM in a randomised controlled trial conducted in 9 European countries. In a subset of three of the participating centers, we performed a substudy investigating possible differences between the groups in 24h glucose values using continuous glucose monitoring (CGM). We aimed to compare average and variability of 24h glucose values measured by CGM in the four study groups.

Materials and methods: Pregnant women at risk of GDM (pre-pregnancy BMI ≥29 Kg/m2), ≤19+6 weeks of gestation, with normal glucose tolerance at baseline (IADPSG criteria) were included. The intervention was conducted by personal coaches, who had received standardized motivational interviewing training, and consisted of five face-to-face interviews and up to four telephone sessions. At 35-37 weeks CGM (iPro2, Medtronic) was performed for three days (minimum CGM information > 60% of the period required for inclusion). Sixty-four out of 198 women were included in the CGM substudy. Statistical analysis: variables are expressed as %, mean ± standard deviation or P50 (P25, P75) according to distribution; Chi-square test, ANOVA and Kruskal-Wallis ANOVA used for comparison.

Results: See table 1

Conclusion: In pregnant women with pre-pregnancy BMI ≥29 Kg/m2, lifestyle interventions did neither modify the average or variability of 24h glucose values measured by CGM at 35-37 weeks of gestation.

Variable	Control group N=16	Healthy eating (HE) N=16	Physical activity (PA) N=12	HE + PA N=20	p
Prepregnancy BMI (Kg/m^2)	32.7 (30.8, 33.7)	32.5 (31.3, 34.4)	31.7 (30.2, 33.0)	32.0 (29.7, 34.1)	0.702
Baseline fasting plasma glucose (mmol/l)	5.1 (4.9, 5.2)	4.8 (4.6, 5.2)	4.8 (4.7, 5.0)	4.8 (4.7, 5.0)	0.145
Diagnosis of GDM or overt DM (%)	33.3	37.5	27.3	16.7	0.560
Average glucose (mmol/L)	5.4 ± 0.4	5.3 ± 0.6	5.3 ± 0.4	5.5 ± 0.4	0.833
Day-time average glucose (mmol/L)	5.4 (5.2,5.8)	5.2 (5.0,5.8)	5.5 (5.2,5.8)	5.4 (5.2,5.9)	0.606
Night-time average glucose (mmol/L)	5.1 (4.7,5.3)	5.1 (4.9-5.6)	5.1 (4.4,5.6)	5.1 (4.7,5.8)	0.928
Glucose variability (arbitrary units)	2.4 (1.6,3.2)	2.0 (1.3,3.3)	2.4 (1.7,4.0)	2.3 (1.2,2.9)	0.883
Glucose stability (mg/dl/min)	0.027 (0.022,0.037)	0.024 (0.020,0.028)	0.024 (0.021,0.030)	0.027 (0.023,0.030)	0.474
Glucose level <3.3 mmol/L (%)	0.1 (0,2.3)	0 (0, 0.6)	3.2 (0, 6.5)	0 (0, 1.9)	0.230
Glucose level >6.7 mmol/L (%)	6.4 (2.8,18.1)	2.0 (1.0,6.6)	10.6 (3.8,16.2)	8.3 (3.4,19.6)	0.283

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Postpartum dysglycaemia among women meeting criteria for overt diabetes in pregnancy

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Background and aims: The International Association for Diabetes in Pregnancy Study Groups, in addition to proposing new gestational diabetes diagnostic criteria in 2010, introduced a new category of overt diabetes in pregnancy (fasting glucose of ≥7 mmol/L, HbA1c of ≥6.5%, or random plasma glucose of ≥11.1 mmol/L confirmed by HbA1c/fasting plasma glucose). However, there is a lack of evidence regarding the postpartum implications of this classification

Materials and methods: From January 2007 to December 2010, pregnant women across five centres were screened for GDM (one-step method; 75g oral glucose tolerance test [OGTT] with fasting, 1-hr and 2-hr values). Women were retrospectively classified as having overt diabetes in pregnancy, hyperglycaemia not meeting overt diabetes criteria, or normal glucose tolerance (NGT) according to IADPSG criteria. All women meeting IADPSG criteria for GDM were invited back for an OGTT at 12 weeks postpartum, and invited for later follow-up up to 5 years postpartum. Women were included in this study if they attended at least once during this time period. American Diabetes Association criteria for the diagnosis of prediabetes (fasting glucose 5.6-6.9 mmol/L, 2-hr glucose 7.8-11.0 mmol/L, HbA1c 39-47 mmol/mol) and diabetes (fasting glucose ≥7 mmol/L, 2-hr glucose ≥11.1 mmol/L, HbA1c ≥48 mmol/mol) were used to classify glycaemic status at follow-up. The chi-squared and Fisher's tests were used to examine differences across groups.

Results: Thirty-eight women met the criteria for overt diabetes on fasting and/or two-hour glucose values on the pregnancy OGTT, and had at least one OGTT postpartum (up to 5 years). In addition, 107 women met overt diabetes criteria by virtue of a single isolated one-hour value of ≥11.1 mmol/L on the pregnancy OGTT. Women with hyperglycaemia in pregnancy without overt diabetes (n=237), and women with NGT who were invited for follow-up glucose testing (n=515), were included for purposes of comparison. Results are detailed in the table below.
Conclusion: Women with overt diabetes in pregnancy on fasting or 2-hr glucose values are at higher risk of postpartum diabetes and prediabetes than women with hyperglycemia in pregnancy not meeting overt diabetes criteria. However, the majority of these women do not have diabetes, either at postpartum or later follow-up. Women with an isolated one-hour glucose value consistent with overt diabetes in pregnancy are at lower risk of progression to prediabetes than those meeting fasting or 2-hr criteria.

	Classification on pregnancy OGTT			
Cumulative incidence (up to 5 years post partum)	NGT in pregnancy (n=515)	Hyperglycaemia in pregnancy (not meeting overt diabetes criteria) (n=237)	Overt diabetes - fasting or 2-hr glucose values (n=38)	One-hr value ≥11.1 mmol/L (n=107)
Prediabetes	5.2% (27)	24.5% (58)	52.6% (20)	27.1% (29)
Diabetes	0% (0)	0.4% (1)	7.9% (3)	6.5% (7)

Table 1. Glycaemic status up to 5 years postpartum according to glycaemic status on the pregnancy OGTT. Figures indicate percentages within each pregnancy OGTT category (absolute numbers in parentheses)
p<0.05 for all comparisons, except for; hyperglycaemia vs. one-hr value only groups (prediabetes p=0.603); and NGT vs hyperglycaemia (DM p=0.315)

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PS 092 Gestational diabetes: screening and predictors

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Analysis of a large German register with data of >11,000 pregnancies with gestational diabetes

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Background and aims: A number of Diabetic specialist practices (DSPs) in Germany document systematically data of diabetic pregnancies in a register to optimise the quality of the care process. We analysed relevant topics in the wake of the introduction of a new gestational-Guideline as well as a change in the maternity directive.
Materials and methods: Data of a total of 11,357 pregnancies with gestational diabetes in the years 2008 to 2014 documented in up to 28 DSPs were analysed, with a continuous increase in documented pregnancies per year of 1,000 in 2008 to 2,456 in 2014.
Results: In the years 2013/14 the GDM was more frequently diagnosed more frequently in the preferred time span between the 25th-28th week of pregnancy than in previous years (33% vs. 27% in 2009/10). 40% of pregnant women were treated withon an insulin therapy in 2013/14 compared to 34% in 2009-2011 ($p<0.0001$). The percentage of overweight women was higher with 28% in 2013/14 than before (26%, $p<0.001$), that of obese women was also higher (35% vs. 32%; $p<0.001$). 4% of these women were diagnosed with overt diabetes during their pregnancy in the years 2013-2014. The increase in the frequency of caesarean sections was not significant (39% versus 38%). 43% of the mothers participated in a postpartum diabetes screening, 40% of these showed pathological results. 71% of the mothers breastfed their children at this time.
Conclusion: This analysis shows that implementing a guideline for the treatment of gestational diabetes helps to improve quality of care: GDM diagnosis and treatment start more often in a timely manner, allowing for more time to profit from therapy. The growing prevalence of adiposity can also be observed among pregnant women, stressing the need to focus on this additional health risk for mother and child. GDM screening identifies families with severely elevated diabetes and cardiovascular risk. However, less than half of the young mothers take advantage of this offer and miss the chance to prevent their chronic disease.
Disclosure: **L. Heinemann:** None.

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Screening of glucose tolerance to detect gestational diabetes in Italy: a population study

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Background and aims: Universal screening for GDM has been used in Italy for over 20 years; since 2012 the National Health Authority recommended selective GDM screening. According to these national guidelines (NGL), not all women are eligible to perform screening test at any time during pregnancy and selective screening (2hr-75g-OGTT) is recommended early in pregnancy (16-18 week) in women at high risk (previous GDM; pre-pregnancy BMI ≥30 kg/m2, glucose value at 1st visit between 5.6-7 mmol/L) and later in pregnancy (24-28 week) in women at medium risk (pre-pregnancy BMI ≥25 and <30 Kg/m2, age ≥35 years, previous macrosomia; positive family history of diabetes an high risk ethnicity), while women at low risk (no risk factors) are excluded. The present study was conceived

to evaluate the compliance to NGL in Tuscany, a region of central Italy.

Materials and methods: This study includes all women, resident in Tuscany, who delivered in year 2014, after excluding those with pregestational diabetes, individualized by the certificates of care at delivery (CEDAP). The database, included information about: onset of pregnancy and gestational age at delivery, place of birth; pregestational body weight, marital status, employment, smoking habit, parity, education degree. The CEDAP dataset was then linked to the regional flux of all specialists' visits including prescriptions of OGTT performed since the 16th gestational week. All pregnant women were classified as eligible and not eligible according to NGL. In the two groups, for each stratum, a logistic regression was performed to evaluate the chance of being tested by the OGTT, after adjusting for maternal age, marital status, education degree, ancestry, employment status, parity, smoking habit, pregestational BMI and first visit's setting.

Results: We identified 26,365 pregnant women: 13,601 (51%) eligible and 12,764 (49%) not eligible. OGTT was performed in 18,169 cases (69%) including 73% of eligible (n.=9973) and 64.2% (n=8196) of not eligible women. In almost all cases of both two groups OGTT was performed late in pregnancy. The rate of OGTT performers increased with age and the class age > 40yr had the higher chance of having an OGTT as compared to those aged lesser than 25yr [RR:1.22 (1.16-1.27); p=0.001]. The chance of being tested was increased in overweight-obese women [RR:1.08 (1.04-1.12); p=0.001], in nulliparous pregnancies [RR:1.03 (1.01-1.04); p=0.04] and among those with a medium-high education degree, as compared to those with a low degree [RR:1.10(1.01-1.22); p=0.03]. The chance of performing an OGTT decreased among women not followed by a specific clinical setting [RR:0.72 (0.62-0.83); p=0.001] and among housewives, as compared to employed or student women [RR:0.94 (0.92-0.96);p=0.001].

Conclusion: Our data show that 51% of pregnant women should be considered eligible for GDM selective screening according to NGL. In this population screening test is largely applied. Moreover NGL are still not correctly applied, considering the time of OGTT execution in high risk women as well as the high prevalence of screening test in non eligible women. Action has to be taken to improve application of new recommendations.

Disclosure: **C. Lencioni:** None.

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Incidence and major characteristics of gestational diabetes mellitus with the new IADPSG criteria: updated data of a Cantonal hospital in Switzerland

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Background and aims: The International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria replace the Carpenter and Coustan (C&C) criteria in a Cantonal Hospital in Switzerland since 2011. These new criteria propose a one-step screening test with a lower glucose concentration threshold and abandon the category of gestational glucose intolerance (GGI). The aim of this study is to assess the incidence of gestational diabetes mellitus (GDM) and birth major complications in the population of the Cantonal Hospital. Results are compared with the data of a previous study done in the Cantonal Hospital and using the C&C criteria.

Materials and methods: We conducted a retrospective study of all pregnant women screened for GDM between the January 1st 2014 and December 31st 2015. Clinical data of the GDM women and their offspring were collected and analyzed from the screening test until the postpartum OGTT control test. We compared these data to women without GDM and their offspring as controls. At least the results are compared with a previous study concerning pregnant women screened in 2004-05.

Results: On 502 pregnant women included, 159 women (31.7%) were diagnosed for GDM; 132 (83%), 18 (11.3%) and 9 (5.7%) women were diagnosed respectively at fasting, at 1-hour and 2-hour after glucose challenge. There was an increase of GDM incidence comparing with C&C criteria (4.8% of GDM and 2.6% of GGI). Mean age for GDM women was 30 years old and 29 years old for non GDM women (p= 0.04). Our population of GDM women was significantly younger comparing to the previously study (p=0.0223). Mean BMI before pregnancy was higher for GDM women compared to non GDM women (25.6 vs 23.7 kg/m2, p=0.0002). Fifty-nine GDM women (37%) needed insulin-treatment. Of these, 39 women (66.1%) were treated with intermediate-acting insulin, 9 (15.3%) with rapid-acting insulin and 11 (18.6%) with both types. The proportion of GDM treated women decreased in the current study (37% in current study vs 70% in previous study, p=0.0233). Proportion of caesarean section was not significantly different with 33.6% of GDM women vs 28% for non GDM women (p=0.226). Proportions of preeclampsia, premature delivery, symptomatic newborn's hypoglycemia and obstetrical trauma were not significantly different in the two groups. The rate of persistent impaired glucose tolerance was not significantly different between the current and the previous study (16% of GDM women in current study vs 18% in previous study, p=0.85).

Conclusion: Our results show a significant increase in GDM incidence of 6.6 times in 10 years in a same population with the new IADPSG criteria (of 4.2 times if GGI women are included). The proportion of birth complications is unchanged. The proportion of insulin-treated women decreases by almost half.

Disclosure: **H. Savopol:** None.

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Pre-pregnancy body mass index or gestational weight gain is a better predictor of gestational diabetes

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Background and aims: The obesity epidemic has fueled an epidemic of prediabetes and diabetes in women of childbearing age. For this reason screening for gestational diabetes (GDM) is essential. The aim of the present study is to assess the pre-pregnancy body mass index (BMI) and weight gain during pregnancy (until 24 week of gestation when screening for GDM is performed) as a predictive factor for GDM development.

Materials and methods: We conducted a retrospective analysis on 7809 pregnant women who delivered for the period 2014-2015 in our university hospital. The weight before pregnancy and those gained during pregnancy was obtained from hospitals medical records. For diagnosis of GDM IADPSG criteria has been used.

Results: The prevalence of GDM was 7% (546). The total weight gain and rate of weight gain decreased with increasing pre-pregnancy BMI. The mean level of pre-pregnancy BMI was 23.4±2.8 kg/m2 and the weight gain during pregnancy was 6.5±1.8kg. Ten percent of women had a pregravid BMI ≥30 kg/m2. Women with GDM had higher pre-pregnancy BMI (29.4±4.7 vs 23.2±2.4) and higher weight gain during pregnancy (8.2±1.8 vs 6.3±1.5) in comparison with women without this condition (p<0.005). Body mass index (BMI) between 25 and 29.9 kg/m2 before pregnancy increases the risk for having GDM 1.629 (p30kg/m2 4,162 (p<0.0001). The area under receiver operating characteristic curve (AUC-ROC) of weight gain during pregnancy to detect GDM was 0.958 (95% CI 0.946-0.970, p<0.0001). Using a value of <10.1kg up to 24 week of gestation could rule out GDM with a sensitivity of 81% and specificity of 89%. Weight gained more than 10.1kg increase 1.394 (p<0,034) risk

for GDM development. Furthermore, pre-pregnancy obesity (BMI of 30 kg/m2) was associated with worse pregnancy outcome, independent from GDM.
Conclusion: Weight loss before pregnancy may improve fertility and reduce the risk of poor maternal-fetal outcomes, especially gestational diabetes. The lack of established national screening program necessitates the active search for pregnant women at high risk for GDM development. Lifestyle interventions that moderate gestational weight gain may reduce the risk of poor pregnancy outcomes, as well as lower the risk for significant postpartum retention.
Disclosure: **M.V. Boyadzhieva:** None.

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The effect of maternal weight gain before the diagnostic OGTT on the risk of gestational diabetes

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Background and aims: Body mass index (BMI) measured in the 1st trimester is an important predictor of gestational diabetes. In contrast, only limited and controversial data is available on the effect of weight gain during the first two trimesters on this association. Therefore we hypothesized that larger BMI change until the OGTT (dBMI) would predict GDM.
Materials and methods: Between 2002 and 2005, n=5335 pregnant women participated in an universal population-based screening at a hospital. After excluding women with pregestational diabetes, twin pregnancies, and with missing body weights, data on n=4397 women was analyzed. Body weight was measured at the first prenatal visit (6-8 weeks of gestation) and at the time of the 75g OGTT (24-28 weeks of gestation). GDM was diagnosed based on both the WHO-1999 (n=296) and the WHO-2014 (n=648) diagnostic criteria. Hierarchical logistic regression models were used to investigate the association between GDM risk and (1) change in BMI and then (2) further adjusted for baseline BMI.
Results: Mean age of the participants was 29.3±4.22 years, baseline weight 63.22±12.24 kg, baseline BMI 22.80±4.25 kg/m2. Their BMI increased by 2.51±1.50 kg/m2 by the time of the OGTT. Later GDM women had a smaller BMI increase compared to controls (WHO-2014: 0.26, 95%CI 0.12-0.40; WHO-1999 0.20, 95%CI 0.02-0.37 kg/m2), and there was a negative correlation between baseline BMI and BMI increase (r=-0.21, p<0.001). In univariate analysis a larger BMI increase seemed to be protective against GDM development (OR: 0.89 95%CI: 0.84-0.94, p<0.001), however this association was completely abolished after adjustment for baseline BMI (OR: 0.97 95%CI: 0.91-1.03, p=0.261). A similar (but weaker) negative association was found between the risk of GDM based on the WHO-1999 recommendation and change in BMI (OR: 0.91 95%CI: 0.84-0.99, p=0.023), that was also reduced to nonsignificance after adjustment for baseline BMI (OR: 0.98 95%CI: 0.90-1.06, p=0.558).
Conclusion: Women with an increased risk of GDM or a higher BMI has a lower weight gain before the diagnostic OGTT compared to normal weight, low risk women. Our results suggest that weight gain between early pregnancy and the 2nd trimester has little effect on the risk of GDM determined by early pregnancy obesity and thus lifestyle counselling at early pregnancy is unlikely to substantially decrease the risk of GDM development.
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Disclosure: **Z. Szili-Janicsek:** None.

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Total bile acid in first trimester pregnancy predicts the risk of gestational diabetes

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Background and aims: To assess whether TBA level in first trimester pregnancy is associated with the occurrence of GDM in Chinese pregnant women.
Materials and methods: In the current study, biochemical parameters including serum TBA of 742 pregnant women were tested within 12 weeks of gestation and compared. Then 268 cases were diagnosed with GDM and 474 were diagnosed with normal glucose tolerance by 75 g oral glucose tolerance test (OGTT) performed at 24-28th weeks of gestation.
Results: Serum TBA levels at early pregnant period were significantly higher in pregnant women with GDM compared with healthy pregnant women (2.3±1.4 μmol/L vs. 1.9±1.0 μmol/L, P < 0.001). The Spearman's correlation analysis showed that TBA was positively associated with HOMA-IR and the occurrence of GDM (both P < 0.05). A binary logistic regression analysis after adjusting for other confounding variables revealed a significant and independent association between TBA and GDM [odds ratio (OR), 1.383; 95% confidence interval, 1.183-1.616, P < 0.001]. The pregnant women were divided into quartiles according to their serum TBA concentrations, and compared to the first quartile, the OR of GDM markedly increased in the fourth TBA quartile (P < 0.05).
Conclusion: Serum TBA is closely linked with insulin resistance and GDM. Monitoring TBA in first trimester is helpful to identify women who are at risk for the subsequent development of GDM.
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Disclosure: **W. Hou:** None.

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Higher plasma levels of relaxin-2 and C-peptide in early pregnancy in patients with gestational diabetes

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Background and aims: Relaxin is an important hormone in shaping the endometrium in early pregnancy. It promotes vascularization and secretion of prolactin as well as insulin-like growth factor binding protein. Relaxin has been suggested to promote insulin sensitivity in studies on mice and negatively associated with insulin levels in human pregnancy. Lower levels of relaxin-2 have been suggested in patients with type 2 diabetes and higher in pregnant women with type 1 diabetes although both studies had very limited statistical power. There is to our knowledge no report on levels of relaxin in gestational diabetes which is peculiar considering the importance of this hormone in both pregnancy and insulin resistance. The aim of this study was to investigate levels of relaxin-2 in patients with gestational diabetes and compare with healthy controls at 12th week of gestation.
Materials and methods: Patients included in this study were diagnosed with GDM at a University Hospital (n=137). Pregnant women without diabetes were included as controls and recruited at health care units in the same area (n=114). Blood samples were drawn at the 12th week of gestation in both patients and controls. Commercially available ELISA were used to measure plasma levels of relaxin-2, C-peptide and adiponectin. Mann-Whitney U-test was used to study differences in mean rank

between the groups and Spearman rank correlation to study association between parameters. Data are reported as median followed by interquartile range in brackets.

Results: We found significantly higher plasma levels of relaxin-2 in women with GDM (833 [404-1525] pg/mL) compared to in controls (468 [332-935] pg/mL; p=0.001) and a positive correlation was found between relaxin-2 and C-peptide (rs=0.20, p=0.002). Also plasma levels of C-peptide were higher in women with GDM (1.2 [0.7-1.8] nmol/L) compared to controls (0.6 [0.4-0.9] nmol/L, p<0.001) whereas adiponectin levels were lower in women with GDM (2.7 [0.9-4.4] μg/mL) compared to in controls (5.2 [3.5-6.8] μg/mL, p<0.001), suggesting lower insulin sensitivity in patients as expected. Although patients with GDM had significantly higher BMI there was no correlation found with relaxin-2.

Conclusion: This is the first study to report significantly higher levels of relaxin-2 in women with gestational diabetes compared to healthy pregnant controls. This is similar to what have been reported in pregnant women with type 1 diabetes. However, it is in contrast to current reports on patients with type 2 diabetes. We found a positive correlation between relaxin-2 and C-peptide which suggests that it could be a compensatory effect to the insulin resistance in GDM. More studies are needed to study the role of relaxin-2 in GDM.

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Impact of maternal obesity on fetal growth independently of gestational diabetes

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Background and aims: Newborn of obese mother are usually considered at risk of macrosomia but the part of maternal obesity is unclear because of gestational diabetes (GDM) often associated.We aim to determine the specific effect of obesity on fetal growth and adiposity, independently of GDM.

Materials and methods: This prospective bicentric case-control study included pregnant women aged 18-40 years. Two groups were constituted: a normal weight group (PNW) (BMI between 18.5-25kg/m2) and an obese group (PO) (BMI≥30 kg/m2). Women with pregestational diabetes were excluded. GDM was screened in the PO group by fasting blood glucose (FBG) in the first trimester. GDM was further screened in the PO and PNW groups by a standard 75g oral glucose tolerance test between 24-28 weeks of gestation (WG) and at 32 WG, and by FBG at 37/38 WG (IADGSP Criteria). All diabetic patients were treated according to the current French recommendations. Neonatal anthropometrics, sum of skinfolds, placenta weight, cord serum C-peptide, leptin and IGF1 were measured. Associations between maternal obesity and neonatal anthropometrics, adiposity, placenta weight and cord serum hormones were assessed using multiple logistic regression analysis. Interactions of sex of the neonates with obesity, diabetes and HbA1c were introduced in the model.

Results: 243 normal weight women (PNW) and 253 obese women (PO) were included. 21% of women were diagnosed with GDM in the PNW group (19% insulin treated) and 46% in the PO group (49% insulin treated). Term of birth, weight Z-score and birth weight were not different between groups (PNW/PO: 39.7 vs 39.4 WG, p=0.13; 3346 vs 3367 g, p=0.6), Cord serum C-peptide and IGF1 were not different either. Skinfolds measure, cord serum leptin and placenta weight, were significantly increased among girls in the PO group compared to the PNW group. In multivariate analysis SK measure and cord serum leptin were significantly increased in the PO group only among girls (SK+1.67mm, p<10-4; leptin + 4.62 ng/m, p<10-4); obesity was not associated with birth weight and weight Z-score but was positively associated with placenta weight (+38.5g, p<0.05). HbA1c was positively related to SK measure, leptin level and weight Z-score. A 1-point increase in HbA1c corresponded respectively to 1.71mm increase in SK (sd=0.44, p=10-3), +3 ng/ml increase in leptin level (p<10-2) and +0.33 increase in weight Z-Score (p<10-2).

Conclusion: Maternal obesity is not associated with increased birth weight but is associated with increased neonatal adiposity only among girls and irrespective of the presence of treated gestational diabetes and gestational weight gain. These data suggest that the effect of altered intrauterine environment is different according to the sex of the fetus. Glycemic control is also correlated to neonatal adiposity and birth weight.

Clinical Trial Registration Number: NCT02681588
Supported by: PHRC

Disclosure: **S. Jacqueminet:** Grants; PHRC.

PS 093 Gestational diabetes: biomarkers and predictors

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Metabolomic differences in response to glucose challenge between gestational diabetes patients diagnosed based on fasting glucose level, oral glucose tolerance test and controls

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Background and aims: Gestational Diabetes Mellitus (GDM) can be diagnosed based on fasting plasma glucose (FPG) and/or oral glucose tolerance test (OGTT). Although both increased fasting glucose (IFG) or glucose intolerance (IGT) are diagnosed as GDM they may differ in origin of mechanisms responsible for dysglycemia. Hepatic insulin resistance and normal muscle insulin sensitivity is characteristic for isolated IFG, whereas in individuals with isolated IGT moderate to severe muscle insulin resistance and normal to slightly reduced hepatic insulin sensitivity are observed. In the present study LC-MS based metabolomics was used to evaluate changes in serum metabolites after glucose challenge performed in women with GDM (diagnosed based on FPG or OGTT) and controls.
Materials and methods: From the group of 1030 women screened for GDM at our hospital in years 2010-2014 we selected 78 women without previous DM or other complications. The study population consisted of 3 groups of patients (age and BMI matched) diagnosed using the new WHO (2013) criteria: diagnosed solely based on FPG (n=23), OGTT (n=26) and controls (n=29). Fasting, 1h and 2h serum samples were taken from each participant during OGTT. Samples were fingerprinted using LC-QTOF-MS. Data were collected in both ESI (+/-) modes (50-1,000 m/z). For each individual and each properly measured metabolite the change between 0h and 2h of OGTT was calculated. Differences in obtained changes between FPG, OGTT, and controls were evaluated by Welch's t test. Identity of statistically significant metabolites (p-value <0.05) was confirmed by MS/MS analysis.
Results: Patients diagnosed based on OGTT showed significantly higher decrease in palmitic (- 37%, p=0.005), arachidonic (- 30%, p=0.01), and docosahexaenoic (DHA) (- 32%, p=0.01) acids in comparison to FPG and controls. On the other hand patients from control and FPG groups showed significant increase in various metabolites in comparison to OGTT group. These metabolites were as follow: dihydroxyeicosatetraenoic acid (+239%, p=0.01 for controls; +98%, p=0.05 for FPG group), several lysophosphatidic acids (LPAs) (+119-92%, p=0.005-0.02 for controls; + 159-107%, p=0.02-0.03 for FPG group), and choline (+118%, p=0.00002 for controls; +95%, p=0.002 for FPG group).
Conclusion: Differences in response to OGTT between studied groups are mainly related to lipid metabolism. Glucose challenge affects metabolism of free fatty acids in women with GDM diagnosed based on OGTT, but not in other groups. Increase in choline and LPAs in control and FPG groups may indicate enhanced activity of phospholipase D in response to glucose in these women. Changes in metabolic profiles after glucose challenge in control and FPG groups are similar, but differ significantly from that observed in patients with increased glucose values during OGTT. The potential impact of observed changes in lipid profiles on outcomes related to mother and foetus during the pregnancy requires further investigations.
Supported by: Polish Ministry of Science and Higher Education (KNOW 2012-2017)
Disclosure: **M. Ciborowski:** None.

941

Glucose intolerance after a recent history of gestational diabetes based on the IADPSG criteria

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Background and aims: More data are needed on the prevalence of glucose intolerance in women with a recent diagnosis of gestational diabetes (GDM) based on the 'International Association of Diabetes and Pregnancy Study Groups' (IADPSG) criteria. Our aim was to evaluate the uptake of our current screening strategy postpartum, the prevalence and the risk factors for glucose intolerance in women with a recent history of GDM in a Western European population.
Materials and methods: Retrospective analysis of the medical files of our university hospital from 01-03-2014 till 08-02-2016 of women with a recent history of GDM diagnosed with the IADPSG criteria. All women with a history of GDM are advised to undergo a 75g oral glucose tolerance test (OGTT) around 12 weeks postpartum. Indices of insulin sensitivity (the Matsuda index and the reciprocal of the homeostasis model assessment of insulin resistance, 1/HOMA-IR) and an index of beta-cell function, the Insulin Secretion-Sensitivity Index-2 (ISSI-2) were calculated based on the OGTT postpartum. Multivariable logistic regression was used to adjust for confounders such as age, BMI, ethnicity and breastfeeding.
Results: Over a period of 23 months, 191 women were identified with a recent history of GDM. Of all women, 29.3% (56) did not attend the scheduled postpartum OGTT. Compared to women who received an OGTT postpartum, women who did not attend the postpartum OGTT, had a higher BMI (28.6 ±6.8 vs. 26.2 ± 5.6, p=0.015), were more often from an ethnic minority background (EMB) (41.1% vs. 25.2%, p=0.029) and smoked more often during pregnancy (14.3% vs. 2.2%, p=0.001). The OGTT postpartum was performed at a median of 27.0 weeks (25.0-28.0). Of all women (135) who received an OGTT postpartum, 42.2% (57) had prediabetes (11.9% impaired fasting glucose, 24.4% impaired glucose tolerance and 5.9% both impaired fasting and impaired glucose tolerance) and 1.5% (2) had overt diabetes. Compared to women with a normal OGTT postpartum, women with glucose intolerance were older (32.5±4.3 vs. 30.8±4.8 years, p=0.049), were more often obese (34.5% vs. 17.3%, p=0.023), had more often excessive weight gain during pregnancy (44.8% vs. 25.3%, p=0.018), were more often from an EMB (33.9% vs. 18.4%, p=0.040), less often breastfed (69.5% vs. 84.2%, p=0.041) and had more often an abnormal fasting glycaemia (55.6% vs. 37.3%, p=0.040) and higher median HbA1c [(5.1% (5.0-5.4) vs. 5.0%(4.8-5.2), p=0.001] at the time of the OGTT. Compared to women with a normal OGTT postpartum, women with glucose intolerance postpartum had a similar insulin sensitivity [Matsuda index 0.656 (0.386-1.224) vs. 0.778 (0.532-1.067), p=0.709; 1/HOMA-IR 0.004 (0.002-0.009) vs. (0.004-0.003-0.007), p=0.384] but a lower beta-cell function, remaining significant after adjustment for confounders [ISSI-2 1.6 (1.2-2.1) vs. 1.9 (1.7-2.4),p=0.002].
Conclusion: Glucose intolerance is frequent in early postpartum in women with GDM based on the IADPSG criteria and these women have an impaired beta-cell function. One third of women did not attend the scheduled OGTT postpartum and these women have an adverse risk profile.
Disclosure: **K. Jegers:** None.

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The association between mid-pregnancy metabolic dysfunction and postpartum glucose intolerance in women with gestational diabetes

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Background and aims: Women with a history of gestational diabetes mellitus (GDM) have a greatly increased risk of conversion to type 2 diabetes over time. Because the performance ratio of postpartum glucose

testing is low, women with postpartum glucose intolerance could not be managed appropriately. If postpartum glucose intolerance is predicted during pregnancy, clinician can examine women with GDM more easily after delivery. The aim of this study was to evaluate whether metabolic dysfunction at mid-pregnancy could be predict postpartum glucose intolerance in women with GDM.

Materials and methods: We enrolled 609 pregnant women diagnosed with GDM from October 2005 to December 2013. Metabolic dysfunction was defined as three or more of the modified National Cholesterol Education Program metabolic syndrome criteria [waist circumference replaced by pre-pregnancy BMI (≥25 kg/m2)] at mid-pregnancy. Postpartum glucose intolerance was defined as fasting plasma glucose ≥ 100 mg/dL or 2-h plasma glucose ≥ 140 mg/dL using 75-g oral glucose tolerance test at 6-12 weeks after delivery.

Results: Mean age was 32.8 ± 3.5 years and mean pre-pregnancy BMI was 22.4 ± 3.6 kg/m2. The prevalence of postpartum glucose intolerance increased with increasing number of metabolic dysfunction components (0 = 48.1%, 1 = 49.6%, 2 = 54.9%, and 3 or more = 72.5%, P for trend < 0.05). Women with metabolic dysfunction were 2.69 times (95% CI 1.39-5.20) more likely to have postpartum glucose intolerance than those without metabolic dysfunction component. Among the components of metabolic dysfunction, pre-pregnancy obesity was the most strong risk factor, even after adjusting for maternal age (OR 2.56; 95% CI 1.60-4.07).

Conclusion: In Korean women with GDM, metabolic dysfunction at mid-pregnancy seems to be an independent risk factor for postpartum glucose intolerance at 6-12 weeks after delivery. Consequently, postpartum glucose testing should be performed to manage postpartum glucose intolerance as early as possible when women with GDM have metabolic dysfunction at mid-pregnancy

Table 1. Prevalence and odds ratio of postpartum glucose intolerance by the number of metabolic dysfunction components at mid-pregnancy

No. of components	N	Prevalence (%)	Adjusted odds ratio[a] (95% CI)	P value
0	81	48.1	1.0	-
1	264	49.6	1.02 (0.62 - 1.70)	0.925
2	184	54.9	1.29 (0.76 - 2.18)	0.341
3 or more	80	72.5	2.69 (1.39 - 5.20)	0.003

[a] Adjusted for maternal age

Disclosure: **K. Kim:** None.

943

HbA_{1c} as a predictor of diabetes after gestational diabetes

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Background and aims: Postpartum follow-up after pregnancy with gestational diabetes mellitus (GDM) is important as these women have a manifold increased risk of progression to type-2 diabetes. However, the uptake of postpartum screening is suboptimal and easy tools to identify those at the highest risk of diabetes are desirable so that intervention can start already in pregnancy. We have previously reported that HbA1c in the upper quartile during pregnancy is associated with a 4-fold increased risk of subsequent diabetes. In the present study we further explored HbA1c as a predictor of diabetes up to five years postpartum.

Materials and methods: Women giving birth in southern Sweden 2003-2005 were prospectively followed. GDM was defined as a 2-h capillary plasma glucose concentration of ≥10.0 mmol/L during a universally applied OGTT in the 28th week of gestation. HbA1c was measured within two weeks from GDM diagnosis. Participants were followed by means of an OGTT at 1-2 years and 5 years after pregnancy; or until the diagnosis of diabetes (WHO 1999). T-test, logistic regression analysis and receiver operating characteristic (ROC) curves were used for statistical evaluations.

Results: 5-year data was available for a total of 196/391 women with GDM (73% European origin, mostly Swedish). After five years, 73 women had been diagnosed with diabetes and were compared with those who had normal glucose tolerance at 5-year follow-up. Mean (95% CI) of HbA1c levels during pregnancy for the respective groups were: 5.5 (5.3-5.7) % (37 (35-39) mmol/mol) and 4.9 (4.8-5.1) % (31 (30-32) mmol/mol), p < 0.0001. The ability of an ROC curve to predict diabetes was fair (AUC 0.720, 95% CI 0.634-0.806): optimal cut-point 5.4% (36 mmol/mol), specificity 92%, sensitivity 45%. Overall, the specificity and positive predictive value was high, but sensitivity and negative predictive value low, for various cut-points: HbA1c ≥ 6.3% (45 mmol/mol), 100%, 100%, 16% and 50%, respectively; HbA1c ≥ 5.7% (39 mmol/mol), 97%, 91%, 30% and 53%, respectively. A logistic regression analysis, testing the predictive value of HbA1c quartiles on the 5-year diabetes risk, showed that women in HbA1c quartile 4 (HbA1c 5.4-8.6% (36-70 mmol/mol, n=46)) had a 7-fold increased risk of post-partum diabetes compared with women from quartiles 1 to 3 (n=150) (OR 7.0, 95% CI 3.3-14.6, p < 0.0001).

Conclusion: The results confirm our previous findings that HbA1c levels in the upper quartile, obtained close to the 28th week of pregnancy, predict diabetes development during the subsequent five years. An HbA1c level corresponding to the prediabetes range outside pregnancy could with high specificity and high positive predictive value capture women with postpartum diabetes, but due to low sensitivity HbA1c does not appear suitable as a screening test to predict diabetes after GDM in all women.

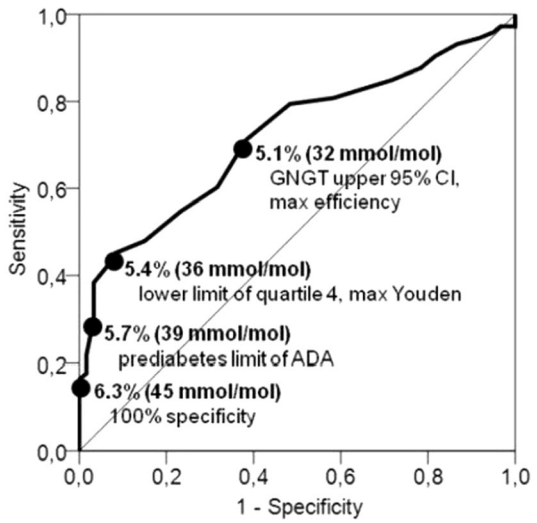

Figure. Predictive accuracy of HbA1c for detecting diabetes 5 years after gestational diabetes using women with normal glucose tolerance at 5-year follow-up as a reference. Various cut-points are indicated.

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Disclosure: **R. Claesson:** None.

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Consequences of reduced insulin sensitivity on adiponectin during course of pregnancy in women with gestational diabetes

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Background and aims: Dysregulation of adipokines could promote the manifestation of gestational diabetes. We sought to assess associations of leptin and adiponectin with insulin sensitivity at early pregnancy and trajectories of adiponectin during the course of gestation until 3 months postpartum.
Materials and methods: 212 pregnant women were included at 16th (IQR:14-18) gestational week (GW) for fasting determination of adiponectin and leptin with subsequent OGTT testing including multiple measurements of glucose, insulin and C-peptide for evaluation of insulin sensitivity and ß-cell function. Follow-ups of adiponectin measurements were performed during GW 24-28, GW 30-34, GW 36-40 and 8-12 weeks after delivery.
Results: In early pregnancy differences in adiponectin and leptin were significant between women with early GDM manifestation (24th GW, n=33) of GDM as well as controls (n=132), whereby those with early GDM showed more distinguishing levels (adiponectin: 7.9±3.7 vs. 9.4 ±3.8 vs. 10.4±4.4 μg/ml, p=0.005; leptin 99.5.1±36.6 vs. 85.4±40.5 vs. 78.1±39.3 μg/ml, p=0.006). Moreover, both adipokines were significantly associated with whole body insulin sensitivity (adiponectin r=0.32, p<0.001; leptin r=-0.48, p<0.001) as well as with the oral disposition index (adiponectin r=0.27, p<0.001; leptin r=-0.30, p<0.001). However, their attribution for GDM prediction was only moderate, when measured during early gestation. During later course of pregnancy trajectories of adiponectin remained constantly low in the GDM group whereas dynamics in NGT showed increased concentrations at beginning of gestation with decreasing tendency until 3rd trimester. At early postpartum GDM women with impaired glucose tolerance had significant lower adiponectin levels than those without disturbances in glucose homeostasis after pregnancy with GDM.
Conclusion: Adiponectin and leptin are associated with early features of deteriorated glucose metabolism in early gestation. Including adiponectin in clinical follow-ups may improve risk stratification during pregnancy and prediction of metabolic long-term development in affected women.
Supported by: Medical Scientific Fund of the Mayor of Vienna (Pr.Nr.:09063) to AK-W
Disclosure: **L. Bozkurt:** None.

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Differences in food intake and genetic variability in taste receptors between pregnant women with and without gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) represents the most frequent metabolic disorder in pregnancy. Since dietary intake plays an important role in obesity development it could also influence the susceptibility to GDM. Very little focus has been given to possible differences in pre-gestational and gestational dietary intake between healthy pregnant women and GDM complicated pregnancies. Taste can play a non-negligible role in food preferences. Previous studies found an association of several single nucleotide polymorphisms (SNPs) in genes for taste receptors (TR) with type 2 diabetes mellitus, however, no study ascertained the possible relationship between different allelic variants of TR genes and GDM. The aim of our study was 1) to characterize dietary habits of pregnant women and to find possible differences in food preferences between healthy pregnant women and those with GDM and 2) to ascertain possible association of several SNPs in TR genes with GDM.
Materials and methods: A total of 357 pregnant women (293 with GDM and 64 with physiologic pregnancy) were included in the study. All subjects underwent oral glucose tolerance test (oGTT) with 75g of glucose between 24-30th week of pregnancy. GDM was diagnosed according to the WHO criteria: FPG ≥ 5.6 mmol/l, 1-hr post-load glucose ≥ 8.9 mmol/l and 2-hr post-load glucose ≥ 7.7 mmol/l (any one of the three above cut-off values qualified for the GDM diagnosis). 88.7% of participants (196 GDM and all controls) filled a food frequency questionnaire covering pregnancy period. A total of 5 SNPs in TR genes - TAS1R2 gene (rs35874116) for sweet TR, TAS2R7 (rs619381) and TAS2R9 (rs3741845) gene for bitter TR, CD36 (rs1527479) gene for "fat" TR and finally a gene SLC2A2 (rs5400) for glucose transporter GLUT2 were selected for genotyping.
Results: Women with GDM exhibited significantly more frequent meat consumption (esp. poultry, pork and smoked meat), dairy products and sweet drink consumption (P=0.02, chi-square test). GG genotype in TAS2R9 gene (SNP rs3741845) was significantly associated with GDM (P=0.0087, chi-square test). No associations were ascertained between alleles or genotypes of SNPs studied and BMI, total weight increment during pregnancy, offspring birth weight or glucose levels during oGTT.
Conclusion: In conclusion, our study showed differences in dietary intake of selected food items between healthy pregnant women and those with GDM and genetic association of bitter taste receptor allele with GDM. Possible relationship between those findings requires further study. Moreover, women in risk of GDM development (i.e. obese older or with diabetic relatives) should be educated about a proper diet at the beginning of pregnancy
Supported by: AZV ČR 16-28040A
Disclosure: **K. Kuricová:** None.

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Serum miRNA profiles in women with gestational diabetes mellitus

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Background and aims: Gestational diabetes mellitus (GDM) is one type of diabetes that presents during pregnancy and significantly increases the risk of a number of adverse consequences for the fetus and mother[1]. MicroRNA (miRNA) is a small non-coding RNA molecule that functions in regulation of gene expression by targeting mRNA to affect its stability and/or translation[2]. MiRNA have recently been demonstrated to abundantly and stably exist in serum and to be potentially disease-specific[3]. However, no reported study investigates the associations between serum miRNA and GDM. To investigate the potential specific miRNA expression profiles of gestational diabetes mellitus (GDM), the profile of miRNA expression was analyzed in serum of GDM and normal pregnant women.
Materials and methods: 42 GDM patients and 30 normal pregnant women in third trimester were selected between July to December in 2013 from our hospital. All subjects' age, body mass index (BMI), gestational age, pregnancy times, heart rate, blood pressure, oral glucose tolerance test (OGTT) results were collected and recorded. The serum of both groups were mixed well respectively. Apply HiSeq high-throughput sequencing technologies to detect the expression of miRNA in serum of GDM patients and the control group. Verify the differentially expressed miRNA with real-time PCR.
Results: There were no significant differences between GDM group and control group in age, BMI, gestational age, pregnant times, heart rate, systolic pressure and diastolic blood pressure. OGTT results of GDM group were greater than the control group at every time point, the differences were statistically significant such as blood glucose of 0 minute(4.41 ±0.54 vs 4.00±0.26, p<0.05), the blood glucose of 30 minutes (8.82±0.94

vs 7.01±0.94,p<0.05), the blood glucose of 60 minutes (9.72±1.45 vs 6.14±1.14,p<0.05), the blood glucose of 120 minutes (8.70±1.38 vs 5.80±0.89,p<0.05). Compared with the control group, miR-144-5p, miR-197, miR-222 were statistically down-regulated in GDM group (p<0.01).

Conclusion: There were differences of miRNA expression in serum of GDM patients and normal pregnant women which may be related to the higher blood glucose in GDM group. Whether this specific miRNA expression profiles in serum of GDM patients could be a diagnostic chip of gestational diabetes need further investigation.

Disclosure: **X. Huang:** None.

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Association study of 77 single nucleotide gene polymorphisms with gestational diabetes and with the fasting and post-challenge glucose levels in pregnancy

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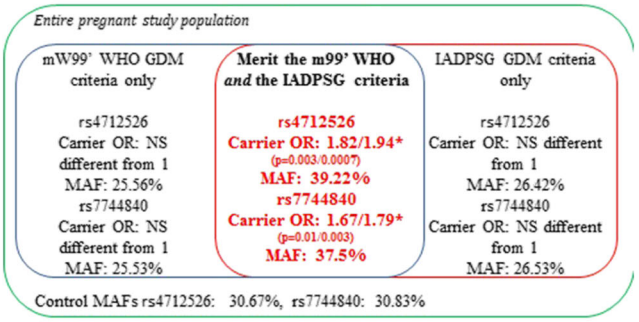
Background and aims: To identify maternal single nucleotide gene polymorphisms (SNPs) associated with gestational diabetes mellitus (GDM) or influencing fasting and/or post-challenge glucose levels in pregnancy.

Materials and methods: A set of 77 SNPs was selected based on reports associated with type 2 diabetes/metabolic traits. Altogether 802 pregnant women were enrolled in one Austrian and two Hungarian centers. After genomic DNA isolation from of 592 samples, KASP genotyping assay was used for bi-allelic discrimination. Logistic regression risk models were used to calculate ORs according to both IADPSG/modified'99WHO criteria based on 75g OGTT values (24-28th gw).

Results: Binary GDM trait associations: rs10830963/G (MTNR1B, OR=1.54/1.50 [IADPSG/m'99WHO], p=0.034/0.043), rs4712526/A (OR=1.62/1.55 p=0.019/0.03) rs7754840/C (OR=1.57/1.49 p=0.03/0.05) of CDKAL1. The rs720390/A* (IGF2BP2, OR=NS/0.51, p=NS/0.007); rs13266634/T (SLC30A8, OR=NS/0.66, p=NS/0.01) and rs7578326/G (LOC646736/IRS1, OR= 0.607/NS p=0.016/NS) variants were associated with lower GDM development risk after adjustments to pre-pregnancy BMI and age. Glycaemic traits: carrying a minor allele of rs10830963 (MTNR1B); rs7903146, rs12255372 and rs12243326 (TCF7L2); rs1799884 (GCK); rs4712526 (CDKAL1); rs12534093 (IGF2BP3) SNPs were associated with increased, while rs13266634 (SLC30A8); rs1801282* (PPARG) with decreased plasma glucose levels at OGTT. Genetic susceptibility appears to be more preponderant in those who merit both the m99'WHO and the IADPSG GDM diagnostic criteria (Fig 1).

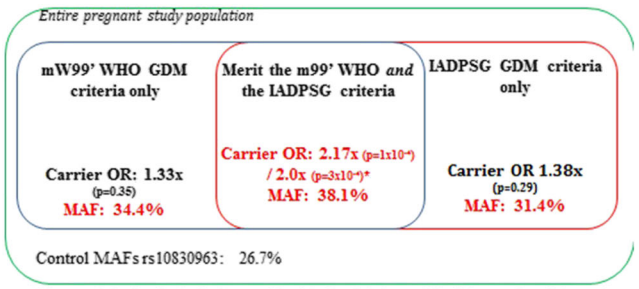
Conclusion: Here we confirm the robust association of rs10830963/G MTNR1B gene variant with GDM and additionally report 2 novel genetic associations (*) at suggestive level. Predicting GDM is not accurate enough (sensitivity and specificity of traditional methods are between 60-81%). Genetic markers do not change over time and may help the pre-pregnancy identification of individuals with increased risk to develop GDM who might benefit from earlier OGTT and disease control than the routine screening at 24-28th gestational weeks.

Minor Allele Frequencies of the rs4712526 and rs7744840 CDKAL1 gene variants in the diseased and control population – according to different diagnostic criteria



ORs were calculated under the dominant model and in this calculation we defined the control population as the group of pregnant individuals who did not merit any of the two diagnostic criteria. In addition OR was calculated for those who merit both diagnostic systems and compared to all the other individuals.*

Minor Allele Frequencies and Odds Ratios of the true casual gene variant, rs10830963 MTNR1B in the diseased and in the control populations – according to different diagnostic criteria



ORs were calculated under the dominant model and in this calculation we defined the control population as the group of pregnant individuals who did not merit any of the two diagnostic criteria. In addition OR was calculated for those who merit both diagnostic systems and compared to all the other individuals.*

Supported by: EFSD New Horizons Collaborative Research Initiative

Disclosure: **K. Rosta:** Grants; EFSD New Horizons Collaborative Research Initiative.

PS 094 The many aspects of diabetic neuropathy

948

Sudomotor function is related with microvascular perfusion in type 1 diabetic patients with long disease duration

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Background and aims: Dysfunction of small fibers is an early clinical manifestation of diabetic neuropathy. SUDOSCAN+ is a device used to noninvasively assess the function of the sweat glands, which are innervated by C-fibers, on the basis of the electrochemical reaction of chlorine ions secreted in sweat due to low voltage current. Laser-Doppler flowmeter evaluates small fiber function due to axon-reflex vasodilatation after heating to 44oC. The aim of the study was to evaluate the electrochemical skin conductance (ESC) in patients with type 1 diabetes (DM1) with long disease duration.
Materials and methods: The study included 416 adult patients with DM1 (207 women and 209 men) aged 41.5 years (IQR: 33-52.5), with HbA1c level of 8% (IQR: 7.2-8.9) and with disease duration of 25 (IQR: 20-32) years. The study group was subdivided into two groups depending on the presence of peripheral neuropathy identified by standard methods. We performed analysis of peak flow during thermal hyperemia (THmax) in laser-Doppler blood flowmeter (PERIFLUX 5000) and ESC with SUDOSCAN+ on the feet (Feet ESC) and hands (Hands ESC). We also evaluated metabolic control of diabetes and presence of its chronic complications. We used AGE Reader device to measure skin autofluorescence (AF) phenomenon, which occurs because of fluorescent properties of AGEs.
Results: We diagnosed peripheral neuropathy in 45% of patients. Patients with peripheral neuropathy, compared to those without clinical neuropathy, had lower Feet ESC [67 (IQR: 43-79) vs 82 (IQR: 76-86)μS; p<0.001] and Hands ESC [54 (IQR: 34.5-67.5) vs 67 (IQR: 58-77)μS, p<0.001] and lower THmax [635 (IQR: 114-1461) vs 1021 (IQR: 314-1748)PU; p=0.002]. We found a negative correlation between Feet and Hands ESC and patients age (Rs=-0.42, p<0.0001; Rs=-0,40, p<0.0001), duration of diabetes (Rs=-0.34, p<0.0001, Rs=-0.32, p<0.0001), skin AF (Rs=-0.39, p<0.0001, Rs=-0,34, p<0.0001) and positive correlation with the eGFR (Rs=0.39, p<0.0001, Rs=0.33, p<0.0001) and THmax (Rs=0.21, p<0.0001, Rs=0.14, p=0.004). In a multivariate regression model, which takes into account age, sex, duration of diabetes, HbA1c, skin AF, eGFR, it was shown that the electrochemical skin conductance is independently associated with the presence of peripheral neuropathy.
Conclusion: Sudomotor function is reduced in adult patients with type 1 diabetes and diabetic neuropathy. The lower increase of microvascular perfusion after heating the greater sudomotor dysfunction is.
Supported by: Diabetes Poland Grant
Disclosure: **A. Gandecka:** None.

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Impaired olfactory function is related to the presence of neuropathy in adults with type 1 diabetes

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Background and aims: Diabetes mellitus contributes to the central nervous system degeneration and cognitive decline. Olfactory dysfunction is potentially suggested to be a clinical manifestation of central diabetic neuropathy. Hyperglycemia and oxidative stress might play an important role in the development of olfactory dysfunction in diabetic subjects. The aim of the study was to assess olfactory function in adult patients with type 1 diabetes (T1DM).
Materials and methods: We included 102 patients with type 1 diabetes (51 men), median age 35 (IQR 28-42.5) years, disease duration 17 (IQR 12.5-25) years, HbA1c 8.15 (IQR 7.25-9)%. The control group consisted of 29 healthy people matched for age and gender. The patients underwent ENT examination with nasal endoscopy to exclude other factors disabling sense of smell. Olfactory function was assessed with "Sniffin' Sticks. For the assessment of odor identification 12 pens with different odors were used and patient should select 1 of 4 presented items which best described each odor for every pen (score 0-12, normosmia: score 11-12). We assessed the metabolic control of diabetes and the presence of diabetic retinopathy, peripheral and autonomic neuropathy and diabetic kidney disease.
Results: Hyposmia was found in 67.6% of patients with type 1 diabetes compared to 55.2% in control group [median odor identification score 10 (IQR 9-11) in both groups]. We found a negative correlation between olfactory identification scores and body mass index (Rs -0.21, p=0.04). Comparison of odor identification scores between groups with and without diagnosed chronic microvascular complications showed lower scores in neuropathy group vs non neuropathy group [8.5 points (IQR 7-10) vs 10 points (IQR 9-11); p=0.02]. In multivariate logistic regression analysis presence of neuropathy was associated with higher odds of olfactory dysfunction (OR 1.5; 95%CI: 1.1-2.2; p=0.01) independently from age, sex, smoking, HbA1c. In multiple linear regression impaired olfactory function was independently associated with neuropathy (beta -0.32; p=0.003).
Conclusion: Higher degree of olfactory dysfunction is observed in patients with type 1 diabetes and diagnosed neuropathy. The mechanisms of this phenomenon and a relation of olfactory function to chronic microvascular complications of type 1 diabetes is still uncertain.
Supported by: Poznan University of Medical Sciences
Disclosure: **A. Duda-Sobczak:** Grants; Poznan University of Medical Sciences.

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Though nerve dysfunctions are associated with pre-diabetes and hypertension, peripheral neuropathy arises at the stage of diabetes

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Background and aims: Several reports showed that peripheral neuropathy (PN) and peripheral nerve dysfunctions (ND) in the legs begin in early stages before diabetes. To clarify the pathogenesis, we examined the prevalence and risk factors of PN and ND in the regional population-based Japanese subjects.
Materials and methods: 624 subjects who received medical screening were divided into four groups according to glucose tolerance; Normal (N) group, preDM group, newly diagnosed diabetes (NDM) group and known DM (KDM) group. They were also divided into three groups by blood pressure; Normal pressure (NP) group, normal hypertension (NHT) group and hypertension (HT) group. Furthermore they were divided into two groups (normolipidemia or dyslipidemia), and another two groups (non-obesity or obesity). Abnormal sensation in the feet (symptom) and Achilles tendon reflexes (ATR) were examined. Additionally, three quantitative nerve function tests, vibratory perception threshold (VT), sensory nerve action potential (AMP) and conduction velocity (CV) in the sural nerve, were evaluated. PN was diagnosed when both of symptom and decreased ATR were positive and/or when nerve conduction abnormality

was confirmed. Risk factors of PN were assessed by comparing PN prevalence between clinically divided groups. A logistic regression analysis to detect the risk factors for PN was also performed. Aggravating factors of ND were evaluated by comparing measured values of VT, AMP, CV between clinically divided groups. Additionally, multiple regression analyses were performed to detect significant aggravating factors of the values of VT, AMP, CV.

Results: Prevalence (%) of PN in N, preDM, NDM, KDM groups were 5.6, 3.3, 15.4, 14.5, respectively. PN prevalence in two diabetic groups was significantly elevated. In contrast no significant association was observed between the PN prevalence and hypertension, dyslipidemia and obesity. By the logistic regression analysis, smoking and glucose tolerance has been extracted as the risk factor of PN. Measured values of 3 quantitative tests (VT, AMP and CV) in 4 groups (N, preDM, NDM and KDM) showed stepwise deterioration in the order of glucose tolerance. These values of preDM group were significantly reduced compared to N group. Actual values of 3 quantitative tests showed stepwise deterioration in the order of hypertensive stages. Measured values of HT group were significantly reduced compared to NT group. On the other hand, there was no significant association between the values of 3 quantitative tests and dyslipedemia or obesity. Multiple regression analysis revealed following three findings; firstly, VT elevation was associated with aging and high stature; secondly, AMP reduction was associated with aging, female, high stature, high BMI and high blood pressure; thirdly, CV slowing was related with aging, high stature, low BMI and high blood pressure.

Conclusion: Diabetes is the most critical factor in development of PN. Smoking is also a risk factor for PN. Aging and high stature seem to be aggravating factors of ND. Hypertension seems to associate with nerve conduction impairment. Good control of blood glucose and blood pressure and non-smoking education are necessary for preventing neuropathy.

Disclosure: **H. Sasaki:** None.

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Physical activity and albuminuria were associated with painful diabetic polyneuropathy in type 2 diabetes in an ethnic Chinese population

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Background and aims: Diabetic neuropathy is a common complication in patients with type 2 diabetes. However, prevalence of painful diabetic polyneuropathy (PDPN) and potential influences of physical activity and albuminuria on development of PDPN have been rarely studied. The aim of this study was to examine the prevalence of PDPN as well as the independent and joint effects of physical activity and albuminuria on it.

Materials and methods: This retrospective study enrolled 2,359 outpatients with type 2 diabetes with completed survey of Douleur Neuropathique en 4 Questions (DN4) questionnaire from January 2013 to October 2013 in one of medical center in central Taiwan. Painful neuropathy was defined as a total score exceeding 3 points. Independent and joint effects of physical activity and albuminuria on PDPN were assessed by fixed effect with logistic regression.

Results: Overall, 179 (7.6%) patients were diagnosed as having PDPN. Both less physical activity and albuminuria were associated with a higher mean DN4 score (1.07 for no exercise, 0.87 for daily exercise duration <=30 minutes, and 0.56 for daily exercise duration >30 minutes, p for trend <0.001; 1.51 for macroalbuminuria, 1.10 for microalbuminuria, and 0.78 for normal UACR, p for trend <0.001). Adjusted analysis showed that the risk of painful neuropathy increased in the groups without physical activity (Odds ratio (OR)= 3.38, 95% CI 1.54-9.79) and daily exercise duration <= 30 minutes (OR= 3.33, 95% CI 1.27-8.73), when compared with the group with daily exercise duration >30 minutes (p for trend 0.006). Comparing with normal UACR, the OR for PDPN were 0.96 (95% CI 0.61~1.50) and 2.31 (95% CI 1.44-3.73) for microalbuminuria and macroalbuminuria respectively (p for trend 0.002). In addition, we observed a joint effect of macroalbuminuria and physical inactivity on PDPN risk (OR= 6.68, 95% CI 2.23-20.04).

Conclusion: Less physical activity and albuminuria, respectively, increased the risk of PDPN and had a joint effect.

Clinical Trial Registration Number: CE14232A

Disclosure: **S. Chiang:** None.

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Impact of glycaemic variability on neuroretina in patients with type 1 diabetes

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Background and aims: Recent studies have identified early neuroretinal abnormalities in diabetic patients, before the onset of microvascular changes. However, the role of glycemic control and daily glucose variability (GV) on the development of retinal neurodegeneration is still not fully clarified. The aim of our study is to evaluate the different impact of overall glycemic load and GV on neurosensory retina.

Materials and methods: 39 patients with type 1 diabetes mellitus (DM1) with no signs or mild non-proliferative diabetic retinopathy (DR), with good metabolic control and without peripheral neuropathy and 13 healthy control subjects (C) were enrolled. All subjects underwent an Optical Coherence Tomography (OCT) Heidelberg Spectralis, with automatic segmentation of all neuroretinal layers and multifocal electroretinogram measuring amplitude (Amp) and implicit time (IT). Measurements of mean of subfoveal, inner and outer nasal (N)/temporal (T)/superior (S)/inferior (I) quadrants for individual layer were also calculated. Metabolic control was evaluated by glycated hemoglobin (HbA1c) measurement, and indexes of GV were calculated from continuous glucose monitoring (CGM)

Results: Retinal nerve fibre layer (RNFL)-N thickness was significantly reduced (DM1 vs. C: -7.7; Bonferroni 95% CI: -0.2; -15.2), while inner nuclear layer (INL) was increased in all quadrants in the DM1 group compared to C (DM1 vs. C: 7.6; 95% CI: 0.8-14.3). Amp was significantly reduced in all quadrants in DM1 group vs. C (p <0.001). To assess the differences between groups and whether such differences were similar or not across quadrants of each layer, a General Linear Model was applied with Group as between-subjects factor and Quadrant as within-subjects factor. A negative correlation between RNFL-N and low blood glucose index (LBGI) (r=-0.382, p=0.034) and positive between INL and continuous overall net glycemic action (CONGA)-1, -2, -4 hours (r=0.40, p=0.025; r=0.39, p=0.031; r=0.41, p=0.021, respectively) were observed in DM1 patients. However, no correlation was found with HbA1c. The correlation analysis was performed by means of Pearson'r. All variables were checked for gaussianity and eventually transformed to obtain a better fit to gaussianity and a reduction of heteroscedasticity.

Conclusion: These results support the concept that diabetes has an early neurodegenerative effect on the retina, which occurs even though the vascular component of DR is minimal. GV, but not overall glycemic load, seems to play a pathogenic role in the early structural damage of neuroretina in DM1 patients.

Disclosure: **F. Picconi:** None.

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Intra-epidermal electrical stimulation to determine pain thresholds in patients with diabetic neuropathy and with and without pain sensation

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Background and aims: Loss of pain sensation in patients with diabetic neuropathy leads to limb amputation, but quantifying pain sensation in such patients is difficult. Intra-epidermal electrical stimulation (IES) has been proposed as a useful method for pain studies because it can selectively activate Aδ- and C-fibers that convey pain sensation without concomitant activation of Aβ-fibers at low current intensities in healthy individuals. Therefore, IES can evaluate degrees of pain sensation by measuring pain threshold, which is a type of current perception threshold. A previous study has found that an elevated pain threshold determined using IES closely correlates with histological changes in the epidermal nerve fibers of diabetic patients. The present study aimed to determine associations between pain threshold and risk factors for diabetic complications.
Materials and methods: We recruited 132 patients (mean age, 59.8 ± 12.3 years; male, n = 93) with type 2 diabetes who had undergone pain threshold tests at the Takahashi Family Clinic. We placed IES electrodes on the dorsal skin of the foot and started stimulation at an intensity that was sufficient for the patient to feel a pricking sensation and then reduced the current in steps of 0.05 mA until sensation was not discernable. Levels of glycated hemoglobin A1c (HbA1c), urine albumin creatinine ratio (UACR) and systolic blood pressure (SBP) were analyzed. Pain perception was assessed by the patients as a feeling of numbness, tingling or coldness of the limbs.
Results: Pain threshold values of patients with and without pain sensation were 0.36 ± 0.25 and 0.21 ± 0.14 mA, ($p < 0.01$), respectively, and values for HbA1c, UACR, and SBP were 6.6 ± 0.7%, 48 ± 126 mg/gcr, and 123.8 ± 19.8 mmHg, respectively. None of these correlated with pain threshold.
Conclusion: The elevated pain threshold in patients with diabetes and pain sensation indicated that the source of pain sensation is located in the central rather than peripheral nervous system.
Disclosure: **N. Takahashi:** None.

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Oxidative stress induced neural neuritin reduction is associated with peripheral nerve dysfunction in diabetic rats

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Background and aims: The true nature of diabetic neuropathy remains to be explored. Oxidative stress is one important factor leading to diabetic peripheral neuropathy. Neurotrophins act as another causal factor implicated in the pathogenesis of diabetic neuropathy. Neuritin is a recently discovered neurotrophin in support of peripheral nerves. In this study, for the first time, we linked oxidative stress to neural neuritin reduction in association with peripheral nerve dysfunction in diabetic rats.
Materials and methods: Hyperglycemia was induced with streptozotocin and monitored in adult Sprague-Dawley rats. Duration of diabetic model was ranged from 2-6 weeks. A single dose of lipoic acid (anti-oxidative agent) was intraperitoneally administrated. Age-matched rats were randomly divided into three groups: normal control, saline-treated control diabetic, and lipoic acid-treated diabetic groups. Sciatic nerve malonaldehyde was measured using TBARS (thiobarbituric acid reactive substance assay). Sciatic nerve neuritin mRNA measurement was measured using Real-time Quantitative PCR and protein was measured using Western blotting. S/MNCVs (sensory/motor nerve velocity conduction) were carried out using electromyography.
Results: At week 2 of the experiment, malonaldehyde increased, neuritin mRNA and peptide contents decreased in sciatic nerves of saline treated-diabetic rats in contrast to normal rats ($P < 0.05$). However, malonaldehyde increase, along with neuritin decrease, was blocked with lipoic acid treatment, compared to saline treatment: malonaldehyde ((259.43 ± 12.91 vs. 328.75 ± 13.21) nmol/g,$P<0.01$), neuiritin mRNA ((0.952 ± 0.023 vs. 0.774 ± 0.01) arbitrary units, $P < 0.05$) and neuiritin protein ((0.541 ± 0.012 vs. 0.46 ± 0.008) arbitrary units, $P < 0.05$)), respectively. Nerve conduction velocities (sensory/motor) were shown to be slow 2 weeks after nerve neuritin down-regulation in saline-, not lipoic acid-, treated rats in contrast to normal rats ($P < 0.05$). With progressively increased nerve malnoaldehyde and decreased neuritin levels, nerve conduction velocities were shown to be further slower through week 6 in saline treated-diabetic rats. In contrast to saline treatment, these parameter changes were delayed with lipoic acid treament, including malonaldehyde ((285.24 ± 13.02 vs. 372.11 ± 13.47) nmol/g,$P < 0.005$), neuiritin mRNA ((0.892 ± 0.012 vs. 0.623± 0.010) arbitrary units, $P < 0.01$), neuiritin protein ((0.485 ± 0.007 vs. 0.33 ± 0.005) arbitrary units, $P < 0.01$), SNCV((25.61 ± 4.01) vs. 20.7 ± 2.79) m/s, $P < 0.05$) and MNCV ((21.47 ± 2.68 vs. 16.26 ± 3.47) m/s. $P < 0.05$), respectively. Throughout the experiment, blood glucose levels were stable and no differences in blood glucose were found between the saline- and lipoic acid- treated groups.
Conclusion: Hyperglycemia-induced oxidative stress affected nerve neuritin expresssion. 0xidative stress-induced down-regulation of neuritin expression was associated with peripheral nerve dysfunctions in diabetic rats.
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Disclosure: **J. Li:** None.

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Modification of the glyoxalase system and the importance of alternative detoxification of methylglyoxal in the context of diabetic neuropathy

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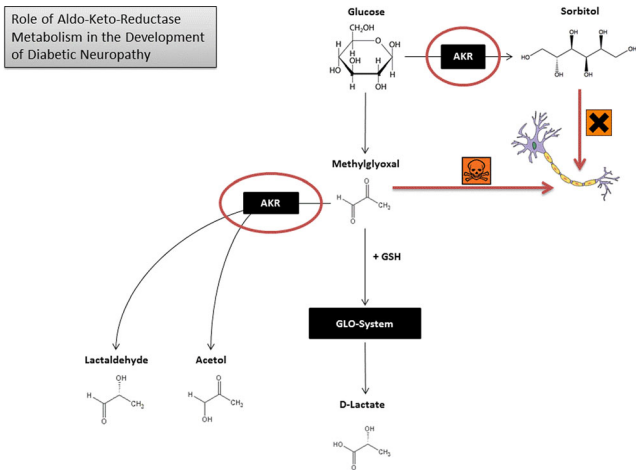
Background and aims: Methylglyoxal (MG) is a by-product of glycolysis and reacts primarily with arginine and lysine residues of proteins, which leads to advanced glycation endproducts (AGE`s). Glycated proteins in neurons (e.g. voltage-gated sodium channel 1.8) can change the mechanical triggers and thereby mediate the neuropathic symptoms in diabetic patients. In healthy organisms the detoxification of MG is accomplished by the highly specific glyoxalase system. However in experimental models of diabetes as well as in patients, this system is altered, potentially leading to an accumulation of MG in the peripheral nervous system. To establish whether this is the case, the pathways of MG detoxification were studied in vitro.
Materials and methods: Murine Schwann cells were cultured under iso- and hyperglycemic conditions. Enzyme kinetics of potentially involved enzymes of interest were determined spectrophotometrically. Intra-/extracellular MG, Acetol, Lactaldehyde and Sorbitol concentrations were measured using liquid chromatography coupled to mass spectrometry. The levels of protein content of MG modifications, Glyoxalase 1 (GLO 1) and Aldo-Keto-Reductase (AKR) 1b3 was determined using Western Blot techniques. Toxicity assays were performed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT-reagent). Knockdown of specific genes of potentially involved enzymes was achieved with validated siRNA and efficacy was confirmed by RT-qPCR.
Results: The silencing of genes for AKR1b3, AKR1a1 and GLO1 caused a significant decrease in LD50 for MG suggesting an involvement of those genes in the detoxification of MG. Michealis-Menten kinetics (Km, Vmax) revealed that hyperglycemic conditions lead to reduced capacity of Glyoxalase 1. Despite this, there was no significant increase in intracellular MG-concentrations and MG-modification (e.g. MG-H1). However, an elevated activity of Aldo-Keto-Reductase (AKR) towards

MG was observed, specifically, by the subtype AKR1b3. This was paralleled by an increase in intracellular acetol and sorbitol concentrations.

Conclusion: The altered kinetic of Glyoxalase 1 lead to the activation of an alternative detoxification of MG through AKR1b3. The conversion of glucose into sorbitol (polyol pathway) seems to be a negative side-effect of this enzyme in a hyperglycemic state. This controversial role of AKR1b3, detoxifying MG to Acetol with high efficacy on one hand, but activating the polyol pathway on the other hand should be investigated in further studies. The results could be a first hint that inhibitors of the AKR-enzymes, which are used in several clinical trials (Tolrestat, Epalrestat etc.) are not the ideal solution for a treatment of diabetic neuropathy.



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Disclosure: **J. Morgenstern:** Grants; Deutsche Forschungsgemeinschaft (DFG; SFB1118), Dietmar Hopp Foundation (DHS), German Center for Diabetes Research (DZD).

PS 095 Autonomic neuropathy: assessment and treatment

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Proposal of a new clinical scoring system to predict cardiac autonomic neuropathy in patients with type 1 diabetes

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Background and aims: Cardiac autonomic neuropathy (CAN) is frequently underdiagnosed. Early identification can improve quality of life. However, clear indications and practical tools for CAN screening are not available. Develop a clinical scoring system for the screening of can in patients with type 1 diabetes (T1D).

Materials and methods: One hundred patients with T1D (67% F) from one university in Brazil (U1) were evaluated for CAN by Poly-Spectrum software using standardized cardiovascular reflex testing and measures of heart rate variability. Clinical characteristics associated to CAN, based on logistic regressions, were used to compute a numerical score for CAN diagnosis. This score was then applied on a second sample of 120 patients with T1D from the another university in Brazil (U2).

Results: Of the 100 patients from U1, CAN was diagnosed in 39 (38.2%). Predictors of CAN in regression models were: hypertension (OR = 6.8; 95% CI 2.76-16.74; p= <0.001), total cholesterol (OR = 1.013; 95% CI 1.003-1.022; p=0.008), triglycerides (OR = 1.012; 95% CI 1.004-1.02; p=0.002), post-prandial sweating (OR = 3.36; 95% CI 1.28-8.79; p=0.01), symptomatic orthostatic hypotension (OR = 2.44; 95% CI 1.06-5.57; p=0.035), retinopathy (OR = 2.04; 95% CI 1.36-3.04; p=0.001 and), nephropathy (OR = 2.47; 95% CI 1.59-3.82; p= <0.001), diastolic blood pressure (OR = 1.06; 95% CI 1.02-1.11; p=0.003), right 10 g monofilament (OR = 5.7; 95% CI 1.66-19.49; p=0.006) and left 10 g monofilament (OR = 5.0; 95% CI 1.44-17.31; p=0.011). Score formula was: CAN-Score = (post-prandial sweating x 5) + retinopathy + nephropathy + (total cholesterol/10) + (triglycerides/10) + (hypertension x 5) + (orthostatic hypotension x 5) + (diastolic blood pressure/10) + (right 10 g monofilament x 2,5) + (left 10 g monofilament x 2,5). Categorical variables (post-prandial sweating/hypertension/orthostatic hypotension/right and left 10 g monofilament) were considered = 1 if present/abnormal and = 0 if absent/normal. Retinopathy was classified as normal = 0, nonproliferative = 1, proliferative = 2, unilateral blindness = 3 and bilateral blindness = 4, and nephropathy as normal = 0, microalbuminuria = 1, macroalbuminuria = 2, chronic kidney disease = 3, hemodialysis = 4 and kidney transplant = 5. The ROC-AUC for this score was 0.872 (95%CI = 0.802-0.942; p< 0.001) with the best cutoff at 44.6 (sensitivity 81.6% and specificity 79%). Sensitivity analysis with 1,000 resamples with replacement (bootstrapping) yielded similar confidence intervals. The population from the U2 consisted of 120 patients; 16 (13.3%) with diagnosis of CAN confirmed by Poly-Spectrum analysis. The CAN-Score cutoff of 44.6 was applied and compared to the diagnosis of CAN by Poly-Spectrum software. This yielded a sensitivity of 75%, specificity 49%, positive predictive value 18.5% and negative predictive value 92.7%. Sensitivity analysis with bootstrapping (1,000 resamples) did not significantly alter these results.

Conclusion: These results support that a simple clinical score is potentially capable of predicting CAN with high sensitivity and a good negative predictive value in T1D patients. Although a positive score will still require formal complementary testing, patients with scores less than 44.6 have a low probability of CAN, thus helping select those actually in need of more evaluations.

Supported by: CNPq

Disclosure: **C. Minatel Riguetto:** None.

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Risk markers for development of small and large nerve fiber lesion in patients with diabetes

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Background and aims: Peripheral neuropathy is one of the major chronic complication of diabetes mellitus(DM). Both small and large nerve fibers can be effected. Small nerve fibers (SNF) are poorly myelinated (type Aδ) and unmyelinated (type C), responsible for the thermal sensation, pain perception and forms an essential part of the autonomic nerves. Large nerve fibers(LNF) are myelinated and contribute to the perception of touch, proprioception and muscle innervation. It is not clear which patient will predominantly develop what type of neuropathy. Therefore our study focused on finding risk markers of both type.
Materials and methods: Patients with DM (both 1.and 2.type) with clinical suspection on peripheral neuropathy were examined during the years 2014-2016 in neurophysiology laboratory. SNF lesion was determined by the thermal threshold testing (TTT) on upper and lower extremities (compared to Czech normal values) and by the cardiac autonomic neuropathy (CAN) testing (including Ewing battery of tests and spectral analysis of heart rate variability). CAN was determined in case of pathology of more then 2 of 7 standard tests. LNF lesion was diagnosed in case of reduction or disappearance of tendon reflexes, tactile and vibration sensation. SNF and LNF lesion presence was correlated with important anamnestic, anthropometric and biochemical markers. We used logistic type of generalized additive models for statistical analysis. Lesion presence probability was modeled depending on various factors and covariants. Semiparametric portion of final model was built on penalized splines using crossvalidation method.
Results: We examined 77 patients with diabetes mellitus (50 patients with type 1 DM, 27 with type 2 DM). SNF pathology was diagnosed in 60 patients, LNF lesion in 46 of them. SNF pathology correlated with age (p=0.0163), DM duration independently on patients age (p=0.0109), smoking (p=0.0026), incidence of retinopathy (p=0.0067). LNF lesion correlated with age (p=0.0337), DM duration (p=0.0294), smoking (p=0.00157), incidence of retinopathy (p=0.0037), glycosylated hemoglobin level (p=0,00029)and triacylglycerole level(p=0.0428).
Conclusion: Risk markers for small and large nerve fibres lesion are age, DM duration, smoking and presence of diabetic retinopathy. Large nerve fibre pathology correlates also with actual compensation of DM and hypertriglyceridemia.
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High prevalence of cardiovascular autonomic neuropathy in young patients with congenital generalised lipodystrophy (Berardinelli-Seip Syndrome)

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Background and aims: Metabolic abnormalities observed in patients with Congenital Generalized Lipodystrophy (CGL) can be accompanied by cardiovascular autonomic neuropathy (CAN). The aim of this study was to evaluate the prevalence of CAN in patients with CGL.
Materials and methods: It was a cross sectional study comparing 10 patients with CGL, 20 patients with type 1 diabetes mellitus (DM1) and 20 healthy controls. We evaluated clinical and laboratory data, 3 spectral analysis components - high frequency (HF), low frequency (LF), and very low frequency (VLF) - of heart rate variability (HRV), sympathetic-vagal balance, time domains of HRV, corrected QT interval (cQT), and 4 cardiovascular reflexes tests (postural hypotension test, orthostatic, respiratory, and valsalva coefficients - 2 abnormal tests: clinic CAN, 1 abnormal test: incipient CAN, and postural hypotension: advanced CAN).
Results: There was no difference in age, sex, BMI, and pubertal stage between groups. Seven patients (70%) with CGL had diabetes. There was no difference in the duration of diabetes and A1c between CGL and DM1 patients. It was observed significant involvement of autonomic modulation, higher baseline HR, BP, and cQT interval in patients with CGL. Prevalence of clinic CAN was 40% in CGL group vs 5% in DM1 group (p=0,031), and incipient CAN was 10% in CGL group vs 25% in DM1 group (p=0,633). One patient (10%) with CGL presented advanced CAN. All tests were normal in the control group. Prevalence of cQT prolongation was 50% in CGL vs 25% in DM1 group (p=0,231) vs 5% in control group (p=0,009). In the CGL group, there was inverse correlation between orthostatic coefficient and triglycerides (r= -0,778; p= 0,008), respiratory coefficient and diastolic BP (r= -0,827; p=0,003), LF and triglycerides (r= -0,648; p= 0,043), HF and HOMA-IR (r= -0,648; p= 0,043); and positive correlation between LF/HF ratio and HOMA-IR (r=0,673; p= 0,033), and insulin (r=0,714; p=0,047).
Conclusion: These findings demonstrate high prevalence of CAN in young patients with CGL, some of them presenting the most severe forms. These data allow us to speculate that insulin resistance has early involvement in the pathogenesis of CAN, irrespective of the onset of hyperglycemia.

Variable	CGL group (n=10)	DM1 group (n=20)	Control group (n=20)	p
Age (years)	12 (6; 30)	14 (9; 30)	12 (5;31)	0,235
Female% (n)	60 (6)	45 (9)	60 (6)	1,000
Body mass index (kg/m²)	19 (16,2; 22,9)	20,9 (16,8; 23,7)	17,2 (14,4; 24,8)	0,338
Basal heart rate (bpm)	90 (70; 109)	83 (62; 106)	71 (53; 94)	0,010
Systolic blood pressure(SBP) (mmHg)	123 (90; 175)	103 (94; 134)	104 (80; 113)	0,003
Diastolic blood pressure (mmHg)	78 (50; 109)	68 (55; 99)	66 (60; 80)	0,030
Durationof diabetes (years)	8 (1; 14)	5 (1; 16)	NA	0,229
Glycohemoglobin(%)	7,2 (4,4; 12,1)	7,8 (5,2; 10)	5,2 (4,2; 5,8)	0,001
Orthostatic coefficient (30:15 ratio)	1,19 (0,98; 1,59)	1,34 (1,04; 1,68)	1,55 (2,26; 2,02)	0,000
Valsalva ratio	1,54 (1,15; 2,34)	1,70 (1,24; 2,52)	1,74 (1,50; 2,42)	0,099
Respiratory coefficient (E : I ratio)	1,33 (1,09; 1,57)	1,52 (1,14; 2,01)	1,60 (1,23; 2,23)	0,001
Postural hypotension test (SBP reduction) (mmHg)	6 (0; 22)	6 (0; 12)	0 (0; 14)	0,000
Very low frequency (VLF)	383 (88; 4250)	1077 (392; 5857)	1988 (688; 18341)	0,001
Low frequency (LF)	329 (139; 2525)	972 (181; 3109)	1916 (343; 5580)	0,001
High frequency (HF)	627 (55; 1840)	1447 (182; 8374)	2993 (270; 9969)	0,001
LF/HF Ratio	1,21 (0,12; 3,70)	0,67 (0,19; 1,99)	0,58 (0,26; 1,70)	0,552
Standard deviation of RR interval (SDNN)	64 (22; 169)	60 (25; 124)	82 (36; 163)	0,085
Square root of the average of RR interval (rMSSD)	57 (12; 237)	52 (17; 170)	85 (18; 180)	0,178
cQTinterval	0,44 (0,41; 0,49)	0,40 (0,38; 0,44)	0,41 (0,39; 0,44)	0,034

Results expressed as median (min; max). Chi-square (Fisher's exact test) and Kruskal-Wallis were used. Program: stata 13. Significant p <0.05.

Disclosure: **C.M.M. Ponte:** None.

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Impaired cardiovascular autonomic function and diminished vibration perception are present among patients with higher future type 2 diabetes risk screened by FINDRISC questionnaire

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Background and aims: Diabetes mellitus and even impaired glucose tolerance are associated with autonomic and sensory nerve disfunction. The Finnish Diabetes Risk Score (FINDRISC) is a validated and one of the most widely used T2DM risk score questionnaire that comprises questions on age, body mass index (BMI), waist circumference, physical activity, diet, history of antihypertensive medication, high blood glucose and family history of diabetes. The aim of our study was to compare autonomic and sensory nerve function, as well as anthropometric data among patients with higher future T2DM risk (minimum 12 points in the FINDRISC questionnaire) with healthy conrol subjects.
Materials and methods: Our study involved 28 patients with higher future T2DM risk (mean age: 60,3 ±8,2, female 15, HbA1c 5,9 [5,6;6,1], BMI 31 [27,9;34,5]) and 20 healthy control subjects (mean age:54,3 ± 1,4 , female 10, HbA1c 5,4 [5,1;5,8], BMI 29,5 [26,3;32,4]). Sensory function was evaluated by Neurometer (Neurotron Inc., Baltimore, USA) device, 128 Hz calibrated tuning fork, Semmes-Weinstein monofilament and Q-sense (Medoc Ltd., Yamat Rishai, Israel) device. Cardiovascular autonomic function was assessed by the five standard cardiovascular reflex tests, 24-hour heart rate variability (HRV) and ambulatory blood pressure monitoring (ABPM).
Results: Patients with higher future T2DM risk had significantly higher vibration perception thresholds on the lower extremities than healthy control subjects (5,8 vs 6,8; p= 0,01). Assessing autonomic function, the total autonomic impairment score was higher (2,1 vs 0,7; p=0,04), while SDANN, a sensitive parameter of heart rate variability was lower among patients with higher future T2DM risk compared to controls (133 vs 184, p=0,02). Moreover, patients with higher future T2DM risk had significantly higher scores in the case of the 6th question of the FINDRISC questionnaire which refers to the use of antihypertensive medication (1,5 vs 0,76; p= 0,01).
Conclusion: Impaired cardiovascular autonomic function and diminished vibration perception at the lower extremities might be present among patients with high risk for the development of type 2 diabetes mellitus screened by the FINDRISC questionnaire, compared to healthy controls. Our results highlight the importance of early neuropathy assessment, as well as for the development of effective risk reduction strategies among these patients.
Disclosure: **O. Vági:** None.

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Cardiovascular autonomic neuropathy and cardiovascular outcomes in DCCT/EDIC study

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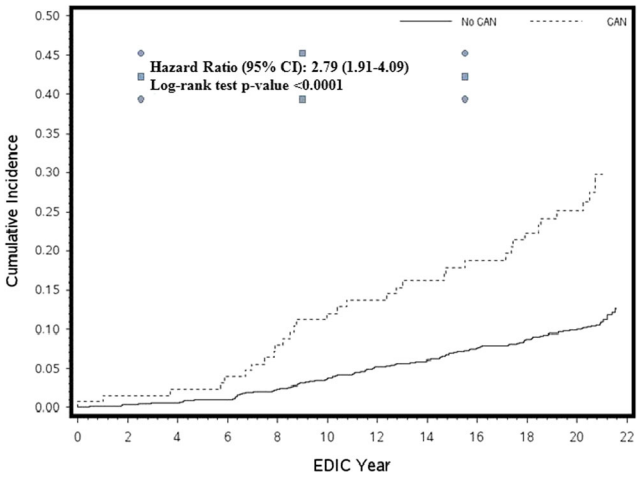
Background and aims: Cardiovascular autonomic neuropathy (CAN) has been shown to be an independent predictor of cardiovascular disease (CVD) mortality in diabetes. We aimed to examine whether CAN is an independent risk factor of CVD events during the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) in participants with type 1 diabetes.
Materials and methods: Standardized cardiovascular autonomic reflex tests (R-R response to paced breathing, Valsalva maneuver, and postural changes in blood pressure) were performed at DCCT baseline, every 2 years throughout DCCT, and at two time points in EDIC. CVD events were ascertained throughout the study and adjudicated by a review committee. Cox proportional hazards models were used to estimate the effect of CAN on subsequent CVD risk.
Results: There were 299 adjudicated events in 165 participants following the DCCT closeout assessment, 132 of 1262 (10%) without CAN experienced 244 CVD events vs. 33 of 131 subjects (25%) with CAN who experienced 55 events (HR=2.79, 95% CI 1.91-4.09 for time to first CVD event) (Figure). In models adjusted for multiple risk factors, the cumulative incidence of the first occurrence of any CVD event during EDIC was significantly higher in participants with CAN at DCCT closeout compared to those without CAN, although the association was no longer significant after further adjustment for the EDIC updated mean HbA1c. When analyzed as a continuous variable, R-R variation was significantly lower at DCCT closeout in participants who experienced a CVD event compared to those who did not (p=0.0012).
Conclusion: In the DCCT/EDIC cohort, individuals diagnosed with CAN at DCCT closeout experienced a higher long-term risk of CVD events during follow-up in EDIC, although this association was not independent of historic glycemic exposure, the principal determinant of long-term CVD risk in type 1 diabetes.



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Disclosure: **R. Pop-Busui:** Grants; NIDDK: U01 DK094176 and U01 DK094157.

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Main predictors of cardiovascular autonomic dysfunction in early stages of glucose intolerance

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Background and aims: The exact nature of the association between different metabolic parameters and peripheral nerve injury in early stages of glucose intolerance remains debatable. The present study aims to evaluate the predictive value of different cardio-metabolic parameters for the presence of cardiovascular autonomic dysfunction (CAD) in early stages of glucose tolerance.
Materials and methods: A total of 478 subjects without previously diagnosed diabetes, of mean age 49.3±13.7 years and mean BMI 31.0±6.2 kg/m2, were enrolled. They were divided according to glucose tolerance: 130 with normal glucose tolerance (NGT), 227 with prediabetes - 125

with impaired fasting glucose (IFG) and 102 with impaired glucose tolerance (IGT), and 121 with newly-diagnosed T2D (NDT2D). Glucose tolerance was studied during OGTT applying 2006 WHO criteria. Anthropometric indices and blood pressure were measured. HbA1c, serum lipids, hsCRP, albumin-to-creatinine ratio (ACR) were assessed. Electrocardiogram was performed. Body fat distribution was estimated by a bioimpedance method (InBody 720, BioSpace). Tissue AGEs accumulation was assessed by skin autofluorescence (AGE-Reader-DiagnOpticsTM). CAD was assessed by ANX-3.0 method applying standard clinical tests. Statistical analysis was performed using SPSS v.21.0.
Results: CAD was found in 12.3% of NGT, 19.8% of prediabetes (13.2% of IFG and 20.6% of IGT), and 32.2% of NDT2D group. There was an enduring trend towards lower sympathetic and parasympathetic tone at rest and during applied clinical tests with the progression of glucose intolerance. Age, waist circumference, visceral fat area, fasting and 120-minute plasma glucose, HbA1c, AGEs, ACR and QTc interval were significantly higher in the presence of CAD as compared to the group without CAD. In a logistic regression analysis the panel of age >53 years (76% sensitivity, 61% specificity), HbA1c >6.0% years (66% sensitivity, 60% specificity), QTc interval >423ms years (65% sensitivity, 61% specificity) and presence of arterial hypertension (83% sensitivity, 55% specificity) was found to significantly increase the risk for the presence of CAD, with predictive value of the model - AUC 0.778 (95%CI: 0.73-0.83), p<0.001.
Conclusion: Our results demonstrate high prevalence of CAD in the early stages of glucose dysmetabolism. Age, HbA1c, QTc interval and presence of arterial hypertension outline as predictive markers for the presence of CAD in this population.
Disclosure: **R. Dimova:** None.

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Efficacy of acupuncture in the treatment of patients with diabetic gastroparesis

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Background and aims: The treatment of patients with diabetic gastroparesis (DG) remains the difficult clinical task. There are some limited data that acupuncture could be of some use for the treatment of these patients. Acupuncture at heterotopic points could improve gastroparesis via excitation of vagal nerves and inhibition of sympathetic nerves. The aim of this study was to compare the efficacy of acupuncture for the management of gastrointestinal symptoms in patients with DG.
Materials and methods: We studied 32 patients with DG who were randomly allocated into 2 groups: a treatment group (n=16 (5M/11F), mean age was 56,4±10,7 years , DM duration - 12,4±6,7 years) assigned to accupuncture 5 times per week 40 minutes each for 10 days, and a control group (n=16 (7M/9F), mean age - 58,4±9,7 years , DM - 13,1±8,6 years). The severity of DG was assessed by Gastroparesis Cardinal Symptom Index (GCSI) questionnaire and Gastric emptying rate (GER) with 13C-octanoic breath test (13C-OBT) before and after the treatment period.
Results: The treatment with acupuncture resulted in the clinically significant improvement of the severity of symptoms in patients with DG while in the control group there were no positive changes of the signs of DG. In the first group patients was associated with a decrease in scores for cardinal symptoms of the GCSI: nausea by 68,4%, retching by 76,8%, vomiting by 86,7%, stomach fullness by 62,5%, not able to finish a normal-sized meal by 21,2%, stomach visibly larger by 13,4%, loss of appetite by 12,8%, feeling excessively full after meals by 64,7% and bloating by 22.5% (p<0.05). Gastric motility was significantly decreased after the end of treatment period - 101.61±3.41 min and 93,2 ± 1.11 min, before after the treatment, respectively, p<0.05. In the control group no significant changes of GER were observed after 2 weeks of observation.
Conclusion: Acupuncture therapy is feasible and effective method for the treatment of patients with DG.
Disclosure: **I.O. Kostitska:** None.

PS 096 Autonomic function tests and risk

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Cardiac autonomic neuropathy is strongly associated with abnormal cardiac repolarisation

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Background and aims: Diabetic cardiac autonomic neuropathy (CAN) is associated with an increased risk of cardiac events and sudden cardiac death. QT variability index (QTVI) is a measure of cardiac repolarisation that is associated with myocardial electrical instability and arrhythmias. We therefore aimed to study the relationships between QTVI and CAN.
Materials and methods: Using O'Brien's tests and baroreceptor sensitivity (BRS) testing, 62 diabetes subjects (37 with T2DM, 38 males) were classified into three groups: 22 with no CAN (No-CAN, 47±15 yrs), 28 subclinical CAN (53±12 yrs) and 12 established-CAN (57±14 yrs). We then analysed QTVI and indices of heart rate variability (HF - parasympathetic and LF - sympathetic) in all subjects.
Results: QTVI was significantly lower in subjects with No-CAN (-0.76 ±0.62) compared to Subclinical-CAN (-0.11±0.56) and Established-CAN (0.28±0.86; ANOVA p<0.003). Parasympathetic and sympathetic activity were significantly higher for No-CAN vs Subclinical-CAN and Established-CAN, LF (2.17±0.58 vs 1.11±0.65 and 0.94±0.52 ms2, respectively; ANOVA p<0.001) and HF (2.03±0.59 vs 0.92±0.60 and 0.98 ±0.53 ms2; ANOVA p<0.001). There was a strong negative correlation between QTVI and sympathetic (ρ=-0.844) and parasympathetic activity (ρ=-0.713; p<0.001). Moreover, BRS significantly (p<0.001) correlated with QTVI (-0.753), LF (0.758) and HF (0.718).
Conclusion: These results demonstrate a strong association between CAN and cardiac repolarisation abnormalities, which are recognised to increase the susceptibility to cardiac events. Alarmingly there is a clear demonstration of significant abnormalities in early subclinical CAN. Further studies are required to examine if early intensive multifactorial risk factor treatment that has been shown to reduce incident CAN will also have an impact on cardiac repolarisation and arrthythmogenic risk.
Supported by: NIDDK
Disclosure: **M. Oleolo:** None.

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Cardiac autonomic function is associated with the coronary microcirculation in type 2 diabetic patients

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Background and aims: Cardiac autonomic dysfunction and cardiac microvascular dysfunction are both diabetes complications associated with increased mortality, but the association between these complications have been difficult to assess. We therefore applied new and sensitive methods to assess this in type 2 diabetes patients.
Materials and methods: In a cross-sectional design, coronary flow reserve (CFR) and coronary artery calcium (CAC) score, assessed by cardiac 82Rb-positron emission tomography/computed tomography (PET/CT), cardiac autonomic reflex tests and heart rate variability (HRV) indices were performed in 55 patients with type 2 diabetes, without clinical cardiovascular disease, and 28 non-

diabetic controls. Cardiac 123I-metaiodobenzylguanidine scintigraphy (123I-MIBG) was conducted and evaluated as the late heart/mediastinum-ratio as measure of sympathetic function, in a subgroup of 29 patients and 14 non-diabetic controls.
Results: Impaired cardiac autonomic function was associated with reduced CFR ($p \leq 0.005$). The HRV measure low-frequency power and the late heart/mediastinum-ratio were positively correlated with CFR after age and heart rate adjustment ($p \leq 0.010$). The late heart/mediastinum-ratio remained correlated with CFR after further adjustment for sex, 24-h systolic blood pressure, HbA1c, urinary albumin excretion and smoking ($p < 0.001$). All measures of cardiac autonomic function, except the late heart/mediastinum-ratio, were negatively associated with CAC after age and heart rate adjustment ($p \leq 0.043$), but after further adjustment significance was lost ($p \geq 0.072$), which could reflect shared risk factors.
Conclusion: In type 2 diabetic patients without clinical cardiovascular disease, we demonstrate a strong independent association between cardiac sympathetic nerve system function, and CFR. We suggest that elevated cardiac sympathetic tone may play an important pathogenetic role in subsequent development of myocardial injury.
Disclosure: **B.J. von Scholten:** None.

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Effect of cardiac autonomic function on ST-segment depression, heart rate and heart rate variability during exercise in children and adolescents with type 1 diabetes

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Background and aims: The aim of this study was to investigate relation between cardiac autonomic function and ECG parameters during and post-exercise in children and adolescents with type 1 diabetes mellitus (T1DM).
Materials and methods: The study included 71 young patients with T1DM aged 9-18 years. Mean duration of the disease was 5.0 years, and the mean HbA1c level was 9.1%. Cardiac autonomic function was assessed using cardiovascular tests (Valsalva maneuver, 30:15, deep breathing) and ECG monitoring with automatic calculation of QT interval and heart rate variability parameters (RMSSD, SDNN). Each patient underwent the physical working capacity test (PWC170) with simultaneous ECG recording.
Results: Asymptomatic ST-segment depression (more than 0,1 mV) during exercise was registered in 10 (45.5%) patients with signs of autonomic dysfunction (CAN+) compared to 9 (18.4%) patients without autonomic dysfunction (CAN-; p=0.042). During the recovery period, asymptomatic ST-segment depression was present in the first minute in 8 (36.4%) CAN+ patients compared to 1 (2%) CAN- patient (p=0.0003) and in the second minute in 5 (22.7%) CAN+ patients compared to 1 (2%) CAN- patient (p=0.0095). CAN+ patients had lower heart rate variability (HRV) and heart rate (HR) values during and post-exercise. During recovery period there was a poor recovery of HRV and HR values in CAN+ patient with abnormal (less than 23 beats/min) HR recovery in 7 patients compared to 4 CAN- patients (31,8% vs 8,2%, CAN+ and CAN- respectively, p=0,028).
Conclusion: Children and adolescents with T1DM and impaired autonomic function have increased prevalence of asymptomatic ST-segment depression during and post-exercise as well as poor post-exercise recovery of HRV and HR. This fact may be responsible for the high cardiovascular morbidity and mortality rate among the patients with T1DM in the mature age.
Disclosure: **D. Laptev:** None.

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Long-term follow-up of autonomic function in type 1 diabetic patients on insulin pump treatment

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Background and aims: The long-term hyperglycemia before insulin pump treatment and the following improvement of the glycemic control might influence the autonomic function in type 1 diabetes (DM). Objectives: The aim of our study was to assess cardiovascular autonomic function at initiation of insulin pump treatment, two months later, as well as 6 years later.
Materials and methods: 13 type 1 DM patients were involved (7 women, 6 men, age: 30.4±2.7 years, duration of DM: 16.5±2.7 years; BMI: 24.2±1; mean±SE). Autonomic neuropathy (AN) was assessed at the first application of insulin pump and 2 months as well as 6 years later by cardiovascular reflex tests (CRT).
Results: Correlations were found between the duration of DM and CRT-s (AN score-duration: $r=0.57$, $p<0.05$; heart rate response to breathing-duration: $r=-0.55$, $p<0.05$). Moderate to severe AN was found, while improvement of total autonomic score was detected two months later (2.85 ±0.3 vs 1.23±0.3, $p<0.01$). The AN score found six years later was identical to the initial value (AN score: 2.85±0.47). The change of CRT-s was not significant during the follow-up period. The mean HbA1c decreased by 0.7% after 2 months (8.85±0.2% vs 8.12±0.3%, p=0.07) and was even lower 6 years later (8.85±0.2% vs 7.85±0.3%, $p<0.05$).
Conclusion: Moderate to severe autonomic neuropathy was detected in type 1 diabetic patients at the initiation of insulin pump treatment. The severity of the parasympathetic involvement correlated with the duration of diabetes. Improvement of autonomic function could be confirmed after short-term treatment with insulin pump. No progression of autonomic nerve dysfunction compared to baseline values was observed after six years of observation.
Disclosure: **T.T. Várkonyi:** None.

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Corneal confocal microscopy and cardiovascular autonomic function tests for detecting diabetic neuropathy in children

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Background and aims: Identification of early markers of diabetic neuropathy by corneal confocal microscopy and cardiovascular autonomic function tests with spectral analysis of heart rate variability in diabetic children.
Materials and methods: 44 patients with type 1 diabetes mellitus (DM) mean disease duration 4,5 (1-5) years, 6 patients with maturity onset diabetes of the young (MODY2, MODY3), mean disease duration 4,1 (1-5) years; 5 patients with type 2 DM, mean disease duration 2,75 (1-4) years; and 30 nondiabetic healthy control subjects were recruited in the study. Informed consent was obtained from all patients. All patients and control subjects underwent an evaluation of neurologic signs (Neuropathy Disability Score), symptoms according to the neuropathy symptom score and autonomic cardiovascular tests with spectral analysis of heart rate variability (the low-frequency component (LF), high-frequency component (HF), the LF/HF ratio). All patients underwent examination with the Heidelberg Retina Tomograph III in vivo corneal confocal microscopy (CCM). CCM was performed with assessment of corneal nerve fiber density (CNFD). Automated image analysis was performed using a specialized computerized software (ACCMetrics). HbA1c, total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides (TG) was measured.

Results: No significant difference was found in vibration, pin prick and temperature perception. CNFD was significantly lower in participants with DM compared to control group (27.7 ± 9.1 vs 35.5 ± 9.4 fibres/mm2; p<0,05). The subjects with type 1 DM showed a lower LF and HF component of heart rate variability, and a lower LF/HF ratio. Modifications in heart rate variability characterized by a reduction in both sympathetic and parasympathetic activity (changes in unmyelinated nerves). Mean HbA1c in patients with type 1 DM was 8.7% (5.5-17.9), type 2 DM - 6% (5,5 - 7,7), MODY - 6% (4,4 - 8,1). Only 10 patients had lipid profile in normal range. CNFD significantly negatively correlated with HbA1c (r=-0,4; p<0,05), TG (r=-0,3; p<0,05), diabetes duration (r=-0,78; p<0,05).
Conclusion: This study demonstrates significant abnormalities in corneal nerve structure in children with different types of diabetes in early stages of the disease (duration up to 5 years), despite normal Neuropathy Disability Score. Prospective studies are needed to show the prognostic value of CCM and autonomic cardiovascular function testing and clarify the optimal way for screening and management of diabetic neuropathy.
Disclosure: **E. Artemova:** None.

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Diabetic cardiovascular autonomic neuropathy predicts recurrent cardiovascular diseases in patients with type 2 diabetes

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Background and aims: Cardiovascular autonomic neuropathy (CAN) is a risk factor for cardiovascular disease (CVD) and mortality in patients with type 2 diabetes. This study evaluated the relationship between CAN and recurrent CVD in type 2 diabetes.
Materials and methods: A total of 206 patients with type 2 diabetes who had a history of CVD within 3 years of enrollment were consecutively recruited from January 2001 to December 2009 and followed−up until December 2015. Cardiovascular autonomic function tests were performed using the following heart rate variability parameters: expiration−to−inspiration ratio, response to Valsalva maneuver, and standing. We estimated the recurrence of CVD events during the follow−up period.
Results: A total of 159 (77.2%) of the 206 patients enrolled completed the follow up, and 78 (49.1%) patients had recurrent episodes of CVD, with an incidence rate of 75.6 per 1,000 patient−years. The mean age and diabetes duration were 62.5 ± 8.7 and 9.2 ± 6.9 years, respectively. Patients who developed recurrent CVD also exhibited hypertension (P = 0.004), diabetic nephropathy (P = 0.012), higher mean systolic blood pressure (P = 0.006), urinary albumin excretion (P = 0.015), and mean triglyceride level (P = 0.035) than did patients without recurrent CVD. Multivariable Cox hazard regression analysis revealed that definite CAN was significantly associated with an increased risk of recurrent CVD (hazard ratio [HR] 3.03; 95% CI 1.39−6.60; P = 0.005) (Table 1).
Conclusion: Definite CAN was an independent predictor for recurrent CVD in patients with type 2 diabetes who had a known prior CVD event.

Table 1. Multivariable Cox hazards regression model for the risk of recurrent cardiovascular diseases

	Model 1	Model 2	Model 3	Model 4
Staging of CAN	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Normal	1.00	1.00	1.00	1.00
Early	2.31 (1.05−5.06) *	2.16 (0.98−4.75)	1.87 (0.84−4.15)	1.93 (0.86−4.36)
Definite	3.56 (1.71−7.42) *	3.16 (1.51−6.63) *	2.74(1.29−5.86) *	3.03 (1.39−6.60) *

Multivariable Cox proportional hazard models were adjusted for the following covariates: model 1: sex, age, and diabetes duration; model 2: model 1 + presence of hypertension; model 3: model 2 + mean SBP; and model 4: model 3 + eGFR, mean LDL−Cand mean HbA_{1C}.
HR, hazard ratios; CAN, cardiovascular autonomic neuropathy; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; LDL−C, low−density lipoprotein cholesterol. *P< 0.05

Disclosure: **L. Kang-Min:** None.

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Dipeptidyl peptidase-4 acitivity is associated with autonomic neuropathy and insulin resistance in type 1 diabetic patients

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Background and aims: Dipeptidyl peptidase-4 (DPP-4) is a protease involved in to metabolism of various adipokines and recently associated with insulin resistance (IR). The influence of adipokines and the contribution of an altered sympathovagal balance to the development of IR has been previously established. We investigated the potential impact of cardiac autonomic neuropathy (CAN) on DPP-4 activity and its relationship with IR and leptin, resistin, tumor necrosis factor α (TNFα) and neuropeptyde Y (NPY) concentration in T1DM.
Materials and methods: Seventy T1DM patients (47 males) aged 50.85 ±1.57 years with a duration of diabetes 26.36±1.97 years were divided into two groups according to median DPP-4 activity(22.14 U/L). Leptin, resistin, NPY and TNFα plasma concentration were analyzed with ELISA while serum DPP-4 activity was determined spectrophotometrically. Insulin sensitivity was estimated using glucose disposal rate (eGDR) equation: 24.31−12.2X(WHR)−3.29X(AHT)−0.57X(HbA1c), where WHR indicates the waist to hip ratio, AHT indicate hypertension, and is expressed as: 0 = no, 1 = yes. Lower eGDR indicates higher IR. CANwas assessed by hearth rate coefficient of variability (HRV CV) at rest and during deep breathing (dbHRVCV), db E/I ratio, 30:15 ratio and Valsalva ratio while sympathovagal balance was evaluated as low- to high-frequency ratio (LF:HF) by spectral analysis on R-R intervals.
Results: The two groups did not differ in age, gender and disease duration.The group with higher DPP-4 activity showed increased prevalence of CAN, higher level of resistin, leptin, TNFα while NPY and eGDRlevels were lower (Table). DPP-4 activity was positively related to TNFα, leptin, resistin and IR and negatively with NPY, standard and spectral indices of CAN:HRV CV at rest, dbHRV CV, db E/I ratio,30:15 ratio and LF:HF ratio (Table). The linear regression analysis showed that DPP-4 activity increase of 1 U/L was associated with eGDR decrease of0.090 mg kg−1 min−1 (p=0.001),NPY of 0.358 pg/mL (p=0.002), dbHRV-CV of0.056 (p<0.001) and LF:HF ratio of0.010 (p=0.019) and TNFα increaseof 0.251 (p=0.014), leptin of 0.085 (p=0.039) and resistinof0.060 (p=0.001) after adjustment for age, gender, disease duration, glycated haemoglobinA1c (HbA1c) and body mass index. In the binary regression mixed model with CAN, gender, disease duration and HbA1c as independent and DPP-4 activity above the median as dependent variable, CAN was the top independent predictor of DPP-4 activitywith prevalence ratio of 1.324 (95% CI 1.110-1.956).

Conclusion: Increased DPP-4 activity is associated with IR and the concentration of adipokines related to IR.DPP-4 activity appears to be influenced by autonomic neuropathy. It might represent a significant factor contributing to the development of IR in type 1 diabetic patients through sympathovagal disbalance. The predictive value of the relationship between DPP-4 activity and autonomic disbalance in the pathogenesis of IR in type 1 diabetes merits to be further investigated

Table. Characteristics of the study participants according to the median DPP-4 activity value of 22.14 U/L

	DPP-4 activity>22.14 U/L N=35	DPP-4 activity<22.14 U/L N=35	P	Correlations: DPP-4 activity (U/L)
CAN (N, %)	15 (42.86%)	9 (25.71%)	0.016	-
HRV CV (%)	4.50±0.55	5.86±0.12	<0.001	r= -0.802; p<0.001
db HRV CV (%)	7.85±0.47	9.14±0.50	<0.001	r=-0.524 ; p=0.007
E/I ratio	1.27±0.21	1.42±0.05	0.001	r=-0.362 ; p=0.022
30/15 ratio	1.42±0.07	1.67±0.14	<0.001	r=-0.415 ; p=0.006
Valsalvaratio	2.17±0.24	2.21±0.16	0.418	r=-0.181 ; p=0.098
LF/HF ratio	1.18±0.12	1.69±0.18	<0.001	r=-0.551 ; p=0.002
TNF α (pg/mL)	11.93±0.97	8.21±0.63	<0.001	r=0.422 ; p=0.001
Resistin (ng/ml)	19.72±0.63	18.88±0.52	<0.001	r=0.438 ; p=0.002
Leptin (ng/ml)	14.67±2.04	13.37±1.56	0.004	r=0.441; p=0.004
NPY (pg/ml)	52.44±3.46	58.94±3.34	<0.001	r=-0.441; p=0.002
eGDR (mg $kg^{-1}min^{-1}$)	6.02±0.31	7.51±0.27	<0.001	r=-0.643; p<0.001

NOTE: values are expressed as mean±S.E.M.

Disclosure: **L. Duvnjak:** None.

PS 097 The diabetic foot and wound healing

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Investigation of the effects and molecular mechanism of adenine for diabetic wound healing

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Background and aims: Chronic wound-derived foot ulcers are major complications of diabetes resulting in lower-limb amputations. Current research suggests that AMP-Activated Protein Kinase (AMPK) modulating energy metabolism may potentially target the strong association between cellular energy supply and various diseases such as diabetes, cancer, and cardiovascular disease. However, the role of AMPK as a therapeutic agent for diabetic foot ulcers still unknown. Our previous findings suggest that adenine represents a novel AMP-activated protein kinase (AMPK) activator, which could ameliorate TNFα-stimulated inflammation in HUVECs and enhance glucose uptake in NIH-3T3 fibroblasts. The mechanism that underlies adenine-induced AMPK activation is via the catabolism by adenine phosphoribosyltransferase (APRT), which increases the cellular AMP levels to promote AMPK phosphorylation. In the study, we evaluate the effects of adenine on wound healing in diabetic mice. Furthermore, we also compare the effects of adenine and other AMPK activators on keratinocytes and fibroblast.

Materials and methods: Normal human epidermal keratinocyte (NHEK) and normal human dermal fibroblast (NHDF) cells were exposed to various concentrations (20, 200, 500 uM) of adenine and cell proliferation was measured by MTT assay; Migration was evaluated by boyden chamber assay. A single full-thickness skin wound with silicone splinting was made on the dorsal thorax of 8 weeks old of db/db mice. Wounds were treated by the carboxymethyl cellulose (CMC) hydrogels with adenine (0.01, 0.02%) or vehicle daily. Body weights and wound closure area were recorded daily on the duration of test. The levels of fasting blood glucose were measured before and the 15th day after operation. In addition, histopathological parameters including macrophage infiltration, fibroblast deposition and re-epithelialization were calculated to evaluate the effects of adenine.

Results: Here we demonstrated that the use of adenine accelerates diabetic wound healing by lowering inflammation and by enhancing re-epithelialization and matrix deposition of the wound, thereby reversing the pathological condition. The mechanism of adenine-mediated AMPK activation in the pathology of diabetic wounds was confirmed further by the injection of siRNA against APRT in the wound margins, which exhibited wounds more resistant to healing. Histology and immunohistochemistry results showed that this treatment contributed to the rapid healing of diabetic skin wounds by promoting granulation tissue formation, extracellular matrix secretion, and re-epithelialization. In vitro experiments in NHEK and NHDF cells also revealed that adenine-induced AMPK activation contributes to wound healing by increasing proliferation in a dose-dependent manner under high glucose conditions. These effects were abolished by co-administration of AMPK antagonist compound C.

Conclusion: It is concluded that adenine promote the proliferation and remodeling phases of wound healing. Based on these findings, we speculate that adenine-induced AMPK activation may be a potential option for the management of diabetic ulcers. These results need to be confirmed by clinical studies with diabetic foot ulcer patients.

Disclosure: **G. Young:** None.

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Epoxidized α-tocotrienol enhances wound healing in the db/db mouse model of diabetes and stimulates in vitro angiogenesis and cell migration

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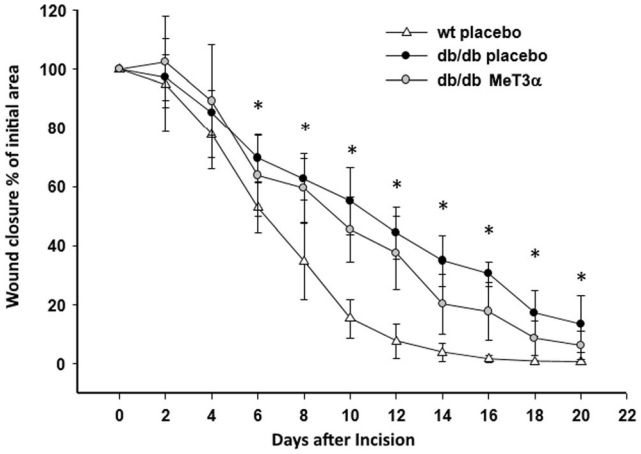
Background and aims: Diabetes mellitus is characterized by hyperglycemia and capillary hypoxia, causing excessive production of free radicals. The resulting oxidative stress is associated with impaired wound healing in diabetic ulcers. Modified tocotrienols were previously shown to have beneficial effects on the biosynthesis of mevalonate pathway lipids and isoprenoids including Coenzyme Q10. We aimed to investigate the effects of epoxidized α-Tocotrienol (MeT3α) in wound healing, its effects on mitochondrial function and mitochondrial superoxide production.
Materials and methods: Microarray and qPCR were performed on HepG2 cells and human dermal fibroblasts (HDF), respectively. In vitro wound healing was studied by scratch assay in HDF cells. Capillary tube formation was measured using human dermal microvascular endothelial cells (HMVEC). db/db diabetic mice were used for in vivo wound healing experiments. Mitochondrial function, including oxygen consumption rate, proton leak and reserve respiratory capacity was measured with SeaHorse XF24-3 Extracellular Flux analyzer. Mitochondrial superoxide production using MitoSox red probe was measured with FACS in primary HDF cultures from type 2 diabetic and healthy subjects.
Results: Microarray profiling analysis in HepG2 and HDF cells treated with MeT3α showed a 20-fold increase of KIF26A 11-fold decrease of lanosterol synthase expressions. Gene expression analysis by qPCR showed significant increases of the growth factors VEGFA (4-fold increase) and PDGFB (5-fold increase) compared to controls. Treatment yielded significant increases in fibroblast migration ($p<0.05$) and HMVEC capillary tube formation ($p<0.01$) were observed. in vivo wound healing assay using db/db mice, a small but significantly enhanced wound closure was observed upon topical MeT3α treatment compared to control (fig. 1). Treatment led to an increase in reserve respiratory capacity in both HDF and HepG2 cells. MeT3α treatment significantly reduced mitochondrial superoxide production in primary HDF cultures from diabetic as well as healthy subjects ($p<0.01$).
Conclusion: MeT3α has beneficial effects on wound healing by increasing gene expression affecting cell growth and motility. Additionally, MeT3α improves mitochondrial function by increasing respiratory reserve capacity while reducing oxidative stress. Future plans aim to elucidate the links between the role of Coenzyme Q10 in diabetic wound healing and the observed effects of MeT3α.



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The relationship between the skin advanced glycation endproducts (AGEs) and skin colour or elasticity in Japanese diabetic and non-diabetic subjects

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Background and aims: The accumulation of skin advanced glycation endproducts (AGEs), possibly quantified as the autoflourescence (AF) value, are related to progression of diabetic microvascular complications with type 1 diabetes mellitus (T1D) and type 2 diabetes (T2D). It has been reported that AGEs accumulation possibly causes changes in skin elasticity or skin color. The aim of this study is to investigate the relationship between skin AF value and skin elasticity or color in diabetic and non-diabetic subjects.
Materials and methods: A total of 228 patients (123 men) with T1D, 85 (62 men) with T2D, and 80 (62 men) non-diabetic controls (nDM) were enrolled. Clinical data were collected from the medical records for diabetes patients (DM), and from the health checkup records for nDM. Skin AF value was evaluated using AGE Reader® which was able to non-invasively assess AGEs accumulation using skin AF under ultraviolet light. Spectrophotometer CM-2600d measures skin color which was calculated as L*a*b* color space values as skin brightness and saturation, melanin index (MI), and hemoglobin index (HI). Skin elasticity was evaluated using cutometer MPA-580. All variables were assessed on the same area on the ventral side of the forearm.
Results: Past glycemic control and diabetic duration were the independent risk factors for skin AF value with T1D. Skin AF value was significantly elevated with progression of diabetic retinopathy with T1D and T2D, and nephropathy with T1D. And the skin elasticity tended to decrease as retinopathy progressed with T1D. Regarding skin color, brightness was lower ($p < 0.05$), while saturation was higher ($p < 0.05$) in DM than in nDM. MI was lower in DM than in nDM. MI and HI were the independent variables for skin brightness and saturation rather than skin AF value. Skin elasticity decreased with aging in all groups, and age was considered as the independent variable in DM ($p < 0.0001$), whereas BMI was taken as the independent variable in type2 DM ($p = 0.0108$) for skin elasticity.

Conclusion: Skin AGEs was strongly influenced by glucose metabolism. Skin color was mainly influenced by melanin, rather than skin AGEs, and skin elasticity was related to age, with no relation to skin AGEs.
Disclosure: **J. Miura:** None.

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Indocyanine green fluorescence angiography in diabetic patients with peripheral arterial disease

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Background and aims: To evaluate diagnostic value of indocyanine green (ICG) angiography (ICGA) with intravenous injection ICG for assessment peripheral blood flow in diabetic patients with peripheral arterial disease (PAD).
Materials and methods: Prospective study included 23 diabetic patients with PAD who underwent percutaneous transluminal angioplasty (PTA) in 23 lower limbs. Diagnosis and treatment of PAD was based on recommendation of IWGDF (Hague, 2015). Transcutaneous oxygen tension (TcpO2) and ICGA were used to assess skin foot perfusion in ulcer site. To perform ICGA, charge-couple device camera, a laser (800 nm) - the SPY system (Novadaq, Bonita Springs, Fla) and intravenous fluorescent ICG were used. Time to maximum intensity (Tmax) in ulcer site during ICGA was recorded. Patency of lower limb arteries was evaluated by duplex ultrasound (DU). The degree of tissue damage was assessed according to Wagner classification.
Results: There were 23 diabetic patients with PAD. The mean age was 66,7,± 9,8 years, HbA1c 8,25±1,53%, diabetes duration 16,5[0,8-43] years, diabetes type 1/2-4/19, man/woman 61/39%. There were comorbidities: arterial hypertension in 93%, myocardial infarction in 18,75%, stroke in 16,6%, dyslipidemia in 70,8%, smoking in anamnesis in 56,25%, chronic kidney disease (stage 3-5) in 23%. Stenosis >50% of vessel diameter and occlusions were located in the iliac/femoral-popliteal axis in 4,34% (n=1), exclusively in the infrapopliteal axis in 39% cases (n=9), and in both femoral-popliteal and infrapopliteal axis in 57% (n=13). All patients were divided into 2 groups according to the severity of PAD: group A - with mild PAD and nonhealing foot ulcers during 6 weeks despite of standard treatment, TcpO2 more 25 and less 40 [28; 36] mmHg; group B - 12 patients with critical limb ischemia (CLI) and foot ulcers: TcpO2 less 25 mm Hg [9;21]. Groups A and B were comparable in comorbidities, severity of lower limb arteries obstructions and degree of tissue damage (Wagner 2-3, p-value less 0,05).
Conclusion: ICGA is a good tool for visual and rapid quantitative assessment of regional foot perfusion. ICGA may be important additional method in PTA decision-making process in discussible cases. It was pilot study and further clinical investigations are required to refine cut-off of ICGA parameters for detection diabetic patients with insufficient ulcer site perfusion to achieve ulcer healing and clarify the indications for revascularization.
Disclosure: **Z. Abdul'vapova:** None.

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KPC-producing Klebsiella pneumoniae infection and gut colonisation as mortality risk factors in patients with diabetic foot infections: a multicentre case-control study

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Background and aims: To evaluate the role of KPC-Kp gut colonization and infection in influencing the mortality rate of diabetic patients with foot infection (DFI) we performed a retrospective, multicenter case-control study.
Materials and methods: We analysed data from DFI patients cared for in 7 different centres. Patients were grouped as follow: Group A, patients with KPC-Kp isolated from infected foot specimen, Group B, patients with KPC-Kp isolated from rectal swab and Group C, control patients (negative for KPC-Kp cultures both from foot lesion and rectal swab).
Results: From December 2010 to July 2015 62 DFI patients with KPC-Kp infections (42) (group A) or KPC-Kp gut colonization (20) (group B) were identified. The control group was represented by 49 pts (Group C). The three groups were similar regarding main demographic and clinical characteristics, except for the Charlson Index, significantly higher in Group A (13.5) and B (6.3) than in Group C (3.2) (p=0.002 Group A vs B and Group B vs C). The mean duration of hospital stay was longer in Group A and B (respectively 40 and 44 days) vs Group C (7 days - p= 0.024 Group A vs B and p=0.018 Group B vs C). The levels of serum markers of Inflammation were significantly higher in Group A and B than in Group C, both Procalcitonin (Group A 1.86 ng/ml, Group B 1.12 ng/ml, Group C 0.72 ng/ml - p=0.025 Group B vs C, p=0.04 Group A vs C) and C Reactive Protein (Group A 13.5 mg/dl, Group B 6.3 mg/dl, Group C 3.2 mg/dl - p=0.002 Group A and group B vs C). Mortality was significantly higher in Group A (30.9%) and Group B (35%) than in Group C (12.1% - p= 0.038 Group A vs C and p= 0.004 Group B vs C). The healing rate of DFI was 33% in Group A (p= 0.03 vs Group C) 30% in Group B (p= 0.002 vs Group C) and 57% in Group C.
Conclusion: In diabetic foot patients gut colonization and foot infection with KPC-Kp are associated with a reduction in healing rate and a significant increased in mortality
Disclosure: **E. Iacopi:** None.

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Foot self-care in patients with and without a diabetic foot syndrome: a cross-sectional study on the knowledge-behaviour-gap and the role of barriers

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Background and aims: Up to 70 per cent of amputations in Germany pertain to people with diabetic foot ulcers. One measure to prevent foot ulcers is foot self-care of patients with diabetes. This study examines the differences between patients with type 2 diabetes and a diabetic foot syndrome (DFS) vs. without a DFS regarding their knowledge about foot care measurement, their foot care behavior, and perceived barriers.
Materials and methods: Self-administered questionnaires were distributed during August and October 2015 in eight diabetes outpatient centres in Lower-Saxony, Germany. All patients with type 2 diabetes and a DFS with a Wagner-classification <="3" were included during the survey period of two weeks per DSP. As a control group, per DSP the first 50 patients attending without a DFS were included. Verbal-cognitive deficits precluding questionnaire self-administration defined an exclusion criterion. Foot care behavior was assessed with the Nottingham Assessment of Functional Footcare. IBM-SPSS v23 was used for multivariate analyses.
Results: Eligible questionnaires were returned by 473 patients (response rate: 77.4%). 178 women participated (38.4%), and the mean age of sample was 63.8 years. Overall, 31.7% of the participants had a DFS. Participants with DFS showed better knowledge of foot care (p<.001) and reported better foot care behavior (p<.001). Participants with a DFS perceived more barriers (e.g. restrictions or absent perception of feet) to keeping their feet healthy (p=.015). A correlation between barriers and behavior was found in neither the group of participants with DFS nor the group of participants without DFS. Barriers also had no moderating influence on the correlation between knowledge and behavior. However, knowledge was associated with behavior for patients with and without DFS (r=.378 [DFS]/r=.273 [without DFS], p=<.001).

Conclusion: Knowledge of foot care compliant to the guidelines is good both in participants with and without DFS, though particularly patients without DFS have a need for further information. In this study population, the association between knowledge and behavior regarding foot care indicates effective patient education in participating DSPs. At the same time, there still is a gap between knowledge and behavior. To close this gap and improve foot care behavior, assistance and support for changing behavior is needed. Transfer of knowledge as a supporting determinant of improved foot care behavior may be increased in educational programs. Focusing on barriers as an inhibiting factor seems to be of secondary importance.

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Disclosure: **B. Kröning:** None.

PS 098 Focus on diabetic foot ulcers: Can we do better?

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Discordance of standard classification systems to detect the risk of foot ulcer among type 2 diabetic subjects

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Background and aims: The risk factors of today are the diseases of tomorrow. Early detection and grading of the risk of foot ulcer can greatly help to prevent the devastating clinical, psychological and socioeconomic consequences of diabetes. Diverse classification systems, however, are followed by various organizations and it creates substantial problem in interpreting the prevalence as well as the risk for diabetic foot ulcer in a community. The primary prevention is linked to common risk factors and their nature of clustering. The present study was designed to find out the discordance of standard (ADA, IWGDF and SCI-DC) classification systems to detect the risk of foot ulcer and to explore the clustering of risk factors among type 2 diabetic subjects.

Materials and methods: A cross-sectional study was conducted among 1200 (M/F, 445/755; age in yrs, 52.23±11.8; BMI, 25.1±4.3kg/m2) diabetic subjects, selected purposively from various health care facilities in Dhaka and Northern Districts of Bangladesh. In case of the risk classification systems, the Task Force of the Foot Care Interested Group of the American Diabetic Association (ADA), International Working Group on the Diabetic Foot (IWGDF) Modified and the Scottish Care Information-Diabetes Collaboration (SCI-DC) ulcer risk scores were used. Sensory neuropathy was assessed by 10-g Semmes-Weinstein monofilament, peripheral arterial diseases by ankle brachial index (ABI), deformity was measured by goniometer and clinical examination. History of ulceration and amputation were also taken.

Results: Of the total subjects, more than half (53.4%) of the respondents had 4 to 6 risk factors and a high majority (85.4%) of them had multiple (≥3) risk factors for foot ulcer. None of the study subjects were risk free for foot ulcer according to the ADA and SCI-DC criteria, but 25.8% (95%CI, 23.3-28.3) were risk free as per IWGDF criteria. The proportion of low risk subjects were as follows: according to ADA, 28.6% (26.0-31.2); IWGDF, 8.6% (7.0-10.2); and SCI-DC, 9.4% (7.7-11.0). Among the subjects 42.9% (40.0-45.7), 37.2% (34.5-39.9) and 11.4% (9.6-13.2) were in moderate risk groups and 28.5% (25.9-31.1), 20.6% (18.3-22.9) and 79.2% (76.9-81.5) subjects were in high risk groups respectively following the above mentioned classifications. Highly significant ($p<0.001$) difference has been found between ADA and IWGDF; ADA and SCI-DC; and IWGDF and SCI-DC classifications. From the Kappa statistics less than chance agreement has been found for ADA and IWGDF (K, -0.057; $p=0.47$); ADA and SCI-DC (K, -0.165; $p<0.001$) whereas only fair agreement found between IWGDF and SCI-DC (K, 0.332; $p<0.001$).

Conclusion: A substantial clustering tendency has been found for foot ulcer risk factors among the diabetic adults. So, intervention should be designed considering multiple risk factors. A clear discordance has also been found between three different standard classification systems to detect the risk of foot ulcer. A consensus for a unique validated classification system and appropriate recommendation for the prevention of foot ulcer are thereby essential.

Disclosure: **P.C. Banik:** None.

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Characteristics of painful diabetic neuropathy and neuropathic symptom score as a predictor of ulceration: results of the national multi center BRAZUPA study

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Background and aims: There is a paucity of data regarding pain characteristics as well as predictors of ulceration in some populations of patients with diabetes. Our aims were to describe common neuropathic symptoms in a large Brazilian population and assess the neuropathy symptom score (NSS) as a predictor of ulceration.
Materials and methods: We evaluated 1455 patients with diabetes and foot at risk in 19 different centres in Brazil in a cross sectional manner from July 2013 to August 2014. Symptoms were considered severe if NSS > 4 and neuropathy present if neuropathy disability score (NDS) > 2.
Results: Overall 67% of patients presented some degree of lower limb discomfort. The most common symptoms were burning/paresthesia (50%) and pruritus (27.3%). Nearly one third (35%) of patients had symptoms at awakening. Symptoms improved with lying down or sitting in 64% of patients, by walking in 24.4% and by standing in 11.7%. Frequency of NSS > 4 in burning/paresthesia was 72.7% vs 57% in the pruritus group (P < 0.001). Patients with daytime only symptoms were less likely to have severe NSS (14% vs 73.6% and 78.9% for day and night and night only, respectively; P < 0.001). Those with symptoms at awakening had NSS > 4 more frequently (84% vs 33.2%; P < 0.001). Of all patients, 50.1% presented NSS > 4 and 49.1% had NDS > 2. Severe symptoms occurred in 42.3% of patients without neuropathy (NDS < 3) and in 63.5% of those with severe neuropathy (NDS > 8). Chi square tests showed that patients with NSS > 4 were more likely to be older (58.5 vs 56.4 years; P=0.012), female (63% vs 56%; P=0.01), from the Northeast/Midwest regions compared to South/Southeast regions (57.3% vs 44.9%; P < 0.001), of non-white ethnicities (45.8% vs 52.9%, 54.5% and 57% for White vs Black, Mixed and Asian, respectively; P < 0.026), had a higher body mass index (BMI) (30 vs 28.9kg/m2; P=0.001), a higher prevalence of retinopathy (49.1% vs 42.3%; P=0.02) and visual deficit (54.1% vs 46.1%; P=0.006). Patients with neuropathic or neuroischemic foot were more likely to have NSS > 4 compared to normal or ischemic foot (69.8% and 64.2% vs 35.2% and 35.9%, respectively; P < 0.001). The overall prevalence of previous ulceration was 23.4%. In multivariate logistic regression, involving both NSS and NDS, moderate (OR=0.32; 95%CI = 0.16-0.64; P=0.001) and severe (OR=0.36; 95%CI = 0.17-0.76; P=0.007) NSS were protective factors for ulceration, whereas mild scores did not significantly affect ulceration risk (OR=0.53; 95%CI = 0.26-1.09; P=0.09). In contrast, mild (OR=4.03; 95%CI = 2.16-7.54; P < 0.001), moderate (OR=2.79; 95%CI = 1.45-5.37; P=0.02) and severe (OR=5.04; 95%CI = 2.29-11.06; P < 0.001) were significant risk factors for ulceration. Other risk factors for ulceration were disease duration (P=0.036), weight (P < 0.001), BMI (P < 0.001), visual deficit (P=0.023), foot at risk classification (P < 0.001) and region of origin (P < 0.001).
Conclusion: Two-thirds of patients with foot at risk presented lower limb discomfort. Higher NSS was found in those with burning/paresthesia, symptoms during the night and at awakening. Less symptomatic patients were more likely to present ulceration, probably due to loss of protective nociception. Higher NDS were associated to higher frequency of ulceration. These results encourage special attention to asymptomatic patients.
Supported by: INCT Diabetes e Obesidade
Disclosure: **A. Moura Neto:** None.

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Clinical characteristic and lower-extremity amputations in patients with arterial stiffness and limb-threatening diabetic foot ulcers

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Background and aims: Peripheral artery disease affects the healing of diabetic foot ulcers (DFUs). This study aimed to analyze the clinical characteristics and limb preservation outcomes of patients with arterial stiffness and limb-threatening DFUs.
Materials and methods: We enrolled 413 consecutive patients admitted to a major diabetic foot center in Taiwan from January 2014 to June 2015 for the treatment of limb-threatening DFUs. The patients were categorized into those with a low ankle-brachial index (ABI) (ABI<0.9, n=134, 42.1%), normal ABI (n=159, 38.5%) and high ABI (ABI>1.4, n=80, 19.4%) groups. Peripheral neuropathy was assessed using the monofilament test. All data analyses were conducted using SPSS version 20 (IBM SPSS Inc., Chicago, IL). For all statistical analyses, P < 0.001 was considered statistically significant.
Results: Of the patients with a high ABI, 37 (46%) were undergoing dialysis. Of all patients undergoing dialysis (n=101), those with a high ABI had a similar neuropathy rate to those with a low ABI (44.4% vs. 51.6%), compared to 63.6% of those with a normal ABI. There was no significant difference between the high and normal ABI groups in the rate of abnormal pulsation (45.9% vs. 47.1%) or resting pain (10.8% vs. 11.8%). For the patients without dialysis (n=312), the rate of neuropathy was higher in the normal ABI group (61.3%) but similar in the high and low ABI groups (37.5 vs. 41.5%). However, the high ABI group had a significantly higher rate of abnormal pulsation than the normal ABI group (25.6 vs. 11.4%, p<0.001). Furthermore, the high ABI group had a higher rate of previous ulcers than the low or normal ABI groups (41.9% vs. 25.4% or 20.7%, respectively). There were no significant differences in major lower-extremity amputations between the high and normal ABI groups (13.9% vs. 17.7% in those undergoing dialysis and 0% vs. 2.1% in those without dialysis).
Conclusion: There was no difference in limb preservation outcomes between the patients with arterial stiffness and those with a normal ABI during in-hospital treatment for limb-threatening foot ulcers. The patients with a high ABI but without dialysis had higher rates of abnormal pulsation and previous foot ulcers than those with a normal ABI.
Disclosure: **S. Hung:** None.

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Albuminuria as predictive risk factor for foot ulceration in patients with diabetes

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Background and aims: Prevention of diabetic foot consists in screening for persons at risk of foot ulceration. While neuropathy and peripheral artery disease (PAD) are well-known ulceration risk factors, association of albuminuria (Alb) with diabetic ulcer occurrence has not yet been clearly described. Although clinical tests to examine feet are simple, in reality this examination is often neglected due to various reasons and patients seek specialised clinic only when ulceration already develops. In the clinical practice a laboratory marker of foot ulceration risk would enhance identification of at-risk patients and enable more effective specialised care. The aim of this study was to examine the association of Alb with risk factors of foot ulceration - neuropathy and PAD in ulcer-naive patients with diabetes.
Materials and methods: In prospective study we included 87 consecutive ulcer-naive patients with diabetes (mean age 66.2 ys ±10.4, 70% men, 93% T2DM, diabetes duration 8,5 ys ±6.4, mean HbA1C 53 mmol/mol IFFC, 25% treated with insulin, 52% non-smokers, treated at our outpatient clinics which went through preventive foot examination at the foot clinic. Each patient filled a structured questionnaire focused at neuropathy symptoms, specialised nurse examined feet for neuropathy (use of 10-g monofilament, biotheziometer), and ischaemia (ABI, pulsation of peripheral arteries).The following clinical characteristics of the patients were obtained from the medical records(BMI, presence of retinopathy, Alb,

level of creatinine, lipids, TSH). Neuropathy was defined as the loss of 10-g monofilament perception and/or reduced vibration perception threshold with a biotheziometer (25V). PAD was defined as the absence of at least one pedal pulse and/or ankle-brachial index below 0.9. Alb was defined as an albumin-to-creatinine ratio of 2.6 in men / 3.6 mg in women /mmol creatinine in a spot urine sample. The patients were divided into two groups according to presence of Alb: group 1 patients with normoalbuminuria (n=63), group 2 patients with Alb (n=24). Clinical characteristics of both groups of patients were statistically compared. The association of Alb and PAD and neuropathy was assessed both individually and in as combined risk (presence of neuropathy, or PAD, or both) using the Mann-Whitney and Fisher tests.

Results: Clinical characteristics of the patients in both groups did not show a significant difference except for BMI (p=0.037) with higher values in patients with Alb. 54.2% patients with albuminuria suffer PAD while only 22.2% patients with normoalbuminuria suffer PAD (p = 0.008). Neuropathy is present in 62.5% patients with albuminuria and 28.6% normoalbuminuria (p=0.006). Combined risk (presence of neuropathy, or PAD, or both) was proved in 83% patients with Alb while only in 41% (p=0.001) patients with normoalbuminuria. Specificity of Alb as screening test of PAD, neuropathy or combined risk is 82%, 83% and 90%, respectively.

Conclusion: We found a statistically significant association between Alb and risk factors of foot ulceration - neuropathy and PAD. Patients with albuminuria are at a relevant risk of foot ulceration thus clinical foot examination is urgent. Frequent foot checks, repeated education and long-term dispensarisation at specialised clinics are the mainstay of ulceration prevention for these patients.

Disclosure: **J. Venerová:** None.

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Initial results from the National Footcare Audit of England and Wales

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Background and aims: There is evidence of considerable variation in the outcome of disease of the foot - both within and between countries. Systematic audit is required to document the extent of variation and can therefore be a key tool in improving overall quality of care. This report presents details of experience gained in the first 12 months of the National Diabetes Footcare Audit (NDFA) of England and Wales.

Materials and methods: The NDFA was launched in July 2014 with the aim that all specialist services might eventually participate. Each is asked to recruit as many as possible of all newly presenting episodes of foot ulceration and to enter key information on-line. Core demographic information on their diabetes history is obtained centrally by database linkage and does not need to be specifically gathered; the only data submitted are (a) the time elapsed between first presentation to a health care professional and first assessment by an expert, (b) the type and severity of the index foot ulcer using the SINBAD system and (c) a single measure of clinical outcome - being alive and ulcer-free at 12 weeks and at 24 weeks. Further outcome data (including hospital admission, incidence of amputation and later mortality) are obtained by electronic linkage to national databases of hospital activity and population statistics.

Results: 5215 ulcer episodes (in 5015 people) were registered by 129 specialist clinical services in the first nine months. When the foot ulcer population was compared with the core national diabetes population, there were more males (70% vs 56%), mean age was higher (67 vs 64 years) and there were fewer people of Asian extraction (3% of T2DM vs 10%). 2804 index ulcers (53.8%) were graded less severe (SINBAD score <3) while 2411 (46.2%) were graded more severe (≥3). Statistically significant relationships were observed between the time to first assessment and ulcer severity, between the time to first assessment and ulcer-free survival at 12 weeks and between ulcer severity and ulcer-free survival at 12 weeks. The outcome was significantly worse when the delay to first expert assessment was 14 days or longer.

Conclusion: These initial results confirm the feasibility of undertaking nationwide online audit of foot ulcers and the early results provide strong support for the current recommendation that all newly occurring ulcers should receive early referral for expert assessment. When numbers are greater and outstanding outcome data are available, it will be possible to make case-mix adjusted comparisons of outcomes between different health economies and geographical areas as well as between individual specialist services.

Disclosure: **R.J. Young:** None.

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The impact of a diabetes task force on diabetic foot care

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Background and aims: An above average rate of major and minor foot amputations in diabetes were reported locally (population 600,000 South Central England). Route cause analyses found that only 5% of patients were being seen in a multidisciplinary diabetic foot clinic (MDFC) prior to amputation and 50% had no podiatry involvement. In early 2015 a Diabetes taskforce was set up by the local clinical commissioning group (CCG) involving stakeholders involved with diabetes foot care. This led to several new implementations including:

1. Pathway redesign for foot care management in patients with diabetes
2. An increase in frequency of MDFC from three per week to five per week (daily)
3. Introduction of an inpatient podiatry for those with diabetic foot disease
4. A programme of education to promote awareness of foot care for patients and healthcare professionals
5. Improved outpatient communication between MDFC and primary care including patient-held letters, same day clinic letters and faxed communication

Materials and methods: Retrospective data was collected for patients referred to the MDFC Sept 1st to Nov 30th 2015 (post-implementation) and between Sept 1st and November 30th 2014 (pre-implementation). Data collected included the source of referral, referral diagnosis, antibiotic initiation in the community, length of wait and attendance rates in the MDFC was also collected. Data for 2014 and 2015 cohort were compared using unpaired t- tests.

Results: 2014

56 new referrals to 28 MDFCs. Mean age 68 years, 64.3% male.

5.4% seen within five working days, 28.6% within 15 working days.

77% attended their appointment. Practice nurses referred 3.6% of patients, podiatrists 51.8% and general practitioners 37.5%. 14% of referrals were inappropriate. 73% of patients with infected foot ulcers had antibiotics initiated at the point of referral. 2015

96 new referrals to 41 MDFCs. Mean age 66 years, 63.5% male.

28.1% seen within five and 85.4% within 15 working days.

87% attended offered appointment. Practice nurses referred 12.5% of patients, podiatrists 59.4% and general practitioners 25%.

6% of referrals were inappropriate. 81% of patients with infected foot ulcers had antibiotics initiated.

Waiting times reduced significantly (p-0.001) post implementation. There was significant increase in appropriateness of referrals (p-0.037) and a non-significant increase in appropriate antibiotic prescriptions (p=0.535) and referrals from practice nurses (p-0.066). In both cohorts combined,

non-attendance/cancellations episodes were significantly associated with longer waiting times (p-0.033).
Conclusion: The Diabetes taskforce implementations have significantly improved foot care for patients with diabetes in our locality by increasing the availability of appointments and reducing waiting times. There appears to be an increasing awareness in primary care of correct referral and prompt initiation of antibiotics for infected foot ulcers. Amputation rates have improved and are now in line with the national average. CCG-led taskforce groups should be considered where there are areas of suboptimal diabetes outcomes.
Disclosure: **C.A. Shinton:** None.

PS 099 The Charcot foot

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Calcaneal quantitative ultrasonometry is useful method for diagnosis of Charcot foot in patients after simultaneous pancreas and kidney transplantation

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Background and aims: Previous studies showed high incidence of Charcot foot (CF) after simultaneous pancreas and kidney transplantation (SPKTX). Measurement of bone mineral density (BMD) especially in the calcaneal area may be helpful in diagnosing of CF in this high risk population. The aim of our study was to compare calcaneal quantitative ultrasonometry (QUS) parameters in patients after SPKTX with/without CF with patients with severe diabetic neuropathy (DN) and with CF patients.
Materials and methods: 72 diabetic patients were enrolled in the present study - 12 patients after SPKTX with inactive CF (SPKTX-CF group); 20 after SPKTX without CF (SPKTX group); 20 patients with severe DN, but without CF (DN group) and 20 patients with inactive CF (CF group). BMD in calcaneal area was measured in both feet by QUS in CF groups; in patients without CF (SPKTX and DN groups), the foot with the worse T score was used for later analysis. Osteoporosis measured by calcaneal QUS was defined as T-score ≤ -1.8. BMD in the lumbar spine and proximal femur was assessed by dual energy X-ray absorptiometry (DEXA) by standard criteria.
Results: There was a significant lower calcaneal BMD in affected foot in SPKTX-CF group in comparison with both DN group (T score -3.3±1.9 vs.-1.1±1.3; $p<0.01$) and SPKTX group (T score -2±1.3; $p=0.03$), but comparable with affected foot in CF group (T score -3.8±1.9; NS). Calcaneal BMD in non-affected foot in SPKTX-CF group was comparable with SPKTX group (T score -2±1.1 vs.-2±1.3) and both were significantly lower than in DN group (both $p<0.05$). The frequency of calcaneal osteoporosis of affected foot in the SPKTX-CF group was comparable with affected foot in the CF group (83% vs. 95%), but patients in the DN group had osteoporosis less often than both previous groups (50%; both $p<0.05$).BMD in the lumbar spine and proximal femur in both SPKTXCF and SPKTX group were significantly lower in comparison with both DN and CF group (all $p<0.05$).
Conclusion: Calcaneal quantitative ultrasonometry is useful method for diagnosis of Charcot foot in patient after simultaneous pancreas and kidney transplantation. Lower bone mineral density persists in all assessed localizations including calcaneal area in patients after simultaneous pancreas and kidney transplantation, which may result in higher risk for Charcot foot after pancreas transplantation.
Supported by: MZO00023001
Disclosure: **R. Bem:** None.

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The Charcot foot: an emerging public health problem for African diabetes patients

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Background and aims: Although the awareness, diagnosis, management of the complications associated with diabetes have improved in African countries over the past decade, surveillance activities in Tanzania and

anecdotal reports from other African countries have suggested increasing prevalence of Charcot foot over the past few years. Charcot foot is a serious condition that can lead to severe deformity, disability, or, ultimately, amputation. This is of concern because of the potential for Charcot foot to set back the positive progress, and outcomes, including reduced rates of leg amputations, achieved by the Step by Step Foot Program in Africa. We therefore carried out this study to (i) characterize the epidemiology and clinical burden of Charcot foot in a large diabetes population in Tanzania; and (ii) evaluate outcomes of persons with the condition.

Materials and methods: This was a prospective analytic cohort study. A case was defined as any person with diabetes who presented to the diabetes clinic during January 2013 through December 2015 (study period) and diagnosed Charcot foot for the first time. Following informed consent, patients were followed up in the outpatient clinic. We carried out detailed clinical assessments, and documented presence of peripheral neuropathy (PN), macrovascular disease and microvascular disease. Education and counseling were part of the follow-up program.

Results: Of 3,271 patients who presented to the clinic during the 3-year study period, 571 (18%) met the case-definition for Charcot Foot; all case-patients had type 2 diabetes. The prevalence for each of the years 2013, 2014, and 2015 was 19/1192 (1.6%), 209/1044 (20%), and 343/1035 (34%), respectively; the increases in the slope of the trendline was statistically significant ($p < 0.001$). Of these, 374 (65%) were male. The characteristics of case-patients were as follows: median age: 56 (range: 14-92) years; median duration of diabetes 10 (range: 0-33) years; median body mass index: 26.6 (range: 18-49) kg/m2. Of the 571 study patients, 547 (96%) had PN; none had microvascular disease. In addition, none of the 147 (26%) persons with macrovascular disease had cerebrovascular disease or IHD. All 571 patients had presented with open foot ulcers—397 (70%) were Wagner stage 2, 72 (12%) were Wagner stage 3, and 102 (18%) were Wagner stage 4. Over 90% of acute these ulcers were in the midfoot region. Common precipitating factors included blisters (36%), callus (13%), prick with sharp object (9.3%), rat bites (9%); blunt trauma (7%), or burns (4%). Delay in seeking medical treatment was common (median 7 [range: 1-360]) days. Management included sloughectomy (513 [90%]) and major limb amputation (21 [4%]).

Conclusion: The prevalence of Charcot Foot disease is increasing in the Tanzanian diabetes patient population. Charcot Foot can lead to severe deformity, disability, and amputation. Because the risk of limb amputation, it is important that patients with diabetes seek immediate care if signs or symptoms appear and avoid delay in seeking medical attention. Early diagnosis of Charcot Foot by care givers is extremely important for successful outcomes. Warmth to the touch, redness, and localized swelling with tenderness in the feet are key clinical findings that might herald a Charcot Foot.

Disclosure: **Z.G. Abbas:** None.

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Charcot foot in diabetic patients and risk of amputation: Is just a matter of neuropathy?

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Background and aims: The prevalence of Charcot neuroarthropathy in the diabetic population (DP) and its relationship with the prevalence of amputations and revascularizations in the lower limbs are important elements in the natural history of diabetic foot. The purpose of this study is to provide national data on the prevalence of hospitalization for Charcot foot in the DP and the incidence of major and minor amputations and revascularizations in our country.

Materials and methods: In the period 2003-2013, on the national hospitalization database have been identified all hospitalizations with Charcot disease associated with diabetes (codes ICD-9-CM 713.0, 713.5, 713.8 with 250. for diabetes diagnosis indicated during the same hospitalization or in any of the patients in the same year). In the same period were also evaluated by ICD9 code in any position, the amputations (841) and intraluminal (39.5,39.90) and surgical ((39.25, 39.29) revascularizations .

Results: The admission rate for Charcot was constant from 2003 to 2013 with values ranging between 0.16 / 1000 and 0.13 / 1000 diabetic patients (p = NS after Poisson regression). The highest prevalence was found in the age group 45-54 and in males who showed a relative risk of 1.81. The days of hospitalization (10.4 ± 10.3gg in 2003; 12.2 ± 13.6gg in 2013) were unchanged over time. Out of 317 patients admitted in 2008 , within 5 years 117 (36.9%) had a hospitalization for amputation or revascularization: there were 90 amputations (28.4%), including 61 minors, 26 major and 3 unspecified, there were also 57 revascularizations of which 52 endoluminal and 5 surgical.

Conclusion: Although not very often coded, the prevalence of Charcot foot remains constant in hospital admissions in our country. The Charcot foot is associated with increased risk of major or minor amputations and, although it has been usually associated with neuropathy only, peripheral arterial disease is not uncommon as well as the need of revascularization.

Disclosure: **R. Anichini:** None.

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Treatment with parathyroid hormone does not enhance clinical resolution and fracture healing of Charcot osteoarthropathy: double blind randomised placebo controlled trial

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Background and aims: There is a growing body of evidence to show that parathyroid hormone (PTH) is an effective anabolic therapy for the enhancement of bone repair, following fracture. The main objective of this study was to investigate whether recombinant human (rh) PTH (1-84) could enhance fracture healing and arrest bone and joint destruction in the acute Charcot foot.

Materials and methods: We carried out a double blind randomised placebo controlled trial in 48 patients with acute Charcot osteoarthropathy. We treated patients with daily subcutaneous injections of rh PTH 1-84 or placebo until clinical resolution of the osteoarthropathy or up to a period of 12 months. All patients received casting therapy and Calcium and Vitamin D3 supplementation. Time to clinical resolution was recorded in months. Serum concentrations of the bone turnover markers amino-terminal propeptide of type I procollagen (P1NP) and carboxyterminal telopeptide of type 1 collagen (CTX) were measured at presentation and then at 3 monthly intervals until clinical resolution or up to a period of 12 months. The rate of change of these bone markers from baseline up to clinical resolution was compared between the active and placebo groups. Semiquantitative bone marrow oedema (BMO) scores and fracture scores were calculated on non-contrast magnetic resonance imaging scans and the rate of change of these scores from presentation and on follow up (at clinical resolution or at 12 months) was compared between the groups.

Results: Logistic regression analysis indicated that there was no statistically significant difference between the active and placebo groups in the percentage of patients with clinical resolution at 6 months (Odds ratio= 0.94; 95% CI 0.30 to 3; P=0.92) and at 12 months (Odds ratio=2.3; 95% CI 0.68 to 7.7; P=0.18). There was no statistically significant difference between the survival (non-resolution patterns) between the active and placebo groups. The log-rank statistic was 0.11 yielding a p-value of 0.74 and the estimated hazard (for resolution) ratio was 1.1 (95% ci

0.57 to 2.1; P=0.78). There was a significant reduction in the serum concentration of P1NP during the study period, (P=0.004). However, the rate of change in P1NP was not significantly different between the active and placebo groups (P=0.13). The serum concentration of CTX remained unchanged from presentation to follow up (P=0.92). Moreover, there was no significant difference in the longitudinal change of this marker between the active and placebo groups. (P=0.25). The total BMO score significantly decreased between presentation and follow up (p<0.001). However, the rate of change in the total BMO score was not significantly different between the active and placebo groups (p=0.95). Similarly, although the total fracture score significantly decreased between presentation and follow up (p=0.001), the rate of change in the total fracture score was not significantly different between the active and placebo groups (p=0.55).

Conclusion: This study has shown that treatment with rh PTH does not enhance time to resolution and fracture healing of the acute Charcot foot. Casting therapy remains the mainstay of Charcot foot management.

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Supported by: Diabetes UK

Disclosure: **N.L. Petrova:** Grants; Diabetes UK.

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How to improve the outcome of Charcot foot? Results of five years follow up: prospective study

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Background and aims: Foot care for Charcot and its contralateral foot is of utmost importance. We tried to identify different risk factors leading to development of complications in the Charcot and its contralateral foot. In this prospective study we tried to see the impact of nullification of the suggested risk factors responsible on better outcome of Charcot, and its contralateral foot.

Materials and methods: Prospective analysis of the impact of compliance with the nullified risk factors on both feet as 1.Compliance with the removable cast walker, 2.compliance with the regular follow up visits, 3.Nullified leg length discrepancy induced by the high rigid outsole of the removable cast walkers, and 4.slowing of the gait speed (24steps ± 3/min). 43 Patients presented ≥ 5 years ago with unilateral Charcot foot and normal contralateral foot were included and subdivided into 2 groups, (Group A) compliant with all nullified risk factors and (Group B) non-compliant with ≥ 1 of the nullified risk factors, of matched age, sex, BMI, and with no significant difference regarding HbA1c and diabetes duration in between. Both feet are then examined for any change happened since January 2010 till February 2016.

Results: ≥ 5 years ago, the Charcot foot showed no significant difference between both groups regarding the history of ulcers 28% (n=5) vs 44% (n=11), recurrent ulceration 11% (n=2) vs 12.5% (n=3), deformity 66.7% (n=12) vs 68% (n=17), ulcer/deformity relationship 80% (n=4) vs 44.4% (n=4), and minor amputation 16.7% (n=3) vs 16% (n=4), in group A vs group B respectively, p value in all was > 0.05. After ≥ 5 years, 5.6% (n=1) vs 20% (n=5) in the Charcot foot, and 0% vs 20% (n=5) in the contralateral foot have new ulcers; 5.6% (n=1) vs 28% (n=7) in the Charcot foot and 11% (n=2) vs 4% (n=1) in the contralateral foot have new deformity; 0% vs 4% (n=1) in the Charcot foot and 5.6% (n=1) vs 4% (n=1) in the contralateral foot have minor amputation and 16.7% (n=3) vs 24% (n=6) in the contralateral foot developed Charcot foot in group A vs group B respectively, as shown in table 1. In summary, there is statistically significant difference regarding the complications happened in the Charcot foot, 11.1% (n=2) vs 44% (n=11) and in the contralateral foot, 16.7% (n=3) vs 48% (n=12) in group A vs group B respectively, (p 6 folds in the Charcot foot (odds ratio 6.3, p = 0.03) and > 4 folds in the contralateral foot (odds ratio 4.6, p = 0.04).

Conclusion: Nullification of leg length discrepancy, slowing of gait speed, compliance with the removable cast walker and regular follow up visits greatly improved the outcome of patients with Charcot foot in our foot clinic.

Table 1: Comparison between compliant and non-compliant groups after 5 years duration

After 5 years	The Charcot foot			The Contralateral foot			
	New Ulcers	New Deformity	New Minor amputation	Ulcers	Deformity	Minor amputation	Charcot
Group.A (n=18)	5.6% (n=1)	5.6% (n=1)	0% (n=0)	0% (n=0)	11% (n=2)	5.6% (n=1)	16.7% (n=3)
Group.B (n=25)	20% (n=5)	28% (n=7)	4% (n=1)	20% (n=5)	4% (n=1)	4% (n=1)	24% (n=6)
p-value	0.02			0.034			

Disclosure: **M.M.M. Motawea:** None.

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Medical treatment evaluation of diabetic foot osteomyelitis using 99mTc-HMPAO-labelled leucocyte scan and predictive factors of outcome

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Background and aims: Evidence is growing on successful treatment of diabetic foot osteomyelitis (OM) with prolonged antibiotic therapy without the need of major surgical procedures. The aim of the present study was to assess the role of 99mTc-HMPAO-labelled Leucocyte Scan (LS) in the evaluation of medical treatment of diabetic foot OM and to identify factors associated with poor outcome.

Materials and methods: Forty seven (47) consecutive diabetic patients (age 62.9±9.3 yrs and diabetes duration 15.2±9.4 yrs) under antibiotic treatment for pedal OM (mean treatment duration 6.9±4.2 months) were included in the study. Ulcer's severity grade, presence of peripheral neuropathy and peripheral vascular disease (PVD) (by means of clinical examination and absence of palpable pedal pulses in one or more sites, respectively), HbA1c and eGFR (estimated Glomerular Filtration Rate) were recorded and analyzed for potential association with outcome of OM. Inflammatory blood markers (Erythrocyte Sedimentation Rate - ESR) , C-Reactive Protein CRP, White Blood Cells (WBCs) count) were also measured. All patients underwent LS of the feet to evaluate response to medical treatment. Focal leucocyte bone uptake on LS was considered as sign of persistent OM, while absence of leucocyte uptake indicated cured OM. Final evaluation was based on long term clinical follow-up or bone biopsy in patients underwent amputation.

Results: 1. Among the 47 cases of diabetic foot OM investigated, 31 cases of OM cured with medical treatment (66%) and 16 cases of persistent OM (34%) were finally diagnosed. 2. Sensitivity, specificity, accuracy, positive and negative predictive value of LS were 87.5%, 100%, 95.7%, 100%, 93.9% respectively. 3. Patients with persistent OM had higher ESR and CRP compared with those with cured OM (69.2±24.9 vs 30±18.8 mm/h and 3.3±4.9 0.28 vs 0.6 ± 0.9 mg/dl, respectively, p<0.05 for both). WBC count was higher but non significantly in patients with active OM. 4. HbA1c, eGFR values, ulcer's severity, and severity of neuropathy (NDS) were not significantly different between the two groups. 5. The prevalence of PVD was higher in patients with persistent OM compared those who were cured (81.3% vs 38.7%).

Conclusion: LS is a reliable imaging modality for assessing response to medical treatment diabetic foot OM. Conservative treatment may be effective for the majority of patients with diabetic foot OM (66% in our study). Elevated ESR and CRP are associated with active OM. Presence of PVD is associated with failure of medical treatment and poor outcome.

Disclosure: **C. Manes:** None.

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Assessment of using a retinal OCT phantom in multi-centre trials assessing macular thickness

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Background and aims: The assessment of macular thickness by optical coherence tomography (OCT) is increasingly being used as an outcome measure in clinical trials. However, it's use as a primary outcome in multi-centre clinical trials is hampered by (1) variations between segmental algorithms that automatically measure macular thickness between manufacturers; (2) Lack of quality control assessments between centres. Methods to overcome variations in the segmental algorithms have been proposed but little has been published to address quality control within multi-centre clinical trials. Aim: As part of the SUMMIT consortium this study aims to determine (A) intra-device OCT reproducibility; (B) between the same type of OCT device variability (Cirrus and Topcon); (C) longitudinal reproducibility using a retinal phantom (RP) and biological standard (BS).
Materials and methods: A commercially available retinal OCT phantom was identified that was (1) adaptable to be used on any type of OCT device; (2) non-fluid filled and practical to circulate between centres; (3) mimics the shape of the macular including the foveal dip. The right eye of a woman (aged 36) with no known retinal pathology was used as the biological standard. Aim A: The RP and BS were assessed three times on 5 different OCT devices (3 Cirrus and 2 Topcon-1000 HD-OCT across 3 centres) using the 512 x 128 macular cube scanning protocol. The intra-device coefficient of variation (CV) was calculated. Aim B: The first scan on each Cirrus or Topcon device was used to assess variability between each type of device (Cirrus and Topcon). Aim C: to assess reproducibility over time the biological standard was assessed 7 times over 5 years and the retinal phantom 4 times over 17 months on the same Cirrus HD-OCT device using a 512 x 128 scanning protocol.
Results: Aim A: Intra-device CVs of fovea or equivalent thickness was < 1.2% and 0.9% with the retinal phantom and biological standard respectively for all 5 devices. Aim B: The CV between the 3 Cirrus HD-OCT devices were 0.41% (mean(SD): 244.0 (1.0)μm) and 1.0% (275(2.6)) with the retinal phantom and biological standard respectively. There was good agreement between the two Topcon devices (retinal phantom: 225 and 224μm; biological standard: 251 and 257μm). Aim C: Longitudinal CV of fovea thickness of the biological standard was 1.3% (275.7 (3.6)μm). CV of the other regions of the ETDRS grid were < 1.2%. Longitudinal reproducibility was 0.4% (244.3 (1.0)μm) for the RP. Both types of OCT devices consistently recognised the outer retinal boundary of the retinal phantom but struggled with the inner retinal boundary resulting in the manual placement of the inner retinal boundary line by the same operator for all scans with the phantom.
Conclusion: The retinal phantom was practical for circulation between centres and adaptable to be used on different types of OCT devices. However, it's use is limited by the manual placement of the inner retinal boundary. Even with this added variable good agreement was demonstrated with the retinal phantom within and between OCT devices. Longitudinal reproducibility of fovea thickness was excellent with the biological standard.
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Disclosure: **K.M. Gooding:** None.

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Ultra-wide-field retinal photography: implications for detection, treatment and study of diabetic retinopathy

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Background and aims: The detection and subsequent treatment of diabetic retinopathy (DR) is increasingly dependent on retinal photography, often using one 45° photograph of the Central Field (CF). We found a patient with mild non-proliferative diabetic retinopathy (NPDR) on a 45° photograph, but proliferative retinopathy (PDR) when examined using a 200° ultra-wide-field (UWF) scanning laser ophthalmoscope. This study examined whether a 45°CF photograph under-estimates the severity of DR.
Materials and methods: We identified 45 consecutive patients with 69/90 eyes graded as having less than moderate NPDR in the Central Field. The Central and UWF retinopathy grading of these 69 eyes were compared. We also determined whether UWF fluorescein angiography(UWFFA) enhanced the detection of high risk retinal characteristics (ie non-perfusion, vascular leakage, neo-vascularisation) in 87/90 eyes. Retinal images (unmasked) were graded by two trained observers and FA was graded by an ophthalmologist.
Results: Ten out of 69 eyes (*) were graded as have more severe DR on UWF, equivalent to a 2 step progression on the ETDRS scale, with two eyes demonstrating PDR (Table 1). The use of UWFFA identified 22 out of 87 eyes with high risk characteristics not detectable by conventional FA, including three additional eyes with PDR.
Conclusion: We conclude that UWF imaging improved the detection of clinically significant DR in 14.9% of our cohort, with two eyes showing PDR requiring laser treatment. Moreover, UWFFA further enhanced detection of high risk retinal characteristics in 25.3% of individuals. UWF imaging could have a significant impact on detection, monitoring and treatment of DR.

n=69	Central		
Peripheral	Nil (n=27)	Minimal (n=3)	Mild (n=39)
Nil	19	1	5
Minimal	1	0	0
Mild	6*	1	29
Moderate	1*	1*	3
Proliferative	0	0	2*

Disclosure: **T. Alharbi:** None.

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Validation of a prediction model to optimise retinopathy screening in type 1 diabetes

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Background and aims: Retinopathy is a well-known complication of diabetes and has become the leading cause of blindness in working-age adults. Eye screening is a powerful tool to early diagnosis of retinopathy, which supports prompt treatment and prevention of blindness. However, more efficient and cost-effective screening strategies are required to keep up with the rising prevalence of diabetes. Prediction models can be used to estimate person's risk of a complication, which may support specialists and improve cost-effectiveness of care. An Icelandic prediction model was developed to estimate personal risk of sight threatening retinopathy (STR) and is applicable for both type 1 and type 2 diabetes as it can be adjusted

by different coefficients for both types. Although several studies have validated this model, these were all performed in a primary care setting and mostly in persons with type 2 diabetes. Therefore, the aim of this study was to validate the Icelandic model for persons with type 1 diabetes in secondary care setting.

Materials and methods: Persons with type 1 diabetes treated in an academic setting were eligible for this study. All persons underwent standardized eye examination including ophthalmoscopy performed by an ophthalmologist experienced with diagnosing diabetic retinopathy. Diabetic retinopathy was graded according to the International Clinical Diabetic Retinopathy Scale. STR was defined as the presence of severe nonproliferative DR, proliferative DR or macular edema. The Icelandic prediction model was used to compute an individual's 5-year risk for STR and recommended screening interval based on diabetes type, gender, presence of DR, duration of diabetes, HbA1c and systolic blood pressure. The model's predictive performance was measured using discrimination which was quantified by calculating the area under the ROC curve (AUC), and calibration which was evaluated by a calibration plot.

Results: 200 Persons with type 1 diabetes were included for the current study. Twenty-two persons (11%) developed STR during a mean follow-up of 83 months. All incidences of STR occurred after the calculated screening date. Applying the model, mean calculated screening interval was 30.6 months for the total study population, which is a 61% reduction of screening frequency compared to annual screening. The AUC was 0.75 (95% CI 0.65 - 0.86) which means the model is able to distinguish between persons that develop STR and those who do not. Calibration showed that the risk predictions were well corresponding with the observed incidence, with a slight overestimation in persons at higher STR risk.

Conclusion: This study shows that a screening program based on the Icelandic model performed well in a secondary care setting and lead to a substantial reduction of screening frequency have potential.

Table. Follow-up duration, incidence of STR, calculated screening interval and cases of STR for total population and stratified by level of retinopathy at baseline

	Total population	Level of retinopathy at baseline	
		No DR	Background DR
N	200	143 (71.5%)	57 (28.5%)
Follow-up (months)	83.4 (21.1)	85.9 (17.3)	77.1 (27.8)
Incidence of STR during follow-up	22 (11.0)	20 (14.0)	2 (3.5)
Screening interval (months)	30.6 (19.0)	39.2 (15.6)	9.1 (3.6)
Cases of STR before calculated screening date	0 (0.0)	0 (0.0)	0 (0.0)

Data are mean (SD) or number (proportions)

Disclosure: **H. Ng:** None.

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Intraindividual variability and circadian rhythm of systemic vascular endothelial growth factors in subjects with normal glucose tolerance and patients with type 2 diabetes

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Background and aims: Increased levels of systemic vascular endothelial growth factors (VEGFs) in patients with diabetes are associated with increased risk of microvessel disease. On the other hand, rapid decrease in VEGF levels after intravitreal antibody application may be associated with acute cardiovascular complications and renal failure. However, individual levels of systemic VEGF vary in a wide range depending on analytical method and quality of diabetes control. So far only scarce information exists on fluctuations over longer periods and over daytime.

Materials and methods: We analysed the intraindividual variance of VEGF-A, VEGF-C and placental growth factor (PLGF) in CTAD plasma as well as VEGF-A in serum over a period of 6 months in patients with stable controlled type 2 diabetes (T2DM) (mean HbA1c 7.07%, SD 0.49%) (10 M, 10 F) and age and sex matched subjects with normal glucose tolerance (NGT). Furthermore, circadian levels of VEGFs were controlled hourly from 7:30 a.m. to 7:30 p.m. under standardized metabolic ward conditions.

Results: VEGF-A in serum remained stable over time in both groups: NGT mean ranged from 602 ± 316 to 658 ± 359 ng/ml; T2DM mean from 554 ± 541 to 588 ± 614 ng/ml. The same applies for VEGF-A in plasma: NGT mean from 26.4 ± 36.8 to 33.6 ± 39.1 ng/ml; T2DM from 16.5 ± 12.9 to 22.6 ± 14.4 ng/ml. VEGF-C in plasma also remained stable in a range of 0.17 ± 0.14 to 0.20 ± 0.24 ng/ml for NGT and from 0.08 ± 0.07 to 0.10 ± 0.08 ng/ml for T2DM. The same applies for PLGF concentrations: NGT mean from 8.77 ± 4.51 to 11.3 ± 5.32 ng/l; T2DM from 6.72 ± 2.34 to 8.60 ± 2.84 ng/l. No circadian change was observed in VEGF-A serum and plasma concentrations. A minor decrease of VEGF-C plasma levels was evident after 5 p.m. in both groups and a significant peak of PLGF concentrations occurred after lunch which was more pronounced in T2DM.

Conclusion: Interindividual VEGF levels vary in a wide range. However, under stable conditions we observe no significant intraindividual variance for VEGF-A in serum and VEGF-A, VEGF-C and PLGF in CTAD plasma over a period of 6 months. All VEGF fractions were considerably lower in patients with well controlled T2DM compared to matched controls with NGT. Whereas VEGF-A exhibited no circadian rhythm, a drop of VEGF-C concentrations was observed after 5 p.m. and a peak in PLGF after lunch. Thus, a single measurement of systemic VEGF levels appears to be a reliable parameter for the individual risk associated with abnormal VEGF concentrations in blood.

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HbA_{1c} levels over time in children with type 1 diabetes and correlation to diabetic retinopathy

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Background and aims: Type 1 diabetes mellitus (T1DM) is a metabolic disease causing chronic hyperglycemia due to beta-cell destruction. Despite adequate treatment with insulin several complications such as diabetic retinopathy (DR) is common. The aim of this study was to investigate if mean HbA1c levels over time affects years to development of DR in children diagnosed with T1DM.

Materials and methods: Medical records from all children and adolescents diagnosed with diabetes during 1993-2001 in our area in the south of Sweden were studied retrospectively and mean HbA1c each year until DR development was investigated. In total 72 patients were included. Gender, p-glucose at diagnosis, age at diagnosis and HbA1c were analysed for possible correlation of years to retinopathy.

Results: Out of the 72 patients included in this study 62 (86.1%) had developed DR at follow-up. When comparing variables from acute onset of T1DM in the group who developed DR (n=62) and the group who had not developed DR (n=10) no significant differences were found between the groups. A negative correlation was found regarding age at diabetes diagnosis and years to DR development ($p<0.001$). No significant correlation between years to retinopathy and gender, HbA1c or p-glucose at diagnosis was found. There was no significant characteristic pattern regarding mean HbA1c levels each year and number of years to DR.

Conclusion: The present study showed no significant relation between mean HbA1c each year and number of years from diabetes diagnosis to DR development. However age at diagnosis seems to play a role in number of years until development of retinopathy.

Disclosure: **C. Nilsson:** None.

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Ranibizumab and diabetic macular oedema (DMO): a retrospective review of patient outcomes

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Background and aims: Ranibizumab is a vascular endothelial growth factor A (VEGF-A) inhibitor working to prevent angiogenesis and reduce vascular permeability in the eye through intravitreal injection. Approved for use in the United Kingdom since February 2013 for DMO, it is given on a basis of 3 initial monthly injections (loading phase) and then as needed (pro re nata) until maximal visual acuity (VA) is reached or macular oedema resolved. Data from consecutive patients with DMO attending an ophthalmology clinic for ranibizumab was reviewed to see if there were characteristics (including but not limited to HbA1c, GFR, diabetes related complications/hospital admissions/severe hypoglycaemia and number of injections) that would identify patients likely to respond to treatment and also to identify areas of diabetes care that could be improved for these potentially high risk individuals.
Materials and methods: Individuals with a minimum of 12 months treatment/follow-up were divided into 'responders' (VA improved by one row on the Snellen chart or more) versus 'non-responders' (VA on same or deteriorated line on the Snellen chart) with baseline characteristics and outcome measures of these groups being compared. Paired t-test and chi-squared were used as described.
Results: 131 individuals (120 type 2 DM, 11 type 1 DM) were treated over a 24 month period. 56 had no previous eye interventions, 52 previous laser therapy, 13 previous intravitreal therapy (IVT) other than ranibizumab, and 10 previous laser and IVT. 124 had recorded VA outcomes, 74 defined as responders, 50 as non-responders. Baseline characteristics are described in the table. Paired t-test showed no significance (p=0.902) between pre and post treatment HbA1c within the responders, and significance (p=0.002) in the difference between pre and post HbA1c within the non-responders. Chi-square test showed no significance (p=0.062) in the change of HbA1c from pre to post HbA1c in the responders versus the non-responders.
Conclusion: Baseline characteristics between the two groups were comparable. The difference between pre and post HbA1c in the non-responders group was statistically significant (in the responders it was not) although the change in HbA1c when compared between the two groups did not quite reach significance (p=0.062) although this may become significant with potential further diversification of control over time. Non-responders required on average more injections and had more complications than the responders group, both results a potentially significant financial and healthcare burden outcome. DMO is the leading cause of sight loss in patients with diabetes, and our results suggest concentrating on HbA1c control during treatment could contribute to success, and consideration of joint services to address these needs is being considered in our locality.

	Responders (n=74)	Non-responders (n=50)
Mean Age (years)	67.32 (43-84)	67.82 (32-88)
Mean Duration of DM (years)	18 (2-35)	17.49 (3-58)
Mean HbA1c (pre)	69.59 (38-143)	64.90 (39-123)
Mean HbA1c (post)	68.43 (37-148)	75.35 (40-138)
Mean GFR (pre)	68.37 (12-90)	67.29 (22-90)
Mean GFR (post)	61.70 (5-90)	61.45 (3-90)
Mean Cholesterol	4.08 (2.39-6.96)	3.99 (2.36-7.08)
Mean number of injections	5.77 (2-14)	7.02 (2-14)
Mean number of complications	1.22 (0-5)	1.62 (0-6)

Disclosure: **E.J. Nicholson:** None.

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Mediators between reactive gliosis and vascular leakage in diabetic retinopathy: a proteomic approach using human retinas

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Background and aims: Retinal neurodegeneration is an early event in the pathogenesis of diabetic retinopathy (DR). Reactive gliosis (also named glial activation) is a main feature of neurodegeneration and could be identified by glial acidic fibrilar protein (GFAP) overexpression in Müller cells. The effect of glial activation on early microvascular impairment remains to be elucidated. To shed light to this issue we have used a proteomic analysis approach aimed at identifying potential mediators of vascular leakage associated with glial activation.
Materials and methods: Human retinal samples were obtained from 5 non-diabetic donors, and 10 type 2 diabetic donors without (n=5; group A) or with (n=5; group B) reactive gliosis. Diabetic donors did not presented microcirculatory abnormalities in the ophthalmoscopic examinations performed during the two years before death. Retinal lysates from each group were pooled and run on an SDS-PAGE gel. Bands were excised and analyzed sequentially by LC/MS using an Orbitrap Mass Spectrometer.
Results: A total of 307 proteins were upregulated and 373 were downregulated in Group B in comparison with Group A. Notably, retinas with reactive gliosis (group B) presented a significant increase of biological markers of vascular leakage, such a as serum albumin and immunoglobulins, thus indicating the presence of early microvascular damage. Among the proteins upregulated in group B it should be noted the abundance of inflammatory mediators (TNF-alpha receptor, Complement C4 factor, ICAM-1), and carbonic anhidrase. Among the downregulated proteins, several isoforms of the sodium/potassium transporting ATPase subunit and Complement factor H (CFH: the soluble inhibitor of the alternative pathway of complement) were identified.
Conclusion: By means of a proteomic analyses several candidates that could play a relevant role in the link between reactive gliosis and vascular leakage have been identified. In addition, our results suggest new pathogenic pathways involved in the early microvascular impairment mediated by neurodegeneration.
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Disclosure: **O. Simó-Servat:** None.

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Is urinary proteomics useful to predict retinopathy in type 2 diabetic patients in the direct 2 study

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Background and aims: Diabetic microvascular complications affect the kidney and the eyes, being a leading cause of renal failure and blindness. Urinary proteomics has shown promise as an early indicator of future development of diabetic nephropathy. Here we investigate if this could also predict progression of diabetic retinopathy.

Materials and methods: In a post-hoc study of the DIRECT 2 study, a randomized, controlled clinical trial of candesartan for prevention of retinopathy, we studied patients with type 2 diabetes and normoalbuminuria (n=792), followed for a mean of 4.7 years. We address the predictive ability of a previously defined CKD risk score based on proteomic measurement of 273 urinary peptides (CE-MS). We also assessed the possibility of a new EYEscore based on discriminative features choosen out of 1161 peptides in a training set of 528 patients. Progression were either 2 step (E2) (primary) or 3 step (E3) change in retinopathy on the concatenated Early Treatment Diabetic Retinopathy Study severity scale.
Results: Progression of retinopathy was seen in 37% of E2 and 19% of E3 patients. None of the peptides were significantly differentially expressed in cases vs control. In Cox models five peptides were associated with E3 but not E2 in the training set but were not validated. The CKD risk score was able to predict E3 (HR 1.54 95% CI 1.02 to 21.34, p=0.042) but not E2 (HR 1.14 p=0.34) during follow-up, independent of treatment (candesartan/placebo), age, gender, systolic BP, baseline UAER, baseline eGFR, HbA1c and diabetes duration.
Conclusion: In this cohort of patients with type 2 diabetes and normoalbuminuria from a large intervention study, the CKD classifier was an independent predictor of severe but not mild progression in retinopathy. It was not possible to develop a retinopathy specific marker panel with clinically relevant accuracy.
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Disclosure: **M.K. Eickhoff:** None.

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Whole genome sequencing of Cohen diabetic rats identifies novel coding-variants in genes contributing to the susceptibility to diabetic retinopathy in humans

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Background and aims: Retinopathy is a common complication of type 2 diabetes (T2D) and is the leading cause of blindness in these patients. Often, damage is detected relatively late, and its direct cause is unknown. Perturbations to retinal pigment epithelium (RPE) function may include oxidative stress and impaired RPE function. The Cohen Diabetic rat is an experimental model of T2D consisting of a sensitive (CDs) rat that develops diabetes when fed a diabetogenic high-sucrose diet (HSD) and a resistant (CDr) rat that maintains normoglycemia on HSD. Prolonged hyperglycemia leads to retinopathy in CDs rats. We aim to define the genetic-basis of retinopathy in CDs-rats and examine the impact of early stages of diabetes on RPE function
Materials and methods: We performed whole genome sequencing on blood-DNA of CDs and CDr rats using Illumina HiSeq 2500 sequencing system followed by variant analysis using in-silico prediction tools. Eyes of early diabetic-CDs and normoglycemic-CDr rats fed HSD were collected. Eye-sections were analyzed by histology and immunostaining. Analysis of RPE protein extracts was performed by Western-blot. Dynamics of RPE uptake and recycling of photoreceptor outer segments (POS) was assessed by phagosome quantification at different time points. Oxidative stress was assessed by protein-carbonylation (Oxiblot). Function of recycling pathways was assessed by measuring the clearance and accumulation of pathway-specific substrates.
Results: We identified a novel 2-bp frameshift-homozygous deletion (p.T571_W573del) in IMPG2 (interphotoreceptor matrix proteoglycan) reported as a retinopathy susceptibility-gene in a GWAS of T2D Mexican Americans. Two novel coding-variants were identified in Ugt2b10 (p.P290S) and Cyp2c7 (p.P94L), both of which belong to the retinol metabolism pathway, targeting all-trans-retinoate. The variants were predicted to be pathogenic by in silico prediction tools. POS uptake by RPE of CDs-rats at the initiation of diabetes was not different from that of CDr rats on HSD but the clearance and turnover of phagosomes was significantly delayed. We also found a severe inhibition ($P<0.05$) of the lysosomal/autophagy degradation pathway while the proteasome-degradation pathway was not affected. The impairment in the phagosome processing was associated with an increased levels of carbonylated proteins and oxidative stress in RPE cells ($P<0.05$) as well as impaired breakdown and clearance of phagosome cargo ($P<0.05$). Importantly, these abnormalities were detected at the initiation of diabetes, prior to photoreceptor cell death.
Conclusion: We identified 3 novel coding-variants in genes involved in retinal development or function, likely contributing to the susceptibility of the CDs-rats to developing diabetic retinopathy. The role of these genes leading to RPE impairment and retinopathy found in prolonged hyperglycemic-CDs rats awaits functional follow-up confirmation. The early impairment of RPE function prior to photoreceptor cell death suggests that phagosome uptake and recycling may be used to assess ocular damage at the early stages of diabetes.
Disclosure: **I. Guberman:** None.

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Functional assessment of retinal neovascularisations

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Background and aims: Diabetic retinopathy (DR) results in vascular damage and impaired neuronal function, whereas the later, proliferative stage of DR (PDR) is characterized by hypoxia and subsequent neovascularization. The resulting impact of these events on retinal function in humans is unknown. Currently, the only therapeutic intervention in PDR is the delay of neovascularization, achieved by photocoagulation to decrease oxygen consumption. However, it has not been determined, if neovascularizations only have adverse effects or are sufficient to perfuse the inner retina.
Materials and methods: Ang2(-/-) mice and a mouse model of retinopathy of prematurity (ROP) were used to represent peripheral and central hypoxia, respectively. Age matched C57Bl/6 mice served as controls. The different characteristics of the pathological angiogenesis were observed by flourescenangiography (FA) and scanning laser ophthalmoscopy (sLO) in each group. The impact on neuronal function was assessed by photopic 7-segment multifocal electroretinography (mfERG) in the area of neovascularization at P10/P17, P25 and P60. A corresponding area was measured in the control group.
Results: Throughout the observation period, no alterations of photoreceptor function (a-wave) in the neovascularized areas were detectable in Ang2(-/-) mice compared to controls (P10: 2.01±0.93 vs 1.58 ±0.49; P25: 1.95±1.2 vs 1.7±0.73; P60: 1.84±0.69 vs 1.2±0.51; [μV/field]; n.s.). Likewise, the inner retina showed no sign of functional deficits (b-wave) compared to the control group (P10: 2.08 ±0.59 vs 2.15±0.83; P25: 2.09±0.72 vs 2.04±0.54; P60: 2.65±0.63 vs 2.24±0.18; [μV/field]; n.s.). Despite the significant differences in neovascularisations in between the ROP model and the Ang2 (-/-) mice at P60 (0.25±0.86 vs. 45.22±16.21; p-value<0,0001) no differences in neuroretinal function were detected (a-wave: 0.93±0.63 vs 1.84±0.69; b-wave: 1.88±0.48 vs 2.65 ±0.63; [μV/field]; ROP vs. Ang(-/-); n.s.).
Conclusion: Our results indicate that despite their instability, neovascularisations enable sufficient perfusion of the inner retina,

preserving neuronal function in these areas. The ROP model recently was demonstrated to develop b-wave impairment on a whole retinal basis. Based on our data we can address this deficit to the hypoxic avascular zone in the retinal centre. Therefore current research should be more focused on the stabilization of neovascularizations in order to protect patients from intravitreal bleeding.
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Impact of reactive metabolites on vascular damage, microglial activation and neuronal function in the diabetic retina

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Background and aims: Reactive metabolites such as methylglyoxal (MG) are involved in the damage of the neurovascular unit (NVU) in diabetic retinopathy (DR). Our aim was to analyze MG-induced damage of the NVU in vivo.
Materials and methods: Diabetes was induced in rats by STZ treatment (35 mg/kg body weight). Microglial activation was analyzed by retinal wholemount staining and quantitation of CD74 and Iba1 expressing cells. MG (50 mM) was applied by drinking water. MG was quantified by HPLC, GlcNAc by western blotting. Acellular capillaries (AC) and pericyte (PC) loss were determined by quantitative retinal morphometry. In addition, STZ-diabetic rats treated with linagliptin (0.083 mg/kg chow) were evaluated. Neuroretinal function was assessed by multifocal electroretinography (mfERG).
Results: Under diabetic conditions, increased retinal levels of MG (from 5.82 to 9.36 pmol/mg tissue) resulted in microglial activation after 4 months (from 6 to 64 CD74+ microglia/10 fields; $P<0.01$). Oral application of MG to non-diabetic rats increased retinal levels of MG (to 8.82 pmol/mg tissue), similar to diabetes. In the retina, this resulted in an increase of GlcNAc formation (by 24%; $P<0.01$), PC dropout (from 2229 to 1718 PC/mm2; $P<0.01$), and loss of neuronal function (B-wave amplitude from 1.12 to 0.73 μV; $P<0.01$). Inversely, reduced retinal MG levels, in STZ-diabetic rats treated with linagliptin, resulted in reductions of vasoregression (from 30 to 19 AC/mm2; $P<0.001$), PC loss (from 1742 to 2421 PC/mm2; $P<0.001$), and microglial activation (from 186 to 126 CD74+/mm2; $P<0.01$).
Conclusion: In the NVU of the retina, levels of reactive metabolites are associated with vascular damage, microglial activation and neuronal function. The underlying pathological mechanisms will be investigated.
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The effect of the dipeptidyl peptidase-4 inhibitor linagliptin on proliferative retinopathy in a mouse model

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Background and aims: The dipeptidyl peptidase-4 inhibitor linagliptin (Lina) is used for the treatment of type 2 diabetes. Previous work demonstrated a protective effect of Lina on the neurovascular unit of the diabetic retina. The aim of the present study was to investigate the effect of Lina in an animal model of oxygen-induced retinopathy (OIR).
Materials and methods: Lina was administered by s. c. injection (10 mg/kg body weight) in the OIR model from postnatal day 12 (p 12) to p 16 in C57Bl/6J wild-type (WT; n=6) and glucagon-like peptide-1 receptor (GLP-1R) knockout (KO) mice (n=6). Plasma levels of active GLP-1 and stromal cell-derived factor (SDF)-1 were quantified by ELISA at p 17. Expression of retinal angiopoietin (Ang)-2, tumor necrosis factor (TNF)-α, and erythropoietin (Epo) was determined by qRT-PCR at p 17. The avascular zone was measured in retinal whole mounts at p 12 and neovascularizations (NV) were quantified at p 17.
Results: The levels of active GLP-1 were significantly increased by Lina treatment in both WT (1.6 to 17.5 pg/ml; $p<0.001$) and GLP-1R KO mice (7.8 to 25.5 pg/ml; $p<0.001$). The levels of Ang-2 and TNF- α were not changed by GLP-1R expression. However, there was a 0.4-fold decrease in Epo levels ($p<0.01$). The levels of proangiogenic SDF-1 were not significantly changed by Lina. Vasoregression was independent of GLP-1R expression, as demonstrated by the unchanged area of the avascular zone in GLP-1R KO compared with WT animals (4.7 vs 5.6 mm2). Treatment of WT animals with Lina reduced NV from (mean ± SEM) 54.8 ± 2.4 to 38.2 ± 1.5 nuclei ($p<0.001$). Moreover, this effect was independent of both systemic GLP-1R expression and levels of growth factors: in GLP-1R KO animals, Lina reduced NV from (mean ± SEM) 42.3 ± 1.7 to 30.7 ± 1.9 nuclei ($p<0.01$), without affecting expression of Ang-2, TNF-α and Epo.
Conclusion: Lina has an antiangiogenic effect in the OIR model independent of GLP-1R status.
Supported by: Boehringer Ingelheim
Disclosure: **N. Dietrich:** Grants; Boehringer Ingelheim.

PS 102 Diabetic retinopathy: the experimental approach

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Tie-2 expression is down-regulated by hyperglycaemia and AGEs in endothelial cells

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Background and aims: Alteration of the blood retinal barrier occurs in several complication of diabetes responsible of vision loss, such as diabetic macular edema and diabetic retinopathy. The angiopoietin growth factor (ANG)/tyrosine kinase receptor (Tie-2) system plays a crucial role in maintaining vessel stabilization: Ang-1 is considered an activator of Tie-2 and promotes remodeling, maturation, barrier differentiation, and stabilization of blood vessels by recruiting pericytes and synthesis of extracellular matrix, whereas Ang-2 is an antagonist of Tie-2 and acts as a dominant negative ligand. Moreover Ang2 overexpression in the absence of VEGF-A leads to vasoregression. Microvascular changes start in the prediabetic state, become more complex with overt diabetes and remain even when glycemic control is reached. The latest condition is probably sustained by Advanced Glycation End-Products (AGEs), a heterogeneous group of compounds derived from the non-enzymatic reaction of reducing sugars with proteins, lipids or nucleic acids. The aim of the current study was to investigate whether exposure of endothelial cells to hyperglycemia and AGEs may alter mechanisms involved in maintaining vessel stabilization.
Materials and methods: Glycated serum (GS), which consists in a pool of AGEs, was prepared by incubating FBS with 50 mmol/l ribose at 37°C for 7 days and used to replace part of FBS in the culture medium. Human microvascular endothelial cell-1 (HMEC-1) were cultured for 24 hours with 16.7 mmol/L glucose (HG) or GS. At the end of the culture we evaluated: cell viability by MTS; mRNA levels of Vascular Endothelial Growth Factor A (VEGF-A) and Angiopoietin2 (ANG2) by RealTime-PCR; TIE-2 protein expression by Western blot; VEGF-A release by ELISA; Human Angiogenesis Antibody Array was employed to evaluate the amount of ANG2, Matrix Metalloprotease-1 (MMP1), and Urokinase Plasminogen Activator Receptor (UPAR) in the culture media.
Results: Viability of HMEC-1 cells was decreased and ROS production was increased after exposure to HG or GS. Moreover culture with HG down-regulates mRNA levels of VEGF-A, increases the release of ANG2 and UPAR in the culture media, and decreases Tie-2 protein expression. Exposure to GS decrease mRNA levels of VEGF-A and its secretion, down-regulates Tie-2 protein expression, enhances the detection of MMP1 and UPAR in the culture media.
Conclusion: Here we show for the first time that both HG and AGEs negatively affect the expression of Tie2, an important regulator of vessel integrity. These results also highlight that AGEs participates to endothelial cell dysfunction even in absence of hyperglycemia, and that, raising levels of degrading enzymes, the effects of AGEs may be worse than those of HG. Down-regulation of Tie2 expression may, therefore, be a mechanism through which hyperglycemia and AGEs impair vessel integrity, and contribute to vasoregression.
Disclosure: **A. Puddu:** None.

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Hypoglycaemia-induced retinal neurodegeneration is associated with mitochondrial ROS production caused by fatty acid oxidation

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Background and aims: We previously reported that hyperglycemia-induced mitochondrial ROS (mtROS) production is a key event in the development of diabetic complications, and overexpression of mitochondrial-specific superoxide dismutase in endothelium could prevent diabetic retinopathy (DR) in mice (eMnSOD-Tg). In DCCT, "early worsening (EW)" of DR appeared to be more frequent and/or more severe under acute improvement in glycemic control. Etiologies of EW of DR are largely unknown. Recent investigations have clarified the semaphorin 3A (Sema3A) is an early diagnostic biomarker of DR. Our aims of this study are 1) to examine whether low glucose exacerbates mtROS, 2) to examine the expression of early diagnostic biomarkers of DR under hypoglycemia stimulation, 3) to determine the way to protect the EW of DR.
Materials and methods: Bovine aortic endothelial cells (EC) were cultured with 2.5 (LG) or 5.5 (NG) mM glucose. Several metabolomic profiling, cellular oxygen consumption rate (OCR) and mtROS were evaluated in EC. STZ-induced diabetic control (STZ-C) mice and eMnSOD-Tg (STZ-Tg) mice were subjected to a recurrent hypoglycemic stimulation (6 times / 2 weeks). Expression of Sema3A and VEGF, and albumin leakage were evaluated in the mice retina.
Results: 1) In EC, mtROS production was increased (148.43 ± 2.49% of that in NG, $p<0.01$) 1 hour after LG condition. The LG-induced mtROS production was suppressed by the overexpression of MnSOD in EC. Recurrent hypoglycemia stimulation significantly increased 8-OHdG staining in retina of STZ-C mice compared with that of STZ-C mice without hypoglycemia. In contrast, hypoglycemic stimulation of STZ-Tg mice did not increase 8-OHdG staining in the retina. 2) On metabolome analysis of EC, decreased levels of intermediate metabolites in glycolysis, pyruvate and lactate (242 vs 287, 1264 vs 2344 pmol/10^6 cells, $p<0.05$) were observed in LG compared with NG. However, the level of acetyl-CoA, ATP or the tricarboxylic acid cycle metabolites was not altered in LG. 3) Compared with NG, LG increased basal OCR of EC after treatment with 50 μM palmitic acid (140 ± 2.02 vs 203 ± 2.62 pmol/min, $p<0.01$). 4) Etomoxir, a carnitine palmitoyl transferase 1 (CPT1) inhibitor which constitutes a rate-limiting step of fatty acid oxidation (FAO), ameliorated hypoglycemia-induced increase of mtROS in EC. *In vivo*, etmoxir reduced the expression of Sema3A and VEGF, and albumin leakage in retina of STZ-C mice induced by recurrent hypoglycemia, which were comparable to STZ-Tg mice with recurrent hypoglycemia.
Conclusion: The findings of this study demonstrate that hypoglycemia can increase mtROS production via increasing fatty acid oxidation. Recurrent hypoglycemia increased ROS production and enhanced pathological retinal neovascularization. Hence, overexpression of MnSOD or etomoxir, a mitochondrial long-chain FAO blockade, decreased ROS production and inhibit pathological functional breakdown in DR. Therefore, hypoglycemia-induced mtROS production may be one of the mechanisms of EW of DR, and our study suggests that pharmacological FAO blockade is useful in preventing early retinal neurodegeneration caused by acute hypoglycemia.
Supported by: the Japan Society for the Promotion of Science
Disclosure: **N. Kajihara:** None.

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Anril mediates diabetes induced endothelial dysfunction through up-regulation of vascular endothelial growth factor

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Background and aims: Long noncoding RNAs (lncRNAs) have key roles in various biological processes as decoys, scaffolds for proteins, enhancers or guides to recruit transcription factors and other proteins for gene regulation. Mutations in lncRNAs can therefore lead to various diseases. One particular lncRNA; ANRIL (antisense non-coding RNA in the INK4 locus) is located at a genetic susceptibility locus for coronary artery diseases and Type 2 Diabetes. Research from our laboratory has dissected out multiple gene regulatory pathways including microRNA

200b (miR200b) and polycomb repressive complex 2(PRC2) involved in diabetic complications (DC). Our principle aim is to understand the role of ANRIL in DC. Specifically we are focused at function of ANRIL in diabetic retinopathy (DR) which entails both endothelial dysfunction and subsequent proliferation. The mechanism would be elucidated through study of the potential role of ANRIL in regulation of vascular endothelial growth factor (VEGF).
Materials and methods: Human retinal endothelial cells (HRECs) were subjected to incubation in high glucose for various durations to mimic diabetes. ANRIL expression was knockdown with siRNA in HRECs and use of knockout ANRIL mouse model. These models were exposed to hyperglycemia and measured for VEGF mRNA and protein expression.
Results: Expression of ANRIL was elevated at 48h in HRECs exposed to high levels of glucose. Knockdown of ANRIL in these retinal cells reduced expression of VEGF (a potent angiogenic factor) at mRNA and protein levels. The effect of reduced ANRIL expression was normalized by addition of exogenous VEGF to the system. In tissues from animal model for chronic diabetes, we noticed increased VEGF levels. Such changes were prevented in the ANRIL knockout mice with diabetes. Mechanistic studies also showed that ANRIL may regulate VEGF expression through interaction with miR200b, transcription co-activator p300 and EZH2 methyl transferase of the PRC2 complex.
Conclusion: Data from this study showed that glucose-induced upregulation of ANRIL is causally related to increased production of angiogenic factors in the HRECs and possibly in the tissues affected in DC. Identification of such mechanisms may have potential implications in the development of RNA based therapies in DR.
Supported by: CDA
Disclosure: **S. Chakrabarti:** None.

1003

Anks6(p.R823W) overexpression in kidney affects retinal degeneration

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Background and aims: Eye disorders associated with chronic kidney disease. In clinic, patients with chronic kidney disease and end-stage renal disease such as in diabetic nephropathy and polycystic kidney disease, also have retinopathy, so called renal-retinal syndrome. Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic disorders, affecting one in 1000 individuals. ADPKD not only affects the renal function, but is also involved in the vasoregression and neurodegeneration in retinas. Anks6 gene consists of 16 exons with 2655 bp coding for an 885-amino acid protein including10 tandem ankyrin repeats at its N-terminus and a sterile α motif (SAM) domain at its C-terminus. Recently it has been shown that overexpression of Anks6 harbouring a missense mutation in the SAM domain in transgenic rats [TGR CMV-Anks6(p.R823W)] causes polycystic kidney disease. The expression level of Anks6(p.R823W) is tightly linked to the severity of the cystic phenotype. However, it is unclear if Anks6(p.R823W) overexpression links to retinal vascular and neuronal degeneration. Therefore, we use the TGR CMV-Anks6(p.R823W) and study the retinal degeneration.
Materials and methods: Anks6(p.R823W) transgenic rats (TGR) and age matched wild type (WT) rats at 5, 9 and 14 months were used for this study. Expression of Anks6(p.R823W) was evaluated by semi-quantitative PCR. Glial activation was measured by immunofluorescent staining. For neurodegeneration analysis, eyes were embedded in paraffin and 3μm sections were stained with periodic acid-Schiff (PAS) and haematoxilin. Retinal thickness was measured and the numbers of nuclei were counted using Olympus microscope with CellF software. For vasoregression analysis, eyes were fixed in 4% formalin and retinas were digested using 3% trypsin for 3 hours. The digestion preparations were stained with PAS and subjected to the retinal morphometry.
Results: Transgenic Anks6(p.R823W) was mainly expressed in the kidney and traced in retina of TGR. Retinal vessels showed vascular fibrosis and vasoregression in Anks6(p.R823W) TG rats. Retinal neurodegeneration started later than in transgenic rats overexpressing a mutated polycystin-2, mainly in ganglion cell, inner nuclear and outer nuclear layer. Vasoregression and vascular fibrosis as well as neuronal degeneration at 5 month are simultaneous. The photoreceptor layer is not defective compared with polycystin-2 mutated transgenic rats.
Conclusion: Anks6 (p.R823W) overexpression in the kidney induces retinal vasoregression and vascular fibrosis as well as late neurodegeneration.
Disclosure: **J. Lin:** None.

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FOXO1 is required for the NDPK B deficiency-induced upregulation of Angiopoietin-2 in endothelial and Müller cells

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Background and aims: Our previous publication demonstrated that NDPK B-deficient mouse retinas exhibited a vasoregression which mimic the pathology of diabetic retinopathy. Angiopoietin-2 was found to be upregulated in the NDPK B-deficient retina as well as in human umbilical vein endothelial cells (HUVECs) depleted of NDPK B. However, the mechanism of Ang-2 upregulation upon NDPK B deficiency is still elusive. FOXO1 has been reported to be an upstream regulator of Ang-2. Crucial upregulation of Ang-2 in the diabetic retina occurs predominantly in endothelial cells and Müller cells. Thus, we studied the impact of FOXO1 on Ang-2 expression in NDPK B-deficient endothelial and Müller cells.
Materials and methods: Retinas of NDPK B-deficient mice, HUVECs and Müller cells isolated from the NDPK B-deficient retinas were used. Modulation of NDPK B, Ang-2 and FOXO1 in vitro was achieved by single or double gene knockdown with NDPK B, Ang-2, or FOXO1 siRNA using Lipofectamine. Suitable non-specific siRNAs served as controls. The expression of NDPK B, Ang-2 and FOXO1 was assessed by Western blot and/or immunofluorescence.
Results: FOXO1 upregulation was observed in NDPK B-depleted retinas. Moreover, FOXO1 was found to be elevated in NDPK B-depleted endothelial cells. Elevated protein levels of FOXO1 were detected in the endothelial nucleus as well as the cytoplasm. Depletion of endothelial Ang-2 did not alter levels of NDPK B and FOXO1. However, in FOXO1-deficient endothelial cells, Ang-2 was significantly decreased whereas NDPK B remained unchanged. Upon silencing both NDPK B and FOXO1, Ang-2 level were significantly suppressed. The data suggest that FOXO1 is mediating the NDPK B deficiency-induced Ang-2 upregulation, and that there is no feedback regulation from Ang-2 to FOXO1 and NDPK B. Interestingly, upregulation of Ang-2 and FOXO1 was also detected in Müller cells isolated from NDPK B-deficient retinas. Concomitant with the observation in endothelial cells, knockdown of FOXO1 eliminated upregulation of Ang-2 in NDPK B-deficient Müller cells.
Conclusion: Our data indicate that the vascular damage in NDPK B-deficient retina might be a result of an endothelial dysfunction caused by increased Ang-2 levels due to FOXO1 upregulation in both endothelial and Müller cells.
Supported by: EFSD supported by Novartis
Disclosure: **Y. Feng:** Grants; EFSD.

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Effects of high glucose and fluid flow shear stress on connexin 43 (Cx43) hemichannel activity in retinal endothelial cells

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Background and aims: Gap junction intercellular communication (GJIC) is essential for maintenance of tissue homeostasis and is thus, a critical factor for regulation of cell growth and cell death. Previously we have reported that reduced Cx43 expression contributes to vascular cell death in high glucose (HG) condition and in the retinas of diabetic rats. However, in this context, the role of non-junctional Cx43 hemichannels (HCs) is currently unknown. Our recent observation suggests that Cx43 HCs play a distinctive role in response to HG in retinal endothelial cells (RRECs) in contrast to their conventional role in GJIC activity. Specifically, under HG condition, RRECs exhibit reduced dye uptake but the underlying mechanism for this process is currently unknown. In this study, we examined whether decreased dye uptake is associated with reduced Cx43 HC activity and/or Cx43 expression. Additionally, to gain insight into the effect of retinal blood flow changes on HC activity, RRECs were analyzed under fluid flow sheer stress (FFSS) in HG condition.
Materials and methods: To determine whether short-term exposure to HG alters Cx43 HC activity and/or Cx43 gene expression, RRECs grown in normal (N) (5 mM glucose) or HG (30 mM glucose) medium for 1, 2, or 3 days were subjected to dye uptake assay, immunostaining with Cx43 E2 antibody specific to Cx43 HC, and Western blot (WB) analysis with membrane-bound hydrophobic protein isolated at each of the three time points. To investigate the effect of retinal blood flow changes on HC activity, RRECs grown in N or HG medium for 1 or 2 days were also subjected to FFSS at 8 dye/cm2 for 15 min and HC activity assayed by ethidium bromide dye uptake.
Results: Non-junctional Cx43 immunostaining representing HCs showed that RRECs grown for 1, 2, or 3 days in HG medium exhibited no significant difference compared to those grown in N medium for the same periods. Similarly, WB analyses showed no significant difference in membrane-bound or total Cx43 protein expression in cells grown for 1, 2, or 3 days in HG medium compared to those grown in N medium for the three time points. RRECs grown in HG medium exhibited a significant decrease in dye uptake at day 1, 2 time points (48±28%, $p<0.01$; 41±29%; $p<0.05$, respectively) compared to those grown in N medium. Additionally, when RRECs were grown in HG medium for 1 or 2 days and subjected to FFSS, a significant decrease in dye uptake was observed (78±20%, $p<0.01$; 70±26%; $p<0.05$, respectively).
Conclusion: Findings from this study indicate that HG reduces Cx43 HC activity, and that this reduction is likely exacerbated in long-term cultures with HG. Furthermore, FFSS has a profound effect in reducing HC activity, which may be associated with retinal blood flow changes in diabetic retinopathy.
Supported by: NIH
Disclosure: **D. Lee:** None.

PS 103 Impact of treatment on diabetic nephropathy

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Effect of empagliflozin on nephropathy in subgroups by age: results from EMPA-REG OUTCOME

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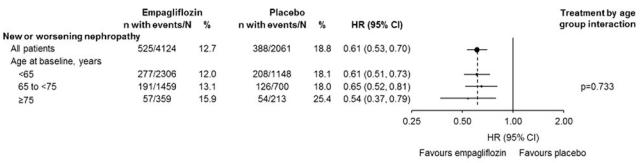
Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin (EMPA) given in addition to standard of care significantly reduced the risk of new or worsening nephropathy versus placebo (PBO) in patients with type 2 diabetes (T2DM) and high CV risk. We investigated the effect of age on the reduction in new or worsening nephropathy with EMPA.
Materials and methods: Patients in EMPA-REG OUTCOME were randomised to receive EMPA 10 mg, EMPA 25 mg, or PBO. New or worsening nephropathy (defined as new onset of macroalbuminuria, doubling of serum creatinine, initiation of renal replacement therapy, or death due to renal disease) was analysed in the pooled EMPA group vs PBO in subgroups by baseline age (<65, 65 to <75, ≥75 years).
Results: A total of 7020 patients were treated. Median observation time was 3.1 years. At baseline, mean (SD) age was 63.1 (8.6) years and 63.2 (8.8) years in the EMPA and PBO groups, respectively, and mean (SD) HbA_{1c} was 8.07 (0.85) % and 8.08 (0.84) % in the EMPA and PBO groups, respectively. The benefit of EMPA vs PBO on new or worsening nephropathy was consistent across age categories (Figure). Across age subgroups, reported adverse events were consistent with the known safety profile of EMPA.
Conclusion: EMPA, in addition to standard of care, reduced the risk of new or worsening nephropathy in patients with T2DM and high CV risk irrespective of age.



Cox regression model including sex, baseline body mass index, baseline HbA_{1c}, baseline estimated glomerular filtration rate, region, treatment, age group and treatment by age group interaction, in patients treated with ≥1 dose of study drug.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **M. von Eynatten:** Employment/Consultancy; Boehringer Ingelheim.

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Empagliflozin and microvascular outcomes in EMPA-REG OUTCOME

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Background and aims: In EMPA-REG OUTCOME, empagliflozin, given at a dose of 10 mg or 25 mg in addition to standard of care, reduced the risk of cardiovascular (CV) outcomes vs placebo (PBO) in patients with type 2 diabetes (T2DM) and high CV risk. We investigate the effect of empagliflozin on microvascular outcomes.
Materials and methods: A pre-specified composite microvascular outcome was defined as time to first initiation of laser therapy for retinopathy, vitreous haemorrhage, diabetes-related blindness, or new or worsening nephropathy (defined in table).
Results: 7020 patients were included in the intent-to-treat analysis. Median observation time was 3.1 years. Mean HbA_{1c} (%) was 8.08, 8.07 and 8.08 at baseline and 7.93, 7.81 and 8.16 (adjusted) at week 206 for empagliflozin 10 mg, 25 mg and PBO, respectively. The composite microvascular outcome occurred in a significantly lower percentage of patients on empagliflozin (pooled; 14.0%) than PBO (20.5%; hazard ratio [HR] 0.62 [95% CI 0.54, 0.70]; p<0.001). HR (95% CI) with empagliflozin versus PBO was 0.69 (0.43, 1.12) (p=0.134) for initiation of laser therapy for retinopathy; 0.93 (0.51, 1.71) (p=0.815) for vitreous haemorrhage; 0.61 (0.53, 0.70) (p<0.001) for new or worsening nephropathy.
Conclusion: Empagliflozin used in addition to standard of care reduced the risk of a composite microvascular outcome in patients with T2DM and high CV risk, driven by a reduction in new or worsening nephropathy.

Outcome	Placebo		Empagliflozin		Hazard ratio (95% CI)	p-value
	n/N (%)	Rate/ 1000 pt-years	n/N (%)	Rate/ 1000 pt-years		
Composite microvascular outcome	424/2068 (20.5)	83.6	577/4132 (14.0)	52.8	0.62 (0.54, 0.70)	<0.001
Initiation of laser therapy for retinopathy	29/2333 (1.2)	4.4	41/4687 (0.9)	3.0	0.69 (0.43, 1.12)	0.134
Vitreous haemorrhage	16/2333 (0.7)	2.4	30/4687 (0.6)	2.2	0.93 (0.51, 1.71)	0.815
Diabetes-related blindness†	2/2333 (0.1)	0.3	4/4687 (0.1)	0.3	–	–
New or worsening nephropathy	388/2061 (18.8)	76.0	525/4124 (12.7)	47.8	0.61 (0.53, 0.70)	<0.001
New onset of macroalbuminuria	330/2033 (16.2)	64.9	459/4091 (11.2)	41.8	0.62 (0.54, 0.72)	<0.001
Doubling of serum creatinine*	60/2323 (2.6)	9.7	70/4645 (1.5)	5.5	0.56 (0.39, 0.79)	<0.001
Initiation of continuous renal replacement therapy	14/2333 (0.6)	2.1	13/4687 (0.3)	1.0	0.45 (0.21, 0.97)	0.041
Death due to renal disease†	0/2333 (0)	0	3/4687 (0.1)	0.2	–	–

Cox regression analysis in patients treated with ≥1 dose of study drug.
*Accompanied by estimated glomerular filtration rate (Modification of Diet in Renal Disease formula) ≤45 ml/min/1.73m^2.
†Hazard ratio and 95% CI were not analysed as the total number of events was <14.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **C. Wanner:** Employment/Consultancy; Boehringer-I, Amgen, Sanofi, GSK. Grants; Genzyme-Sanofi. Honorarium; Boehringer-I, Amgen, Sanofi.

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Evaluation of renal protection by Dipeptidyl Peptidase 4 inhibitor therapy in diabetic patients: relation with serum surfactant proteins

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Background and aims: Surfactant protein (SP)-A and SP-D are used as biomarkers for diagnosing Interstitial pneumonitis (IP) and evaluating disease activity. It has been reported that surfactant proteins may be associated with insulin resistance and may have a protective effect on proximal renal tubular cells, suggesting that these biomarkers of IP may also reflect various aspects of general health. In patients receiving DPP4 inhibitors (DP) therapy, the correlations of these biomarkers with combined thiazolidinedione (TZD) therapy and CKD categories (KDIGO 2012) were evaluated.
Materials and methods: Among diabetic patients in whom SP-A (ng/ml) and SP-D (μg/l) were measured, 84 patients were enrolled in this study (52 men and 32 women aged 65.5 ± 9.9 years; BMI: 24.9 ± 4.0). The mean HbA1c of the subjects was 7.9 ± 1.5%. In the DP group (N = 59, 70.2%), the mean duration of DP use was 2.6 ± 1.1 years. Nineteen patients (23.8%) were taking TZDs and 14 patients were on combined therapy. In addition, 19 patients were not using either DPs or TZDs. Forty-two patients (50%) were smokers. Correlations of these biomarkers with the smoking history, duration of DP use, and TZD treatment were analyzed.
Results: 1. Biomarkers: It was found that 52.3% of the patients had normal levels of all three biomarkers. In addition, SP-A was elevated in 36.9% of the patients (SA group), SP-D was high in 15.5% of the patients (SD group), and both SP-A and SP-D were elevated in 6% of the patients. 2. Correlations of biomarkers with other parameters (all patients): No correlations were observed between any of these biomarkers and age, duration of DP use, HbA1c, Albumin creatinine ratio(ACR mg/g Cr), BMI, or blood pressure. However, there were significant positive correlations between all of the biomarkers and the Brinkman Index (BI) ($p < 0.05$). In addition to the BI, ACR was higher in the SA group (98.3 ± 44.1) than in the patients without elevation of SP-A (30.1 ± 9.1) ($p < 0.05$). 3. Influence of DP and TZD use: The SP-D level was higher in the DP group (71.1 ± 7.6) than in patients not using DPs (47.2 ± 7.2) ($p < 0.1$). In addition, the KL-6 level was 277.7 ± 23.8 in the DP + TZD group and 199.0 ± 27.5 in the TZD group ($p < 0.1$). Furthermore, the SP-D level was 84.2 ± 15.4 in the DP + TZD group, 67.0 ± 8.8 in the DP group, 30.4 ± 6.9 in the TZD group ($p < 0.05$), and 52.5 ± 9.0 in the group not using DPs or TZDs ($p < 0.1$). Compared with TZD monotherapy, the 2 markers were increased by DP and TZD combination therapy, though remaining within the normal range. There was no correlation between SP-A and TZD use. In addition, there was no influence of combination therapy on SP-A or ACR. 4. Stratified analysis by CKD classification (DP group): Both SP-D and SP-A levels were increased in patients with a lower eGFR. eGFR: (SP-D)G1 49.8 ±6.5, G2 76.2 ± 11.2, G3 80.5 ±17.2 (G1 vs G3 $p<0.1$), (SP-A)G1 36.1 ±3.0, G2 43.5 ± 4.0, G3 54.8 ±9.2 (G1 vs G3 $p<0.05$). In particular, analysis by stratified ACR showed that SP-A became significantly higher as urinary protein excretion increased. ACR: (SP-D)A1vs A2 vs A3 (not significant), (SP-A)A1 41.3 ±3.4, A2 42.5 ±4.6, A3 63.6 ±14.6 (A1 vs A3 $p<0.05$, A2 vs A3 $p<0.1$).
Conclusion: Stratified evaluation by the CKD classification showed that the eGFR was correlated with SP-A and SP-D, and ACR was correlated with SP-A. We hypothesize that biomarkers were probably also elevated to provide protection against renal tubular damage caused by systemic vascular injury or increased vascular permeability due to smoking or hyperglycemia.
Disclosure: **C. Watanabe:** None.

1009

Effects of pioglitazone add-on treatment on blood glucose levels and insulin resistance in patients with type 2 diabetic nephropathy receiving sitagliptin

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Background and aims: Type 2 diabetic patients with advanced chronic kidney disease (CKD) usually need multidrug therapy to control blood glucose (BG) levels but the options of agents are limited because of impaired renal functions. Pioglitazone, a thiazolidinedione derivative, is one of agents for the treatment of diabetes and prevention of organ damage in diabetic patients. However, pioglitazone may have adverse effects including body weight gain and body fluid accumulation. Thus, this agent has not often been used in patients with advanced CKD and little is known about the effect of pioglitazone on the glucose metabolism in these patients.

Materials and methods: The study was designed to compare the effects of pioglitazone (15 mg/day) on the BG control with the effects of doubling doses of sitagliptin, one of dipeptidyl-peptidase 4 (DPP4) inhibitors. Fifty-one patients (urinary albumin excretion 0.3-2.0 g/day and creatinine clearance < 60 mL/min/1.73 m2), whose glycoalbumin did not reach the therapeutic goal of 16% by sitagliptin (15 mg/day) alone, were randomly divided into two groups. Twenty-six patients (aged 60 ± 9 years, 15 men and 11 women) had pioglitazone add-on treatment as the pioglitazone group. The rest of patients (61 ± 11 years, 13 men and 12 women) had doubling the doses (30 mg) of sitagliptin (the sitagliptin group). Fasting BG, glycoalbumin, homeostasis model assessment insulin resistance (HOMA-IR) as an index of insulin resistance, and brain natriuretic peptide (BNP), an index of cardiac function, were measured at 0, 4, 8, and 12 weeks after the treatment.
Results: The pioglitazone group showed a significant (P=0.01) decrease in fasting BG from 162±15 to 133±12 at 4 weeks after adding pioglitazone and maintained similar levels during the remaining treatment period. In the sitagliptin group, fasting BG also decreased from 166±13 to 144 ±20 (P=0.01) at 8 weeks after doubling the doses of sitagliptin. Glycoalbumin declined during the study period in the pioglitazone group (from 19.5±2.6% to 17.3±1.6%, P=0.04) and in the sitagliptin group (from 19.6±1.6 to 17.6±1.4%, P=0.04). However, the number of patients who fulfilled the therapeutic goal of glycoalbumin, 16%, was higher (12 out of 26) in the pioglitazone group than the sitagliptin group (8 out of 25, P=0.04) at 12 weeks. HOMA-IR declined (P=0.03) in the pioglitazone group (from 2.6±0.5 to 2.0±0.8) but did not changed in the sitagliptin group (from 2.8±0.7 to 3.1±0.8, P=0.21). No significant increases in BNP were observed in both groups during treatment nor did hypoglycemic attack occur in the two groups. Body weight remained unchanged from 63±3 to 63±4 kg (P=0.83) in the pioglitazone group and from 64±2 to 65±4 kg (P=0.17) in the sitagliptin group during the study period.
Conclusion: These results suggest that adding pioglitazone decreased BG safely and more effectively than increasing the dose of sitagliptin. Furthermore, insulin resistances in patients with type 2 diabetic nephropathy were improved. Achievement of the recommended BG control is considered to have impact on renal disease progression. Thus, coadministration of pioglitazone with DPP4 inhibitors may minimize organ damage.
Disclosure: **G. Yasuda:** None.

1010

Efficacy of statins in patients with diabetic nephropathy: a meta-analysis of randomised controlled trials

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Background and aims: To evaluate the potential therapeutic role of statins in diabetic nephropathy.
Materials and methods: Trials were retrieved through PubMed, Embase, Web of Science and China National Knowledge Infrastructure (CNKI). The languages were restricted to English and Chinese.
Results: Fourteen trials were identified including 2866 patients with diabetic nephropathy, 1430 in statin and 1436 in control group. Overall, patients receiving statins showed a greater reduction in albuminuria [standardized mean difference(SMD), −0.46; 95%CI,−0.68 to −0.25, P<0.0001] and urinary albumin excretion rates (SMD, -1.68; 95%CI, -3.23 to -0.12, P=0.03) than those in control group. The reduction of albuminuria was much greater in patients of type 2 diabetes mellitus with diabetic nephropathy(SMD, -0.56; 95%CI, -0.80 to -0.32, P<0.00001) and the decrease was significant during the 1 to 3 years period of statin therapy (SMD, -0.57; 95%CI, -0.95 to -0.19, P=0.003). In subgroup analysis, the efficacy of statins on renal function was statistically significant in studies of participants with pathologic albuminuria: the SMD of albuminuria in change from baseline was -0.71(95%CI, -1.09 to -0.33, P=0.0003)in patients with urinary protein excretion 30 to 300 mg/day, -0.37 (95%CI, -0.67 to -0.06, P=0.02) in patients with urinary protein excretion more than 300 mg/day and -0.29 (95%CI, -0.78 to 0.21, P=0.26) in patients with urinary protein excretion less than 30 mg/day. However, statin did not significantly reduce estimated glomerular filtration rate, serum creatinine and blood urea nitrogen compared with control group.
Conclusion: Statins decrease the albuminuria and urinary albumin excretion rates significantly. The efficacy of statins on renal function is time dependent and better in type 2 diabetic patients with nephropathy.
Supported by: National Natural Science Foundation of China Grants (81070637)
Disclosure: **X. Shen:** None.

1011

The use of green tea polyphenols for treating residual albuminuria in diabetic nephropathy: a double-blind randomised clinical trial

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Background and aims: Prior research has shown that in experimental diabetes mellitus, green tea reduces albuminuria by decreasing podocyte apoptosis through activation of the WNT pathway. In the present study, we tested the effect of green tea polyphenols (GTP) on residual albuminuria of diabetic subjects with nephropathy. To test the hypothesis that GTP by activating WNT pathway reduces podocyte apoptosis and albuminuria, we quantified plasma DKK-1, a WNT inhibitor, and performed in vitro studies using immortalized human podocytes (iHPs).
Materials and methods: We conducted a 12 weeks, randomised, double-blind, placebo-controlled study in 42 diabetic subjects with a urinary albumin-creatinine ratio (UACR) > 30 mg/g, despite administration of the maximal recommended dose of renin-angiotensin (RAS) blockade. The primary outcome was a reduction in the UACR. Patients were randomly assigned to 2 equal groups to receive GTP (800 mg of epigallocatechin gallate) or placebo daily for 12 weeks.
Results: Treatment with GTP reduced UACR (geometric mean, 95% CIs) by 41%, -0.64, 0.96, while the placebo group saw a 2%, -0.13, 0.45 increase in UACR (p=0.019). Basal plasma DKK-1 levels did not differ between 2 groups and it was significantly reduced by GTP treatment (p=0.004). Podocyte apoptosis (p=0.001) and in vitro albumin permeability (p<0.001) were higher in iHPs exposed to plasma from diabetic subjects (PDS) compared to podocytes treated with plasma from normal individuals. These abnormalities were abrogated when iHPs were exposed to PDS treated with GTP. The protection from apoptosis conferred by GTP was lost when iHPs were pretreated with DKK-1, a WNT inhibitor.
Conclusion: GTP administration reduces albuminuria in diabetic patients receiving the maximal recommended dose of RAS blockade. Reduction in podocyte apoptosis by activation of the WNT pathway may have contributed to this effect.
Clinical Trial Registration Number: NCT01923597
Supported by: FAPESP e CNPq
Disclosure: **C. Borges:** None.

1012

Metformin prevents from severe kidney failure, vascular calcification and high bone turnover disease in a rat model for chronic kidney disease-mineral and bone disorder

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Background and aims: Chronic impairment of the kidney causes systemic dysregulation of the mineral metabolism and coincides with vascular calcification and bone disorders which is called 'Chronic Kidney Disease-Mineral and Bone Disorder' (CKD-MBD). Metformin, an oral anti-hyperglycemic agent used for type 2 diabetes mellitus as a standard treatment, has been shown to have beneficial effects on kidney fibrosis and atherosclerosis. This study aims to investigate the effect of metformin on renal function and structure, arteries and the bone in a rat model of CKD-MBD.
Materials and methods: To induce CKD, rats received a 0.25% adenine/low vitamin K diet for 8 weeks. Animals were daily treated with 200 mg/kg metformin or vehicle by oral gavage from 1 week after CKD induction onwards until week 8. Renal function, histology, fibrosis and inflammation were assessed. The calcium content in the aorta, carotid and femoral arteries was determined and both static and dynamic bone parameters were measured.
Results: Severe, stable CKD along with serious hyperphosphatemia and hypocalcemia had developed in vehicle treated rats which led to calcification in the arteries and high bone turnover disease. Metformin treatment protected adenine dosed rats from the evolution towards severe CKD, with significant lower serum creatinine levels throughout the study as compared to vehicle CKD rats and serum phosphorus and calcium concentrations remained within the normal range. The kidney of the metformin group showed significant less cellular infiltration, fibrosis and inflammation. The renal tubulointerstitial area percent, comprising both extracellular matrix and infiltrating cells, was 35% lower in metformin treated animals ($p < 0.05$). The mRNA expression of the pro-inflammatory cell adhesion molecule VCAM1 was markedly decreased in CKD rats exposed to metformin. Metformin treatment also led to a significantly decreased expression of the inflammatory cytokines TNFα and IL-1β in the kidney. In addition, TGF-β and Collagen 1a1 expression was significantly reduced in CKD rats treated with metformin as compared to vehicles. Metformin also prevented the development of vascular calcification and inhibited the progression towards high bone turnover disease.
Conclusion: Metformin treatment protected against the development of severe adenine-induced renal failure and preserved the calcium phosphorus homeostasis which presumably prevented the onset of vascular calcification and the development of high bone turnover disease.
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Disclosure: **P. D'Haese:** Grants; This work was supported by a research grant from Merck KGaA.

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A new hope for the treatment of diabetic nephropathy based on histamine H4R antagonism

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Background and aims: JNJ39758979 is an orally bioavailable highly selective histamine H4 receptor (H4R) antagonist originally developed for non diabetic-related indications. Although the hypothesis for a direct involvement of histamine in kidney function has been present for decades but largely undeveloped, our new data on the renal discovery of the histamine H4R, point out at this receptor as as a new promising pharmacological target for diabetic nephropathy.
Materials and methods: Diabetes was induced in forty DBA/2J 7-8 week-old male mice by using the multiple low-dose streptozotocyn (STZ)-injection protocol. After the onset of diabetes, animals were randomized to receive by oral gavage vehicle alone or JNJ39758979 (25, 50, 100 mg/kg/day p.o.; 4 x 10 mice). Urine were fortnightly processed for standard urinalysis. At week 15, mice were sacrificed and blood and kidneys were collected for biochemical and morphological analysis. Results were analyzed by Prism 5 software from Graphpad (CA, USA) by the one-way ANOVA and the post-hoc Newman-Keuls multiple comparison were performed. To determine significant differences between means, the threshold for statistical significance was set to P-values < 0.05.
Results: All the STZ-treated animals showed glycaemic levels over 200 mg/dl within 14 days. The hyperglycemia remained severe throughout the observation period, with diabetic animals displaying a significant weight gain reduction over time (P<0,05 vs control) despite the increase in food consumption. JNJ39758979 did not significantly affect either glycaemic status or body weight, but showed a trend in reducing STZ-induced water intake. Urine collection suggested a dose-dependent inhibitory effect of JNJ39758979 on the urine volume excreted in a 24 h sample period, with animals treated for 15 weeks at 100 mg/kg excreting 10 ml/24 h urine vs 28 ml/24 h (P<0,05) by STZ-treated alone animals. JNJ39758979 displayed a dose-dependent effect on proteinuria with a maximum reduction of 58% at 100 mg/kg. Notably this dose was also effective in reducing the albuminuria, the ACR and the Creatinine Clearance (P<0,05). The beneficial effects of JNJ39758979 on renal function paralleled comparable effect on renal morphological integrity, with treated animals displaying a modest but statistically significant matrix mesangial expansion in comparison with STZ-treated animals (P<0,05). Notably, the drug was demonstrated to be significantly effective against fibrosis: with JNJ39758979 100 mg/kg inducing a reduction in the Syrius red positivity of 33% (P<0,05).
Conclusion: In our model of STZ-induced diabetic nephropathy, JNJ39758979 was effective in preserving renal function and morphological integrity. Thereby, our data provide the first evidence to support the proposal for JNJ39758979 as a novel strategy to counteract diabetic nephropathy.
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Disclosure: **A.C. Rosa:** Non-financial support; The JNJ39758979 has been provided through an MTA agreement with the Jansen Research & Development.

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New modelling approaches for the estimation of GFR in diabetes type 2 patients

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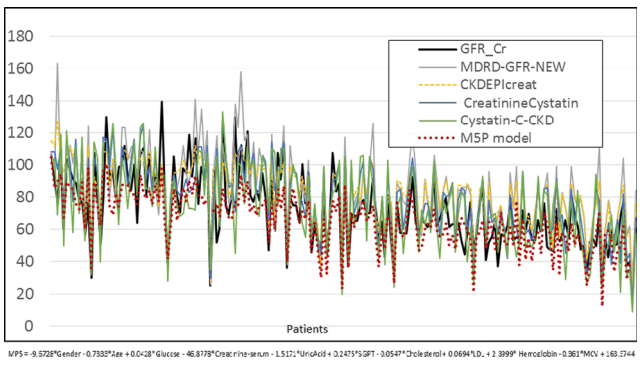
Background and aims: Glomerular filtration rate (GFR) is the best overall index of kidney function. Several equations based mainly on serum creatinine values but also containing additional demographic and clinical variables have been developed, in order to more reliably estimate GFR. The aim of this study was to compare several GFR equations (MDRD, CKD-EPI, CKD-EPI creatinine-cystatin equation, CKD-EPI cystatin C equation) with the gold standard GFR measured by 51Cr-EDTA as well as to develop a new model that could effectively estimate the GFR-51Cr-EDTA values, taking into account several laboratory and clinical characteristics.
Materials and methods: 192 consecutive type 2 diabetic patients whose GFR had been estimated by 51Cr-EDTA method were recruited from our outpatient diabetic clinic. Several laboratory and clinical characteristic have been recorded. The database of these patients was used for developing a new GFR model based on Computational Intelligence method such as the M5P algorithm, a reconstruction of Quinlan's M5 algorithm that induces trees of regression models and combines a conventional decision tree with the possibility of linear regression functions at the nodes. 10-fold cross validation was used as a training and evaluation method, while the WEKA computational environment was employed.
Results: The new model (expressed as a linear equation) was found to have the best correlation coefficient (r=0.83; p<0.001) among all 5 models, while it has the tendency to slightly under predict values. The correlation coefficient concerning the four existing models were 0.76 for MDRD, 0.79 for CKD-EPI, 0.78 for CKD-EPI creatinine-cystatin and 0.69 for CKD-EPI cystatin C equation (p<0.001) (Figure).
Conclusion: It is evident that the M5P-based model has the best correlation coefficient among all 5 models, while it has the tendency to slightly under predict values. Overall, the new model outperforms all four existing GFR models, yet its generalization power remains to be investigated with the aid of additional patient datasets. The most important part of this short investigation is that it establishes a methodology which may lead, if properly checked, to better GFR estimations for various diabetic patient populations.



Disclosure: **N. Kakaletsis:** None.

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Deterioration of eGFR during a year is different among each stage of diabetic nephropathy: the interim report of PRe-STOPPER study

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Background and aims: Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD). A rapid decline in kidney function can lead to renal failure in DN. Therefore, it is much important to prevent the aggravation of DN as soon as possible. However, we do not know how kidney function is changed in each stage of DN. We should investigate and clarify the change of kidney function in DN in more detail for the prevention of its development.
Materials and methods: We have collected the clinical data in patients with DN from 17 hospitals in Japan. We focussed on the estimated glomerular filtration rate (eGFR) and evaluated the change of eGFR during a year in "Pursuance of retrospective eGFR study for strategic trial for the prevention of progression of overt diabetic nephropathy, PRe-STOPPER" study. We then analyzed the data in the interim report of "PRe-STOPPER" study [n=10,630, age 66.4±13.5y, HbA1c 7.2±1.3%, duration of diabetes mellitus 9.8±9.0y and eGFR 67.0±24.7ml/min/1.73m^2]. Annual eGFR decline ($\Delta eGFR_{slope0\text{-}1y}$) was determined by linear regression analysis of eGFR values in each subject. We investigated whether $\Delta eGFR_{slope0\text{-}1y}$ was different among each stages of $eGFR_{0m}$ [$eGFR_{0m}$<15, 15≦$eGFR_{0m}$<30, 30≦$eGFR_{0m}$<45, 45≦$eGFR_{0m}$<60, 60≦$eGFR_{0m}$<75, 75≦$eGFR_{0m}$<90 and 90≦$eGFR_{0m}$] at the beginning of the study. The severe deterioration of kidney function was defined as $\Delta eGFR_{slope0\text{-}1y}$ of -5.0 or below.
Results: $\Delta eGFR_{slope0\text{-}1y}$ has significant correlation with the average HbA1c during a year ($HbA1c_{Ave.}$) [r = -0.1, p<0.001] and it's %CV ($HbA1c_{\%CV}$) [r = -0.1, p<0.001] in all stage of $eGFR_{0m}$. $\Delta eGFR_{slope0\text{-}1y}$ in 75≦$eGFR_{0m}$ was significantly lower [p<0.01] than that in $eGFR_{0m}$<75. The deterioration of $\Delta eGFR_{slope0\text{-}1y}$ has been gradually improved from 90≦$eGFR_{0m}$ to 45≦$eGFR_{0m}$<60. However, $\Delta eGFR_{slope0\text{-}1y}$ was getting worse in proportion to $eGFR_{0m}$ in 15≦$eGFR_{0m}$<45. In addition, %CV of $\Delta eGFR_{slope0\text{-}1y}$ in 30≦$eGFR_{0m}$<45 was particularly higher than that in the other $eGFR_{0m}$. Relative risk (RR.) for the severe deterioration of $\Delta eGFR_{slope0\text{-}1y}$ was observed at $eGFR_{0m}$=25.9 [RR.=2.1, p<0.01] in 15≦$eGFR_{0m}$<30. $HbA1c_{0m}$, $HbA1c_{Ave.}$ and $HbA1c_{\%CV}$ were significantly higher [p<0.001, p<0.05, p<0.001, respectively] in the group whose $\Delta eGFR_{slope0\text{-}1y}$ was severely deteriorated [$\Delta eGFR_{slope0\text{-}1y}$ <-5.0] in $eGFR_{0m}$<60.
Conclusion: Deterioration of DN, annual eGFR decline in each kidney stage was clarified from the interim report of "PRe-STOPPER" study. We believe that we should take care the change of kidney function in DN with $eGFR_{0m}$<45 and 75≦$eGFR_{0m}$, particularly 15≦$eGFR_{0m}$<45. Annual eGFR decline might be divided into 2 groups, "deterioration" and "no deterioration", in 30≦$eGFR_{0m}$<45 due to %CV of $\Delta eGFR_{slope0\text{-}1y}$ was especially higher in 30≦$eGFR_{0m}$<45. Poor glycemic control was significantly related to the deterioration of $\Delta eGFR_{slope0\text{-}1y}$. Therefore, we should perform the better glycemic control for the patient with DN whose eGFR was above than 30. Maintaining much better level of blood glucose was much important, and it might prevent the development of DN and could reduce the risk of ESRD.
Disclosure: **M. Miuchi:** None.

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Urinary metabolomics predict response to spironolactone therapy in patients with type 2 diabetes and hypertension

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Background and aims: The mineralocorticoid blocker spironolactone significantly reduces albuminuria (UACR) in patients with type 2 diabetes mellitus, with a large variability in response between individuals. Finding new biomarkers that predict the response in UACR to spironolactone may tailor optimal therapy. We therefore tested whether an *a priori* defined set of metabolites predicts UACR response to spironolactone.
Materials and methods: Data and samples were used from a randomized placebo controlled double blind clinical trial. Patients with type 2 diabetes mellitus and resistant hypertension were randomly allocated to spironolactone 25 to 50 mg/day (n=44) or placebo (n=44) for 16 weeks, adjunct to renin-angiotensin-system inhibition. We first conducted a systems medicine approach to select *a priori* 18 candidate metabolites (biogenic amines and organic acids) for predicting response to spironolactone. The approach selects candidate metabolites by matching a diabetic kidney disease molecular model with a spironolactone molecular model describing its mechanism of action. We subsequently performed urinary metabolomics by LC-MS to validate whether these candidate metabolites predicted UACR response. Linear regression was used to develop a urine metabolite score at baseline. The performance of this score for prediction of UACR response was assessed by analysis of covariance.
Results: Spironolactone reduced UACR relative to placebo by -42%, but with large variability (5th and 95th percentile -93 to +212). The baseline urine metabolite score was inversely correlated with baseline UACR (Pearson's r -0.30, p=0.004). The baseline urine metabolite score predicted UACR response to spironolactone therapy (p=0.03 for interaction between the score and spironolactone). When analyzed by tertiles of the score, placebo adjusted UACR reduction was only significant in the lowest tertile (-62% (95%CI -82 to -23); p=0.012).
Conclusion: An *a priori* defined set of 18 urinary biogenic amines and organic acids predicts UACR response to spironolactone therapy in type 2 diabetes mellitus. These findings suggest that this score can be used to predict the albuminuria response to spironolactone in type 2 diabetes mellitus.
Clinical Trial Registration Number: NCT01062763
Supported by: Novo Nordisk Foundation Grant number NNF14SA0003
Disclosure: **M.J. Pena:** Grants; Novo Nordisk Foundation Grant number NNF14SA0003.

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Time to treatment intensification and its association with subsequent microvascular outcomes among patients with type 2 diabetes

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Background and aims: Previous research has shown that earlier treatment intensification among patients with Type 2 diabetes mellitus (T2DM) and HbA1c levels ≥7% despite monotherapy is associated with shorter time to subsequent glycemic control, which could potentially result in better long-term outcomes. This study assessed the association between timing of treatment intensification and subsequent microvascular outcomes among patients with T2DM in the UK.
Materials and methods: Patients aged 18-79 years diagnosed with T2DM who used metformin (met) or sulfonylurea (SU) for ≥3 months were selected using the UK Clinical Practice Research Datalink (1/2000 - 12/2014). The first record with HbA1c ≥7% after ≥3 months of met/SU monotherapy was the index event. Intensification was defined as initiating ≥1 non-insulin antidiabetic medication in addition to met/SU after index. The cohort was stratified into 4 groups based on time from index to intensification: <6 months, 6 to <12 months, 12 to <24 months, and 24 to <36 months. Multivariate cox proportional hazard models were used to compare the incidence of albuminuria, eGFR<60 mL/min/1.73m^2, doubling of serum creatinine, retinopathy, and neuropathy after intensification between groups (<6 months as reference group). Models were adjusted for the following covariates, assessed during 6 months prior to index: age, gender, body mass index, HbA1c level, Charlson comorbidity index, duration of monotherapy, use of antihypertensive, statins, or antidepressants, and serum creatinine levels prior to intensification.
Results: Of the 40,036 patients included in the sample (mean age: 61 years, ~57% male), 17% intensified <6 months after index, 9% in 6 to <12 months, 13% in 12 to <24 months, and 8% in 24 to <36 months. The mean HbA1c at index was 8.9 for the <6 months group, 8.3 for 6 to <12 months group, 8.0 for 12 to <24 months group, and 7.9 for the 24 to <36 group. The 24 to <36 months group had the highest rates of the following complications (per 1000 person-years): doubling of serum creatinine (7.7), retinopathy (55.8), and neuropathy (14.7); however, the <6 months group had the highest rate of incident albuminuria (94.9). After adjusting for differences in patient characteristics, earlier intensification was associated with significantly lower risk of incident retinopathy but significantly higher risk of incident albuminuria (Table 1).
Conclusion: This study suggests mixed results on the association of timing of intensification and incidence of microvascular complications among T2DM patients. Future research should evaluate the potential role of durability of response to intensification, in terms of glycemic control, as an explanatory variable.

Table 1: Likelihood of developing microvascular complications among patients intensifying within 36 months after index, stratified by time to intensification

	Adjusted HR	(95% CI)
Albuminuria		
6 to <12 months	0.90	(0.82, 0.99)
12 to <24 months	0.91	(0.83, 0.99)
24 to <36 months	0.91	(0.82, 1.01)
eGFR <60 mL/min/1.73m^2		
6 to <12 months	1.02	(0.88, 1.19)
12 to <24 months	0.94	(0.82, 1.09)
24 to <36 months	1.02	(0.86, 1.2)
Doubling of serum creatinine		
6 to <12 months	1.06	(0.76, 1.48)
12 to <24 months	1.09	(0.82, 1.46)
24 to <36 months	1.25	(0.91, 1.73)
Retinopathy		
6 to <12 months	0.96	(0.85, 1.07)
12 to <24 months	1.04	(0.94, 1.15)
24 to <36 months	1.22	(1.09, 1.37)
Neuropathy		
6 to <12 months	1.12	(0.92, 1.38)
12 to <24 months	0.86	(0.71, 1.05)
24 to <36 months	1.06	(0.85, 1.32)

Notes:
eGFR = estimated glomerular filtration rate; Intensifying <6 months was considered as the reference cohort for all comparisons.

Supported by: BMS, AZ
Disclosure: **U. Desai:** Employment/Consultancy; I am an employee of Analysis Group, Inc., a company that received research funding from Bristol-Myers Squibb and AstraZeneca for this study.

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The healthcare costs and utilisation associated with diabetic nephropathy in adult patients with type 2 diabetes in the US

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Background and aims: Diabetic nephropathy (DN) is a progressive condition affecting up to 40% of patients with type 2 diabetes mellitus

(T2DM). Severity of DN is commonly assessed using urine albumin (UA) levels, typically classified as microalbuminuria (MiA) or macroalbuminuria (MaA). Although DN is the leading cause of kidney failure worldwide, few studies have assessed the economic burden among patients with varying DN severity. This study evaluated the real-world healthcare resource utilization (HRU) and costs associated with MiA or MaA among adult patients with T2DM in the US.

Materials and methods: Data were extracted from the Truven MarketScan claims database between 2003 and 2014. Patients aged ≥18 years who had ≥2 T2DM diagnoses and ≥2 UA tests were categorized into 3 study cohorts (normal, MiA, MaA) based on a randomly selected UA test result (index date). Patient characteristics were assessed during the year prior to the index date (baseline). All-cause HRU and associated costs including inpatient (IP), emergency room (ER), outpatient (OP), and other medical services were measured from the index date to the earliest of 24 months post-index or the end of continuous enrollment (study period) and summarized at the per-patient-per-year level. Incidence rate ratios (IRRs) of HRU and cost differences were estimated using multivariable generalized linear models adjusting for age, gender, region, and insurance type at the index date. Costs were inflated to 2014 USD using the medical care component of the Consumer Price Index.

Results: A total of 23,235 patients were classified into the normal (n=18,409), MiA (n=3,863), and MaA (n=963) cohorts. At baseline, MiA and MaA patients were slightly older (normal: 54; MiA: 55; MaA: 56 years) and more likely to be male (normal: 53%; MiA: 60%; MaA: 63%). During the study period, increasing disease severity was associated with significantly increased use of healthcare resources, across IP (adjusted IRR [aIRR]: MiA vs normal: 1.51 [95% confidence interval: 1.37-1.65]; MaA vs MiA: 1.78 [1.50-2.09]; both p<0.01), ER (aIRR: MiA vs normal: 1.29 [1.16-1.42]; MaA vs MiA: 1.25 [1.04-1.49]; both p<0.05), OP (aIRR: MiA vs normal: 1.05 [1.02-1.08]; MaA vs MiA: 1.29 [1.20-1.39]; both p<0.01), and other medical services visits (aIRR: MiA vs normal: 1.29 [1.22-1.37]; MaA vs MiA: 1.61 [1.39-1.88]; both p<0.01). Patients with MiA and MaA also incurred significantly higher total healthcare costs compared to the normal cohort, with adjusted annual cost differences of $3,506 and $12,565, respectively (Table 1; both p<0.01). IP costs accounted for approximately 50% of the total cost differences.

Conclusion: Increasing severity of DN was associated with significantly higher all-cause HRU and healthcare costs in adult patients with T2DM in the US.

Table 1. Comparison of all-cause healthcare costs among study cohorts

	Annual healthcare costs (USD, 2014) mean ± standard deviation			Adjusted cost difference		
	Normal [A]	MiA [B]	MaA [C]	[B] vs [A]	[C] vs [A]	[C] vs [B]
Total healthcare costs	12,098 ± 19,667	15,565 ± 29,257	24,899 ± 46,856	3,506 *	12,565 ‡	9,059 ‡
Total pharmaceutical costs	3,950 ± 5,509	4,486 ± 5,328	5,558 ± 8,993	518 *	1,424 ‡	906 ‡
Total medical costs	8,148 ± 18,002	11,079 ± 27,818	19,340 ± 44,917	2,972 *	11,168 ‡	8,196 ‡
IP	2,675 ± 12,668	4,353 ± 19,896	9,313 ± 34,766	1,591 *	6,253 ‡	4,662 ‡
ER	700 ± 3,437	1,075 ± 7,398	1,204 ± 3,507	355 *	524 ‡	169 ‡
OP	4,025 ± 7,420	4,619 ± 11,745	6,841 ± 16,139	691 *	2,866 ‡	2,175 ‡
Other medical services	748 ± 2,589	1,033 ± 3,556	1,982 ± 6,314	282 *	1,252 ‡	970 ‡

* $p<0.05$ for [B] vs [A]; ‡ $p<0.05$ for [C] vs [A]; ‡ $p<0.05$ for [C] vs [B]

Supported by: Takeda Pharmaceuticals International, Inc.

Disclosure: **H. Yang:** Employment/Consultancy; Analysis Group, Inc.

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Influence of coexisting diabetes on arterial pressure control in patients in late period after kidney transplantation

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Background and aims: Hypertension (HT) is the strongest, independent risk factor of cardiovascular complications in patients with Chronic Kidney Disease (CKD). Patients with diabetes (DM) are in greater risk of cardiovascular diseases - including HT. Adequate blood pressure control is very important for the proper function of the graft and occurrence of complications in patients after kidney transplantation (KTx). Aim: Comparison of the prevalence of hypertension and its control in patients after KTx in late period after surgery in dependence of coexisting diabetes.

Materials and methods: The study included 82 patients after renal transplant - diabetes was diagnosed in 47 cases. At baseline, groups did not differ in averages serum creatinine level and time after transplantation. The follow-up period was 4 years, during this time patients were regularly controlled on routine ambulatory check-ups. Combined end-point consisted of returning to hemodialysis therapy and/or death of patient. The value of blood pressure was taken as an average of the last 3 ambulatory measurements outpatient setting.

Results: Average time after KTx in the study group was 87.2 months. Combined end-point during the follow-up period was observed in 16 patients. HT was present in the vast majority of patients - 97.9% in group with DM (group 1) and in 97.1% in group without DM (group 2) - (X2=1.76; p=0.184). In 35 patients (42.7%), mean arterial blood pressure from the last 3 visits (at the time of enrollment to the study) exceeds 140/90 mmHg - 48.9% in the first group, 34.3% in the group 2. Patients with DM were treated with larger number of antihypertensive drugs (3.06 vs. 2.69) and had higher systolic blood pressure (SBP) - (145.8 vs. 140.1), but the differences were not significant (p=0.241 and p=0.253). Diastolic blood pressure (DBP) values were similar in both groups (87.8 vs. 88.0 mmHg; p = 0.922). Patients with observed cumulative end-point, at baseline were characterized by higher SBP (157.2 vs. 140.0; p=0.004), DBP (92.5 vs. 86.7; p=0.049) and number of antihypertensive drugs (3.94 vs. 2.65; p<0.001). Among patients who completed a full follow-up period, even after 4 years there was no significant difference between group 1 and 2 in the values of SBP, DBP, and the number of antihypertensive drugs. However, patients with DM had still higher SBP (135.39 vs. 132.97; p = 0.541)and used more antihypertensive drugs (2.92 vs. 2.70; p = 0.570). After four years of enrollment to the study blood pressure values exceeding 140/90 mmHg were observed in 36.1% of patients in the group 1 and in 20% of patients from group 2.

Conclusion: Patients after KTx with coexisting diabetes are characterized by worse control of blond pressure and are treated with larger number of antihypertensive drugs. The presence of higher blood pressure values is connected with higher risk of complications in patients in late period after KTx.

Disclosure: **P. Miarka:** None.

PS 105 Diabetic nephropathy: progression and outcomes

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Disease progression of diabetic nephropathy in adults with type 2 diabetes in the US

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Background and aims: The severity of diabetic nephropathy (DN), a significant complication of type 2 diabetes mellitus (T2DM), is commonly classified by urine albumin (UA) levels as microalbuminuria (MiA) or macroalbuminuria (MaA). This study quantified the risk of disease progression associated with MiA and MaA among US adults with T2DM.
Materials and methods: Adults (age 18+) with T2DM and ≥2 UA tests were identified in Truven MarketScan claims databases (2003-2014). Patients were categorized into 3 study groups (normal, MiA, MaA) based on a randomly selected UA test result (index) and were required to be without end stage renal disease during the year before index (baseline). Patient characteristics were assessed during baseline. Times to disease progression, dialysis/hemodialysis, and renal transplantation were assessed from index to the earliest inpatient mortality or the end of continuous enrollment or data availability. Disease progression was defined as an event indicating a more severe stage of DN compared to index severity. Outcomes between groups were compared using Cox proportional hazard models adjusting for age, gender, region, and insurance type at index.
Results: 18,409 normal, 3,863 MiA, and 963 MaA patients were included in this study. The median follow-up time was approximately 2 years across cohorts. MiA and MaA patients were older and had a higher proportion of males. The MaA cohort had a significantly higher risk of progression compared to the normal and MiA cohorts (adjusted hazard ratio [aHR]: MaA vs normal: 1.44 [95% confidence interval: 1.21-1.72]; MaA vs MiA: 1.31 [1.08-1.60]; both $p<0.01$). Risk of requiring dialysis/hemodialysis increased significantly with disease severity (aHR: MiA vs normal: 4.23 [2.45-7.30]; MaA vs MiA: 9.49 [5.87-15.36]; both $p<0.01$). Five-year renal transplantation rates were 0%, <1%, and 2% in the normal, MiA, and MaA cohorts, respectively.
Conclusion: Increasing severity of DN was associated with a significantly higher risk of disease progression or requiring dialysis/hemodialysis in adult patients with T2DM.
Supported by: Takeda Pharmaceuticals International, Inc
Disclosure: **Z. Zhou:** Employment/Consultancy; Analysis Group.

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The natural history of diabetes nephropathy in Korea: a Korean National Diabetes Programme prospective cohort study

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Background and aims: Stage-to-stage progression in Korean patients with type 2 diabetes has not been previously evaluated. Therefore, we investigated the natural history of DM nephropathy in Koreans with type 2 diabetes.
Materials and methods: The Korean National Diabetes Program (KNDP) is a prospective, multicenter observational cohort study on type 2 diabetic patients in Korea. The study commenced in May 2006 and follow-up continued until March 2014. Of all subjects who underwent assessment of diabetic complications in five centers, we analyzed data on 1,472 for whom urine albumin levels and glomerular filtration rates were available. Diabetic nephropathy was classified into five stages: normal, microalbuminuria, macroalbuminuria, chronic kidney disease (CKD), and end-stage renal disease (ESRD). We calculated the annual stage-to-stage transition rates.
Results: The median duration of diabetes was 5.6 years and the median follow-up time was 67 months. At baseline, the mean fasting glucose and HbA1c levels were 144 ± 53 mg/dL and 7.7 ± 1.7%, respectively. Of all patients, 342 (27%) had diabetic retinopathy and 6% and 5.5% had cardiovascular and cerebrovascular disease, respectively. The annual rates of direct transition from an absence of nephropathy to microalbuminuria, macroalbuminuria, and CKD were 4.83% (4.26-5.44%), 0.53% (0.36-0.74%), and 1.07% (0.83-1.36%), respectively. The respective annual rates of direct progression from microalbuminuria to macroalbuminuria or CKD were 2.67% (1.85-3.73%) and 2.24% (1.51-3.20%). Patients with macroalbuminuria developed CKD at an annual rate of 5.51% (3.27-8.71%), and 2.22% (1.18-3.79%) of CKD patients progressed annually to ESRD.
Conclusion: The annual nephropathic stage-to-stage transition rates in Korean patients with type 2 diabetes were higher than those noted in other ethnic groups. Early diagnosis and appropriate management of Korean patients with type 2 diabetes is thus particularly important to prevent development of microvascular diabetic complications.
Disclosure: **J. Jeon:** None.

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Diabetes and risk of incident chronic kidney disease in women compared with men: a systematic review and meta-analysis

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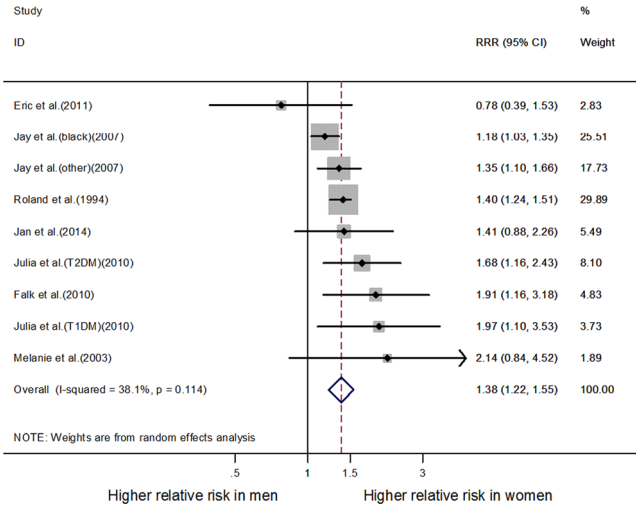
Background and aims: Diabetes Mellitus (DM) is a strong risk factor for chronic kidney disease (CKD) and it remains unclear whether sex differences exist in incidence of CKD among diabetic patients. This study was conducted to evaluate the relative effect of diabetes on the risk of CKD in women compared with men.
Materials and methods: We systematically searched Embase, PubMed and the Cochrane Library up to October 2015 for sex-specific relationship of DM and risk of CKD. We generated pooled estimates across studies using random-effects meta-analysis after log transformation with inverse variance weighting.
Results: Ten studies with data for over five million participants were included. The pooled adjusted risk ratio of CKD associated with DM was 3.34 (95% CI 2.27, 4.93) in women and 2.84 (95% CI 1.73, 4.68) in men. The data showed no differences in diabetes-related CKD risk between the sexes (pooled adjusted relative risk ratio was 1.14 [95% CI 0.97, 1.34]) excepted for end-stage renal disease (ESRD)— the pooled adjusted relative risk ratio was 1.38 (95% CI 1.22, 1.55, P =0.114, I^2=38.1%).
Conclusion: There was no evidence of a sex difference in the association between DM and CKD. However, the excess risk for ESRD was higher in women with diabetes than in men, from which we assume that women could accelerate the disease progression. Further studies are needed to support this idea and elucidate the underlying mechanisms.

Figure 1 Pooled adjusted women-to-men relative risk ratio (RRR) for incident end stage renal disease, comparing individuals with diabetes versus those without diabetes



Disclosure: **Y. Shen:** None.

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The impact of hyperuricaemia on mortality and renal outcome across different chronic kidney disease stages

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Background and aims: Studies on the relationships between hyperuricemia and mortality or renal replacement therapies (RRT) have yielded inconsistent results. Our study aimed to examine the association between hyperuricemia and subsequent mortality and renal outcomes in patients with different stages of chronic kidney disease (CKD).
Materials and methods: In this retrospective observational cohort study, 4,381 patients with CKD stage 3, 4, and 5, with or without DM, were enrolled from 2007/01/01 to 2014/12/31 in a medical center in central Taiwan. They were categorized into three groups (CKD 3, CKD 4, CKD 5) according to baseline eGFR. In each group, patients were further divided into two subgroups by baseline mean uric acid (UA) levels > 7mg/dL or < 7mg/dL. Mortality and renal outcomes indicated by RRT were analyzed by the Cox proportional hazards models.
Results: During a median follow-up period of 2.5 years, there were 356 deaths (8.1%) and 932 patients receiving RRT (21.3%). The risks of mortality and receiving RRT in CKD stage 3 normouricemic subgroup (CKD 3, UA <7mg/dL) were set as references. Multivariate adjusted hazard ratio (HR) of mortality for each subgroup was 1.18 (CI 0.79-1.76, CKD 3, UA ≥7 mg/dL), 1.53 (CI 0.92-2.53, CKD 4, UA <7mg/dL), 2.42 (CI 1.63-3.59, CKD 4, UA≥7 mg/dL), 1.04 (CI 0.43-2.49, CKD 5, UA <7mg/dL), and 2.46 (CI 1.58-3.83, CKD 5, UA≥7 mg/dL). And the corresponding HR of receiving RRT was 1.27 (CI 0.78-2.08), 4.11 (CI 2.50- 6.76), 5.68 (CI 3.67-8.80), 21.54 (CI 13.56- 34.22), and 32.38 (CI 21.18-49.50), respectively.
Conclusion: Our study showed that mortality did not increase significantly across CKD stage 3 to 5 in subjects with UA less than 7 mg/dL, but did increase significantly in subjects with UA greater or equal to 7mg/dL in stage 4 and 5. In addition, hyperuricemic subgroups in CKD stage 4 and 5 were associated with significantly higher RRT risks.
Disclosure: **T. Yu:** None.

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Plasma copeptin in children and adolescents with type 1 diabetes in comparison to healthy controls

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Background and aims: In a cohort of children and adolescents with type 1 diabetes mellitus the trial tested the hypothesis that copeptin levels are associated with kidney function, biometrical data and quality of diabetes control.
Materials and methods: A total of 141 subjects were recruited to participate in the trial: 80 patients with type 1 diabetes [13.0±3.4 years, HbA1c 7.85±1.42%] and 61 healthy controls [12.4±2.8 years]. Clinical and socioeconomic data were assessed. A sandwich immunoassay (B.R.A.H.M.S. GmbH/Thermo Fisher Scientific, Hennigsdorf/Berlin, Germany) was used for measuring plasma copeptin levels.
Results: The mean concentration of copeptin in the diabetic patients was 4.75±3.46 pmol/l. There was a strong inverse correlation between copeptin and GFR (r=-0.86, p=0.021), as well as with total cholesterol (r=-0.23, p=0.041), LDL-cholesterol (r=-0.24, p=0.036), but not with serum creatinine, albuminuria, HbA1c, blood glucose, MAGE, CRP, systolic or diastolic blood pressure or age, diabetes duration, weight, height and BMI. Comparing patients with a diabetes duration of ≥7 years (n=45) with those with a diabetes duration <7 years (n=35), patients with a longer duration of diabetes had higher copeptin levels (5.24±2.26 vs 4.13±2.86, p=0.045). Performing multivariate analyses only GFR could be identified as a parameter associated with copeptin (R-square=0.05, ß=-0.23, p=0.032). In the healthy controls mean copeptin concentration was 5.56±3.15 pmol/l. The copeptin concentration and GFR were inversely correlated as well (r=-0.61, p=0.034). However, other correlation and multivariate analyses revealed no further significant results. Comparing patients with type 1 diabetes mellitus with the healthy controls, the diabetes patients revealed no significant difference with respect to copeptin (p=0.24), serum creatinine (49.8±11.9 vs 50.4±11.0 µmol/l, p=0.53) or GFR (102.4 ± 23.3 vs 104.5 ± 19.1ml/min, p=0.47). On the other hand, patients with type 1 diabetes had lower concentrations of CRP (1.66±3.91 vs 3.21±3.04 µg/ml, p=0,013), triglycerides (0.88±0.53 vs 1.13±0.60 mmol/l, p=0.010), and a lower ratio of LDL-/HLD-cholesterol (1.73±0.69 vs 2.32±0.80, p<0.001), as well as lower body weight (51.3±18.0 vs 60.3±15.7 kg, p=0.002) and BMI (19.7±3.8 vs 23.2 ±2.9 kg/m2, p<0.001). In contrast to the controls, the diabetes patients had higher blood glucose levels at the time of examination (8.2±3.8 vs 4.7±0.5 mmol/l, p<0.001), higher HDL-cholesterol levels (1.59±0.34 vs 1.26±0.24 mmol/l, p<0.001), as well as higher education and higher educational levels of the mothers.
Conclusion: The present trial revealed a clear association between GFR and copeptin in children and adolescents with type 1 diabetes mellitus. Hence similarly to adults as well as healthy subjects, copeptin can be considered a marker of renal function. Results from this study also suggest that levels of copeptin may not only be related to stress, behavioral and lifestyle factors but potentially to inflammatory activity and the lipid profile as well.
Disclosure: **R. Schiel:** None.

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Secondary hyperparathyroidism and osteopenic syndrome in patients with diabetes type 1 and type 2 and chronic kidney disease

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Background and aims: Study features of calcium-phosphorus metabolism and osteoporosis in secondary hyperparathyroidism in patients suffering from type 1 and type 2 DM with chronic kidney disease at stages 3 and 4.

Materials and methods: 402 patients with DM type 1 and type 2 were observed and CKD stages 3 and 4, the study was carried out at RSSPMCE, diabetic nephropathy department. Serum Ca, P, alkaline phosphatase levels and radio immune analysis (RIA) was used to determine level of a parathormone (PTG) in plasma. Bone mineral density was examined with two photonic R-densitometry. According to clinical guidelines on diagnosing osteoporosis and osteopenia, the conclusions were made based on the values of T-criterion - number of standard deviations (SD) from age norm: rate -2,5 SD or less was regarded as osteoporosis, T-score > -2.5 SD but ≤ - 1,0 SD was defined as osteopenia and T-score <- 1,0 SD was accepted as normal.
Results: Among the 402 patients with diabetes type 1 and type 2 70 patients had GFR below 65 ml/min, which comprised 17.4% of total. Of these, SHPT (secondary hyperparathyroidism) was diagnosed in 30 patients, which comprised 42.8% of cases. The frequency of SHPT among patients with type 1 diabetes comprised 4.3% (n = 3) and in patients with type 2 diabetes - 38.6% (n = 27). Mean concentration of parathormone (PTH) comprised 82.7 pg/ml, total Ca - 1.9 mmol/L and P - 1.8 mmol/L. Alkaline phosphatase values ranged between 135 - 846 U/L and was 328 U/L on average. Study of gender prevalence of SHPT showed that this pathology was more common in women (53.3%) compared to men (46.6%), but there was no significant differences among them. In addition, we studied the prevalence of disorders of bone mineral density (DBMD) in the form of osteopenia and osteoporosis in patients with SHPT. DBMD was present in 100% of cases. Examined data showed that osteopenia occurrence was 66.6% (n = 20) and osteoporosis - 33.3% (n = 10). There was no significant difference in the frequency of osteopenia by gender (55.5% ± 5.7 and 44.5% ± 5.2, in men and women, accordingly). At the same time, the frequency of osteoporosis was significantly higher among women than among men (70% ± 5.3 and 30% ± 3.4, accordingly).
Conclusion: 1. Secondary hyperparathyroidism was diagnosed almost in every fifth patient with diabetes, whose GFR was less than 65 ml/min. 2. Secondary hyperparathyroidism leads to disturbance of bone mineral density in either form - osteopenia or osteoporosis in 100% of patients with. 3. The average values of calcium-phosphorus metabolism, such as alkaline phosphatase, calcium and phosphorus were beyond the normal ranges.
Clinical Trial Registration Number: 15.12.2
Disclosure: **N. Alikhanova:** None.

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Long-term incidence of microvascular events in relation to incident type 2 diabetes in obese patients with prediabetes treated by bariatric surgery or usual care

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Background and aims: Many obese patients with prediabetes develop diabetes but we have previously shown that the risk of diabetes is markedly reduced by bariatric surgery. To extend these findings, our aim was to examine if bariatric surgery also prevents long-term (up to 26 years) microvascular diabetes complications in patients with prediabetes in the Swedish Obese Subjects (SOS) study, and if the prevention of complications is more pronounced in patients who do not develop diabetes.
Materials and methods: Microvascular events (retinopathy, nephropathy and neuropathy) in SOS study participants with baseline prediabetes (impaired fasting glucose), treated by bariatric surgery (n=301) or usual care (n=290) were traced in nationwide registers. Median follow up was 19 years.
Results: Bariatric surgery was associated with reduced incidence of microvascular events [incidence rate 3.9 and 18.7 per 1000 person years in the surgery and control groups, respectively; HR=0.19 (0.12-0.31), p<0.001]. At or before the 15-year examination, the fraction of patients with prediabetes who developed diabetes was 15.6% in the surgery group and 54.5% in the control group. Those with incident diabetes had higher incidence of microvascular events compared to those who had not developed diabetes (9.3 vs. 2.9 per 1000 person years, respectively; p=0.009 in the surgery group and 22.6 vs. 14.0 per 1000 person years, respectively; p=0.028 in the control group). Bariatric surgery was associated with reduced incidence of microvascular events both in prediabetics that developed diabetes (HR=0.36; p=0.007) and in those who did not (HR=0.20; p<0.001).
Conclusion: We conclude that bariatric surgery reduces the long-term incidence of microvascular disease in obese patients with prediabetes.
Clinical Trial Registration Number: NCT01479452
Supported by: Swedish research council, National Institute of Health (NIH), ALF-grants, D
Disclosure: **P. Svensson:** None.

PS 106 Biomarkers of diabetic nephropathy

1027

Predictive biomarkers for chronic kidney disease in diabetes

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Background and aims: Microalbuminuria is not precise predictor for early stage of chronic kidney disease (CKD) in diabetes that emphasizes the need for more specific predictive markers. In order to define the utility of reliable early biomarkers we examined a panel of plazma (kidney injury molecule type 1(KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), osteopontin, cystatin C) and urinary (collagen type IV, nephrin, podocin, uromodulin, NGAL, KIM-1) peptides.
Materials and methods: We examined 160 patients with type 1 (T1, n=80) and type 2 (T2, n=80) DM and healthy controls (20 and 15, matched for age). DM patients divided into 3 subgroups based on albumin excretion rate (AER) in the morning urine spot with normo- (NA, n1), micro- (MAU, n2) and macroalbuminuria (MA, n3). Biomarkers were measured by enzyme-linked immunosorbent assay ELISA in the morning urine and fasting plasma; AER assessed by immunoturbodimetry assay; glomerular filtration rate (eGFR) estimated by standard CKD-EPI formula. Statistical analyses performed by STATISTICA 8.0. Differences tested by one-way analyses of variance ANOVA Kruskal-Wallis H test, correlations were analyzed using Spearman correlation coefficients. Statistical significance accepted at $p<0.05$.
Results: Clinical characteristic of DM patients presented in a table below. Significant differences of biomarkers between healthy controls and DM subgroups were found in podocin which increased prior to MAU in T1 [T1: 0.08/0,2/0,6/0,08, p<0,05; T2: 0.2/0,19/0,59/0,08, p<0.05]; uromodulin decrease starting at NA stage [T1: 3282/1868/2574/1298, p<0.05; T2: 2108/1642/1487/1477, p<0.05]; collagen increased progressively due to CKD progression in both T1: 3,4/3,8/6,08/8,6; p<0,05 and T2: 1,4/1,78/1,7/3,86, p<0.05. Significant increase of plazma markers observed prior to MAU in T1: cystatin C [632;1031;1022;2391; p<0,05] and osteopontin [59;107;90;153, p<0.05], in T2: KIM-1 [144/163/287/498, p<0.05] and NGAL [24/24/36/44, p<0.05] that increased progressively according to CKD. There were positive correlations of collagen with AER (r=0,48;p=0,05) and serum creatinine (r=0,51;p=0,05), podocin with serum creatinine (r= 0,36, p=0,05) and eGFR (r=-0,28, p=0,05); uromodulin with eGFR (r=0,39, p=0,05) in T1, for plazma markers: osteopontin with serum creatinine (r=0,35, p=0,05), cystatin C with serum creatinine and eGFR (r=0,47, p=0,05; r= -0,49, p=0,05, respectively). While in T2 podocin correlated with AER (r=0,25, p=0,05), uromodulin with serum creatinine (r= - 0,33, p=0,05).
Conclusion: Our findings of biomarkers changes prior to MAU, progressed due to CKD stage and correlated with eGFR and AER let to suppose urinary podocin and uromodulin, plazma (osteopontin, cystatin C, KIM-1, NGAL) as promising and simple markers for the preclinical diagnostics of diabetic kidney injury alone or in addition to classical singes of CKD.

	T1 DM (n=80)				T2 DM (n=80)			
	n_1=45 (0-19 mg/L)	n_2=20 (20-199 mg/L)	n_3=15 (>200 mg/L)	p value	n_1=48 (0-19 mg/L)	n_2=20 (20-199 mg/L)	n_3=12 (>200 mg/L)	p value
age (years)	33	35	31	0,6	64	60	63	0,7
DM duration (years)	14	22	22	0.7	10	11	20	0,7
Systolic BP (mmHg)	120	120	130	0.8	115	130	135	0,5
Diastolic BP (mmHg)	80	80	70	0.5	70	80	80	0,7
HbA1c (%)	8.6	8.5	8.1	0.9	8,2	9,8	7,9	0,03
Creatinine (µmol/l)	75	72	194	0.0008	69	76	82	0,03
eGFR(ml/min/1.73 m2)	103	98	36	0.002	89	60	63	0,03
Total cholesterol (mmol/l)	4.8	4.9	5.4	0.4	5,3	5,1	5,4	0,8
Uric acid (µmol/l)	270	274	337	0.04	333	422	446	0,2

Clinical characteristic of T1 and T2 DM groups

Disclosure: **Z. Zuraeva:** None.

1028

Association between the serum adipocyte fatty acid binding protein levels and microalbuminuria in a Chinese hyperglycaemic population

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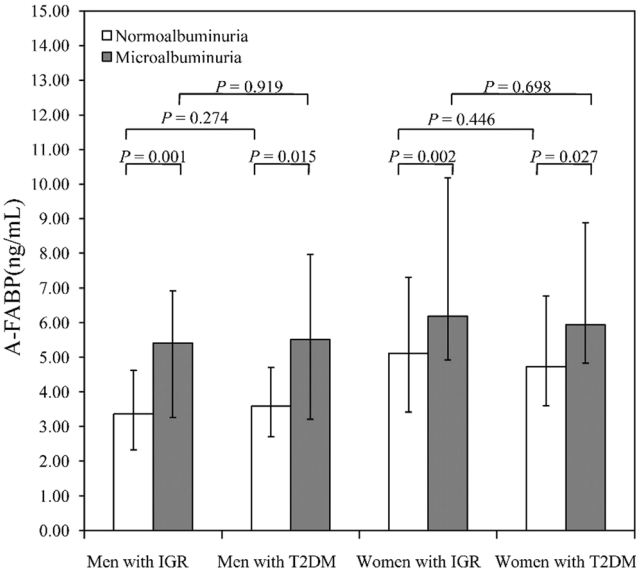
Background and aims: Microalbuminuria predisposes patients with type 2 diabetes mellitus (T2DM) to developing micro- and macrovascular complications. Adipocyte fatty acid binding protein (A-FABP) was suggested as a serum biomarker for albuminuria in diabetes patients. The present study aimed to investigate the relationship between serum A-FABP levels and microalbuminuria in a hyperglycemic population.
Materials and methods: Serum A-FABP levels were measured by sandwich enzyme-linked immunosorbent assay. Microalbuminuria was defined as a urinary albumin to creatinine ratio (UACR) between 30-300 mg/g.
Results: A total of 939 subjects, including 436 men and 503 women, were enrolled in the study. Subjects with microalbuminuria had higher serum levels of A-FABP than those without microalbuminuria, and this results was observed for men with impaired glucose regulation (IGR), men with newly-diagnosed T2DM, women with IGR, or women with newly-diagnosed T2DM (all $P < 0.05$, figure 1). Spearman correlation analyses revealed that serum A-FABP levels were positively associated with the UACR in both men and women ($P = 0.005$ and $P < 0.001$, respectively). Multiple stepwise regression analysis identified serum A-FABP levels as an independent and positive factor associated with UACR in both men (standardized $\beta = 0.256$, $P < 0.001$) and women (standardized $\beta = 0.155$, $P = 0.001$). The independent and positive relationship between serum A-FABP levels and the UACR remained significant in each subgroup (all $P < 0.01$).
Conclusion: In hyperglycemic population, increased serum A-FABP levels contributed to the presence of microalbuminuria. The serum A-FABP levels were independently and positively associated with the UACR.'



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Disclosure: **X. Hu:** None.

1029

Dramatically elevated pancreatic stone protein/regenerating protein (PSP/reg) in diabetic nephropathy

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Background and aims: This study aims to investigate the predictive value of serum PSP/reg for diabetic nephropathy (DN).
Materials and methods: A total of 138 participants were enrolled in this study. All the participants were screened from healthy people, inpatients and outpatients at our university hospital from February 2011 to February 2012. The subjects were divided into four groups, including healthy control (Group A: n=50; 26 males, 24 females; age range 54 ± 9 years), newly diagnosed T2DM (Group B: n=36; 21 males, 15 females; age range 56 ± 8 years), long-term T2DM without complications (Group C: n = 25, 15 males, 10 females; age range 55 ± 8 years), and long-termT2DM with DN (Group B: n=27; 13 males, 14 females; age range 55 ± 11 years). PSP/reg serum values were measured by a newly developed enzyme-linked immunosorbent assay (ELISA). Data in multiple groups were compared with one-way analysis of variance (ANOVA). Sperman's and Pearson's correlation analysis were used to detect the correlation between PSP/reg and clinical index, and receiver operating characteristic (ROC) curve was ploted to assess diagnostic efficiency of PSP/reg.
Results: There was no significant difference in age, sex and body mass index among different groups (P > 0.05). However, significant variations were observed in fasting plasma glucose (FPG), 2-h plasma glucose (2hPG), hemoglobin A1c (HbA1c), PSP/reg, glomerular filtration rate (GFR) and creatinine clearance (Ccr) (P < 0.05). Compared with PSP/reg in group A (14.21 ± 3.33ng/mL), B (18.13 ± 6.80ng/mL) and C (23.58 ± 7.19ng/mL), PSP/reg level in group D (56.92 ± 21.39 ng/mL) was significantly increased (P < 0.01). PSP/reg levels were positively correlated with systolic pressure (r = 0.136, P < 0.01), FPG (r = 0.215, P < 0.01), 2hPG (r = 0.198, P < 0.01) and HbA1c (r = 0.382, P < 0.01), while negatively correlated with GFR (r = -0.533, P < 0.01) and Ccr (r = -0.421, P < 0.01). In the ROC analysis, the area under the curve of PSP/reg for presence of DN was 0.902 [95% CI (0.737, 0.737)]. The valuable threshold for predicting incidence of DN was 32 ng/ml. The sensitivity was 69% and specificity 93% (positive likelihood ratio: 2.09, negative likelihood ratio: 0.73).
Conclusion: PSP/reg was up-regulated in T2DM patients, and dramatically elevated in DN patients in particular. Therefore, PSP/reg might be useful as an assistive biomarker for early diagnosis of DN.
Supported by: NSFC
Disclosure: **M.M. Zhi:** None.

1030

The lysosomal protease cathepsin L as a potential link between blood glucose levels and albuminuria in type 2 diabetic patients

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Background and aims: A link between altered lysosomal degradation in the kidney and albuminuria in diabetes has been established previously. The lysosomal protease Cathepsin L (CTSL) is the strongest cysteine protease and can be induced by AGE-protein adducts, thus reducing their toxicity. AGEs are elevated in diabetes and elevated levels of CTSL could be a way to deal with an increased load of AGE modified proteins. In line with this observation an increased modification of kidney collagen with AGEs paralleled by a decreased digestibility of collagen is associated with and correlates strongly with an increased expression of CTSL in diabetic rat kidneys. Aim of the current study was to determine whether CTSL shows changes indicative of such adaptive mechanisms in type 2 diabetic patients. To achieve this we determined whether CTSL levels in urine correlate with clinical parameters of diabetes and diabetic nephropathy like fasting glucose levels and albumin creatinine ratio.
Materials and methods: Blood and Urine from type 2 diabetic patients was analysed for the following clinical chemistry parameters: fasting glucose level, HbA1c, high sensitive C-reactive protein (hsCRP), albumin creatinine ratio (ACR) and glomerular filtration rate (GFR). Urine from patients was analysed for CTSL content by isotopic dilution LC MS/MS after trypsin digestion of precipitated urinary protein. The proteotypic CTSL peptide (CTSL238-260) was detected with good sensitivity and linearity at a range from 0-250 fmol (r2=0.9987; limit of quantitation of 1 fmol).
Results: Clinical chemistry parameters and CTSL urine levels were analysed for correlations. Both ACR and hsCRP values were log transformed prior to analysis. HbA1c and fasting glucose level displayed a strong positive correlation (r2=0.352, p<0.0001). Weaker positive correlations were found for hsCRP and ACR (r2=0.035, p=0.030) while an inverse correlation was present between ACR and GFR (r2=0.043, p=0.016). A very weak positive correlation between fasting glucose levels and GFR was present (r2=0.016, p=0.030). CTSL levels in urine displayed a very strong positive correlation with fasting glucose levels (r2=0.500, p=0.016) and a strong positive correlation with ACR (r2=0.395, p=0.052). Interestingly correlations between CTSL and fasting glucose levels were stronger than those between HbA1c and fasting glucose levels (r2=0.500 vs. r2=0.352). No other correlations were detected.
Conclusion: Here, we show that in urine from type 2 diabetic patients high CTSL levels correlate with a higher fasting glucose level and an increased ACR. This could be indicative of an adaptive response in diabetic patients to increase proteolytic capacity to deal with increased proteolytic demand due to a higher number of modified proteins. In order to determine whether the higher urine levels of CTSL are reflective of higher CTSL levels in diabetic kidneys the next steps are to determine CTSL levels in kidney biopsies of diabetic patients. Correlations of clinical parameters in particular between HbA1c and fasting glucose levels are similar to previous reports.
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Disclosure: **S. Brings:** None.

1031

Change in urinary angiotensinogen excretion and progression of diabetic kidney disease

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Background and aims: Urinary angiotensinogen (AGT) was identified as a possible specific biomarker of intrarenal RAS status in diabetes mellitus. This study was designed to determine that the change in urinary AGT excretion was associated with the change in renal function in patients with early diabetic kidney disease (DKD) in type 2 diabetes.
Materials and methods: Urinary AGT levels were measured in 118 type 2 diabetic patients with estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73m2 without the use of RAS blockers at baseline. A total 91 patients were followed up for 35 months (range, 10-52 months). Urinary AGT was normalized to urinary creatinine. The change in urinary AGT (AGT1-AGT0) was calculated by urinary AGT at one year later (AGT1) minus urinary AGT at baseline (AGT0).
Results: The change in urinary AGT levels (AGT1-AGT0) significantly correlated with the annual rates of eGFR decline (r = 0.335, p = 0.001). AGT1 levels of thirty seven patients decreased compared with their AGT0 levels (AGTDec group) and AGT1 levels increased compared with AGT0 levels in 54 patients (AGTInc group). During follow-up, average annual rate of eGFR decline was higher in the AGTInc group than AGTDec group (AGTInc group = 4.98 ± 11.08 ; AGTDec group = 0.22 ± 11.12 mL/min/1.73 m2 per year, p = 0.047). The difference remained significantly after adjusting clinical factors (p = 0.002 by anaylsis in covariance). In the AGTInc group, the change in urinary AGT levels also significantly correlated with annual rates of eGFR decline after adjusting for all clinical variables (β = 0.315, p = 0.019). In the AGTDec group, the correlation was disappeared(β = 0.212, p = 0.207).
Conclusion: The increased change in urinary AGT levels correlated with rapid decline in eGFR in patients with early DKD in type 2 diabetes.
Disclosure: **Y. Yi:** None.

1032

The relationship of fetuin-a with cardiac disease and markers of mineral and bone metabolism in type 1 diabetes patients

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Background and aims: To estimate the effect of fetuin-A on the cardiovascular system (CVS), and its relationship with markers of mineral and bone metabolism in patients with a long history of type 1 diabetes (T1D).
Materials and methods: We investigated 80 patients with a long history type 1 diabetes (more than 20 years) and CKD on the different stages. We have estimated anthropometric parameters, clinical and biochemical blood tests, determination of urinary albumin excretion, markers of mineral and bone metabolism (calcium, phosphorus, parathyroid hormone, vitamin D, fibroblast growth factor 23 (FGF 23), inhibitor of vascular calcification - fetuin-a, factors of cardiac diseases (atrial natriuretic protein (NT pro-BNP), matrix metalloproteinase-9 (MMP-9)). All patients underwent ambulatory blood pressure monitoring, echocardiography, made a multi spiral computed tomography of heart with Agatston index definition.
Results: We identified a negative correlation between the level of fetuin-A and the age of patients (r = -0,366; p 0,05), duration of diabetes; (r = -0,332; p 0,05), diastolic blood pressure (r = -0,331; r 0,05), interventricular septum (r = -0,28; r 0,05) and rear wall left ventricular (r = -0,25; r 0,05), left ventricular myocardial mass (LVM) (r = -0,370; r 0,05), LV myocardial mass index (L VMI) (r = - 0.381; r 0,05), Agatston index (r = -0,642; r 0,05) and MMP-9 (r = -0,433; r 0,05). Revealed the existence of a direct relationship fetuin-A with FGF-23 (r = 0,594; r 0,05).
Conclusion: We determined the inhibitory effect of fetuin-A on development of vascular calcification and LV remodeling in patients with type 1 diabetes. FGF-23 potentiating effect on fetuin-a production by osteocytes was confirmed.
Disclosure: **M. Arutyunova:** None.

1033

The carnosine metabolism of the human kidney under diabetes

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Background and aims: Carnosine is able to bind and inactivate reactive metabolites accumulating under diabetes mellitus. Carnosine levels are regulated by carnosinase (CN1). Female Type 2 diabetic patients with lower CN1 activity have a lower risk of developing diabetic nephropathy (DN); exogenic carnosine protects diabetic mice (db/db) from DN. Recently, we could demonstrate an intrinsic carnosine metabolism in the kidney of healthy humans. We now characterize the renal carnosine metabolism of diabetic patients with and without nephropathy.
Materials and methods: CN1 expression was measured via qRT-PCR, carnosine concentration via HPLC and CN1 activity with a fluorescence based assay in kidney homogenates of 27 diabetic patients without DN, 28 diabetic patients with DN and 18 controls.
Results: CN1 expression is not altered in diabetic kidneys compared to controls. Renal CN1 activity is significantly higher in diabetic patients than in healthy individuals, in women (0.75±0.33 vs. 0.44±0.16 nmol/mg/h; p< 0.05), as well as in men (0.58±0.24 vs 0.38±0.08 nmol/mg/h; p< 0.01). In diabetic patients with DN CN1 expression in the proximal tubules correlates positively with increasing proteinuria.
Conclusion: The renal carnosine metabolism is strongly altered in patients with diabetes mellitus. CN1 activity is increased and renal carnosine concentrations are reduced. These alterations of the carnosine metabolism, mitigating protective function against reactive metabolites, may contribute to the development of DN.
Supported by: SFB 1118
Disclosure: **T. Weigand:** None.

PS 107 Mechanistic aspects of diabetic nephropathy

1034

Nox5 aggravates renal damage in diabetic nephropathy

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Background and aims: Reactive oxygen species (ROS) play crucial role in the pathogenesis of diabetic nephropathy. The newly discovered NADPH oxidase isoform, Nox5, could play a role in DN. However, there is a paucity of information about Nox5 in animal models of DN since Nox5 is absent in rodents. We aimed to examine the role of Nox5 in a model of human inducible Nox5 transgenic (Tg) mice expressing Nox5 selectively in vascular smooth muscle cells (mesangial cells (SM22+ Nox5+)) in the setting of diabetes.
Materials and methods: Nox5 negative (SM22+Nox5-) and Nox5 positive (SM22+Nox5+) transgenic mice were rendered diabetic via streptozotocin (55mg/kg/body weight) injections. At week 10 urine samples were collected for the assessment of albuminuria. Animals were culled and kidneys were removed for the assessment of structural damage as well as gene and protein expression of markers of inflammation, fibrosis and oxidative stress.
Results: Nox5 negative diabetic mice had more than 10 fold increased albuminuria which was further increased by 30% in Nox5 positive transgenic diabetic mice (control & diabetic SM22+Nox5-: 177 ± 19 & 3036 ± 394 μg/24hrs and control & diabetic SM22+Nox5+ : 147 ± 14 & 4273 ± 537 μg/24 hrs). In addition, Nox5 positive diabetic mice appeared to have increased glomerulosclerosis and mesangial area (%) (control & diabetic SM22+Nox5-: 20.5±1.6 & 33.6±1.5%; control & diabetic SM22Nox5+ : 22.5±1.6 & 40.0±2.4%) when compared to Nox5 negative diabetic mice. Furthermore, there was a further increase in glomerular expression of markers of inflammation (MCP-1 and F4/80) and oxidative stress (nitrotyrosine) in Nox5 positive diabetic mice when compared to Nox5 negative diabetic mice.
Conclusion: Our findings of a further increase in albuminuria and glomerulosclerosis as well as increased expression of markers of fibrosis, inflammation and oxidative stress in Nox5 transgenic diabetic mice suggest a deleterious effect of Nox5 in the context of DN. These findings emphasize the role of Nox5 as a target for new renoprotective agents in diabetes.
Supported by: JDRF
Disclosure: **J.C. Jha:** None.

1035

Defense mechanisms against proteotoxic stress triggered by reactive dicarbonyls are up-regulated in diabetic nephropathy

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Background and aims: Methylglyoxal (MG) is a reactive metabolite, which is formed non-enzymatically during glycolysis and increases at a higher glycolytic flux. Not surprisingly, it is elevated in patients with diabetic late complications. Because MG production during glycolysis is inevitable, cells have developed complex defense strategies against MG. So far, most of those mechanisms are poorly understood. However, it becomes more and more evident that MG-modification of proteins, leading to protein-misfolding and -aggregation, is detrimental to cells. To date, the consequences of cellular protein damage as well as the fate of modified proteins are largely unknown. Our studies focus on the following questions: a) what cellular defense mechanisms against increasing MG concentrations exist; b) what proteins are targets of MG-modifications and how they are further processed.
Materials and methods: To study cellular defense mechanisms against MG, we used yeast and mouse cardiac endothelial cells (MCECs) as in vivo models. MG-induced protein aggregates were visualized by fluorescence microscopy as well as electron microscopy. After extraction from yeast cells, aggregates were analyzed via mass spectrometry. To identify different cellular defense mechanisms against MG, we carried out a genome-wide mRNA sequencing approach in yeast. Key components of those protective pathways were verified in MCECs by RT-PCR and specific siRNA knockdowns. Selected candidates were then analyzed in kidney tissue from patients with diabetic nephropathy using immunohistochemistry.
Results: In yeast cells and in MCECs, low concentrations of MG as well as high levels of glucose, can induce a cell protective response against toxic concentrations of MG. A similar response was also identified in mesangial and tubular epithelial cells. This defense mechanism is multifactorial and we were able to identify major transcription factors driving the response. Loss of key-effectors that protect against MG-induced protein damage, like the heat shock protein 70 (Hsp70) and vms1 (VCP/Cdc48-associated Mitochondrial Stress-responsive), resulted in increased cellular MG-sensitivity. In line with this, increasing intracellular MG-concentrations resulted in elevated modified protein levels and aggregates at the site of mitochondria. Furthermore, mass spectrometry analysis identified molecular chaperones and mitochondrial proteins in MG-induced aggregates isolated from yeast cells. The accumulation of Hsp70 in the kidneys of patients with diabetic nephropathy is a first possible hint that indicates the relevance of our findings for diabetic late complications.
Conclusion: Low concentrations of MG or high levels of glucose can initiate cellular defense mechanisms that prepare the cell for further increasing MG-stress. This protective response is a multifactorial system. The effectors of the MG-defense include heat shock proteins and proteins involved in mitochondria-associated protein degradation, which can deal with MG-induced protein misfolding. Furthermore, Hsp70 was found to accumulate in diabetic nephropathy, suggesting that those systems are also activated in diabetes, but fail at a certain point to prevent from the progression into diabetic late complications.
Supported by: Deutsche Forschungsgemeinschaft (SFB 1118), Dietmar-Hopp-Foundation, DZD
Disclosure: **J. Zemva:** None.

1036

HIF-1 attenuates oxidative stress and ameliorates renal function in diabetes

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Background and aims: Diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD) and is a major cause of mortality in patients with diabetes. Increased reactive oxygen species (ROS) production from mitochondria is proposed as pathogenic mediator for DN. Recently, hypoxia has been shown to play an important pathogenic role for the development of DN. Hypoxia-inducible factor-1 (HIF-1) is a master regulator of the adaptive cellular responses to hypoxia. Under normoxic conditions, HIF-1α is hydroxylated by prolyl hydroxylase domain proteins (PHD). The hydroxylated HIF-1α is then recognized by the

von Hippel-Lindeau (VHL) protein, which targets HIF-1α for proteasomal degradation. Under hypoxic conditions, HIF-1α is stabilized and binds to the hypoxia response element (HRE) in the promoter of HIF-1 target genes and activates gene transcription. Despite profound hypoxia in diabetic organs, HIF-1 signalling is inhibited in diabetes. However, the role of HIF-1 in the development of DN is still controversial. In this study, we intend to investigate the role of HIF-1 in regulating mitochondrial ROS production and renal function in diabetes.

Materials and methods: Renal mIMCD-3 (ATCC) were cultured in normoxia or hypoxia, and exposed to either normal or high glucose levels for 24h. Mitochondrial ROS production was detected by FACS after incubation with MitoSOX, and apoptosis was detected by FACS using Annexin V / 7-AAD kit. Endogenous HIF-1 function was detected as HRE-driven luciferase reporter activity. HIF-1 target gene expression was detected using Real-Time PCR. DMOG (Dimethyloxalyl Glycine) was used to stabilize HIF-1α both in vitro and in vivo. Db/db diabetes mice were obtained from Janvier Labs. Kidney-specific VHL knockout mice were generated by breeding VHL loxP/loxP mice with Cdh16-cre transgenic mice. Diabetes was induced with streptozotocin. Albuminuria and renal ROS production were detected using ELISA methods.

Results: Mitochondrial ROS production and apoptosis increased when renal mIMCD-3 cells were exposed to high glucose levels in hypoxia. High glucose levels suppressed endogenous HIF-1 function in hypoxia as shown by reduced HRE-driven luciferase reporter activity and HIF-1 target gene expression. Recovery of HIF-1 function, through either inhibition of PHD with DMOG or by silencing VHL, attenuated the mitochondrial ROS production and the rate of apoptosis under concomitant exposure to hypoxia and hyperglycemia. These results suggest a potential renoprotective role of HIF-1 in diabetes. In order to explore the role of HIF-1 for DN we have induced HIF-1 in diabetic animals using either pharmacological approach with DMOG or genetic approach by knocking down VHL specifically in kidney. DMOG treatment of db/db mice for one month reduced albuminuria and attenuated ROS production in the kidney. Similar beneficial effects on diabetes-induced albuminuria and renal ROS production was observed in kidney-specific VHL knockout mice where HIF-1 is activated in the kidney, confirming the renoprotective role of HIF-1 in diabetes nephropathy.

Conclusion: HIF-1 attenuates renal oxidative stress and ameliorates renal function in diabetes. Increasing renal HIF-1 function is a promising therapeutic strategy for diabetes nephropathy.

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Disclosure: **X. Zheng:** None.

1037

Peripherally restricted cannabinoid-1 receptor reverses diabetic nephropathy by regulating proximal tubule glut2

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Background and aims: Diabetic nephropathy (DN) is a worldwide, progressive kidney disease that affects both type 1 and 2 diabetic patients. Overactivity of the endocannabinoid/cannabinoid-1 receptor (CB1R) system contributes to the development of DN, whereas chronic treatment with globally acting CB1R antagonists improves renal function in murine models of DN. However, the clinical use of these inhibitors is halted due to centrally-mediated neuropsychiatric side effects. Recently, the development of peripherally restricted CB1R antagonists have revised the clinical potential of CB1R blockade for the treatment of both diabetes and obesity. Yet, their therapeutic use and downstream molecular mechanism in type 1 DN has not been elucidated.

Materials and methods: We tested the renal effect of a peripherally restricted CB1R antagonist, JD5037, in a streptozotocin (STZ)-induced DN mouse model. Physiological, biochemical, histopathological and molecular biology analyses were used to compare its efficacy with the globally acting parent compound, SLV319. Primary human renal proximal tubule cells (RPTCs), and MDCK II cells expressing a C-terminal GLUT2-mCherry fusion protein were used to assess the underlying molecular mechanism by which CB1R is involved in DN.

Results: The DN-induced structural and functional injuries in the kidney, along with the enhanced fibrosis and inflammation were completely attenuated by a selective blockade of CB1Rs in periphery, and were equally effective with SLV319 treatment. The improved kidney function by JD5037 was associated with a significant reduction in the expression of the facilitative glucose transporter GLUT2 in the brush border membrane (BBM) of the RPTCs. JD5037 blocked the high-glucose- or CB1R-induced translocation of GLUT2 to the BBM in RPTCs, via the inhibition of a Ca2+/PKC-β1 signaling pathway.

Conclusion: These results demonstrate, for the first time, that RPTC-CB1R regulates the hyperglycemia-induced renal dysfunction and the development of DN via modulating GLUT2 dynamics in these cells. Moreover, this study highlights the therapeutic relevance of blocking CB1Rs in periphery for the management of diabetic kidney disease, and may further support the clinical development and testing of peripherally restricted CB1R antagonists for this pathology.

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Regulation of proximal tubule GLUT2 by cannabinoiod-1 receptor in the diabetic kidney

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Background and aims: Diabetic nephropathy (DN) is a worldwide, progressive kidney disease that affects both type 1 and 2 diabetic patients. Overactivity of the endocannabinoid/cannabinoid-1 receptor (CB1R) system contributes to the development of DN, and chronic treatment with globally-acting CB1R antagonists improves renal function in murine models of DN. However, their clinical use is halted because of centrally-mediated adverse neuropsychiatric effects. Recently, the development of peripherally-restricted CB1R antagonists, such as JD5037, have revised the potential clinical use of CB1R antagonism for the treatment of the metabolic syndrome. However, its therapeutic efficacy and molecular mechanism in type 1 DN has not elucidated yet.

Materials and methods: In order to examine the influence of peripherally-restricted CB1R antagonism on DN, we have designed a streptozotocin (STZ) -induced DN mouse model in which mice were given consecutive STZ injections for 5 days. Diabetic mice were treated daily with JD5037 (3 mg/kg, po) and its brain penetrant parent compound, SLV319 (3 mg/kg, po) or vehicle for 16 weeks, and their controls were given vehicle. Physiological and biochemical parameters were obtained from urine and blood. Histopathological, immunohistochemical, protein and mRNA analysis were performed on the pancreas and kidney. Primary human renal proximal tubule cells (RPTCs) and MDCK II cells expressing C-terminal GLUT2-mCherry fusion protein were used to assess the mechanism in which CB1R antagonism is involved.

Results: Here, we report that in a streptozotocin-induced DN mouse model the increased structural and functional injuries in the kidney, along with enhanced fibrosis and inflammation were completely attenuated by a selective blockade of CB1Rs in periphery. Daily chronic treatment with JD5037 was equieffective as its brain penetrant parent compound, SLV319. The improved kidney function by JD5037 was associated with

a significant reduction in the expression of the glucose transporter GLUT2 in the brash border membrane (BBM) of the renal proximal tubule cells (RPTCs). In primary human RPTCs, JD5037 blocked the high glucose- or CB1R-induced translocation of GLUT2 to the BBM via the inhibition of Ca2+/PKC-β1 signaling pathway.

Conclusion: These results demonstrate for the first time that RPTC-CB1R regulates the hyperglycemia-induced renal dysfunction and the development of DN via affecting GLUT2 dynamics in these cells. Moreover, this study highlights the therapeutic relevance of blocking CB1Rs in periphery for the management of diabetic kidney disease, and may further support the clinical development and testing of peripherally-restricted CB1R antagonists in this pathology.

Supported by: EFSD/Sanofi to Joseph Tam

Disclosure: **L. Hinden:** Grants; European Foundation for the Study of Diabetes (EFSD) to Joseph Tam (Grant. no. 94904).

1039

Glycine attenuates renal oxidative stress in streptozotocin-induced diabetic rats

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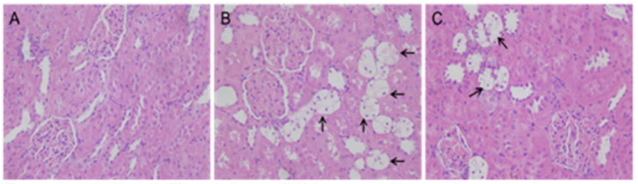
Background and aims: As a member of NADPH oxidase (Nox) family, Nox4 contributes largely to renal oxidative stress, an underlying mechanism of diabetic nephropathy. On the other hand, glycine, as the simplest amino acid in human body, has been reported to ameliorate diabetic complications, although the mechanism remains unclear. In this study, we aimed to investigate whether glycine can attenuate renal injury by suppressing Nox4 expression in diabetic rats.

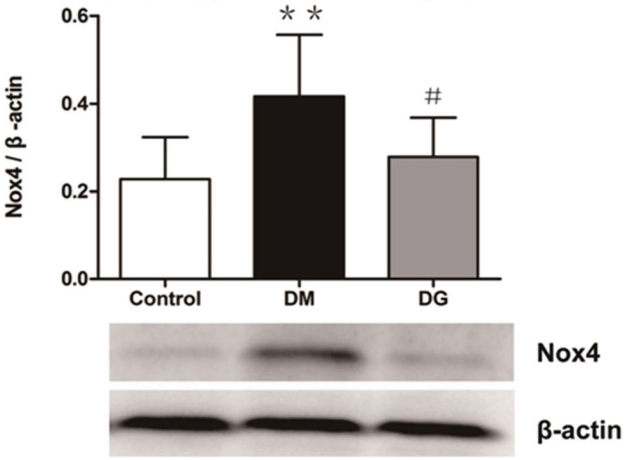
Materials and methods: The STZ-induced diabetic rats were treated with or without glycine (1% in drinking water) from 10 to 30 weeks. The concentration of glycine in plasma and kidney homogenates was measured by using Gas Chromatograph Mass Spectrometer. Routine HE (haematoxylin-eosin) staining was applied to assess renal morphology. Renal Nox4 expression was determined by western blot and immunohistochemistry.

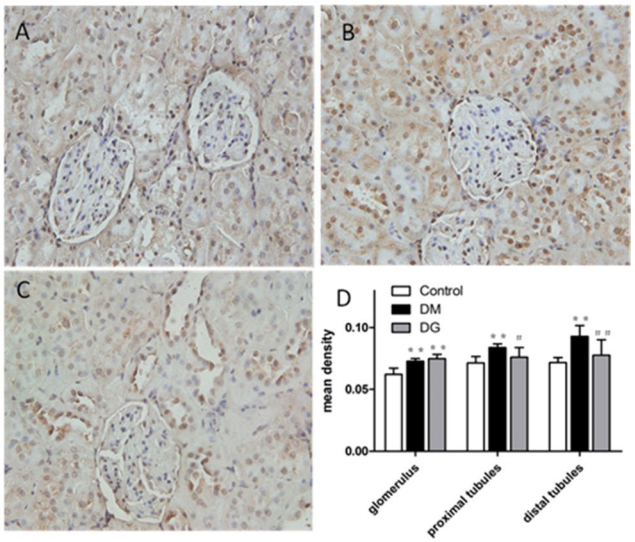
Results: GC-MS analysis showed that the levels of glycine decreased in the plasma and kidney homogenates of STZ-induced diabetic rats and were elevated by oral glycine administration ($p<0.01$, $p<0.05$ respectively). Renal HE staining of the diabetic rats showed multiple tubular dilation and extensive vacuolization, which were markedly restored by glycine treatment. Western blot analysis demonstrated that Nox4 expression increased in diabetic rats ($p<0.01$), but was attenuated after glycine treatment ($p<0.05$). Furthermore, the suppressive effects of glycine on renal Nox4 expression were observed in the proximal ($p<0.01$) and distal ($p<0.05$) tubules by immunohistochemistry.

Conclusion: Our study shows for the first time that oral glycine administration suppresses renal Nox4 expression in STZ-induced diabetic rats, thus protecting against oxidative stress in diabetic nephropathy. The insufficiency of glycine in the diabetic rats may provide insights of applying glycine supplementation to clinical practice, in an attempt to protect the antioxidant defense against the depletion resulted from diabetes mellitus.



Representative HE staining of kidney sections. A: control group. B: diabetic group. C: glycine-treated diabetic group





Western-blot and immunohistochemistry analysis of Nox 4. A: control group. B: diabetic group (DM). C: glycine-treated diabetic group (DG)
* * $p<0.01$ vs. Control; # $p<0.05$ vs. DM; ## $p<0.01$ vs. DM

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Disclosure: **Z. Wang:** None.

1040

Characterisation and targeting of CDA1/CDA1BP1 axis in diabetic nephropathy

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Background and aims: We have previously shown that Cell Division Autoantigen 1 (CDA1) plays a key role in diabetic nephropathy (DN) by enhancing TGF-β signaling. Based on a large body of experimental data including studies in diabetic CDA1 KO mice we have previously

demonstrated that CDA1 is likely to be a safe and effective target to attenuate renal fibrosis in DN. Thus, we aimed initially to identify the key regulatory mechanism responsible for the profibrotic activity of CDA1 in order to provide an opportunity to develop a CDA1 inhibitor as a novel therapy to treat DN.

Materials and methods: A CDA1 interacting protein, designated as CDA1 binding protein 1 (CDA1BP1), was identified by Yeast 2-hybrid screening. Subsequently, its role in regulating the profibrotic effect of CDA1 in vitro as well as in a streptozotocin (STZ) induced DN model was explored using CDA1BP1 KO mice. A newly developed peptide inhibitor of CDA1/CDA1BP1 interaction was then used to treat diabetic ApoE KO mice as a delayed intervention for the last 10 weeks of a 20 week protocol of STZ diabetes. Renal parameters were examined with a focus on sclerotic parameters including trichrome staining to assess the extracellular matrix (ECM) accumulation, immunohistochemical (IHC) staining for various types of collagens and mRNA expression levels for ECM proteins, as determined by quantitative real-time RT-PCR.

Results: Renal gene expression of collagens I, III, fibronectin and TNF-α were elevated by ~3-fold in diabetic WT mice after 20 weeks of diabetes (n=8 (Con), n=20 (Dia), $p<0.001$). These parameters were significantly attenuated by >50% in diabetic CDA1BP1 KO mice (n=9), ($p<0.05$ vs diabetic WT). IHC staining for collagen III and Masson's Trichrome staining for ECM accumulation also showed a >3-fold increase in diabetic WT mice ($p<0.001$ vs non-diabetic WT), which was significantly attenuated by ~50% in diabetic CDA1BP1 KO mice ($p<0.001$ vs diabetic WT). Similarly, renal gene expression of collagens I, III, MCP1 and TNF-α were found to be increased by 2-3 fold in ApoE KO mice after 20 weeks of diabetes ($p<0.05$ or $p<0.001$, n=8-10), which were significantly abrogated ($p<0.05$, n=8) by treatment with the CDA1/CDA1BP1 inhibitory peptide (IP injections at 10 mg/kg twice a week for the last 10 weeks) to levels similar to those seen in control mice. IHC staining of collagen III showed a marked increase in diabetic ApoE KO mice (11.6±2.1-fold, n=9, $p<0.001$ vs non-diabetic ApoE KO mice, 1.0±0.16-fold, n=8), which was attenuated by approximately 75% in the peptide treatment group ($p<0.05$, 3.0±0.3-fold, n=7). Masson's Trichrome staining was increased by 2.5-fold by diabetes ($p<0.05$, n=7-9), which was attenuated by >60% in the peptide treated diabetic group ($p<0.05$, n=7-9).

Conclusion: Genetic deletion of CDA1BP1, as was also seen previously in CDA1 KO mice, attenuates diabetes associated increases in various renal markers of kidney fibrosis in a mouse model of diabetic nephropathy, supporting the putative role of CDA1BP1 as a critical CDA1 partner protein. A pharmacological approach in diabetic mice using a novel peptide inhibitor of the CDA1/CDA1BP1 interaction also retarded development of renal fibrosis in experimental diabetes. These genetic knockout and pharmacological findings demonstrate the feasibility and likely benefits of targeting this profibrotic axis in DN.

Supported by: grants from NHMRC and JDRF.

Disclosure: **Z. Chai:** None.

1041

Angiotensin II type 2 receptor localises to mitochondria of renal tubules and modifies mitochondrial function in early stages of type 1 diabetes in rats

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Background and aims: Diabetic nephropathy (DN) is the leading cause of end-stage renal failure. The incidence and severity of DN are closely associated with increased ROS production due to mitochondrial dysfunction in renal tubular epithelial cells. DN is most effectively treated by blocking angiotensin II (Ang II), which is a dominant regulator of renal function. In DN, local renal levels of Ang II and its cell membrane receptors (AT_1R and AT_2R) are strongly elevated. The AT_1R transmits most of the well-known Ang II actions, while the AT_2R often antagonizes these effects, indicating a protective role. Recently, functionally active Ang II receptors were found, not only on the cell membrane, but also on subcellular compartments. The aim of this study was to clarify the presence of AT_2R in the mitochondria of renal tubular epithelial cells and their functional role in the early stages of type 1 diabetes.

Materials and methods: The present study was performed in transgenic rats which have a 30-fold overexpression of AT_2R in renal tubules (TGR). Their wild type littermates (WT) were used as controls. AT_2Rs were quantified in pure mitochondrial fractions (validated by electron microscopy) isolated from the kidney cortex of TGR using the well acknowledged method of receptor binding studies. The presence of mitochondrial AT_2R was further confirmed by immunohistochemistry (IHC) on TGR kidney sections. The specificity of the AT_2R antibody was previously validated using the kidney sections of AT_2R knockout mice. Mitochondrial functions (oxygen consumption, and ATP and superoxide production) were evaluated in mitochondrial fractions isolated from TGR and WT kidneys, which were either untreated or subjected to STZ-induced diabetes for 28 days.

Results: Pure mitochondrial fractions obtained from TGR kidneys, demonstrated a maximal number of binding sites (Bmax) for AT_2R of 1500 fmol/mg protein. This constitutes 63% of the Bmax for AT_2R measured in the transgenic plasma membrane fraction. The estimates for ligand binding affinity (KD) in the mitochondrial and plasma membrane fraction were in the same range (395 pM and 300 pM, respectively). Co-localization of AT_2R with the mitochondrial membrane protein, VDAC-1, in TGR kidney sections was confirmed by IHC. In the early stages of type 1 diabetes (28 days after STZ injection), mitochondrial oxygen consumption was significantly increased in both, TGR and WT rats, relative to their untreated control groups ($p<0,05$). However, this increase was significantly blunted in TGR ($p<0,05$). The mitochondrial ATP/OCR ratio decreased in diabetic kidneys, but this decline was significantly smaller in TGR than in WT rats (control: TGR $130x10^6$, WT $137x10^6$; diabetic: TGR $56x10^6$, WT $24x10^6$). Although the mitochondrial superoxide production in state 2 of respiration increased in diabetic kidneys ($p<0,02$), there was no difference between the genotypes.

Conclusion: AT_2R localizes to the mitochondria of renal tubules and appears to preserve mitochondrial function in the early stages of STZ-induced diabetes.

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Disclosure: **T. Micakovic:** None.

PS 108 Hypertension revisited

1042

Liver enzymes and hepatic insulin resistance are predictive of incident hypertension

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Background and aims: An association between liver enzymes with diabetes and cardiovascular risk has been established. However, their association with incident hypertension has not been characterized. Furthermore the relationship between hepatic insulin resistance and the development of hypertension remains poorly understood. The aim of the present study was to investigate whether liver markers are related with incident hypertension, independently of other metabolic risk factors, including peripheral insulin resistance. We also investigated the relation between hepatic insulin resistance and incident hypertension.
Materials and methods: We studied 2565 normotensive participants from the D.E.S.I.R. cohort and 1053 non-diabetic and normotensive individuals from the RISC cohort. Hepatic insulin resistance was investigated in a subgroup of 393 participants who had a measure of endogenous glucose production in the fasting state.
Results: In the D.E.S.I.R. cohort, the incidence of hypertension over nine years increased progressively across the quartiles groups of both gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) at baseline, but not of asparate transaminase. After adjustment for sex, age, waist circumference, fasting glucose, smoking and alcohol intake, only GGT was significantly related with incident hypertension [OR: 1.21; 95% confidence interval (1.10-1.34); P=0.0001]. Fatty liver index (FLI), as considered as a continuous value, was also predictive of hypertension. Furthermore, having a FLI ≥60 at baseline was associated with an increased risk of incident hypertension after 9 years in the multivariate model [odds ratio: 1.25 (1.07-1.45), P=0.004]. In the RISC cohort, baseline GGT [OR: 1.25; 95% CI (1.03-1.52); P=0.026] but not ALT nor the fatty liver index were associated with 3-year incident hypertension, after adjustment for sex, center, age, waist, smoking and alcohol intake. This association was independent of the clamp-derived measure of insulin sensitivity. Hepatic insulin resistance index was positively related with the risk of 3-year incident hypertension [standardized odds ratio: 1.54 (1.07-2.21), P=0.02] in the multiple regression and this was independent of the fatty liver index. However, further adjustment for GGT (but not for ALT) altered the association between the hepatic insulin resistance index and hypertension risk, which was no longer statistically significant.
Conclusion: In a healthy population, GGT levels, including within the normal range, were associated with the risk of incident hypertension. Enhanced hepatic insulin resistance predicted the onset of hypertension and may be a link between liver markers and the development of hypertension.
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Disclosure: **F. Bonnet:** None.

1043

Early blood pressure alterations are associated with pro-inflammatory markers in type 1 diabetes

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Background and aims: High blood pressure (BP) in patients with diabetes mellitus type 1 (T1DM) is currently considered one of the principal risk factors for the development and progression of micro-vascular complications and cardiovascular disease. The aim of this study was to evaluate the relationship between early BP changes (detected using ambulatory blood pressure monitoring; ABPM) with different markers of inflammation and endothelial dysfunction in patients with type 1 diabetes mellitus (T1DM).
Materials and methods: We designed an observational cross-sectional in 85 T1DM patients, clinically normotensive and with normo-albuminuria. We analyzed the relationships between ABPM-measured BP alterations over 24h with the inflammatory cytokines (IL-6, TNF-α, VEGF) and the markers of endothelial damage (VCAM, ICAM and PAI). ABPM was performed over 24h and masked hypertension was considered if: 1) mean systolic pressure (sbp) was >130 mmHg in the 24 hours and daytime periods and > 120 mmHg in the nighttime period and/or mean diastolic pressure (dbp) >80 mmHg or 70 mmHg in the same periods respectively. Non dipper pattern was defined as nocturnal sbp or dbp <10% relative to the diurnal mean value.
Results: Of the 85 patients included in the analysis, 55,3% (n=47) were women with an average age of 27,9±6.1 years and a length of disease of 12,3 ±6.5 years. Despite being recorded as normotensive, 27 (31.8%) subjects presented with an average of pathological BP. VEGF levels were significantly elevated in the patients with an altered mean diurnal values compared to normotensives [112.33 (72.87 - 213.53) pg/mL vs 71.03 (37.71 - 107.92) pg/mL; p=0.007]. Further, VEGF levels correlated significantly with the parameters of diurnal BP and of 24h values. IL-6 concentration was a risk factor in the patients with hypertension (OR=1.406; p=0.027). There were no modifications in the levels of markers of endothelial damage.
Conclusion: In patients with T1DM with early alterations in BP, there is an inflammatory status that is manifested as elevated levels of Il-6 and VEGF. The physio-pathological and clinical significance of these findings would require the conduct of further long-term prospective studies, with a wide population base.
Supported by: SED
Disclosure: **J. Tamayo-Serrato:** Grants; Spanish Diabetes Society.

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Changes in intrarenal catecholamines in diabetes and hypertension

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Background and aims: We and others have found greater levels of renal noradrenaline (NA) in hypertensive rodents as compared to normotensive controls. Using the hypertensive Schlager mouse model, we have previously found correspondingly greater cortical tubular staining for the neural marker tyrosine hydroxylase (TH) in hypertensive BPH/2J Schlager mice. Changes in intrarenal nerves in diabetes have not previously been investigated. Therefore we aimed to investigate the effects of diabetes upon neural staining and catecholamine content with and without concomitant hypertension.
Materials and methods: After 10 weeks of study, hypertensive BPH/2J and normotensive BPN/3J Schlager mice with and without concomitant streptozocin induced diabetes (5x 55 mg/kg i.p.) were placed in metabolic cages for 24hr and had kidneys harvested. In a separate group of animals BP telemetry probes were implanted.
Results: Induction of diabetes did not change the hypertensive status of BPH/2J mice (MAP 129 ± 2 vs 131 ± 3 mmHg for non-diabetic vs diabetic BPH respectively, n=5/gp). Diabetic mice showed significantly greater albuminuria, with diabetic hypertensive animals showing significantly greater albuminuria than normotensive diabetic animals. Glomerular mesangial expansion was significantly greater in diabetic animals compared to respective controls with no difference between hypertensive and normotensive diabetic animals. Similarly plasma cystatin C

was significantly lower in diabetic animals, with no difference between hypertensive and normotensive diabetic animals. NA and dopamine levels were significantly greater in hypertensive mice but interestingly normo- and hypertensive diabetic animals had significantly less NA and dopamine levels compared to animals with hypertension alone. Hypertensive animals had significantly more cortical tubular TH staining than normotensive animals however this was not seen in diabetic hypertensive animals with reductions seen in proximal tubules in particular.
Conclusion: These data indicates that diabetes alters renal catecholamine content and distribution in a manner which is independent of hypertensive status and suggests that diabetes alters neural function in the kidney.
Supported by: NHMRC, DART, ADS
Disclosure: **A.M.D. Watson:** Grants; NHMRC, DART, Diabetes Australia.

1045

Co-existing hypertension as independent risk factor for diabetic retinopathy among type 2 diabetes

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Background and aims: Diabetic retinopathy is exacerbated by, or associated with, coexistent systemic hypertension. Epidemiological studies clearly identify hypertension as an independent risk factor for diabetic retinopathy .Systemic hypertension aggravates diabetic retinopathy and other coexisting ocular disorders. To assess coexisting hypertension as a risk factor for diabetic retinopathy (DR) in type 2 diabetes mellitus.
Materials and methods: A total of 977 type-2 diabetic patients recruited retrospectively from Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorder (BIRDEM) based on hospital records for determining the incidence of diabetic retinopathy (DR), who were naive type-2 diabetes during 1993. At the end of 15 year follow-up 495 patients were diagnosed as DR by retinal colour photography. Data on diabetes and lipid profile with serum creatinine, blood pressure and biophysical measures were obtained at baseline, 5, 10 and 15 years follow-up. Binary logistic regression model was used to assess the associations of co-existing hypertension with retinopathy risk adjusting for clinical, biochemical and anthropometric variables.
Results: In all three follow-up points, including at baseline, mean systolic and diastolic blood pressure were found to significantly higher among patients who developed DR (P<.001). Binary logistic regression model confirmed coexisting hypertension (OR 3.2; 95% CI 2.2 -4.7) as a significant risk factor of DR adjusting for possible confounders; serum creatinine (OR 21.9; 95% CI 14.0 -35.4) and glycemic control status (OR 17.2; 95% CI 9.4 - 31.4). Interaction between hypertension, glycemic control and raised serum creatinine did not appear to be statistically significant (P>0.05).
Conclusion: Co-existing hypertension is a significant risk factor of DR irrespective of nephropathy or glycolic control status among Type 2 diabetes mellitus.
Supported by: NOMA Grant
Disclosure: **K.R. Ahmed:** None.

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Nocturnal blood pressure is related to the development of microvascular complications and hypertension in patients with type 1 diabetes

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Background and aims: Ambulatory blood pressure monitoring (ABPM) can detect sub-clinical alterations of blood pressure levels, such as the non-dipper pattern or masked hypertension. These alterations can be missed when office blood pressure measurements are made. The aim of this study was to evaluate the relationship between early blood pressure alterations (detected by ABPM) and the development of microvascular complications and arterial hypertension in patients with type 1 diabetes (T1DM) clinically normotensive.
Materials and methods: We designed a prospective observational study of 85 patients, clinically normotensive and without microalbuminuria, monitored over 7 years. ABPM was performed over 24h and masked hypertension was considered if: 1) mean systolic pressure (sbp) was >130 mmHg in the 24 hours and daytime periods and > 120 mmHg in the nighttime period and/or mean diastolic pressure (dbp) >80 mmHg or 70 mmHg in the same periods respectively. Non dipper pattern was defined as nocturnal sbp or dbp <10% relative to the diurnal mean value. We evaluated the development/progression of microalbuminuria, retinopathy and hypertension during the following period.
Results: Of the 85 patients included in the analysis, 55,3% (n=47) were women with an average age of 27,9±6.1 years and a length of disease of 12,3±6.5 years. Initially, 20 patients (24%) were diagnosed with masked hypertension and 31 (37%) with non-dipper pattern as the only pathological findings. 69 patients completed the seven-year study. At 7 years: 1) twenty-seven patients (32%) had progression of retinopathy related to the nocturnal diastolic blood pressure (BPD) (OR:1.122; p=0.034) and final non-dipper pattern (OR:5.857; p=0.005); 2) seven patients (10%) developed microalbuminuria for which nocturnal systolic blood pressure (BPS) was a risk factor (OR:1.129; p=0.007); 3) five of the normotensive patients (9%) progressed to hypertension; historic HbA1c (OR:2.767; p=0.046) and nocturnal BPD (OR:1.243; p=0.046) being the related risk factors. BPD level ≥65 mmHg was associated with an increase in progression of retinopathy and hypertension.
Conclusion: In T1DM patients there is an elevated prevalence of BP alterations, detected using ABPM. Alterations in nocturnal BP predispose to development/progression of microvascular complications and overt hypertension.
Supported by: SED
Disclosure: **I. Mateo-Gavira:** Grants; Spanish Diabetes Society.

1047

Wrist circumference is associated with systolic blood pressure in a population of overweight/obese children and adolescents

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Background and aims: Insulin resistance, according to many pathophysiological models is one of the most important cardiovascular (CV) risk factors. In a previous study, we demonstrated that the wrist circumference is a clinical marker for insulin-resistance in overweight/obese children and adolescents. Hypertension is another relevant cardiovascular risk factor and obesity is one of its major determinants in children. Various indexes of obesity, such as body mass index (BMI), waist circumference (WC), waist-to-hip ratio and neck circumference, are associated with a high risk of hypertension. The aim of the present study was to investigate a possible association between the wrist circumference and systolic (S) and diastolic (D) blood pressure (BP) in a population of overweight/obese children and adolescents.
Materials and methods: N=1133 overweight/obese children and adolescents (580 boys and 553 girls) were consecutively enrolled. In all children and adolescents, body weight, height, SBP, DBP,wrist circumference, SDS-BMI, fasting glucose, fasting insulin levels, and lipid profiles were

evaluated at entry. Insulin resistance was estimated according to the homeostasis model assessment of insulin resistance (HOMA-IR). Subjects were evaluated by a doctor for the pubertal stages. Shapiro-Wilk test was used to verify the normality of distribution of continuous variables. The dependent variables for this study were SBP and diastolic DBP; independent variables were SDS-BMI and wrist circumference adjusted for Tanner stage. Multivariate linear regression analyses were used to investigate the influence of independent variables on the variance of blood pressure. All analyses were performed using Statistical Analysis Software (SAS v.9.3).

Results: The frequency of hypertension was 22.6% in males and 28.2% in females (p=0.048). Results of the multivariate regression analysis performed in the 1133 children and adolescents stratified according to gender, using wrist circumference and SDS-BMI as independent variable and blood pressure as the dependent variables showed that SBP was significantly associated with wrist circumference and SDS-BMI both in males and females (p≤0.04 for both comparison). We found no association between DBP and wrist circumference in both gender. Wrist circumference and SDS-BMI together explained 21% of the variance of SBP in males and 18% in females. To evaluate the contribution of wrist circumference and SDS-BMI, respectively, to explained variance (R2) of SBP index, we used the backward method. The total variance of SBP was explained by wrist circumference for 17% and by SDS-BMI for 2.7% in males; and by wrist circumference for 14% and SDS-BMI 1% in females.

Conclusion: The wrist circumference in overweight/obese children and adolescents is correlated with SBP, confirming that this bone anthropometric marker could be useful for the prediction of cardiovascular risk being correlated with insulin resistance and its deleterious effects.

Disclosure: **G. Campagna:** None.

1048

Treatment of hypertension in type 2 diabetes and non-diabetic individuals; a population-based cohort study

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Background and aims: Hypertension is a prevalent disorder, also in people with type 2 diabetes. Approximately half of the deaths from stroke or CVD is attributable to hypertension. Early screening and diagnosis of hypertension is important to reduce these mortality risks. We assessed actual blood pressure (BP) levels in a large population-based cohort study, and evaluated BP course during ageing in healthy participants and those treated with BP-lowering agents, as well as in subjects with type 2 diabetes.

Materials and methods: Data of western European participants, aged 40-80 yrs, were available from the Dutch Lifelines Cohort study. Participants were categorized into four age decades. Normal blood pressure levels within these age groups were calculated from non-diabetic individuals not treated with BP-lowering drugs. In addition, mean systolic and diastolic BP was calculated for each age group depending on diabetes and BP-treatment status. Type 2 diabetes was already known in 1,396 individuals, and was newly-diagnosed in 669 individuals based on fasting plasma glucose level ≥ 7.0 mmol/l during screening. To assess need for treatment in type 2 diabetics not on BP-lowering medication we applied a cut-off BP level of ≥140/90 mmHg, based on the 2014 Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 8).

Results: In total, 48,846 non-diabetic individuals, and 2,065 with type 2 diabetes, were available for analysis. In non-diabetic individuals, BP increased gradually in each age group, with levels of 124/74 mmHg in participants aged 40-50 yrs, and 139/75 mmHg in those aged 70-80 yrs. Use of BP-lowering drugs did not result in BP levels comparable to healthy individuals except in the participants aged 70-80 years. In type 2 diabetics not using BP-lowering drugs, higher BP levels in the age groups between 40 and 70 yrs were seen, except for those aged 70-80 yrs who had comparable BP vs non-diabetics. In contrast to non-diabetic individuals, type 2 diabetics who were medically treated, had BP levels comparable to the (elevated) levels of type 2 diabetics not treated. Furthermore, 32% of the people with known diabetes, and 41% of those with newly-diagnosed diabetes (p=0.006 vs. known diabetes), had elevated BP ≥140/90 mmHg but were not treated with BP-lowering drugs.

Conclusion: Non-diabetic individuals with hypertension do not reach the normal values of BP for their specific age group when treated with BP-lowering drugs. Type 2 diabetic individuals have higher BP compared to non-diabetic individuals, except for the oldest age group, and there appears to be considerable undertreatment of elevated BP in people with type 2 diabetes.

Average systolic/diastolic blood pressure levels (mmHg) according to age and disease group

No diabetes	Use of BP-lowering drugs**		
Age (yrs)	0	1	2
40-50	124/74	131/79*	133/80*
50-60	127/76#	132/79*	132/78*
60-70	132/76#	137/77#*	135/77#*
70-80	139/75#	141/76#	141/76#
Type 2 diabetes	**Use of BP-lowering drugs****		
Age (yrs)	0	1	2
40-50	132/78	135/79	134/77
50-60	134/79	134/77	133/76
60-70	138/77$	139/75&	137/76
70-80	139/76$	141/74&	144/74&

** number of different classes of BP-lowering drugs
# p<0.001 vs 40-50 yrs; $ p<0.005 vs 40-50 yrs
& p<0.05 vs 40-50 yrs; * p<0.005 vs no treatment

Disclosure: **B.H.R. Wolffenbuttel:** None.

1049

Systolic blood pressure and cardiovascular events in type 2 diabetes: the lower the better

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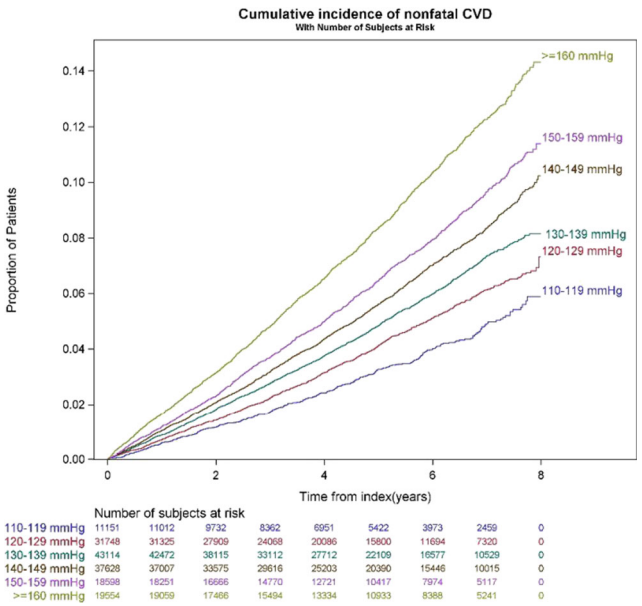
Background and aims: Recent hypertension guidelines have raised the target blood pressure for patient with diabetes, but in view of recent findings from the Systolic Blood Pressure Intervention Trial (SPRINT) which, however, did not include individuals with diabetes these recommendations may no longer be valid. We aimed to assess the long-term prognosis for patients with type 2 diabetes whose systolic blood pressure meet the new guidelines of below 140 mmHg. We used several methods to minimise confounding by concomitant disease.

Materials and methods: We used the Swedish National Diabetes Register for an observational study. Inclusion criteria were type 2 diabetes, duration of at least one year, age 75 or younger and no previous cardiovascular or other major disease. Patients were included from January 2006 to December 2012 and monitored until December 2013.

Baseline systolic blood pressure was measured and clinical events were obtained from the Hospital Discharge and Death Registers, with respect to acute myocardial infarction (AMI), stroke, a composite of AMI and stroke (CVD), coronary heart disease, heart failure and total mortality. Cox proportional hazards regression was used to estimate hazard ratios for the relation between systolic blood pressure and the outcomes, while clinical characteristics and drug prescription data were used as covariates.

Results: This observational study of 187,106 individuals with type 2 diabetes and no major previous diseases found no indications of a J-shaped relationship between systolic blood pressure and the endpoints, with the exception of heart failure and total mortality. The lowest systolic blood pressure group (110-119 mm Hg) had 20% lower risk of nonfatal myocardial infarction (hazard ratio, 0.80, 95% confidence interval [CI], 0.68 to 0.95; P=0.01) and 16% lower risk of nonfatal CVD (hazard ratio, 0.84, 95% CI, 0.74 to 0.95; P=0.006) than the reference group (130-139 mm Hg).

Conclusion: Our data support the findings of randomised trials that systolic blood pressure of 120 mm Hg or less is superior to higher blood pressure levels for preventing cardiovascular events. A recommendation of systolic blood pressure below 130 mm Hg for patients with type 2 diabetes would appear to be more appropriate and we believe that current guidelines should be changed accordingly.



Disclosure: **S. Adamsson Eryd:** None.

PS 109 Mediators and treatments for complications

1050

Insulin therapy for diabetes does not modify the effect of patiromer on serum potassium in hyperkalaemic patients with type 2 diabetes on RAAS inhibitors

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Background and aims: Intravenous insulin increases skeletal muscle uptake of potassium (K) leading to reductions in serum K (sK), an effect used to treat hyperkalemia (HK) that is not altered in insulin resistant states. However, there are no reports on the effect of daily subcutaneous insulin injections for diabetes on sK in patients with HK. Patiromer, a sodium-free non-absorbed potassium-binding polymer that uses calcium as the counter-exchange ion, is approved in the U.S. for the treatment of HK. A prespecified subgroup analysis of the Phase 3 OPAL-HK study showed no difference in the mean reduction from baseline in sK at Week 4 with patiromer in patients with HK and chronic kidney disease (CKD), with and without DM type 2 (DM2) (p=0.77 for interaction). Here we analyzed pooled data from patients with DM2 in OPAL-HK and a Phase 2 study (AMETHYST-DN) to determine if daily insulin use modifies patiromer's effects on sK-lowering.

Materials and methods: This was a post-hoc pooled analysis of DM2 patients treated with patiromer (N=443) in two studies of the treatment of HK in CKD. In AMETHYST-DN, a randomized, open-label, 52-week study, all (n=304) patients had DM. In the 4-week, single-blind, initial treatment phase of OPAL-HK, 139 patients (57%) had DM2. Entry sK (based on local laboratory values) was >5.0 to <6.0 mEq/L and 5.1 to <6.5 mEq/L, respectively, and patiromer starting doses were 8.4-33.6 g/d and 8.4-16.8 g/d, respectively. Difference between DM2 patients using insulin (DM2+ins) vs those not using insulin (DM2-ins) for change in sK (central laboratory) from baseline through 4 weeks (when all patients were on patiromer) was analyzed using MMRM adjusted for baseline sK, study, and patiromer starting dose. Data are shown as mean±SEM.

Results: In the pooled analysis, 177 (40%) patients used insulin; of these, 87.6% used a short- or rapid-acting insulin. Compared to DM2-ins, DM2+ins had higher baseline mean sK (Table), HbA1c (7.80±0.12 vs 6.96±0.09%) and time since DM2 diagnosis (18.3±0.63 vs 10.4±0.42 yr); stage of CKD, eGFR, and BMI were similar between groups. At Week 4 the LS-mean for change from baseline in sK and proportion of subjects who achieved target range sK (3.8-5.0 mEq/L) were similar in DM2+ins and DM2-ins (Table). The frequency of sK values <3.5 mEq/L with patiromer was 2.0% in DM2 patients (1.1% in DM+ins and 2.6% DM-ins). Patiromer was generally well tolerated. At least one adverse event (AE) was experienced by 28.4% of DM2 patients. The most common individual AE was constipation (DM2 6.1%; DM2+ins 7.3%, DM-ins 5.3%); all constipation events were mild or moderate in severity.

Conclusion: Patiromer reduces serum K in hyperkalemic patients with CKD and diabetes irrespective of daily insulin use.

	DM2+insulin (N=177)	DM2-insulin (N=266)
Baseline sK, mEq/L	5.44±0.030*	5.36±0.023
Change sK at 4 weeks, mEq/L	-0.84±0.037†	-0.78±0.030†
Proportion (%) with sK 3.8–5.0 mEq/L at 4 weeks (95% CI)	85.4% (79.0, 90.5)	86.7% (81.8, 90.7)

*p<0.05 vs DM2-ins by analysis of variance; †p<0.0001 change from baseline by mixed-effect model repeated measure (MMRM), adjusted for baseline sK, study, and patiromer starting dose.

Clinical Trial Registration Number: NCT01371747; NCT01810939
Supported by: Relypsa, Inc.
Disclosure: **P. Rossignol:** Employment/Consultancy; Relypsa Inc., AstraZeneca, Novartis, Stealth Peptides, Vifor-Fresenius Medical Care Renal Pharma. Other; Co-founder of CardioRenal.

1051

Mitochondrial uncoupling combined with pyruvate dehydrogenase activity ameliorated hyperglycaemia

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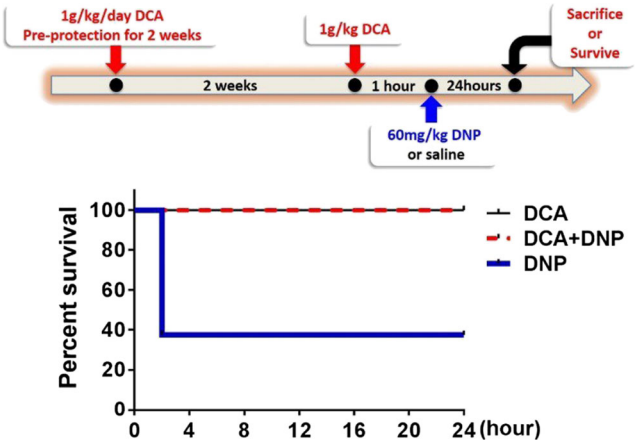
Background and aims: DNP, a classical mitochondrial uncoupler, performed hypoglycemic and hypolipidemic effect and had been applied in clinic but was withdrawn because of a combination of hyperthermia, hyperkalemia, hyperlactatemia and the risk of death. The side effect was clarified to closely associated with excessive promotion of lipid oxidation leading to ketones body release and fever, and the compensatory ATP generation by glycolysis contribution to lactate release. Pyruvate dehydrogenase complex (PDC) is another key switch point of glucose and lipid metabolism. Increasing activity of PDC would promote glucose but not lipid oxidation, inhibit ketone and lactate release and result in a significant hypoglycemia. Our study aim to clarify that the enhanced activity of PDC could partially reverse the side effect inspired by DNP in vitro and vivo, and a novel molecular with mitochondria uncoupling and PDC activity were identified to ameliorate hyperglycemia.
Materials and methods: The survivors of mice (n=8) were observed in 24 hours after single-dose treated with DNP (60mg/kg) on two-week pretreated with DCA (PDC activator, 1g/kg) or not. Glucose oxidation and lipid oxidation were obtained by isotope labeling in myotubes. Lactate was measurement after incubated with compounds in 12 hours. ob/ob mice(n=8) were oral administrated with 20mg/kg 1093 for five weeks, and glucose tolerance and insulin sensitivity were detected during treatment.
Results: DCA blocked the lipid oxidation (0.73±0.025-fold vs 1.38 ±0.032-fold, p<0.001) and lactate release (0.82±0.021-fold vs 1.12 ±0.035-fold, p<0.001) induced by DNP in myotubes. But the effect of DNP on glucose oxidation was not affected by DCA in myotubes (1.31 ±0.032-fold vs 1.35±0.14-fold, p>0.05). DCA protected mice from death caused by single-dose treated with DNP in 24 hours (100% vs 37.5%, p<0.01). Based on a series of screening of oxygen consumption, lactate release and PDC activity in vitro, we found that 1093 might be a mitochondrial uncoupler combined with enhancing PDC activity in myotubes. 1093 promoted glucose oxidation (p<0.05) other than glycolysis and had no effect on lipid oxidation in myotubes. Long-term treatment with 1093 in ob/ob mice, glucose tolerance (2712±397.6 vs 1947±88.48, p<0.05) and insulin sensitivity (1412±247.4 vs 910.0±82.79, p<0.05) were significantly improved and no changes in serum lactate (24.73±4.89 vs 27.43 ±3.17mg/dl, p>0.05).
Conclusion: Our findings showed DCA could partially reverse the side effect caused by DNP in vitro and in vivo. Through a series of compound screening, we found 1093 was a mitochondrial uncoupler combined with enhancing PDC activity and promoted glucose metabolism. These results indicated that mitochondrial uncoupling combined with PDC activity seems to be a safe and effective strategy to ameliorating hyperglycemia.



Disclosure: **H. Jiang:** None.

1052

Oxyntomodulin analogue for the treatment of diabetes and obesity

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Background and aims: Oxyntomodulin (OXM) is a peptide hormone released from the L-cells of the small intestine in proportion to nutrient ingestion. OXM is a glucagon-like peptide 1 receptor (GLP1R)/glucagon receptor (GCGR) dual agonist that regulates glucose homeostasis and body weight through incretin effects and increased energy expenditure. We investigate metabolic effects of a long acting GLP1R/GCGR dual agonist in rodent models.
Materials and methods: A long acting dual GLP1R/GCGR co-agonist, LY2944876 (LY), a corresponding GLP1R agonist, LSN2954023, matched for GLP1R agonist potency and pharmacokinetics, and a GLP1R agonist, LSN2534554 were developed in house. LY or LSN2534554 was administered by subcutaneous (SC) injection to lean mice. An IPGTT was performed 16 hours following drug administration. LY or LSN2954023 was administered every 3 days by SC injection to diet-induced obese (DIO) mice for 4 weeks. Food intake and body weight were monitored. An IPGTT was performed at the end of 4 weeks study. Blood and liver samples were collected for analysis. LY or LSN2954023 or LSN2534554 was administered by SC injection to DIO mice. Food was removed at the time of drug administration. Blood and liver samples were collected 16 hours following drug administration for analysis.
Results: A dose-dependent statistically significant increase in insulin secretion and decrease in glucose excursion were seen when LY was tested in lean mice. To explore the metabolic effects of LY in a model of obesity and insulin resistance, LY or LSN2954023 was administered at 6.7 and 20 nmol/kg to DIO mice for 4 weeks. LY exhibited superior efficacy compared with LSN2954023 on food intake reduction and weight loss. Body composition analysis confirmed that the decrease in body weight was primarily due to decrease in fat mass. An IPGTT performed on day 29 revealed that glucose tolerance in high dose LY group was improved and comparable to the LSN2954023 groups. Chronic LY treatment

significantly decreased liver triglycerides, plasma insulin, cholesterol and leptin. To investigate metabolic effects independent of weight change, LY was administrated once to DIO mice. Acute treatment with LY demonstrated statistically significant reductions in hepatic malonyl-CoA and acetyl-CoA levels and ChREBP gene expression, and a statistically significant increase in hepatic FGF21 gene expression. Acute treatment with LY also statistically significant decreased plasma triglyceride levels and increased plasma FGF21 levels. These changes were not observed in the LSN2954023- or LSN2534554- treated group.
Conclusion: These results demonstrate that GLP1R/GCGR co-agonist is effective in glucose control and weight reduction. The findings suggest that OXM analogs are potential therapies to offer non-inferior glycemic control, superior weight management capability and potential beneficial lipid profiles.
Disclosure: **Y. Chen:** None.

1053

Adverse effects of methylglyoxal on metabolic syndrome parameters
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Background and aims: Biogenesis of reactive dicarbonyls such as methylglyoxal is related to glycaemic and lipid status in metabolic syndrome and type 2 diabetes. Plasma methylglyoxal levels are elevated in diabetic patients, mainly those with nephropathy or cardiac failure. Excessive generation of dicarbonyls leads to the production of advanced glycation end products (AGE), activates inflammatory processes, increases oxidative stress and can play a key role in the development of vascular complications. However, the underlying mechanisms remain unclear. In our study, we examined the effect of methylglyoxal administration on the parameters of metabolic syndrome in hypertriglyceridaemic rats (HHTg) with insulin resistance and fatty liver.
Materials and methods: Adult male rats of HHTg strain were fed a standard diet. Methylglyoxal was administered intragastrically three times a week in a dose of 0.5 mg/ kg b.wt. for 4 weeks. Concentrations of glutathione and urine albumin were determined using the HPLC-method. Gene expression was measured by quantitative RT-PCR.
Results: Compared with controls, methylglyoxal-treated HHTg rats exhibited increased non-fasting serum levels of glucose (8.8±0.2 vs 7.1±0.2 mmol/l, $p<0.01$), insulin (0.515±0.030 vs 0.246±0.026 μmol/l, $p<0.05$) and impaired glucose tolerance (AUC 0-120, $p<0.05$). However, there were no differences in serum triglycerides, adiponectin or ectopic triglyceride accumulation in the liver and skeletal muscle. In the liver, methylglyoxal resulted in decreased levels of glutathione ($p<0.05$) and increased lipoperoxidation ($p<0.05$) measured as TBARS. In the kidney cortex, methylglyoxal administration did not affect activity or relative mRNA expression of glyoxalase-1, which is involved in the degradation pathway of methylglyoxal. Methylglyoxal administration significantly elevated microalbuminuria (42.8±7.3 vs 13.8±2.9 mg/g creatinine, $p<0.01$) and urine lactate ($p<0.05$) in HHTg rats. Markedly decreased levels of glutathione (1.08±0.06 vs 0.84±0.02 μmol/mg, $p<0.01$) together with reduced activity of glutathione-dependent enzymes can contribute to the impairment of kidney function after methylglyoxal administration. In visceral adipose tissue, methylglyoxal-treated rats exhibited decreased relative mRNA expression of transcription factor Nrf2 ($p<0.05$), which controls antioxidant and lipogenic genes, and strongly deteriorated fatty acid composition in phospholipids. The proportion of saturated fatty acids, mainly palmitic ($p<0.05$) and myristic acid ($p<0.05$), were significantly increased together with significantly decreased proportion of PUFA n-3 ($p<0.001$).
Conclusion: Methylglyoxal administration in rats with severe hypertriglyceridaemia aggravated glucose tolerance, induced oxidative stress in the kidney and liver and elevated microalbuminuria. Methylglyoxal also caused lipid alterations in visceral adipose tissue, which may promote insulin resistance. Our results support the hypothesis, that methylglyoxal may be involved in the pathogenesis of disorders associated with metabolic syndrome and type 2 diabetes.
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Disclosure: **H. Malinska:** None.

1054

Effect of acute hyperinsulinaemia and postprandial state on the endothelial function in metabolically healthy volunteers with overweight or obesity
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Background and aims: Endothelial dysfunction has been linked to the pathogenesis of cardiovascular disease. Mechanisms involved in the regulation of vascular reactivity are not fully understood. The aim of the study was to test the effects of isolated acute hyperinsulinemia induced by hyperinsulinemic euglycemic clamp and postprandial state after meal test on the vascular reactivity, and examine the relations to the degree of oxidative stress (OS) and levels of selected gastrointestinal peptides (GIP).
Materials and methods: We enrolled 30 volunteers with overweight or obesity and with normal glucose metabolism. All subject underwent the hyperinsulinemic euglycemic clamp combined with indirect calorimetry and meal test using standard breakfast. Parameters of the endothelial function - the peripheral augmentation Index (AI) and the reactive hyperemia index (RHI) were measured by finger pulse plethysmography before and during the clamp and before and after the meal test. Insulin sensitivity was estimated as the metabolic clearance of glucose (MCG), the metabolic flexibility as the increase in the respiratory quotient during the clamp (ΔRQ). Parameters of OS and selected GIP in serum were measured during the meal test. T-test and the Pearson correlation coefficient were used for the statistical analysis.
Results: AI increased during the clamp (ΔAI: 10.45 ± 5.12; $p<0.01$) and during the meal test (ΔAI: 7.97 ± 7.66; $p<0.001$). RHI decreased during clamp (ΔRHI: -0.091 ± 0.03; $p<0.05$), while it did not change during the meal test. We found a positive relationship between MCG and ΔRHI ($r = 0.32$; $p<0.001$) and negative correlation between ΔAI and ΔRQ (r 0.24; $p<0.001$) during the clamp. The glucagon-like peptide 1 (GLP-1) at 120 min of the meal test correlated positively with RHI ($r = 0.38$; $p<0.001$), and negatively with AI ($r = -0.36$; $p<0.001$). The change in activity of superoxide dismutase (ΔSOD) was positively related to ΔRHI ($r = 0.21$; $p<0.001$) and negatively to ΔAI ($r = -0.51$; $p<0.001$).
Conclusion: Hyperinsulinemia and postprandial metabolic changes impair endothelial function in metabolically healthy volunteers with overweight and obesity. OS is a possible mediator. The impairment of endothelial function is less pronounced in individuals with higher insulin sensitivity, higher metabolic flexibility and higher postprandial rise of GLP-1.
Clinical Trial Registration Number: NCT01946347
Supported by: AZV15-27431A
Disclosure: **J. Veleba:** None.

1055

Probiotic Lisosan G preserves functional properties of human endothelial progenitor cells exposed to lipopolysaccharides
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Background and aims: Endogenous signals (such as oxidized-LDL) and exogenous signals (such as lipopolysaccharides, LPS) derived by the gut microbiota orchestrate inflammatory responses and contribute to plaque evolution in obesity, metabolic syndrome and diabetes. The involvement of LPS as a potentially important source of vascular inflammation in the induction of atherogenesis has been postulated. Several strategies have been developed to change gut microbiota. For instance, probiotics can modify gut microbiota. With this background, we have evaluated the effects of the probiotic Lisosan G (LG) on human endothelial progenitor cells (EPCs) exposed to LPS.
Materials and methods: Early EPCs, obtained from peripheral blood mononuclear cell (PBMC) of healthy subjects, pre-treated with LG 0.7 mg/ml for 1 hour, and/or exposed to LPS 10 μg/ml for the next 23 hours, have been evaluated at 5-hour and 24-hour for: 1. cells viability; and 2. reactive oxigen species (ROS) production; at 24-hour for: 3. adhesion to fibronectin; and 4. expression of IL-6, COX-2, ICAM-1, ET-1, Casp-3, Casp-9, CHOP, SOD2, CAT, GPx1; at 5-hour and at 24-hour for: 5. the nuclear factor NF-kB translocation.
Results: First, cells viability at 5-hour and 24-hour: no differences in LG-/LPS- (the control experimental condition, C), vs. LG+/LPS-, LG-/LPS+ and LG+/LPS+. Adhesion at 24-hour: it was higher (p=0.021) in LG+/LPS+ vs. C, marginally higher (p=0.09) vs. other experimental conditions. ROS production: differences have been observed both at 5h (p=0.0026) and 24h (p=0.051); reduced in LG+/LPS- vs. C (p=0.033 and p=0.119, at 5h and 24h, respectively); marginally increased in LG-/LPS+ vs. C at 5h and 24h; reduced in LG+/LPS+ vs. LG-/LPS+ (p=0.0043 and p=0.067, at 5h and 24h, respectively). At 24h, as compared to C, LPS (LG-/LPS+) rises expression of IL-6, COX-2, ICAM-1 (p=0.0001 for all), ET-1 (p=0.0003), Casp-3 e Casp-9 (p=0.005), CHOP (p=0.0004), SOD2 (p=0.0001) and GPx1 (p=0.0073). LG+/LPS+ decreases IL-6 and ICAM-1 (both p=0.0001), normalizes COX-2 (p=0.0001), ET-1 (p=0.0002), Casp-3 (p=0.0001), Casp-9 (p=0.0001) and CHOP (p=0.0001), further increases SOD2 (p=0.0017) and GPx1 (p=0.0048). Finally, LPS pauperises CAT (p=0.012), with LG normalizing its expression. In C and in LG+/LPS-, NF-kB was mainly localized into the citosol, in LG-/LPS+ mainly within the nucleus; in LG+/LPS+ nuclear translocation of NF-kB was reduced.
Conclusion: In human EPCs, LPS increases ROS, up-regulates pro-inflammatory tone, pro-apoptotic factors and anti-oxidants. Lisosan G protects human EPCs exposed to LPS reducing ROS, down-regulating pro-inflammatory and pro-apoptotic factors and strengthening anti-oxidant defenses through NF-kB translocation.
Supported by: Regione Toscana, Grant n. D55E11002680005
Disclosure: **L. Giusti:** None.

1056

Novel targets involved in the restoration of recurrent hypoglycaemia induced defective counter-regulation following acute high intensity exercise

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Background and aims: Recurrent hypoglycemia (RH) is a major limitation to glycaemic control in individuals with Type 1 Diabetes. This can result in suppression of the normal counter-regulatory hormonal and physiological response (CRR) to hypoglycemia, increasing the risk of severe hypoglycemia. We have previously demonstrated that acute high intensity exercise (HI) restored defective CRR potentially through a process of "dis-habituation". The molecular mechanisms through which this improvement occurred remain unclear. Glucose sensing neurons within the hypothalamus play a fundamental role in glycaemic control. This study tested the hypothesis that changes in key proteins within the hypothalamus may be responsible for the improvement in CRR following acute HI exercise.
Materials and methods: Hypothalamic samples from male Sprague Dawley rats (250-300g; n=4/group) previously exposed to RH (1U/kg insulin i.p; 3 times per week for 4 weeks) and (ii) low-intensity exercise (LI: Total 25 min: 5m/min) or (ii) high-intensity exercise (HI: Total 25min; 5min (5m/min) accelerating (2m/min) to final 1min (15m/min) were processed, labeled with iTRAQ 8-plex reagent and fractionated using high pH fractionation and a strong cation exchanger for subsequent LC-MS/MS analysis. Peptide identification and protein quantification was performed using PEAKS 7.0 (database =ipi-RAT 2012-09-27) with false detection rate (FDR) set to 1% at the identified peptide spectrum match level. Normalisation was performed in PEAKS 7.0 as default. Top hits were validated by Western blot analysis.
Results: A total of 4085 proteins were identified (FDR at 0.1%) with quantification data available on 1866 proteins containing unique peptides/quantifiable reporter ions. Using a cut-off of 30% changes and p value (Bonferroni-Hochberg method) of 0.05, 25 proteins were differentially expressed. Five proteins; DNAJB3 (Hsp40), BSN-Bassoon, DPP10, MTX2 and Adenoline cyclase 9 (ac9) passed the fold change filter and manual curation criteria. The proteins identified share similar properties involved in synaptic transmission either through formation of presynaptic complexes (BSN-Bassoon), translocation of complexes to the surface membrane (MTX2 and DPP10) or glycolysis and cell proliferation (Hsp40 and ac9).
Conclusion: Differential expression of 5 key proteins within the hypothalamus highlight novel pathways that may play a role in exercise induced restoration of defective CRR following RH.
Supported by: JDRF and Diabetes UK
Disclosure: **A.D. McNeilly:** None.

PS 110 Mechanisms of complications: pathways and interventions

1057

Recombinant ACE2 reduces angiotensin II and supresses atherosclerosis associated with experimental diabetes in apolipoprotein E knockout mice

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Background and aims: Angiotensin Converting Enzyme 2 (ACE2) opposes the actions of Angiotensin II (Ang II), by degrading it to the vasculoprotective peptide, Angiotensin 1-7 (Ang 1-7). ACE2 expression is reduced in the diabetic vasculature coinciding with activation of the renin angiotensin system. Given the important role of Ang II/Ang 1-7 in atherogenesis, we investigated the effects of ACE2 replenishment on the development of atherosclerosis associated with experimental diabetes.
Materials and methods: Diabetes was induced in male apolipoprotein E knockopiut mice using streptozotocin (55mg/kg/ in 5 daily doses). Mice were then randomized to receive murine recombinant ACE2 (1 mg/kg/day IP) or vehicle for 6 weeks. Circulating ACE2 activity and angiotensin levels were estimated at 1 weeks and 6 weeks and atherosclerotic burden was quantified after 6 weeks using en face staining.
Results: Recombinant ACE2 significantly increased ACE2 activity and significantly reduced Ang II levels in plasma and tissue without lowering blood pressure. Plaque accumulation was increased in diabetic mice when compared to non-diabetic mice associated with increased vascular expression of adhesion molecules and inflammatory cytokines, including IL-6, MCP-1 and VCAM-1. Treatment with murine recombinant ACE2 significantly reduced plaque area and markers of vascular inflammation.
Conclusion: ACE2 deficiency is associated with up-regulation of atherogenesis and enhanced responsiveness to pro-inflammatory stimuli. As diabetes is associated with relative ACE2 deficiency, replacement of circulating ACE2 using recombinant protein is a logical strategy. In addition, we now demonstrate for the first time that ACE2 repletion is effective as a strategy to reduce atherosclerosis associated with diabetes.
Disclosure: **M.C. Thomas:** None.

1058

A salvage pathway of SGK1 in eNOS activation in coronary cells

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Background and aims: Endothelial dysfunction results from the combination of different vascular risk factors such as hypertension, diabetes, and dyslipidemia. Several molecular pathways underlays this process and when deregulated by noxious stimuli, such as hyperglycemia, lead to arterial impairment. These pathways include mainly intracellular signaling like PI3K-AKT1 and when activated by insulin stimulation, induce Endothelial Nitric Oxide Synthase (eNOS) phosphorylation and consequent nitric oxide (NO) production, which is critical in vascular homeostasis. In a recent work, we have demonstrated that along with AKT1, Serum and glucocorticoid-inducible kinase 1 (SGK1), which have a highly homology with AKT1, was able to regulate the production of NO and to protect endothelium from hyperglycemia. Moreover, while it is established the role of AKT1 in the activation of eNOS and NO production, it is not clear the role of SGK1 in this pathway. Therefore, the aim of this study was to evaluate the role of SGK1 in the activation of eNOS and NO production by using an in vitro model of Human Coronary Artery Endothelial Cells (HCAEC).
Materials and methods: HCAECs were infected with different constructs of SGK1: 1. SGK1wt (full length protein, ubiquitinated and degraded by proteasome), 2. SGK1Δ60 (not ubiquitinated, not degraded, and more active protein), and 3. SGK1Δ60KD (kinase-inactive); or treated with a specific proteasome inhibitors (ALLN, Lactacystin) that prevent the degradation of SGK1 by proteasome mechanism. In order to determine the specific role of AKT1 and SGK1 in eNOS activation, we evaluated eNOS phosphorylation at Ser1177 by western blot analysis and NO production by NO detection assay, in these constructs stimulated with or without insulin and/or selective AKT1 and SGK1 inhibitors administered alone or in combination. Furthermore, we evaluated apoptosis levels in HCAECs treated with TNF-alpha by FACS analysis.
Results: We showed even in the HCAEC that SGK1 was degraded by proteasome and that proteasome inhibitors blunted SGK1 degradation inducing an increase in SGK1 cytoplasm expression and activity. Phosphorylation of eNOS in Ser1177 and NO production after insulin stimulation were significantly increased in cells overexpressing SGK1 and with SGK1 highest activity (SGK1Δ60 (n=3), or treated with proteasome inhibitors (n=3)), compared to the other constructs (SGK1wt (n=3), SGK1Δ60KD (n=3)) ($p<0.05$). Interesting, AKT1 inhibitor, was able only partially to reduce the phosphorylation of eNOS, as well as the SGK1 inhibitor in each construct but especially in SGK1Δ60, suggesting that SGK1 may be an alternative pathway in NO production when AKT1 in blunted. In addition, SGK1Δ60 HCAECs have reduced apoptotic levels after TNF-alpha stimulation, confirming that increasing the NO bioavailability may increase cellular survival.
Conclusion: These results propose a novel important role for SGK1 in the modulation of eNOS mediated NO production, which can have an AKT1 compensatory effect in regulating cell/tissue NO levels. These results may open new therapeutic strategy against cardiovascular diseases.
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Disclosure: **D. Pastore:** None.

1059

Novel insights into the effects of insulin-like growth factor 1 and endothelin signalling on vascular redox state in patients with type 2 diabetes

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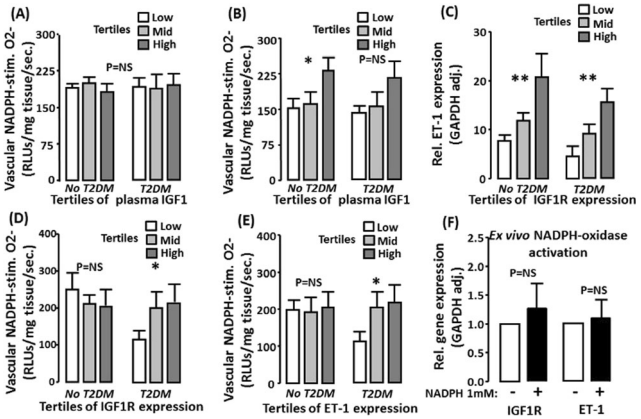
Background and aims: Oxidative stress participates in the vascular complications of Type 2 Diabetes Mellitus (T2DM). Endothelin 1 (ET1) and insulin-like growth factor 1 (IGF1) affect redox hubs, but their effect on vascular redox state in T2DM patients is unknown. We explored the role of ET1 and IGF1 signalling on vascular redox regulation in T2DM patients with coronary atherosclerosis.
Materials and methods: Internal mammary artery (IMA) segments were obtained from 486 patients undergoing coronary bypass surgery (146 T2DM) and used for superoxide (O2-) measurement ex vivo. Plasma ET1 and IGF1 were measured by ELISA.
Results: High plasma ET1 (not IGF1) was associated with elevated vascular NADPH oxidase activity (A, B), while vascular IGF1R and ET1 expression were strongly correlated (C). Vessels with increased IGF1R and ET1 expression had higher NADPH oxidase activity only in T2DM patients (D, E). To test the direction of these associations, we induced O2- generation in IMAs by incubating with NADPH 1mM ex vivo, showing no effect on IGF1R or ET1 expression (F).
Conclusion: We demonstrate for the first time in humans that elevated vascular IGF1R expression and plasma or vascular ET1 may drive

vascular NADPH oxidase activity selectively in T2DM patients. Our findings suggest dysregulation of vascular IGF1R and ET1 pathways in T2DM, identifying them as therapeutic targets to prevent vascular complications in such patients.



Supported by: Sanofi Aventis Deutschland GmbH
Disclosure: **I. Akoumianakis:** Grants; Sanofi Aventis Deutschland GmbH.

1060

Transplantation of mobilised human mononuclear cells into nude rats improved diabetic peripheral neuropathy and nerve regeneration

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Background and aims: Current medicines for diabetic peripheral neuropathy (DPN) control not the mechanism but the symptoms. Stem cell therapy has shown regenerative effects on damaged cells from limb ischemia, and some animal stem cells have been also reported to have favorable effects on DPN. Therefore, we explored the effects of human mobilized mononuclear cells (hMNC) in rats with DPN and examined the mechanisms.
Materials and methods: hMNC were isolated and cryopreserved from a healthy volunteer after G-CSF mobilization. Sprague Dawley nude rats were induced to diabetes (DM) by streptozotocin injection and then 8 weeks later, 1 x 106 hMNC were transplanted into muscles of Rt. hind limb. Into the contralateral Lt. limb, normal saline was injected as a control. Before and at 4 weeks after transplantation, nerve conduction velocities (NCV) and laser-doppler perfusion imaging of both limbs were assessed. Immunohistochemical staining for PGP9.5 in the foot epidermis was performed to measure nerve regeneration. Expression of growth factors with angiogenic and neurotrophic properties were examined in the muscle by RT-PCR, and the grafts were monitored using 3T-MRI in vivo. Immortalized mouse Schwann cell line, IMS32 cells were cultured to examine effects of growth factors on myelination markers.
Results: The DM rats showed decreased NCV and perfusion compared to non-DM rats. NCV and perfusion improved in the hMNC-transplanted limbs compared to the saline-injected limbs in the DM rats. However, density of intra-epidermal nerve fibers was not significantly different between the limbs. According to MRI imaging, iron-tagged hMNC were observed as long as 5 weeks after transplantation, but the grafts didn't exist so long. mRNA expression of HGF in the hMNC treated muscle increased compared to that in the saline-injected muscle, and HGF treatment significantly enhanced Mpz transcript in IMS32 cells.
Conclusion: This study suggested that hMNC had a potential to improve DPN, and improved blood perfusion and induction of growth factors could be involved in the process.
Supported by: IRICT
Disclosure: **S. Min:** Grants; IRICT.

1061

Cardiac function and energy metabolism are preserved in diabetic mice overexpressing GTP cyclohydrolase I

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Background and aims: Diabetic cardiomyopathy is defined as the presence of ventricular dysfunction in the absence of underlying vascular disease or hypertension. Although its pathophysiology is not completely understood, mitochondrial dysfunction and decreased cardiac energetics are associated with impaired diastolic function in this condition. We have previously demonstrated in a murine model of type I diabetes (T1DM) that it is possible to prevent diastolic dysfunction by increasing the levels of tetrahydrobiopterin and nitric oxide (NO) via GTP cyclohydrolase I (GCH1) overexpression. Because NO activates glucose transport and may regulate metabolism and mitochondrial bioenergetics via S-nitrosation, the aim of our study was to test whether the cardioprotective effect of GCH1 was mediated by an improvement in cardiac energetics.
Materials and methods: The experiments were conducted in accordance with the Animals (Scientific Procedures) Act (UK, 1986). PCr/ATP ratio was measured in perfused hearts using 31P-NMR and oxygen consumption rate (OCR) of intact cardiomyocytes using a phosphorescent probe. Data was evaluated using 2-way ANOVA followed by Bonferroni's multiple comparison test.
Results: We induced T1DM in mice with specific cardiac overexpression of GCH1 (TG) and their wildtype (WT) littermates via multiple streptozotocin injections. WT diabetic mice presented decreased PCr/ATP ratio (1.32±0.1 vs 1.73 ±0.1, $p<0.05$, n=11 per group) and mitochondrial Creatine Kinase (CK) activity (1.56±0.1 vs 1.98 ±0.1, $p<0.05$, n= 10 per group) when compared with non diabetic WT mice, indicating impaired cardiac energetics. In contrast, TG diabetic mice were protected and maintained PCr/ATP ratio and CK activity comparable to non diabetic WT. The protection could not be explained by an increase in mitochondrial content, as the activity of citrate synthase enzyme and the level of mitochondrial voltage dependent anion channels were the same across the groups. Mitochondrial uncoupling protein 3 (UCP3) levels in the WT diabetic hearts were 2.5 fold greater compared to non diabetics ($p<0.001$, n=12 per group), indicating a switch towards fatty acid oxidation. However, TG diabetic mice did not show this increase, suggesting an avoidance of the cardiac metabolic inflexibility observed in T1DM. Moreover, TG diabetic hearts presented an increase in the protein expression of the insulin independent glucose transporter 1 (GLUT-1) compared to WT diabetic hearts (1.71±0.1 vs 1.28±0.1, $p<0.05$, n=12 per group). The OCR indicated a 30% increase in glucose utilisation in TG diabetic vs WT diabetic mice ($p<0.002$, n=7 per group), an effect that was inhibited by STF-31 (20 μmol/L), a specific GLUT-1 inhibitor. The increase in glucose utilisation in the TG vs WT (6.3±0.4 vs 4.9±0.4, $p<0.05$, n=7 per group) was mediated by NO, since the incubation with NOS inhibitor L-NAME (1mmol/L) abolished the differences between groups.
Conclusion: The specific cardiac overexpression of GCH1 preserves the function and energy metabolism of the heart in the presence of T1DM, in association with an improved glucose utilisation and metabolic flexibility. Our study gives an important contribution to the comprehension of the pathophysiology of diabetic cardiomyopathy, opening potential scenarios of new treatments.
Supported by: BHF
Disclosure: **S.F. Mafrici:** None.

1062

Vascular GLP-1 receptor expression is decreased under diabetic conditions: TCF7L2 is a possible regulator of GLP-1 receptor expression in artery

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Background and aims: It is known that incretin signal exerts anti-arteriosclerotic effects in vascular cells via GLP-1 receptor. We previously reported that GLP-1 receptor expression in the intima and media was down-regulated in obese human. On the other hand, GLP-1 receptor expression in pancreatic β-cells is reduced under diabetic conditions. The aim of this study is to clarify whether diabetic condition influences vascular GLP-1 receptor expression and which factor could regulate GLP-1 receptor expression in artery.
Materials and methods: We examined the biochemical data and artery wall thickening using 18-week-old male db/db mice compared to db/m mice. Excised thoracic artery was specifically collected, and vascular endothelial cells were cultured. Gene expression of various factors in the intima and media was analyzed by real time RT-PCR. Primer pairs encoding genes associated with GLP-1 receptor and factors related to arteriosclerosis were prepared. We exposed HUVEC to siRNA directed to TCF7L2 (siTCF7L2) or scrambled control siRNA and cultured for 24 hours. And real-time RT-PCR with Sybr Green was performed. Each gene expression was semi-quantified by the comparative Ct method with each result in β-actin as a control. We performed immunostaining using anti-GLP-1 receptor antibody in thoracic artery. These results were expressed as mean ± SE. A Wilcoxon test was used to test the difference between 2 groups with $p < 0.05$ regarded as significant.
Results: Arteriosclerosis index [(area of artery outer periphery - area of artery cavity) / area of artery outer periphery)] was significantly higher in db/db mice compared to db/m mice (db/db / db/m = 0.43 / 0.37). Between the db/db and db/m group, there were significant differences in body weight (37.8 ± 1.5 g, 31.4 ± 0.7 g), FBG (287.2 ± 6.6 mg/dl, 67.0 ± 47 mg/dl), insulin (1.35 ± 0.10 ng/ml, 0.34 ± 0.02 ng/ml), triglyceride (188.9 ± 13.1mg/dl, 118.9 ± 3.0 mg/dl), free fatty acid (1.45 ± 0.03 mEq/L, 1.08 ± 0.05 mEq/L), respectively ($p < 0.05$). GLP-1 receptor and TCF7L2 gene expressions in vascular endothelial cells were significantly lower in db/db mice compared to db/m mice. Immunostainig with antibody against GLP-1 receptor showed that GLP-1 receptor expression was significantly lower in endothelial cells and smooth muscle cells in db/db mice compared to db/m mice. Furthermore, siTCF7L2 decreased TCF7L2 mRNA levels by 71% compared with control and such reduction of TCF7L2 resulted in the downregulation of GLP-1 receptor gene expressions in HUVEC ($p = 0.0005$).
Conclusion: Vascular GLP-1 receptor was decreased in obese type 2 diabetes model mice. Since it has been reported that GLP-1 receptor expression is decreased by glucolipotoxicity in pancreatic β-cells, we think that vascular GLP-1 receptor expression is also decreased through the similar mechanism. Furthermore, it is likely that TCF7L2 is a regulator of GLP-1 receptor expression in artery as reported in β-cells.
Disclosure: **T. Kimura:** None.

1063

Exendin-4 induces autophagy via SIRT1/LKB1/AMPK pathway to protect vascular endothelial cells from oxidative stress damage

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Background and aims: Vascular endothelial cells (ECs) injury induced by oxidative stress plays a central role in the pathogenesis of diabetic vascular complications. Regulation of autophagy could protect ECs from oxidative stress damage. Exendin-4 has been found to exert direct beneficial effects on endothelial function in addition to its insulinotropic action. Whether autophagy is involved in the cytoprotective effect of Exendin-4 is unknown. The present study aims to investigate the effect of Exendin-4 on ECs autophagy and its impact on oxidative stress-induced ECs apoptosis.
Materials and methods: Cultured human aortic vascular ECs (HAECs) were divided into the following groups: control group, Exendin-4 group, H2O2 group and Exendin-4+H2O2 group. Autophagosomes were observed by electron microscopy. The apoptosis rate was evaluated by flow cytometry. The expression levels of LC3-II, p-62, RAb7, cleaved-caspase-3, Bcl-2, SIRT1, LKB1, p-LKB1, AMPK, p-AMPK, mTOR and p-mTOR were determined by western blotting. Localization of the target protein was detected by immunofluorescence.
Results: H2O2 promoted apoptosis of HAECs in a dose dependent manner. Exendin-4 markedly inhibited the H2O2-induced HAECs apoptosis ($p<0.05$) with decreased cleaved-caspase-3 level and increased Bcl-2 expression. Meanwhile, Exendin-4 significantly upregulated LC3-II expression and the number of autophagosomes with declined p-62 level and elevated Rab7 expression. Western blotting and immunofluorescence showed that Exendin-4 obviously increased the expression of SIRT1, promoted the transfer of LKB1 from the nucleus to the cytoplasm, activated cytoplasmic LKB1 and AMPK by phosphorylation, and reduced the expression of p-mTOR. Inhibition of autophagy with 3-MA or the SIRT1/LKB1/AMPK pathway with SIRT1 inhibitor nicotinamide could reduce the protective effect of Exendin-4 on the oxidative stress-induced HAECs apoptosis.
Conclusion: Exendin-4 could protect vascular ECs from oxidative stress-induced apoptosis through inducing autophagy via SIRT1/LKB1/AMPK pathway.
Supported by: NSFC(81261120566)
Disclosure: **X. Wu:** None.

1064

GLP-1 receptor independent effects of DPP-4 inhibition in mice with 5/6 nephrectomy

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Background and aims: Dipeptidyl peptidase (DPP)-4 inhibitors delay chronic kidney disease (CKD) progression in experimental CKD models. The underlying molecular pathways are not well defined so far. It is hypothesised that the renoprotective effects are mediated via the glucagon-like peptide (GLP)-1/GLP-1 receptor pathway. To test this hypothesis, we analysed the effects of the DPP-4 inhibitor linagliptin in preventing CKD progression in GLP-1 receptor knockout mice with 5/6 nephrectomy (5/6 Nx) or sham operation in comparison with linagliptin-treated wildtype mice with 5/6 Nx or sham operation.
Materials and methods: Placebo-treated wildtype and GLP-1 receptor knockout mice with 5/6 Nx served as controls. Mice were treated for 12 weeks.
Results: Plasma DPP-4 activity was decreased in all linagliptin-treated groups. Blood pressure was similar in all groups and not affected by linagliptin treatment. Plasma concentrations of active GLP-1 and active gastric inhibitory polypeptide increased substantially in all linagliptin-treated groups. It is of note that the increase in active GLP-1 levels after linagliptin treatment was more pronounced in the GLP-1 receptor knockout mice compared with wildtype mice. In GLP-1 receptor knockout mice and wildtype mice, 5/6 Nx led to the development of renal interstitial fibrosis, increased cystatin C concentrations, and increased urinary albumin excretion. Treatment with linagliptin abolished the development of interstitial fibrosis, the increase in cystatin C concentrations, and the

increase in urinary albumin excretion. Compared with non-treated GLP-1 receptor knockout control mice, we detected using mass spectrometry 197 altered peptide signals following linagliptin treatment in GLP-1 receptor knockout mice with 5/6 Nx.
Conclusion: The beneficial renal effects of linagliptin in mice with 5/6 Nx are independent of the GLP-1/GLP-1 receptor pathway. Sequencing of the differentially regulated peptides detected by mass spectrometry will provide insights into the underlying GLP-1 receptor independent renoprotective mechanisms of linagliptin.
Supported by: Boehringer Ingelheim
Disclosure: **B. Hocher:** Grants; Boehringer Ingelheim.

PS 111 Usual and unusual associations in diabetes complications

1065

Poor glycaemic control is related to excess risks of hospitalisation for infection in patients with type 2 diabetes: the Hong Kong Diabetes Registry

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Background and aims: Infections occur more commonly in people with diabetes compared to the general population, and are linked to impaired immune response and comorbidities. Large-scale studies that examined the relationship of glycaemic control with infection are limited, and results from available literatures are conflicting. We evaluated the risk association of HbA1c with hospitalisation for infection in a prospective cohort of Chinese adults with type 2 diabetes in Hong Kong.
Materials and methods: Between 1 July 1994 and 31 May 2015, 22,978 patients underwent detailed assessment of metabolic control and diabetes complications. Patients were followed for occurrence of infection requiring hospitalisation as identified using discharge diagnosis coded by International Classification of Disease, Ninth Revision. Multivariate Cox regression model with time-dependent HbA1c was constructed to estimate the hazard ratio (HR) of HbA1c with incident infection.
Results: Over a median follow-up period of 4.8 (interquartile range: 2.8, 10.3) years, 4,671 (20.3%) patients were hospitalised for any infection types, with respiratory infection (n=2,180), urinary tract infection (n=1,079), skin infection (n=970) and gastrointestinal tract infection (n=625) being the most common sites. Patients who were hospitalised for infection were older, of female predominance, and had longer duration of diabetes. Baseline HbA1c, blood pressures and total cholesterol were higher but body mass index (BMI) lower in keeping with older age and longer disease exposure in this group, who also had higher frequencies of micro- and macrovascular complications. In multivariate Cox regression model, time-dependent HbA1c was associated with any infection with HR 1.08 (95% confidence interval [CI]:1.06-1.10, $p<0.001$), adjusted for age, gender, diabetes duration, smoking status, BMI, estimated glomerular filtration rate, and history of cardiovascular diseases. When infection site was considered separately, HbA1c remained significantly associated with urinary tract infection (HR 1.08 [95% CI:1.02-1.13], $p<0.001$) and skin infection (HR 1.18 [95% CI:1.14-1.22], $p<0.001$) but not respiratory or gastrointestinal tract infection. Given that age is a risk factor for infection, we further examined whether the association of HbA1c with infection varied with age by including an interaction term of HbA1c and age in Cox regression model. Adjusted for co-variables, the interaction term was related to incident infection of any sites with HR 0.99 (95% CI:0.99-0.99, $p<0.001$) indicating that the association between HbA1c and infection was greater in younger compared to older age group.
Conclusion: Poor glycaemic control is associated with increased risk of serious infection in particular infection of urinary tract and skin. The risk relationship between HbA1c and infection is more prominent in younger than older patients.
Supported by: Li Ka Shing Institute of Health Sciences
Disclosure: **A.O.Y. Luk:** None.

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Plasma clusterin and the CLU gene rs11136000 variant are associated with mild cognitive impairment in type 2 diabetic patients

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Background and aims: Type 2 diabetes mellitus (T2DM) is related with an elevated risk of mild cognitive impairment (MCI). Plasma clusterin was reported associated with the early process of alzheimer's disease (AD) pathology and longitudinal brain atrophy in subjects with MCI. The rs11136000 SNP within the CLU gene was also associated with the risk of AD. We aimed to investigate the associations between plasma clusterin, rs11136000 genotype and type 2 diabetes-associated MCI.
Materials and methods: A total of 231 T2DM patients, including 126 MCI and 105 cognitive-healthy controls enrolled in this study. Demographic parameters were collected and neuropsychological were conducted. Plasma clusterin and CLU rs11136000 genotype were examined.
Results: Plasma clusterin was significantly higher in MCI patients than control group ($p = 0.007$). In subjects with MCI, plasma level was negatively correlated with Montreal Cognitive Assessment (MoCA) and Auditory Verbal Learning Test_delayed recall (AVLT_DR) scores ($p = 0.027$; $p = 0.020$, respectively). After adjustment for age, education level, and gender, rs11136000 TT genotype carriers have a reduced MCI risk than CC genotype carriers ($p = 0.043$). Multivariable regression model showed that education years, diabetes duration, HDL-c, and plasma clusterin levels are associated with prevalence of MCI in T2DM patients.
Conclusion: Plasma clusterin was associated with the presence of MCI and may reflect a protective response in T2DM patients. A beneficial effect of TT genotype on MCI may exist. Further investigation should be conducted to determine the role of clusterin during the process of cognitive decline.
Clinical Trial Registration Number: ChiCTR-OCC-15006060
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Disclosure: **S. Wang:** None.

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Incidence of urinary tract and genital infections among patients with and without diabetes

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Background and aims: Type 2 diabetes (T2DM) is associated with increased risk of infection. We aimed to compare the incidence of urinary tract infections (UTI) and genital infections (GI) among a T2DM cohort and a matched comparison group without T2DM.
Materials and methods: We included T2DM patients from electronic medical records (EMRs) of Kaiser Permanente Northwest during a 7-year window (2006-2012). The cohort included 39,301 people with T2DM, of which 23,067 had their diabetes identified prior to 2006 (prevalent cases) and 16,234 were first identified during the 7-year window (incident cases). Those with a diabetes recognition date prior to 2006 were assigned an index date of 1 January 2006. For cases identified on or after that date, the actual diagnosis date was considered the index date. We matched the 39,301 non-T2DM patients matched on age, sex, index year, and availability of a serum creatinine measurement in the index year (to account for health and propensity to use services). Non-T2DM patients were assigned the same index date as their matched T2DM patients. Patients were followed from index date through 2014, or until death or disenrollment. We calculated incidence per 1,000 person-years of UTI by first excluding anyone who had a UTI diagnosis 6 months prior to their index date and similarly calculated GI incidence among those without a GI diagnosis 6 months prior to index date. We adjusted incidence rates for age, sex, race, chronic kidney disease, and diuretic use.
Results: Incident T2DM patients had a mean age of 58.3 (SD 12.7) years; 48% women. Mean age of prevalent T2DM patients was 61.6 (12.6); 48% women; mean diabetes duration of prevalent cases at index date was 5.8 (4.7) years. As shown in the table, incident T2DM patients had a UTI rate of 49.9/1,000 person-years (PY; 95% CI 48.1-51.7), age/sex matched non-T2DM patients, the rate ratio was 1.16 (1.11-1.22). GI incidence was 66.1 (64.0-68.2) among incident T2DM (rate ratio 1.14, 1.09-1.19). Incidence of UTI and GI was higher among prevalent T2DM cases. UTI incidence was 56.4 (55.0-57.8) with a rate ratio of 1.28 (1.23-1.32), and incidence of GI was 69.5 (68.0-71.1) with a rate ratio of 1.25 (1.21-1.29). Incidence of UTI and GI was strongly associated with female sex and presence of chronic kidney disease.
Conclusion: UTI and GI are common among T2DM and occur at higher rates compared with age/sex matched non-T2DM patients. Risk of these conditions is elevated among T2DM cases and becomes greater with longer diabetes duration.

Figure. Incidence of Urinary Tract and Genital Infections among Incident and Prevalent Cases of Diabetes and Age/Sex Matched Non-Diabetic Comparison Subjects

	UTI		GI	
	T2DM	No T2DM	T2DM	No T2DM
Incident T2DM				
Incidence/1,000 p-y	49.9	42.9	66.1	57.8
95% CI	48.1 - 51.7	41.3 - 44.5	64.0 - 68.2	55.9 - 59.8
Rate Ratio (95% CI)	1.16 (1.11 - 1.22)		1.14 (1.09 - 1.19)	
p value	<0.001		<0.001	
Prevalent T2DM				
Incidence/1,000 p-y	56.4	44.2	69.5	55.7
95% CI	55.0 - 57.8	43.0 - 45.4	68.0 - 71.1	54.4 - 57.1
Rate Ratio (95% CI)	1.28 (1.23 - 1.32)		1.25 (1.21 - 1.29)	
p value	<0.001		<0.001	

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Chronic disease hypogonadism not age-related: hypogonadism in diabetic male patients

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Background and aims: Hypogonadism is common in old age and in chronic conditions such as diabetes. Hypogonadism may change body composition, increases insulin resistance, deteriorates metabolic control, induces dyslipidaemia, precipitates heart disease and increases bone frailty. Hypogonadism interferes with sexual life, changes behaviour and performance and decreases the quality of life. Assessing gonadic function is therefore part of the medical care of diabetic patients. We report results regarding gonadic function in diabetic male patients assisted at a tertiary out patient medical center.
Materials and methods: All diabetic male patients assisted by a single doctor at a tertiary out patient center were included. Besides the usual care, total testosterone, FSH, LH, estradiol, SHBG and prolactin were obtained in a morning blood sample. Clinical and analytical data were introduced in a specific database. Chi-square, T-student test and Anova were used to compare groups and the relation between continuous variables was explored with multiple regression analysis.
Results: The database comprised 614 patients, including 327 (53%) male patients. Gonadic function was assessed in 127 (39%) of such male patients. Patients were either type 1 (18%) or type 2 (72%) diabetes mellitus. They were old (60±15 years), overweight (28.4±4.8 kg/m2), with long standing disease (18±11 years) and fair metabolic control (7.9±1.5). Microvascular disease was common (30-50%) as well as high blood pressure (76%) and dyslipidaemia (56%).Testosterone values were not normally distributed - 359±185 ng/dL [19 - 1467 ng/dL]. Seventy-five patients (59%) presented evidence for hypogonadism, being serious (<240 ng/dL) in 29 (23%) and mild (<360 ng/dl) in 46 (36%). Hypogonadism was almost always central (normal FSH and LH < 10U/L) (92%). This was true regarding serious hypogonadism (86%) and even more so regarding mild hypogonadism (96%). Testosterone values were not

significantly related to age, were not significantly different in patients under or over age 50 years, and the frequency of hypogonadism was also not significantly different in those over or under 50 years. Total testosterone was inversely related to BMI (r=-0.250, p<0.01) and to metabolic control (r=-0.153, p<0.1). Total testosterone levels were significantly lower in those with peripheral neuropathy than in those without (299±100 vs 384±206, t=2.440, df=125, p<0.05) as well in those without high blood pressure (334±135 vs. 434±279, t=2.679, df=125, p<0.01).

Conclusion: Hypogonadism may adversely affect metabolic control and cardiovascular risk in diabetic patients and decreases the quality of life. We found a very high prevalence of hypogonadism in diabetic patients, either mild or severe. This very common hypogonadism was almost always central and not age-related. Testosterone levels were inversely related to obesity, metabolic control, high blood pressure levels and the presence of peripheral neuropathy. Hypogonadism in diabetic patients is a chronic condition associated state and the presence of diabetes overrides the relevance of age as a determinant factor of testosterone levels. Hypogonadism in diabetic patients is related to body composition, metabolic control and cardiovascular disease.

Disclosure: **A.F. Martins:** None.

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Association of autoimmune thyroiditis with sarcopenia in patients with type 1 diabetes

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Background and aims: Aging is an inevitable phenomenon. The age-associated decrease of muscle mass and function is called sarcopenia. Sarcopenia has been reported to be associated with disabilities, mobility disorders, fractures, and increased risk of death. Owing to the increase in the elderly population in the world, sarcopenia has had an increasing impact on healthcare. Some studies reported an association between sarcopenia and insulin resistance in patients with type 2 diabetes. However, only a few studies have evaluated sarcopenia in patients with type 1 diabetes. In this study, we focused on autoimmune thyroiditis, which sometimes co-occurs with type 1 diabetes, and evaluated any association of autoimmune thyroiditis with sarcopenia in patients with type 1 diabetes.

Materials and methods: We examined 43 patients with type 1 diabetes in this cross-sectional study. Regional muscle mass was measured by using dual-energy X-ray absorptiometry. Sarcopenia was defined on the basis of the International Working Group on Sarcopenia criteria, with a cut-off of muscle mass for men and women of 7.23 kg/m2 and 5.67 kg/m2, respectively. All the patients were tested for free T4 (FT4), thyroid stimulating hormone (TSH), thyroglobulin antibody (TgAb), peroxidase antibody (TPOAb), HA1c, and GAD autoantibodies (GADAb). Type 1 diabetes was defined using the American Diabetes Association criteria.

Results: Nineteen of the 43 patients were diagnosed with sarcopenia. The mean age of patients with and without sarcopenia was 67.9 and 68.2 years, respectively. Both TgAb and TPOAb levels were higher in patients without sarcopenia (TgAb: 84.22 ± 26.18 IU/ml vs. 18.24 ± 4.47 IU/ml, respectively; p = 0.008; TPOAb: 76.09 ± 33.06 IU/ml vs. 25.94 ± 13.24 IU/ml, respectively; p = 0.034). However, there were no significant differences in FT4 levels between patients with and without sarcopenia (1.02 ± 0.03 ng/dl vs. 1.16 ± 0.12 ng/dl, respectively; p = 0.44) or TSH (1.63 ± 0.19 μIU/ml vs. 2.25 ± 0.42 μIU/ml, respectively; p = 0.75), indicating no difference in thyroid function. There were also no significant differences between patients with and without sarcopenia in HbA1c (8.26 ± 0.29% vs. 8.29 ± 0.35%, respectively; p = 0.73), GADAb (46.17 ± 29.30 U/ml vs. 110 ± 40.48 U/ml, respectively; p = 0.23), onset of diabetes (50.32 ± 4.26 vs. 51.57 ± 2.67, respectively; p = 0.70), duration of diabetes (17.58 ± 2.82 vs. 18.79 ± 3.66, respectively; p = 0.85), or dose of daily insulin (17.58 ± 2.82 U/day vs. 29.96 ± 3.58 U/day, respectively; p = 0.09).

Conclusion: In this study, we found that patients with type 1 diabetes with high levels of TgAb and/or TPOAb are at low risk for sarcopenia. The combination of autoimmune thyroiditis and type 1 diabetes is one of the features of autoimmune polyendocrine syndrome. There might be genetic factors correlating sarcopenia to type 1 diabetes, because autoimmune polyendocrine syndrome is related to gene mutations and HLA type. Further study is required to elucidate the mechanism of sarcopenia in type 1 diabetes.

Disclosure: **T. Takahashi:** None.

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Role of the "resistin pathway" on cardiovascular risk factors and major cardiovascular events

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Background and aims: Insulin resistance (IR) and low grade inflammation are prime movers in the development of cardiovascular disease (CVD). Among other cytokines linked to both conditions is resistin, whose levels have been associated with atherosclerosis and related clinical phenotypes, including major cardiovascular events (MACE). Recently, the existence of a "resistin pathway" affecting the expression of other cytokines, including IL-1β, IL-6, IL-8, IL-12 and TNF-α, has been proposed. Whether such deleterious effect is exerted only by resistin itself, or, in contrast, is due also to the above-mentioned resistin induced cytokines belonging to the "resistin pathway" has never addressed. Thus, our aims were: i) to explore the plausibility of an in vivo "resistin pathway" by testing in both serum and blood cell mRNA from healthy, non-diabetic, individuals the correlations between resistin levels and those of IL-1β, IL-6, IL-8, IL-12 and TNF-α; ii) to create a multi-marker "resistin pathway" score, both from serum (sRPS) and mRNA data (eRPS) and to test the association with several IR-related cardiovascular (CV) risk factors (i.e. BMI, waist circumference, HOMAIR, triglycerides, HDL-cholesterol, systolic and diastolic blood pressure) in the same individuals; iii) to test the association between sRPS and incident MACE in diabetic patients with established coronary artery disease (CAD), followed over time.

Materials and methods: Serum cytokines levels were measured by using commercial ELISA or multiplex detection kits in 280 unrelated healthy blood donors with a wide range of BMI from the Gargano study 2 (GS2) and in 353 diabetic patients with CAD from the Gargano Heart Study (GHS)-prospective design (follow-up 5.4±2.5 years; 71 MACE). Cytokines expression in white blood cells were also measured in GS2 by RT-PCR.

Results: In GS2 participants, serum resistin was strongly and significantly associated with all serum cytokines (p values ranging 3.0x10-4-4x10-24), but IL-12. Also resistin expression level was significantly correlated (p values ranging 1.1x10-4-3.5x10-14) with those of all cytokines, but IL-12B, thus supporting the existence in vivo of a "resistin pathway", not comprising IL-12. In order to study serum and expression levels of the "resistin pathway" as a whole, both a sRPS and an eRPS were created by summing the estimate of each cytokine obtained by fitting the multivariable regression models for outcomes of interest. In GS2 individuals: a) sRPS and eRPS were correlated (r2=0.024, p=0.034); b) sRPS was associated with all IR-related cardiovascular risk factors (p=3.x10-2-6x10-4). Also eRPS was associated with all IR-related CV risk factors, but systolic blood pressure (p=2.x10-2-7x10-5). In the GHS-prospective design, for each SD increase of sRPS, the HR (95% CI) for MACE was 1.6 (1.3-2.0). This association remained significant (p=7x10-6) after adjusting for sex, age, smoking habits and BMI.

Conclusion: We here report that a) serum resistin is correlated with serum IL-1β, IL-6, IL-8 and TNF-α; b) such correlations are, at least partly regulated at transcriptional level; c) all these cytokines exert a joint effect on several IR-related CV risk factors in healthy individuals and on MACE

in patients with T2D and CAD. Taken together, our study points to the existence in vivo of a "resistin pathway" that plays a deleterious role on CVD in high risk patients.

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Disclosure: **C. Menzaghi:** None.

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Polymorphisms in the gene promoters of IL4, IL6, IL10 and TNFA associated with serum levels of cytokines in type 2 diabetic subjects

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Background and aims: A growing body of evidence indicates that chronic low-grade inflammation is involved in the pathogenesis of diabetic vascular complications. Although the mechanisms of enhanced inflammatory response in diabetes have not been precisely defined yet, the role of immunogenetic factors could be supposed. The aim of the study was to assess the associations of single nucleotide polymorphisms (SNPs) in the gene promoters of IL4, IL6, IL10 and TNFA with serum levels of cytokines in type 2 diabetic subjects.

Materials and methods: We studied 145 Caucasoid patients with type 2 diabetes, 112 F / 33 M, from 43 to 70 years of age, HbA1c 8.1, 7.2 - 9.6% (median, 25th-75th percentile). Seven SNPs, including IL4: -590 C/T (rs2243250), IL6: -174 G/C (rs1800795), IL10: -592 A/C (rs1800872) and -1082 A/G (rs1800896), TNFA: -238 A/G (rs361525), -308 G/A (rs1800629) and -863 C/A (rs1800630), were revealed by real-time PCR. The levels of IL-6 and TNF-α were assessed by Multiplex analysis, concentrations of IL-4 and IL-10 were determined by ELISA and compared to control (20 healthy subjects matched by sex and age).

Results: Serum level of IL-6 was elevated and IL-4 level was decreased in observed diabetic patients compared to controls (53.3, 30.4 - 99.5 vs. 28.4, 15.2 - 113.2 pg/ml, p=0.04; 0.38, 0.2 - 0.55 vs. 0.72, 0.43 - 0.84 pg/ml, p=0.005, respectively). Concentrations of TNF-α and IL-10 demonstrated a tendency to increase in diabetic group (29.6, 19.3 - 64.4 vs. 23.2, 12.1 - 39.1 pg/ml, p=0.07; 2.05, 1.74 - 2.58 vs. 1.68, 1.58 - 2.26 pg/ml, p=0.09 respectively). The IL6 (-174) GG genotype was associated with higher IL-6 levels as compared to CC and CG genotype (p=0.01 and p=0.004). The TNFA (-863) CC and CA carries had higher TNF-α levels compared to those with AA genotype (p=0.04). The concentration of IL-10 was lower in patients with IL10 (-592) CC compared to AA genotype (p=0.01). The level of IL-10 exceeded control in patients with IL10 (-592) AA genotype only (p=0.02). The presence of CC at IL4 -590 position was associated with lower IL-4 concentration (p=0.02). Other SNPs did not show associations with appropriate cytokine levels.

Conclusion: The SNPs in the promoters of IL4 (rs1800795), IL6 (rs1800795), IL10 (rs1800872) and TNFA (rs1800630) can affect the levels of pro-inflammatory and anti-inflammatory cytokines in type 2 diabetic subjects.

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Disclosure: **N.V. Tyan:** None.

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Creating phenotypic clusters for the purpose of identifying a personalised HbA_{1c} target in patients with type 2 diabetes

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Background and aims: Personalized HbA1c targets may serve to improve treatment guidelines and outcomes. No previous study has created patient clusters for the purpose of predicting future risk of disease in correlation with underlying HbA1c. The purpose of this longitudinal study was to cluster patients with T2DM into phenotypically similar risk groups.

Materials and methods: We used an electronic medical database from an integrated health care system, serving over 4.3 million members. We created a cohort of 85,783 patients with a 3-7 year history of T2DM as of 1 January 2010 (index date). We trained two logistic regression models, adjusted for demographic, laboratory, and clinical profiles, to predict the relative prevalence of macrovascular complications or hypoglycaemia as of index date. This process yielded four supervised clusters: low/low, high/low, low/high, and high/high risk of macrovascular complications or hypoglycaemia, respectively.

Results: The macrovascular complications model had an area under the curve (AUC) of 0.81 (sensitivity 75.5%, specificity 71.8%). The hypoglycaemia model had an AUC of 0.73 (sensitivity 59.7%, specificity 75.1%). The first cluster contained 49,182 patients with mean age 57.9 with low rates of pre-existing co-morbidities. The second cluster contained 21,970 patients with mean age 69.7 who were predominantly male with high Framingham score. The third cluster contained 6,054 patients with mean age 64.1 who were predominantly female with high rates of diabetes-specific complications, and the highest rate of insulin use. The fourth cluster contained 8,577 patients with average age 71.6, high rates of anaemia, and high rates of both cardiac outcomes and insulin use.

Conclusion: Supervised clustering can successfully create phenotypically distinct groups of patients who may benefit from different guidelines, with adequate sample size and follow up. Longitudinal cohorts can serve to correlate observed risk of future outcomes with underlying HbA1c in order to model an optimal personalized target HbA1c.

Table 1: Demographic Characteristics of Cohort as of 1 Jan 2010

Characteristics	Cluster A (L/L)	Cluster B (H/L)	Cluster C (L/H)	Cluster D (H/H)
Total (N)	49,182	21,970	6,054	8,577
Age (mean, SD)	57.9, 12.7	69.7, 12.7	64.1, 14.3	71.6, 11.4
Male Sex (n,%)	19970 (40.6%)	15738 (71.6%)	837 (13.8%)	4109 (47.9%)
Purchase of fast-acting insulin (n,%)	343 (0.7%)	68 (0.3%)	598 (9.9%)	811 (9.5%)
Presence of Anaemia (n,%)	6867 (14.0%)	5369 (24.4%)	1418 (23.4%)	3690 (43.0%)
Diabetic Neuropathy (n,%)	5754 (11.7%)	2854 (13.0%)	3104 (51.3%)	4017 (46.8%)

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Disclosure: **M. Leventer-Roberts:** Grants; Funding for this study was provided by Eli Lilly and Company.

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Diabetes is an independent cause of relative hypoxia in heart failure

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Background and aims: Although it is well known that diabetes worsens the prognosis of heart failure, the factors that actually determine this conditions are poorly understood. Hypoxia is a frequent complication in heart failure: whether is due to decrease in cardiac output, respiratory abnormalities or respiratory control instability (Cheyne-Stokes respiration) its presence is a well recongised worsening factor, which contributes to sympathetic activation and negative prognosis. Recent studies carried out in small number of subjects suggested that diabetic patients without heart failure have reduced oxygen saturation. We then hypothesised that diabetes could represent an independent cause of hypoxia in heart failure.
Materials and methods: In 582 consecutive patients with heart failure, 450 without diabetes (59.2±10.1yr, 381 male), and 132 with type 2 diabetes (61.3 ±8.4yr, 118 male) we evaluated the mean, minimum, maximum oxygen saturation (SaO2) and its variability (SaO2 standard deviation [SaO2-SD]) during a 20 minute monitoring in supine resting conditions. We also evaluated the possible SaO2 determinants (New York Heart Association [NYHA] class, left ventricular ejection fraction [LVEF], creatinine, brain natriuretic peptide, peak oxygen consumption [VO2peak]), obtained within the previous 7 days. Data were analysed by t-test comparisons and multiple regression analysis.
Results: SaO2 measures were all significantly reduced in diabetic as compared to non-diabetic patients (mean: 95.18±2.46% vs 95.84 ±2.01%, p=0.002, minimum: 91.87±4.13% vs 93.12±3.47%, p=0.0005, maximum: 97.09±2.14% vs 97.49±1.81% p=0.03), whereas SaO2-SD was significantly increased (1.15±0.96% vs 0.92±0.80%, p=0.005). Diabetic patients were significantly older, had higher creatinine levels, and lower hemoglobin and cholesterol levels (p<0.02 for all). In a subset of 309 patients submitted to cardiopulmonary stress test, diabetic patients also had significantly lower VO2peak (p<0.0001). When multiple regression analysis was carried out to assess the association between SaO2 (mean and min) and clinical variables we found that diabetes, age, body mass index, LVEF, creatinine, were significant and independent predictors while sex was significantly associated only with mean SaO2.
Conclusion: Diabetes per se lowers SaO2 in heart failure as an independent factor. The seemingly small difference observed, still in the normoxic range (relative hypoxia), is nevertheless important as it underlines a much greater difference in arterial oxygen pressure, due to the s-shaped haemoglobin dissociation curve. The increased SaO2-SD and the lower minimum SaO2 in patients with diabetes indicate a higher respiratory instability and higher risks of periodic breathing, a well known negative prognostic factor in heart failure. These results demonstrate that diabetes per se leads to a condition of relative hypoxia. Relative hypoxia appears a previously unrecognised cause whereby diabetes is a worsening factor in heart failure.
Disclosure: **L. Bernardi:** None.

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Effect of sarpogrelate, a selective 5-HT$_{2A}$ receptor antagonist, on characteristics of coronary artery disease in patients with type 2 diabetes

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Background and aims: Sarpogrelate, a 5-hydroxytryptamine type 2A antagonist, is an antiplatelet agent. It may be a potential agent in treatment of macrovascular complication in diabetes. We performed a prospective interventional study to evaluate the effect of sarpogrelate compared with aspirin in Korean diabetic patients with subclinical coronary atherosclerosis.
Materials and methods: Forty diabetic patients (26 men, ages 58.6 ± 6.8 years) who had mild to moderate atheroma evaluated with coronary multidetector-row CT (MDCT) were randomly assigned to either sarpogrelate 300 mg/day plus aspirin 100 mg/day (SPG+ASA group) or aspirin 100 mg/day only (ASA group) (n = 20 each) for 6 months. Coronary artery calcium score (CACS) and coronary artery stenosis and plaque volume were investigated. Primary outcome was change of coronary artery disease assessed by coronary MDCT. Secondary outcomes included change in risk factors of atherosclerosis such as glucose and lipid metabolism and subclinical atherosclerosis assessed by ankle-brachial index and pulse wave velocity.
Results: The CACS was decreased in SPG+ASA group (197.2 ± 231.3 to 188.7 ± 196.9) whereas it increased in ASA group (96.1 ± 152.5 to 99.1 ± 147.3), but differences were not statistically significant (p = 0.781 and p = 0.595, respectively). In both groups, there were a small insignificant decrease in maximal coronary stenosis (34.6 ± 19.5 to 33.3 ± 16.8%, p = 0.410 in SPG+ASA group and 30.9 ± 16.7 to 28.4 ± 13.6%, p = 0.279 in ASA group). The total plaque volume decreased from 82.4 ± 63.4 to 74.8 ± 63.2 mm^3 in SPG+ASA group whereas it increased from 62.4 ± 64.4 to 67.4 ± 70.0 mm^3 in ASA group and both were statistically significant (p = 0.009 and p = 0.001, respectively). Furthermore, in SPG+ASA group, non-calcified plaque volume decreased significantly (15.6 ± 19.8 to 11.8 ± 16.6 mm^3, p = 0.032) and calcified plaque volume also decreased but statistically insignificant (66.7 ± 58.8 to 62.9 ± 57.5 mm^3, p = 0.084). However, in ASA group, there was insignificant differences in non-calcified plaque volume (p = 0.968). There were no significant changes in glucose and lipid metabolism and subclinical atherosclerosis in both groups (p > 0.005 for all).
Conclusion: The present study demonstrates that sarpogrelate treatment decrease in atheromatous plaque volume in diabetic patients. Regression of atheroma was more prominent in non-calcified plaque than in calcified plaque. Longer studies with large scale are needed to validate these results.
Clinical Trial Registration Number: NCT02607436
Disclosure: **D. Lee:** None.

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Impact of glucose tolerance status on development of coronary artery disease among working aged men in Japan

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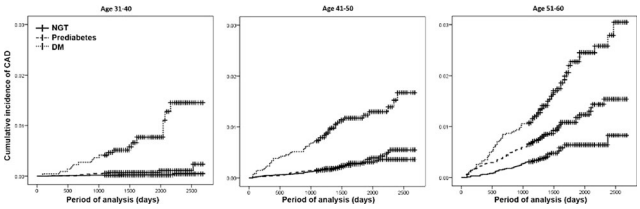
Background and aims: Coronary artery disease (CAD) during working age is a relevant and urgent issue because of not only its association to immature death but also enormous socioeconomic impact. Diabetes mellitus (DM) is well-known to play one of the most important roles for its development. However longitudinal study comparing its incidence and risk factors in comparison to those with normal glucose tolerance is scarce. Moreover, very little is known about risk of those with prediabetes. Therefore, we investigate the impact of glucose tolerance status on the development of CAD in the working age population.
Materials and methods: We retrospectively reviewed data on male employees in Japan who had data on health insurance claims provided by a Japanese big claim database instrument. Patients aged 31-60 years followed for at least 3 years from 1 April 2008 to 31 March 2012 were included and followed up to 31 July 2015. Among 168,676 men who had collected

data, we analysed data on 111,621 men, who were free of CAD at baseline and who also had data on health examination. Patients with coronary artery diseases were determined by both a claim for ICD-10 code I and medical practice for coronary artery disease and myocardial ischemia (percutaneous coronary intervention or coronary artery bypass graft). Cox proportional hazards regression model was used to identify variables related to the incidence of CAD. Unadjusted overall time to the development of CAD was described by Kaplan-Meier analysis with log-rank testing. Cox proportional hazards regression model was used to identify variables related to the incidence of CAD.

Results: The median follow-up period was 4.1 years. During the study period, 33, 172, 231 CAD events occurred in each age category. Cumulative incidence of CAD in each age groups according to glucose tolerance categories are shown in Figure, which demonstrates that the incidence of CAD in the DM group was significantly higher than that in the NGT or prediabetes groups (all p<0.01 DM versus NGT or prediabetes groups). DM was a significant factor that remained in multivariate analysis in all age categories, whereas prediabetes was associated with a 2.9 fold in aged 31-40 years, 0.9 fold in in aged 41-50 years, and 1.6 fold in aged 41-50 years increased risk for CAD, respectively. In multivariate analysis that exclude age as a covariate, the HR for CAD for DM aged 31-40 years and that for NGT aged 51-60 years was close (18.2 (7.15-46.4), 19.4 (8.28-45.4)). Similarly, the HR for CAD for DM aged 41-50 years was equal to the value for prediabetes aged 51-60 years (25.4 (10.9-59.2), 30.8 (13.5-70.4).

Conclusion: In conclusion, diabetes is an independent risk factor for CAD during working aged men. The risk for CAD for diabetes aged 31-40 years was equal to that for NGT aged 51-60 years. Clinicians may need to pay attention to those individuals at high risk for CAD.



Disclosure: **K. Fujihara:** None.

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Effects of lixisenatide on natriuretic peptides in patients with diabetes and acute coronary syndrome

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Background and aims: Some glucose-lowering medications affect the risk of heart failure in patients with diabetes. The glucagon-like peptide 1-receptor agonist lixisenatide did not significantly decrease hospitalization for heart failure (hazard ratio=0.96) or the rate of major cardiovascular events in the ELIXA trial (NCT#01147250). We evaluated the effect of lixisenatide on natriuretic peptides in that study.

Materials and methods: The ELIXA double-blinded trial included 6068 patients with type 2 diabetes and an acute coronary syndrome within the previous 180 days who were randomly assigned to receive lixisenatide or placebo in addition to standard of care. B-type natriuretic peptide (BNP, n=5435) and N-terminal pro-BNP (NT-pro-BNP, n=5481) were measured at randomization and at 24 weeks. Data were log-transformed for analyses.

Results: Geometric means for NT-pro-BNP at baseline were 37.6 and 38.0 pmol/L in the lixisenatide and placebo groups respectively, and decreased to 24.1 and 26.1 pmol/L at 24 weeks (p=0.001 for the comparison of change over time with lixisenatide vs placebo). Similar results were observed for BNP (103.9 and 105.9 mg/L at baseline and 80.3 and 85.6 mg/L at 24 weeks; p=0.011). When patients with a history of heart failure were analyzed in the lixisenatide and placebo groups, NT-pro-BNP values (n=1215) were 64.7 and 59.4 pmol/L at baseline and 45.0 and 46.1 pmol/L at 24 weeks (p=0.048), whereas BNP (n=1204) was 171.6 and 155.5 mg/L at baseline and 130.5 and 136.3 mg/L at follow-up (p=0.006). In those without prior heart failure, NT-pro-BNP concentrations were 32.1 and 33.5 pmol/L at baseline and 20.1 and 22.2 pmol/L at 24 weeks (p=0.006); BNP was 89.9 and 95.0 mg/L at baseline and 69.8 and 75.1 mg/L at follow-up (p=0.12).

Conclusion: Lixisenatide reduces NT-pro-BNP and BNP in patients with diabetes and an acute coronary syndrome within the previous 6 months.

Clinical Trial Registration Number: NCT01147250

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Disclosure: **J. Tardif:** Other; The study was funded by Sanofi.

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SIRT1 rs7896005 polymorphism may be related to coronary artery disease in Caucasians with type 2 diabetes, without affecting all-cause mortality

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Background and aims: SIRT1 exerts a number of effects involved in the aging processes and lifespan determination. More recently, SIRT1 has been claimed to be involved in cardiovascular risk as well. The SIRT1 gene is very polymorph with at least 21 SNPs so far identified. Three-four SNPs tag all 21 SNPs with rs10509291 and rs7896005 being nominally associated with type 2 diabetes (T2DM). Less information is available about the relationship between SIRT1 gene variants and diabetic complications.

Materials and methods: We have genotyped the rs7896005 (A/G; A is ancestral) SNP, which captures most of the variation across the SIRT1 locus, in 961 T2DM to investigate associations with: 1. the prevalence of micro- and cardiovascular (CVD) complications and 2. all-cause mortality over a median follow-up of 12.3 years (IQR 12.0-12.8).

Results: Genotypes distribution was: AA, n. 85 (8.8%); AG, n. 415 (43.2%); GG, n. 461 (48.0%). MAF was 0.3040 and Hardy-Weinberg equilibrium met (p=0.5394). About 10% of the samples were randomly selected for repeated assays with a results concordance of 100%. There was no difference across genotypes for age, diabetes duration (DD), BMI, fasting glucose and HbA1c, blood pressure and lipids, urinary A/C ratio and eGFR (CKD-EPI). Also, there were no differences in genders, active smokers, and family history for diabetes or CVD, prevalence of hypertension or dyslipidemia. GG was more frequent among subjects with peripheral polyneuropathy (54.8% vs. 45.8%; p=0.032, Kendall's test). A similar difference was numerically present between subjects with macro- vs. normo-albuminuria (61.8% vs. 47.3%; p=0.076), with no difference for retinopathy or CKD stage. Myocardial infarction (MI) and any coronary artery disease (CHD; not coronary revascularization procedures) were nominally more frequent in GG homozygous (6.9% and 16.9%, respectively) than in AG (4.1% and 13.3%) and AA homozygous (1.2% and 5.9%; p=0.011 and p=0.010, respectively). Consistently, peripheral

artery disease was more common in GG (16.3%) than in AG (11.8%) and in AA (8.2%; p=0.015), with no association with cerebrovascular events. By logistic regression analysis including male gender, age, DD, hypertension, dyslipidemia, retinopathy, A/C ratio strata and CKD stage, GG remained an independent predictor of MI (OR 7.376), and both GG and AG for any CHD (OR 4.102 and 2.865, respectively). Over the 12-year follow-up, all-cause mortality was 19.3% (195 out of 961; M: 124/565, 21.9%; F: 61/396, 15.4%) with no difference among genotypes: AA: 18/85, 21.2%; AG: 74/415, 17.8%; GG 93/461, 20.2% (p=0.633).

Conclusion: This study suggests that the rs7896005 A/G SNP of SIRT1 might play a role in the risk of coronary artery disease in T2DM, although no association was apparent with all-cause mortality. This results calls for larger association studies as well as studies to ascertain the mechanisms through which this genetic variant could result in increased CHD risk.

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Disclosure: **D. Lucchesi:** None.

1078

Insulin resistance in valvular interstitial cells: an in vitro model for type 2 diabetes in degenerative aortic valve disease

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Background and aims: Degenerative aortic valve disease (DAVD) is characterized by fibrosis and gross calcification in later stages. Type 2 diabetes (T2D) is one of the major risk factors for the development and progression of DAVD. However, underlying mechanisms are largely unknown. Although the incidence of T2D as well as DAVD increases, a reliable in vitro model of diabetic disorder in DAVD is missing. Simulation of hyperglycaemic conditions in combination with induction of insulin resistance in primary valvular interstitial cells (VICs) may be the first step for a better understanding of the impact of diabetes on valvular degeneration.

Materials and methods: VICs isolated from ovine aortic valves were cultured in hyperglycaemic medium (4.5 g/l DMEM, 10% FCS). To induce insulin resistance, the cells were treated every 72 hours with 100 nM insulin in presence or absence of 10 mM β-glycerolphosphate (β-GP), a compound inducing chondro-osteogenic transformation of VICs. After 13 d in differentiation medium, the cells were cultured for 4 h in fasting medium without FCS. Subsequently, a 10 min acute insulin stimulus (100 nM) was performed in one part of the cells to trigger the phosphorylation of AKT (Ser473). The other part of cells remained untreated as control for the basal state of AKT phosphorylation during cultivation. AKT and pAKT amounts were quantified by western blot analyses and normalized against β-tubulin amounts. Data were collected during n=3 independent experiments using VICs from different sheep and are expressed as fold of untreated VICs with acute insulin. Differences among the treatments were evaluated by one way ANOVA with Tukey's multiple comparisons test.

Results: The acute insulin stimulation of untreated VICs led to a significant increase of AKT phosphorylation in relation to the basal level of AKT phosphorylation without stimulation (0.025±0.005 fold of acute insulin stimulation, p<0.0001). Repeated insulin treatments during cell culture impaired insulin signaling by 63% at the level of AKT phosphorylation p<0.0001), which represents a reduced insulin sensitivity of VICs due to continuous hyperinsulinemia. Additionally, the treatment with β-GP reduced the AKT phosphorylation amount by 27% in comparison to untreated cells (p=0.0009), whereas a combination of chronic insulin and β-GP did not exceed the effect of insulin stimulation alone (0.42±0.06 vs. 0.37±0.07). The basal AKT phosphorylation level and the amount of unphosphorylated AKT were not influenced by any treatment modality tested here.

Conclusion: The adverse effect of T2D on the integrity of aortic valve has been shown by several clinical trials. However, mechanistic processes which lead to a chondro-osteogenic transformation of VICs under diabetic conditions are scarcely investigated. The stimulation protocol presented here demonstrates for the first time that primary VICs are sensitive to insulin and may become insulin resistant in response to continuous elevated insulin concentrations. Interestingly, chondro-osteogenic transformation is already sufficient to induce an evident decrease in the insulin response. Our model provides a unique tool to investigate the cellular mechanisms of DAVD in course of T2D under controlled conditions.

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Impaired energy metabolism, cardiac and endothelial function in the female type 2 diabetic Goto-Kakizaki rat heart following ischeamia-reperfusion injury

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Background and aims: Type 2 diabetic women have greater relative risk of morbidity and mortality by cardiovascular disease than non-diabetic women. Particularly, type 2 diabetes doubles the risk of myocardial infarction in women, but mechanisms involved are still not clear. Consequently, we have investigated the tolerance of type 2 diabetic Goto-Kakizaki (GK) female rat hearts to ischemia-reperfusion injury. We used a multiparametric approach allowing simultaneous measurement of energy metabolism, cardiac and endothelial function.

Materials and methods: 8-month-old female GK rats (n=15) and their age-matched respective Wistar Controls (n=12) were used for this study. Isolated rat hearts were perfused with 0.4 mM palmitate, 3% albumin, 11 mM glucose, 3 U/L insulin, 0.8 mM lactate and 0.2 mM pyruvate for 24 minutes before switching to 1.2 mM palmitate during 32 minutes low-flow (0.5 mL/min/g wet wt) ischemia. Next, flow was restored with 0.4 mM palmitate buffer for 32 minutes. High-energy phosphates and intracellular pH were measured during the experimental time course by 31P magnetic resonance spectroscopy with simultaneous measurement of contractile function. Coronary flow was measured before and after ischemia. Glucose and free fatty acids (FFAs) were measured in plasma. Nitric oxide and apoptosis pathways were studied by determination of eNOS, iNOS, Akt, PAkt and Caspase-3 protein expression in freeze-clamped tissues at the end of experiments. Creatine kinase and lactate dehydrogenase activities were also used as markers of myocardial damage.

Results: Glucose was significantly higher in GK versus Controls (p<0.0001) and FFAs were similar in both groups. Heart to body weight ratio was significantly higher in GK group versus Controls (p<0.0001) due to an increased heart weight (p<0.0001). Rate pressure product, index of cardiac performance, was significantly lower in GK before ischemia and during reperfusion (p<0.0001) compared with Controls. PCr, ATP and pHi were significantly decreased in GK during ischemia (p<0.05; p<0.001 and p<0.0001 respectively) and reperfusion (p<0.01; p<0.001 and p<0.01 respectively) compared with Controls. Coronary flow was lower before ischemia and during reperfusion in GK group versus Controls (p<0.05 and p<0.001). eNOS, Akt and PAkt protein expression in hearts were significantly decreased in GK compared with Controls (p<0.001; p<0.01 and p<0.0001 respectively). iNOS and Caspase-3 were not expressed in GK and Control hearts. Creatine kinase and lactate dehydrogenase activities were not different in both groups.

Conclusion: Female type 2 diabetic GK rat hearts exhibit greater sensitivity to ischemia-reperfusion injury, characterized by a decrease of cardiac function and coronary flow, associated with impairment of energy metabolism and nitric oxide pathway. These results may partly explain why type 2 diabetic women are more sensitive to cardiovascular diseases.

Disclosure: **N. Fourny:** None.

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Beta$_1$-, and beta$_2$-adrenoceptor responsiveness in the heart during type 2 diabetes

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Background and aims: Cardiac autonomic dysfunction is one of the common and serious complications of diabetes, and is one of the leading mechanisms of impaired function of the diabetic heart. Recently, we showed that the sympathetic drive to the heart was elevated in diabetes, but its β-adrenoceptor (AR) responsiveness was reduced. The specific contribution of the β_1- and β_2-AR subtypes to the autonomic function of the heart in diabetes is however unknown. The aim of this study was to investigate the functional responses of β_1- and β_2-AR subtypes in the type 2 diabetic heart.
Materials and methods: We used an *in vivo* and *ex vivo* approach in 20-week-old male Zucker Diabetic Fatty rats (diabetes) and their non-diabetic littermates (control)(total *n*=32). For the *in vivo* approach, rats under anaesthesia were instrumented with a pressure sensor in the abdominal aorta to measure arterial blood pressure and derive heart rate (HR), and with a venous access port in the femoral vein to administer drugs. The HR response to a non-specific β-AR agonist (isoproterenol (ISO), 0.1-300 μg/kg) was determined in conscious rats before and after β_1-AR blockade (atenolol, 2000 μg/kg). For the *ex vivo* approach, the maximum speed of contraction (+dP/dt$_{max}$) and relaxation (-dP/dt$_{max}$) were determined in isolated Langendorff-perfused control and diabetic hearts. The myocardial β-AR responsiveness was assessed by administration of a non-specific β-AR agonist (ISO, $1x10^{-10}$ to $3x10^{-8}$ mol/l), before and after specific β_1-AR blockade (CGP-20712A, $3x10^{-8}$ mol/l) or specific β_2-AR blockade (ICI-118,551, $5x10^{-8}$ mol/l).
Results: The β_1-AR HR responsiveness was increased *in vivo* in rats with diabetes, however the intrinsic *ex vivo* β_1-AR HR response was not different (ΔHR from baseline: *in vivo* at 300 μg/kg ISO: control 135 ± 24 vs. diabetes 205 ± 10 bpm, $p<0.05$; *ex vivo* at $3x10^{-8}$ mol/l ISO: control 101 ± 32 vs. diabetes 128 ± 24 mmHg, $p>0.05$, *n*=8 per group). The contractile (+dP/dt$_{max}$) and relaxation (-dP/dt$_{max}$) responses to non-specific β-AR stimulation were reduced in isolated diabetic hearts ($3x10^{-8}$ mol/l ISO: +dP/dt$_{max}$ control 7612 ± 491 vs. diabetes 6602 ± 321 mmHg/s; -dP/dt$_{max}$ control -4447 ± 262 vs. diabetes -3900 ± 174 mmHg/s; both $p<0.05$, *n*=8 per group). Specific β_1-AR blockade completely abolished the β-AR-induced changes in +dP/dt$_{max}$ and -dP/dt$_{max}$ in both control and diabetic hearts. Interestingly, specific β_1-AR blockade reduced the relaxation response in control hearts, and reduced both contractile and relaxation responses in the diabetic hearts.
Conclusion: Our combined *in vivo* and *ex vivo* approach shows that the β-AR-induced HR response is regulated by the β_1-AR subtype; a response increased in type 2 diabetic rats and related to adaptations extrinsic to the heart. In contrast, β-AR responsiveness of contractility and relaxation was reduced in type 2 diabetic hearts. This seemed to relate solely to the β_1-AR subtype because activation of β_2-AR did not increase contraction itself, however β_2-ARs did indirectly support β_1-AR subtype function in the diabetic heart. These findings show that the β_1-AR subtype remains an important target to modulate functional β-AR responses, but also suggest that the β_2-AR subtype might be an interesting novel target to improve contraction and relaxation of the diabetic heart. This knowledge is vital for development of human-based studies to target the complications in the growing cohort of diabetic patients with heart disease.
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Disclosure: **R.R. Lamberts:** Grants; OSMS Deans Bequest, OMRF Laurenson Award.

PS 113 Novel vascular risk markers

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Telomere length, vascular aging and glycaemic variability in patients with type 2 diabetes

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Background and aims: Glycemic variability (GV) may be involved in vascular aging in type 2 diabetes mellitus (T2DM) patients, but pathogenic mechanisms of this effect are not well established. It is known that telomere length (TL) shortening is a biomarker of cellular aging and associated with vascular changes. The aim of our study was to investigate the association between glycemic variability, vascular aging and telomere length in T2DM patients.
Materials and methods: The study group included 50 T2DM patients with mean age 58.4±7.9 years, median diabetes duration of 1.0 year and with HbA1c of 7.27 ± 0.69%. All subjects were measured for TL by quantitative PCR; oxidative stress marked by malondialdehyde; inflammation marked by C-reactive protein (CRP), fibrinogen; arterial stiffness evaluated by carotid-femoral pulse wave velocity (PWV); carotid intima-media thickness (IMT), plaque presence determined by ultrasonography in carotid arteries. The mean amplitude of glycemic excursion (MAGE), the standard deviation of blood glucose values (SD) and the continuous overlapping net glycemic action (CONGA) were calculated from continuous glucose monitoring system data for assessing GV. Statistical analyses were performed using SAS 9.1 (SAS Institute, Cary, NC, USA).
Results: The majority of patients with T2DM had subclinical atherosclerosis (78.1%) and shortened telomeres (less than the median of TL 9.75) (71.4%). Correlation analysis showed significant association between IMT and TL (r=-0.39, p=0.006), IMT and GV parameters: MAGE (r=0.42, p=0.007), SD (r=0.41, p=0.009), CONGA (r=0.27, p=0.093) and for MAGE this relationship remained significant in multiple linear regression analysis (β=0.040, p=0.020, multiple R2 = 0.290). Multiple linear regression analysis also revealed significant independent association between plaque presence and CONGA (β=1.088, p=0.040, multiple R2 = 0.457). It was not revealed relationship between vascular stiffness (PWV) and GV. TL was correlated only with CONGA (r=-0.31, p=0.054), but long telomeres (QIV, more than the TL 10.00) were associated with all GV parameters: MAGE (r=-0.39, p=0.015), SD (r=-0.43, p=0.007), CONGA (r=-0.42, p=0.008). Association GV (CONGA) and oxidative stress (malondialdehyde) was shown in correlation analysis (r=0.33, p=0.043). It was not revealed correlation between markers of chronic inflammation and GV.
Conclusion: Glycemic variability is associated with subclinical atherosclerosis and TL shortening in T2DM patients. Glycemic variability increases the oxidative stress. Thus we can suppose that oxidative stress caused by the glycemic variability plays a principal role in TL shortening and vascular aging in T2DM patients.
Disclosure: **N.V. Brailova:** None.

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Annual decline of TBPI is an independent predictor of cardiovascular events in Japanese type 2 diabetes patients

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Background and aims: Toe Brachial Pressure Index (TBPI), relating with Ankle Brachial Pressure Index (ABPI) is a noninvasive and convenient tool for evaluating peripheral arterial disease. Comparing to ABPI, it is still not common examination as an independent predictor of cardiovascular disease (CVD) in T2DM. TBPI can measure lower limbs arterial pressure more precisely than ABPI, since calcifications of the tibial artery have been often observed in T2DM. The role of TBPI as a predictor of CVD is still unclear.
Materials and methods: In a prospective study of 248 T2DM, we evaluated whether TBPI at baseline, or annual decline of TBPI observed for five years, were predictor of newly-occurred CVD events. Then T2DM with normal TBPI at baseline were selected, and divided into two groups by the mean value of TBI decline, compared and evaluated their clinical features. We excluded patients with severe diabetic neuropathy to avoid the sympathetic nerve dysfunction in evaluating blood pressure of the big toes.
Results: At baseline, mean age was 69.5±9.0 years (mean±SD), duration of diabetes 15.5±9.8 years, HbA1c 7.3±1.0%, prevalence of albuminuria 43.0%, retinopathy 52%, and TBPI 7.5±1.0. Baseline TBPI had no significant relationship with prevalence of CVD events (p=0.19). Mean value of annual TBI decline was -0.03 per a year. T2DM with a high rate of annual TBPI decline (H-TBPI: mean -0.052±0.01) had a higher prevalence of CVD events (25.9% vs 2.5%, p<0.01) than T2DM with low annual TBPI decline (L-TBPI: mean -0.010±0.01). As other clinical features, H-TBPI had a high prevalence of albuminuria (p<0.05) and history of hypertension (p<0.05) compared with L-TBPI. Annual decline of TBPI was an independent predictor of CVD events (p=0.03, odds ratio=1.4) after adjustment for age, duration, blood pressure, gender, albuminuria and retinopathy.
Conclusion: According to our study, annual decline of TBPI was an independent predictor of CVD events in T2DM whereas TBPI at baseline was not sufficient for predicting CVD events. Annual decline of TBPI may be useful marker for early detection of subclinical CVD.
Disclosure: **M. Furuta:** None.

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The visceral adiposity index predicts cardiovascular events both in coronary artery disease patients with and in coronary artery disease patients without diabetes

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Background and aims: The visceral adiposity index (VAI) is a validated tool for the evaluation of visceral adiposity, using waist circumference, serum triglycerides, age and gender to diagnose this metabolic abnormality. It has recently been associated with cardiovascular risk in primary care patients. Our aim was to investigate the association of the VAI with mortality in patients with established CAD.
Materials and methods: We calculated the VAI in 1472 consecutive patients with angiographically proven stable CAD according to the Amato formula. T2DM was defined according to the ADA definition. The incidence of vascular events was recorded over 10 years.
Results: At baseline, the VAI was significantly higher in CAD patients with T2DM than in those without diabetes (362±330 vs. 247±224; p <0.001). Prospectively, 539 vascular events occurred; the event rate were significantly higher in patients with T2DM than in those who did not have diabetes (44.8% vs. 33.7%; p<0.001). The VAI significantly predicted cardiovascular events in CAD patients with T2DM (standardized adjusted hazard ratio (HR) 1.16 [1.01-1.33]; p=0.037) as well as in those without T2DM (HR 1.14 [1.02- 1.27]; p=0.018).
Conclusion: We conclude that the VAI predicts cardiovascular events both in CAD patients with and in CAD patients without diabetes.
Disclosure: **D. Zanolin:** None.

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Evaluation of the creatinine uromodulin ratio as new serum marker for cardiovascular events

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Background and aims: Uromodulin, a protein exclusively produced by the kidney, has recently been demonstrated to be lower in subjects with declined renal function and the ratio creatinine/uromodulin has been proposed as a novel and superior kidney biomarker in serum of healthy subjects. Given the link between kidney function and cardiovascular disease, the prospective value of that new biomarker is thought to be of high clinical relevance but has not been addressed yet.
Materials and methods: We thus evaluated the association between serum levels of uromodulin with the presence of CAD and the cardiovascular risk in 529 angiographically characterized patients. Cardiovascular events have been recorded up to 8 years.
Results: Serum uromodulin concentration did not significantly differ between patients with and without angiographically determined CAD (165.4±78.9 vs. 164.2±75.3ng/ml ng/ml vs. p=0.934) but in patients with CAD, it correlated significantly and inversely with the extent of stenoses (r=-0.191, p=0.001). Apart from that, in the total population, there was a significant and inverse correlation with proBNP (r=-0.164, p=0.002). With respect to the full follow-up time of up to 8 years (6.6±1.8 years, mean±SD), first vascular events occurred in 27% of the study population. Applying the ratio creatinine / uromodulin in serum, we observed an adjusted HR of 1.27 [95%CI 1.10-1.47] and a p=0.001 with with age, sex, bmi, LDL- and HDL cholesterol, triglycerides, presence of CAD and hypertension, T2DM, and smoking status as well as statin treatment as covariates.
Conclusion: We conclude, that serum uromodulin in combination with serum creatinine is a novel predictive tool for cardiovascular event risk.
Disclosure: **A. Leiherer:** None.

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Hypoargininaemia predicts microvascular complications in type 2 diabetes

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Background and aims: Increased oxidative stress in diabetes increases nitric oxide (NO) oxidation and low L-arginine (Arg) could further reduce NO and impair vascular function, thus accelerating, in the long run, vascular complications. We therefore measured Arg and asymmetric dimethylarginine (ADMA) levels in patients with type 2 diabetes mellitus (T2DM) and healthy controls. Additionally, we followed the diabetic individuals over time to see if Arg and ADMA predicted T2DM complications.
Materials and methods: We examined baseline serum Arg and ADMA levels in a cohort of 105 subjects with type 2 diabetes and compared them to an age- and weight matched non-diabetic group of 137 individuals who served as a reference population. Additionally, we assessed whether or not Arg and/or ADMA predicted macro-and microvascular complications over 6 years of follow-up
Results: Serum Arg was lower in individuals with T2DM compared to controls (64±28 vs.75±31 μmol/l; p=0.009) and inversely related to HgbA1c (r=-0.2; p=0.002). Over follow-up, we observe that subjects with T2DM in the lowest quartile of Arg had increased risk for the subsequent evolution of nephropathy, peripheral neuropathy and

composite microvascular complications (OR 5.5; 95% CI-1.9-16; p=0.002). The highest ADMA quartile was associated with increased risk for both microvascular (OR=4.5; 95% CI-1.4-14.1;p=0.009) and 6.5 year incident macrovascular complications (OR=8.3; 95% CI 1.9-35.5; p=0.004).
Conclusion: Arg levels are lower in individuals with T2DM than in matched controls. Both low Arg and high ADMA, independent of each other and adjusted for classical risk factors predict the incidence of microvascular complications.
Disclosure: **T. Ganz:** None.

1086

Cation channels of the TRPC family contribute to development of nephropathy and retinopathy in the STZ model

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Background and aims: The pathogenesis of hyperglycemia-dependent diabetic longterm complications involves the accumulation of reactive metabolites which were recently shown to affect cation channels such as Nav1.8 and TRPA1 channels as targets and essential effector molecules in signaling pathways involved in the disease process. Likewise, other members of the Transient Receptor Potential (TRP) cation channel family including members of TRPC and TRPM family are regulated in their activity by reactive metabolites including reactive oxygen species (ROS). In this study we performed a screening using several TRPC- and TRPM-deficient mouse lines to elucidate a causal relevance of such cation channels for development of diabetic retinopathy and nephropathy.
Materials and methods: We used Nanostring based mRNA expression analysis method to determine mRNA levels of various TRPC channels. Mice were made hyperglycemic by Streptozotocin (STZ) treatment. Mice received 1 injection/day for 5 days with dosage of 60mg/kg KG. Mice were maintained at a glucose level range of 300-500 mg/dl by insulin glargine treatment. Markers for retinopathy and nephropathy were analyzed after 30 weeks of treatment. Measurements of glomerular filtration rates were done using FITC-labelled Sinistrin. Morphometric analysis of mesangial matrix was performed on PAS stained glomeruli (non treated WT n=5 and TRPC KO n=6; STZ treated WT and TRPC QKO, n=6 for each).
Results: Nanostring-based expression analysis revealed abundant expression of TRPC1, TRPC3, TRPC4 and TRPC6 in the mouse retina with TRPC6 being upregulated under diabetic conditions, and STZ treatment-induced increase of acellular capillaries was significantly reduced in mice lacking four of the seven TRPC cation channel proteins (TRPC QKO mice). Although TRPC QKO mice showed comparable levels of hyperglycemia (HBA1C measurements), the STZ treatment-evoked increase in mesangial matrix area was significantly reduced. Accordingly, measurements of glomerular filtration using FITC-labeled Sinistrin showed protection with regard to STZ-evoked hyperfiltration in TRPC QKO. Recent findings about the regulation of TRPC channels by reactive metabolites in primary mesangial and endothelial cells will be discussed.
Conclusion: In our study we have found that cation channels of the TRPC family essentially contribute to the development of diabetic long term complications in the STZ model possibly by interference with signaling pathways triggered by reactive metabolites.
Supported by: DFG Funding from SFB1118 (Sonderforschungsbereich 1118)
Disclosure: **R. Sachdeva:** None.

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Role of glycaemic variability in the changes in microcirculatory blood flow in patients with impaired glucose tolerance or type 2 diabetes

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Background and aims: Glycemic variability may play a role in diabetic microangiopathic complications. We recently showed that glycemic variability is more marked in non diabetic obese patients with slightly elevated HbA1c levels. We aimed to examine the relations between glycemic variability and microcirculatory cutaneous blood flow (CBF) in patients with impaired glucose intolerance (IGT) or type 2 diabetes (T2D).
Materials and methods: We included 34 patients, 19 with IGT (HbA1c 5.1±0.61%) and 15 with T2D on oral hypoglycemic treatments (HbA1c 7.1±0.7%), with normal blood pressure and free of cardio-vascular history. CBF (mean and standard deviation, SD-CBF) was measured by laser doppler (Periflux®) on the forearm during 3 minutes, one hour after a standard breakfast including 75g of carbohydrates. During the post-prandial period mean glucose was calculated and glycemic variability (standard deviation SD-glucose, CONGA and J-index) evaluated during 3 hours after breakfast using CGMS.
Results: Compared with IGT patients, T2Ds had higher mean glucose, CONGA and J-index, and lower mean CBF and SD-CBF ($p<0.03$ to <0.0001). In the total population, mean CBF correlated negatively with SD-glucose and J-index ($r=-0.41$, $p<0.01$; $r=-0.34$, $p<0.05$, even after adjustment for age and BMI), but not with mean glucose. Mean CBF also correlated with HbA1c ($r=-0.40$, $p<0.01$). In multivariate analysis mean CBF remained significantly correlated with SD-glucose and J-index independently from HbA1c only in IGTs. SD-CBF did not correlate significantly with glycemic parameters.
Conclusion: Microcirculatory CBF is lower in T2Ds than in IGTs. Glycemic variability seems to play a greater role than mean glucose and long-term hyperglycemia on peripheral microcirculation, mostly in patients with IGT.
Disclosure: **A. Rezki:** None.

1088

Validation in the real life of a prognostic model developed to predict major vascular outcomes in type 1 diabetes

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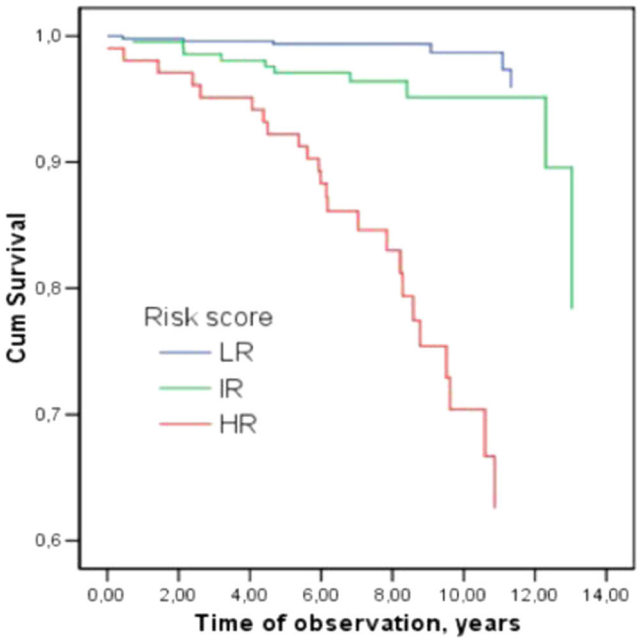
Background and aims: EURODIAB PCS assessed risk for major outcomes (CHD, stroke, ESRD, amputation, blindness and all-cause death) in type 1 diabetes (T1DM) by a model based on age, HbA1c, waist-hip ratio (WHR), urinary albumin to creatinine ratio (ACR) and HDL, identifing 3 groups at low- (LR, score up to 15), intermediate- (IR, score 16 to 20) and high- (HR, score 20 or higher) absolute risk.
Materials and methods: The performance of this model against all-cause mortality was tested in a mean follow-up of 8.25±2.34 years (median 7.58 years; IQR 6.47-9.75) in a cohort of 774 T1DM: 407 M and 367 F, age 40.2±11.7 years, DD 19.3±12.2 years; HbA1c 7.8±1.2% (range 5.2-15.1); ACR (median) 0.49 mg/mmol (0.03-142.6); HDL 62.5±15.2 mg/dl (8-103), WHR 0.92±0.06 (0.74-1.14).
Results: Risk score distribution was: LR n. 466 (60.2%), IR n. 205 (26.5%) and HR n. 103 (13.3%) in the whole cohort, and LR n. 461 (62.9%), IR n. 195 (26.6%) and HR n. 77 (10.5%) after exclusion of 41 T1DM (5.3%) who already had major outcomes. Major outcomes were more frequent in HR (26/103; 25.2%) and IR (10/205; 4.9%) than in LR (5/466; 1.1%; $p<0.0001$). Distribution of risk was similar in M and F ($p=0.113$); IR and HR increased with quartiles of DD ($p<0.0001$). Main factors associated with IR&HR were age, HbA1c and ACR ($p<0.0001$ for all). Several risk factors were increasing from LR to IR and HR: sBP 120

±14, 132±17, 145±20; dBP 72±8, 75±9, 75±10 mmHg; LDL cholesterol 113±29, 122±30, 120±29 mg/dl; triglycerides 69 (55-91), 82 (63-106), 101 (69-137) mg/dl; fibrinogen 320±64, 353±63, 376±67 mg/dl; uric acid 3.6±1.0, 3.8±1.9, 4.8±4.7 mg/dl (p<0.001 for all). Were decreasing eGFR (CKD-EPI) 108±15, 99±13, 83±20 ml/min/1.73 m2 (p<0.001) and rate of current smokers 31.9, 29.7, 19.0% (p=0.037); advanced retinopathy increased (6.2, 23.6 and 42.9%, p<0.0001). Mean follow-up was similar in the 3 risk groups (p=0.394). By Kaplan-Meier analysis, all-cause mortality was 5.2% in the whole cohort: higher in HR subjects (23.3%, 24/103) than in IR (4.9%, 10/205) and in LR individuals (1.3%, 6/466; p=0.0001, figure); 22.1%, 5.1% and 1.3%, respectively after exclusion of T1DM with previous outcomes (p=0.0001). By logistic regression the score (p<0.0001; IR score, OR 3.96; HR score, OR 20.41), not gender, DD, previous CVD and retinopathy enter as independent covariate of all-cause mortality (model 1). In model 2, including BMI, lipids, sBP, dBP, smoking and eGFR other than variables of model 1, only to be current smokers (OR 3.94, p=0.009) and to have reduced eGFR (CKD stage 2b: OR 5.00, p=0.004; CKD stage ≥3: OR 6.86, p=0.001) add significantly to the risk score (p<0.0001) in accounting for all-cause mortality.

Conclusion: In our cohort of T1DM, 30-40% of subjects has high (10%) or intermediate (25%) absolute risk for major vascular outcomes with an all-cause mortality of about 3% per year in the HR subgroup. The EURODIAB risk score is confimed as a useful tool for estimating all-cause mortality risk in subjects with type 1 diabetes.



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Disclosure: **M. Garofolo:** None.

PS 114 Diabetic complications: the unusual suspects

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Effect of glycaemic control on pulmonary function: data from a 3-months interventional study

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Background and aims: There is growing evidence suggesting a deleterious effect of type 2 diabetes (T2D) on lung function and sleep breathing. However, only one previous study from our group has showed how glycemic control improvement significantly reduces the increased number of nocturnal oxygen desaturations that exist in type 2 diabetes. Now, we show data about the impact of 3-months glycemic control improvement on lung function.

Materials and methods: Prospective interventional study in 49 patients with T2D and no pulmonary disease (59.2% men; age 59.6±10.4 yrs.; T2D duration 11.8 ±6.2 yrs.; BMI 30.8±6.6 kg/m2, and HbA1c 9.7 ±1.5%). During a 3-months period subjects underwent blood glucose intensification according to our routine medical practice, giving priority to treatments with neutral or negative weight effect. At the end of this period, HbA1c and BMI decreased to 7.1±1.0% and 30.2±5.9 kg/m2 (p<0.001 and p=0.062 vs. baseline, respectively).

Results: Univariate analysis showed that the magnitude of change in HbA1c negatively correlated with increases in lung function parameters, particularly those related to small airways of lung: maximum mid-forced expiratory flow (FEF25-75%; r=-0.395, p=0.005), forced expiratory volume and forced vital capacity ratio (FEV1/FVC; r=-0.375, p=0.008), and instantaneous forced expiratory flow 50% (FEF50%; r=-0,390, p=0,006). Multiple linear regression analysis showed that the decrease in HbA1c, but not the decrease in BMI, was independently associated with the increases in FEF25-75% (R2=15.6%), FEV1/FVC (R2=14.1%), and FEF50% (R=15.2%). Finally, weight loss had a beneficial effect in pulmonary parameters different from previous, as forced vital capacity and FEV1.

Conclusion: This is the first clinical evidence of a positive effect of glycemic control improvement on lung function. Although the mechanisms are not yet fully understood, our findings support the concept that the lung is a new target of diabetic complications.

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Disclosure: **L.P. Gutiérrez:** None.

1090

Serum surfactant D protein as a new marker for screening pulmonary disease in patients with type 2 diabetes. A case-control study

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Background and aims: There is growing evidence to suggest an association between type 2 diabetes and impaired pulmonary function. In this regard, experimental studies have shown that glucagon-like peptide 1 (GLP-1) plays a role in the stimulation of surfactant production by human type II pneumocytes. Pulmonary surfactant is a surface-active lipoprotein complex that reduces surface tension, increase pulmonary compliance, and prevent collapse of the lung at the end of expiration. Therefore, it can be hypothesized that the diminished incretin effect in type 2 diabetes may be associated with a defect in the surfactant layer. As when the alveolocapillary barrier is damaged surfactant proteins can leak into the

bloodstream, it is reasonable to assume that higher levels of serum surfactant D protein (sSP-D) and serum surfactant A protein (sSP-A) will be present in more concentration in patients with type 2 diabetes.
Materials and methods: We designed a case-control study between 49 subjects with type 2 diabetes and 98 non-diabetic subjects, matched by age, gender, BMI, and waist circumference. Both SP-D and SP-A, the major surfactant-associated proteins, as well as total GLP-1, were measured in serum samples by enzyme-linked immunosorbent assay. Data from an overnight sleep study were available from half of subjects.
Results: Patients with type 2 diabetes exhibited higher concentrations of sSP-D in comparison with control subjects [133.0 (35.4 to 815.8) vs. 97.6 (23.5 to 336.2) ng/mL, p=0.006]. However, no differences in sSP-A [27.4 (5.2 to 449.2) vs. 23.5 (0.03 to 105.3) ng/mL, p=0.198] neither in GLP-1 [91.1 (31.6 to 802.6) vs. 83.8 (6.1 to 262.6) nmol/L, p=0.127] were observed between groups. As it has been previously reported in subjects with pulmonary diseases, a significant negative correlation between sSP-D and BMI was detected (r=-0.193, p=0.021). In addition, among control subjects, a significative negative correlation was detected between sSP-D and the apnea hypopnea index (r=-0.403, p=0.022), as well as between sSP-A and fasting plasma glucose (r=0.211, p=0.041); both correlations disappeared when patients with type 2 diabetes were included. However, no correlation between total GLP-1 and serum surfactant proteins were observed. Finally, a stepwise regression analysis showed that the presence of type 2 diabetes (but not gender, age, BMI, nor polysomnography measures) independently predicted the sSP-D concentrations (R2=0.193).
Conclusion: We have shown that higher sSP-D concentrations are present in patients with type 2 diabetes. Therefore, sSP-D appears as a potential marker reflecting defects in the bronchiolar surfactant layer in patients with type 2 diabetes. This biomarker can be used for assessing strategies meant to reduce type 2 diabetes-induced lung injury.
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Disclosure: **A. Lecube:** None.

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Impaired lung function as a diabetes related complication

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Background and aims: A link between impaired lung function and pulmonary fibrosis with diabetes mellitus has been described in retrospective studies and meta-analysis. So far, the pathomechanism is unknown. Thus, we initiated a prospective observation study to investigate the impaired lung function and pulmonary fibrosis in healthy controls patients with pre-diabetes and patients with diabetes mellitus (type 1 and 2) with and without diabetes related complications. Aim of the current study is to define pulmonary fibrosis as a diabetes related complication in cross-sectional and longitudinal observation. Furthermore, risk factors, correlation with other diabetes related complications and pathomechanism should be identified.
Materials and methods: One hundred sixty four participants (controls n=21; pre-diabetes n=43; diabetes n=100) underwent clinical examination and lung function test with measurement of vital capacity (VC), total lung capacity (TLC), forced expiratory volume (FEV1) and single breath diffusion capacity (DLCo). Additionally glucose control parameter (HbA1c), kidney function (eGFR and urinary albumin-creatinine-ratio; u-ACR) were measured. Furthermore, measurement of advanced-glycation-end products under the skin (skin-AGE) was performed by auto fluorescence measurement. Patients with known heart insufficiency and known pulmonary disease (e.g. asthma, COPD, pulmonary hypertension) were excluded from analysis. First cross-sectional statistical analyses were performed via Pearson correlation coefficient and one-way-ANOVA with SPSS 22.
Results: One-way-ANOVA has shown a significant differences in vital capacity (VC) [p<0.01); single breath diffusion capacity (DLCo) [p<0.01] and total lung capacity (TLC) [p<0.05] between healthy controls, pre-diabetic and diabetic patients with continuous decrease of all parameters in direction towards diabetes. Correlation analyses has shown significant associations between HbA1c with decreased TLC (r=-0.35; p<0.01) and impaired DLCo (r=-0.23; p<0.05). Furthermore, increased urinary albumin excretion (u-ACR) was significant associated with decreased TLC (r=-0.55; p<0.001) and impaired DLCo (r=-0.40; p<0.01). Additionally, increased skin-AGE values were associated with impaired DLCo (r=-0.29; p<0.05). There were no significant differences for forced expiratory volume (FEV1) within the participants.
Conclusion: This is the first on-going study which investigates impaired lung function and pulmonary fibrosis in patients with pre-diabetes and diabetes. We could show significant associations for impaired lung function depend on diabetes mellitus, glucose control, urinary albumin excretion and skin-AGE formation.
Supported by: DZD Grant 2016
Disclosure: **S. Kopf:** Grants; German Center for Diabetes Research (DZD) Grant 2016 for "Pulmonary impairment and fibrosis in Patients with Diabetes mellitus".

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Prevalence and risk of falls among older people with diabetes

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Background and aims: Falls are a leading cause of fractures, hospitalization, loss of independence and impaired quality of life. Many of the established risk factors for falls are more common among people with diabetes. The aim of this study is to estimate the prevalence of falls among older people with diabetes and identify the risk factors which may mediate the relationship between diabetes and falls using nationally representative data from The Irish Longitudinal Study on Ageing (TILDA).
Materials and methods: Data from the 1st wave of TILDA were analysed. TILDA is a prospective cohort study conducted among community-dwelling adults aged ≥50 years in the Republic of Ireland, recruited using multistage stratified clustered sampling. A 62% response rate was achieved among eligible households. During a computer assisted personal interview, participants' self-reported falls in the previous year. Multivariable logistic regression models were used to determine the association between diabetes and the risk of falling.
Results: Of the 8175 participants aged ≥50 years, 634 self-reported having diabetes (7.8%, 95% CI: 7-8%). The prevalence of falls in this group was 24% (n=152, 95% CI: 21-27) compared to 19% among the general population (n=1431, 95% CI: 18-20%) (p=0.002). When stratified by gender, a significant association was only evident among females (p=0.001). When stratified by age, there was a statistically significant association between diabetes and falls among those aged 60-69 years (p=0.029) and 70-79 years (p=0.04). There was no statistically significant association between the presence of any macro- or microvascular complications and falls, however when the complications were analysed separately neuropathy was significantly associated with falls in people with diabetes (p=0.001) (Figure 1). Diabetes was significantly associated with an increased risk of falls in the age- and sex-adjusted models (OR=1.30, 95%CI=1.07-1.58, p=0.008). When mediating factors (polypharmacy, lifestyle factors including exercise, blood pressure, impaired cognition, strength and balance) were included the model, diabetes was still associated with an increased risk (OR=1.36, 95%CI=0.99-1.86) however, this relationship was no longer statistically significant.
Conclusion: One-quarter of adults with diabetes experienced a fall in the preceding year. This population may benefit from a multifactorial falls risk assessment which could be incorporated into the regular structured review recommended for people with diabetes.

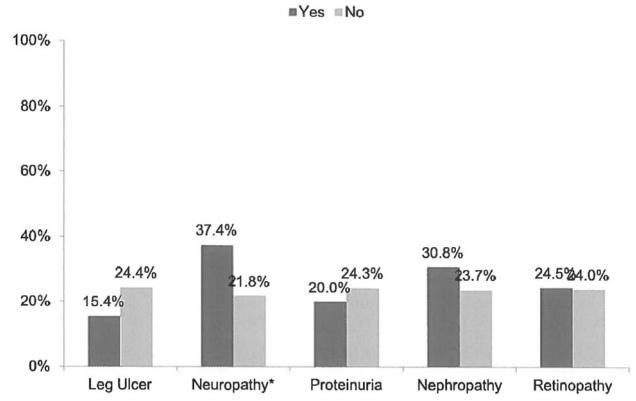

Figure 1. Prevalence of falls among people with diabetes according to micro- and macrovascular complications status

Supported by: Centre for Ageing Research & Development Ireland, CARDI
Disclosure: **S. McHugh:** None.

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Glyoxalase 1 inducer protects human periodontal ligament fibroblasts from dicarbonyl stress and dysfunction in model hyperglycaemia in vitro

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Background and aims: Periodontal ligament (PDL) inflammation or periodontitis is a common complication of diabetes characterised by gradual destruction of connective tissue fibres attaching teeth to the alveolar bone. Poorly controlled hyperglycaemia in patients with diabetes tends to produce a more severe form of periodontitis than in non-diabetic subjects. Systemic changes caused by diabetes reduce the host resistance to periodontal tissue breakdown. Periodontal ligament fibroblasts (PDLFs) are the major cells of the PDL, binding to collagen-I fibrils of the PDL, and remodelling and maintaining the ligament. Dysfunction of PDLFs is a likely major cause of severe periodontitis in diabetes. Glyoxalase 1 (Glo1) is part of the glyoxalase metabolic pathway which catalyses the metabolism of the reactive metabolite and glycating agent, methylglyoxal (MG), to D-lactate and thereby prevents formation of advanced glycation endproducts (AGEs). High glucose concentration induced decrease activity of Glo1, increased MG or dicarbonyl stress, MG modification of collagen IV and cell detachment. We reasoned that PDLFs may suffer dicarbonyl stress in high glucose concentration. The aim of this study was to assess if high glucose concentration induces dicarbonyl stress in human PDLFs in vitro and collagen detachment, and if this could be prevented by small molecule Glo1 inducer - a binary combination of small molecule activators of transcription factor Nrf2 that synergise to increase expression of Glo1 via functional antioxidant response element.
Materials and methods: Primary human periodontal ligament fibroblasts (hPDLFs) were purchased from ScienCell, Carlsbad, USA. They were cultured in Modified Eagles Medium (MEM)supplemented with L-alanyl-L-glutamine and 10% FBS at 37oC. For experiments performed in low and high glucose conditions, culture media was supplemented with 8 mM and 25 mM glucose, respectively, and incubated for 3 days. Activity of Glo1 was assayed by conventional spectrophotometric assay, Glo1 protein by Western blotting and Glo1 mRNA by RT-PCR with beta-actin as reference standard. Concentrations of MG and AGEs were assayed by stable isotopic dilution analysis liquid chromatography-tandem mass spectrometry. Glucose consumption and D-lactate formation was deduced by assay at the start and end of cultures. Glo1 inducer compounds were added at 10 micromolar concentration. Binding of hPDLFs to collagen-I was investigated.
Results: The activity of Glo1 in low glucose culture was 985 ± 148 mU per mg protein (n = 5) and was decreased ca. 45% by high glucose conditions (P<0.001). Glo1 mRNA level was unchanged but Glo1 protein was decreased. MG concentration of the medium and cells was increased ca. 41% and 60%, respectively, in the high glucose conditions, compared to low glucose control (P<0.01). The production of D-lactate was increased 42% and the consumption of glucose was increased 93% in high glucose conditions (P<0.001). Cellular protein content of MG-derived AGE, MG-H1, was also increased: 0.330 ± 0.163 versus 0.763 ± 0.201, P<0.01. There was a ca. 30% decrease in adhesion of the hPDLFs to collagen-I in high glucose concentration (P<0.05). Glo1 inducers corrected all of these changes.
Conclusion: Dicarbonyl stress is induced in hPDLFs by high glucose concentrations and contributes to cell dysfunction. This was prevented by Glo1 inducer.
Supported by: A.A.D.A: PhD Studensthip, Ministry of Education, Saudi Arabia
Disclosure: **A.A.D. Ashour:** Grants; Yes.

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Vitamin D3 improves bones biomechanical properties through NF-kB - VDR-related pathway in diabetes

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Background and aims: Vitamin D3 (D3) deficiency is known to be associated with the development of low bone mineral density related to primary osteoporosis and other disorders, including diabetes. The present study was design to determine the relationship between vitamin D3 availability, VDR expression and RANKL/RANK/NF-kB signaling pathway in the activation of bone resorption and loss of bone architecture associated with type 1 diabetes.
Materials and methods: Diabetes was induced in male C57BL/J6 mice by i.p. injection of multiple low dose streptozotocin (40 mg/kg b.w.). Control and diabetic mice were treated with or without D3 (15 IU/mouse per os, for 8 weeks). Serum 25-hydroxyvitamin D3 (25OHD3), TRAP (tartrate resistant acid phosphatase), RANKL and OPG were assessed by ELISA. The protein levels of VDR, RANKL, RANK, OPG, phosphorylated NF-kB/p65 (phosho-p65) and osteocalcin (OC) in bone tissue were assayed by Western blotting. The biomechanical properties were analyzed with the three-point bending test. μCT analysis was performed on the proximal tibia by using Skyscan 1072 scanner.
Results: Serum level of 25OHD3, the main circulating metabolite of D3, was shown to be reduced to 22.5±1.8 in diabetes vs. 41.7±2.8 nmol/l in control, that reflects reliably vitamin D3 deficiency (p<0.05). These changes were accompanied by significant changes in bone biomechanical properties. Diabetes caused a decrease in maximum load, toughness and stiffness of diabetic tibias by 2.1-, 3.8- and 2.3-fold respectively (p<0.05). Histomorphometry of tibias showed respectively 3.0-, 2.1- and 1.3-fold decreases in bone volume per tissue volume (BV/TV), trabecular number (Tb.N) and cortical thickness (Cort.Th) in diabetes vs. control (p<0.05), indicative of impaired tibial architecture induced by diabetes. Assessment of bone resorption markers, such as TRAP and bone-related cytokines (RANKL, OPG), in blood serum demonstrated that their levels were respectively 48, 23 and 40% higher in diabetic vs. control animals (p<0.05). Diabetes led to up-regulation of phosho-p65, RANKL, RANK (2.3-, 1.72-, 1.51- fold respectively) and down-regulation of OC, OPG and VDR (1.5-, 1.6- and 1.8-fold respectively) in bone tissue of diabetic mice compared with control (p<0.05). Full restoration of

circulatory 25OHD3 level was achieved after D3 treatment and normalization of vitamin D3 bioavailability resulted in increased VDR expression in bone tissue. Treatment with vitamin D3 improved diabetes-induced structural and biomechanical abnormalities in bone tissue that strongly correlated with the normalization of RANKL/RANK/OPG system and diminishing of NF-kB-mediated bone resorption. A significant decrease in serum levels of TRAP, RANKL and OPG was also found after D3 administration.
Conclusion: The findings suggest a significant role of RANKL/RANK/NF-kB signaling pathway activation in the induction of bone resorption in diabetes that occurs on the background of vitamin D3 deficiency and impaired D3 signaling via VDR. It was demonstrated that diabetes-related impairments may efficiently be corrected by vitamin D3 treatment.
Disclosure: **D. Labudzynskyi:** None.

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Normal bone mineral density despite increased bone turnover and hypovitaminosis D in old type 2 diabetic patients

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Background and aims: Old diabetic patients may be at an increased risk of falls and everyday trauma, because of hypoglycaemia, vision defects and neuropathy. Fractures may be more common in diabetic patients. Assessing bone frailty and exploring factors for bone dynamics are therefore of the outmost importance in old diabetic patients.
Materials and methods: All diabetic patients older than 65 years assisted at the outpatient diabetic department of a public tertiary hospital by a single doctor were included. Data was entered on a specific database. Besides the usual clinical and analytical parameters, serum calcium, phosphate, magnesium, parathormone (PTH), 25-hydroxyvitamin D (25OHD), procollagen type 1 N terminal polypeptide (P1NP), and carboxy-terminal cross-linking telopeptide of type I collagen (beta-CrossLaps, beta-CTX) obtained at a morning blood sampling after overnight fast, bone mineral density at the lumbar spine (L1-L4) and femural neck were also included. Chi-square, T-student test and Anova were used to compare groups and the relation between continuous variables was explored with multiple regression analysis.
Results: Two hundred sixty-eight consecutive diabetic patients older than 65 years were included. Patients were both male and female (52/48%), old age (74±6 years), overweight (BMI 28.7±4.5 kg/m2), with long standing disease (19±11 years), treated with insulin and/or oral drugs and with fair metabolic control (HbA1c - 7.7±1.5%). Microvascular disease was common (39-50%) as well as high blood pressure (83%) and dyslipidemia (60%). Serum calcium, phosphate and magnesium were always normal, despite a very high prevalence of Hypovitaminosis D (85%) and Hyperparathyroidism (26%). P1NP and beta-CTX were increased, with increased bone formation (39%) and/or increased bone resorption (48%). Age-adjusted bone mineral density was increased - Lumbar spine z-score +0.8±1.6 [-2.0 to +5.7], femoral neck z-score +0.5±1.0 [-1.7 to + 3.2]. P1NP and beta-CTX were directly related ($r=+0.486$, $p<0.001$).Bone mineral density at the column and femoral neck were directly related ($r=+0.736$, $p<0.001$). Age ($r=-0.366$, $p<0.01$), PTH ($r=-0.260$, $p<0.03$) and beta-CTX ($r=-0.477$, $p<0.001$) were inversely related to bone mineral density at the femoral neck, but not years since diagnosis, metabolic control or the presence of nephropathy.
Conclusion: In this study of a large group of old type 2 diabetic patients assisted at a tertiary out patient center, we found that despite old age, long standing disease and very common hypovitaminosis D, patients presented normal age-adjusted bone mineral density. We also found evidence for an increased bone turnover in these patients. Only age, PTH and beta-CTX were found to be relevant negative factors for bone mineral density at the femoral neck, but not factors related to diabetes including nephropathy. The increased risk of osteoporotic fractures in type 2 diabetic patients, unlike the same risk for type 1diabetic patients, can not be explained by low bone mineral density, reflecting, rather than reduced bone mass, qualitative changes in skeletal tissue.
Disclosure: **J.M. Martins:** None.

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Lower bone mineral density in Japanese type 2 diabetic patients and effect of GIP on RANKL mRNA expression in cultured osteoblastic cells

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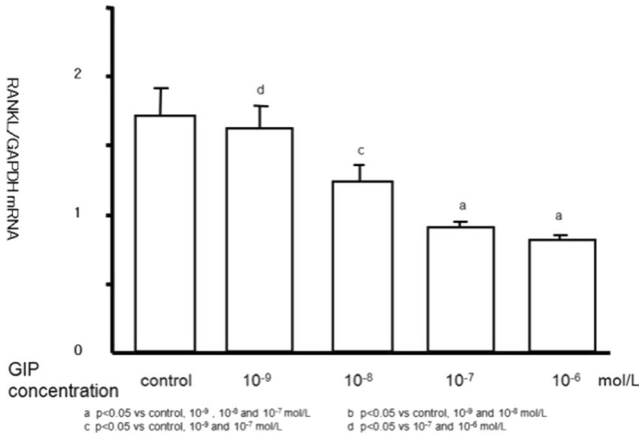
Background and aims: Skeletal fragility is considered among the complications associated with type 2 diabetes. Type 2 diabetic patients have increased fracture risk and lower bone mineral density (BMD). However, there are few reports concerning BMD and type 2 diabetes in Asian populations. In this study we examined whether or not BMD is lower in Japanese type 2 diabetic patients. Receptor activator of nuclear factor NF-kB ligand (RANCL) produced by osteoblastic cells is the essential factor for osteoclast formation, activation and survival, thus resulting in bone resorption and bone loss. From this point of view denosumab was developed as anti-RANCL antibody for the treatment of osteoporosis. On the other hand, DPP-4 inhibitors which improve high plasma glucose levels through increasing incretin such as glucose-dependent insulinotropic polypeptide (GIP) have been commonly used for the treatment of type 2 diabetes mellitus. Little information is available about incretin and bone metabolism. We focused on GIP and examined whether or not GIP affects RANKL expression in cultured osteoblastic cells to elucidate the relation between GIP and bone metabolism.
Materials and methods: We evaluated 18 diabetic patients and 72 non-diabetic subjects (60-69 years, female), and 32 diabetic patients and 272 non-diabetic subjects (70-79 years, female). Patients who took thiazolidine were excluded from this study since it causes bone loss. Lumbar spine BMD and femoral (proximal part) BMD were evaluated with dual energy X-ray absorptiometry (DXA) according to the guideline of Japan Osteoporosis Society 2015. BMD was expressed as the percent ratio to the young adult mean BMD (20-29 years of age). Human osteoblastic cells from Promo Cell Co. USA were cultured by the previously reported method, and then were incubated for 24 h with GIP at the concentration of 0, 10-9,10-8, 10-7 and 10-6 mol/L. To evaluate the expression of RANCL m-RNA in cultured human osteoblastic cells, RT-PCR procedure was performed.
Results: In sixties diabetic patients had significantly ($p<0.01$) lower lumbar spine BMD than non-diabetic controls (73.7% vs 83.0%). In seventies diabetic patients tended to have lower lumbar spine BMD than non-diabetic controls (76.1% vs 81.9%). In sixties diabetic patients had significantly ($p<0.01$) lower femoral BMD than non-diabetic controls (73.4% vs 82.2%). In seventies diabetic patients had significantly ($p<0.05$) lower femoral BMD than non-diabetic controls (72.8% vs 78.9%). In cultured human osteoblastic cells GIP significantly ($p<0.05$) stimulated the expression of GIP receptor mRNA in a dose-dependent manner, and GIP significantly ($p<0.05$) suppressed the expression of RANKL mRNA in a dose-dependent manner(Figure).
Conclusion: This clinical study clearly showed that type 2 diabetic patients had lower BMD than the age-matched non-diabetic subjects in Japan. GIP suppressed the expression of RANKL. This finding suggests that GIP contributes to protect bone loss in type 2 diabetic patients through its lowering effect of RANCL.

Figure. Effect of GIP on RANCL mRNA Expression in Human Osteoblastic Cells



Disclosure: **M. Eto:** None.

PS 115 Mechanisms of complications from glycation to inflammation

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Methylglyoxal affects angiogenesis in vitro in MAEC cells via a mechanism involving HoxA5

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Background and aims: Diabetes mellitus (DM) is characterized by chronic hyperglycemia, a primary factor in the development of diabetic vascular complications. The presence of abnormal angiogenesis may cause or contribute to diabetes associated complications. Hyperglycemia induces cellular damage through the activation of several molecular pathways including the activation of the transcription factor nuclear-kB (NFκB) in the endothelial cells. The major Advanced Glycation Endproducts (AGEs) precursor in endothelial cells is Methylglyoxal (MG), a highly reactive dicarbonyl formed primarily from the intermediates of glycolysis and detoxified by the glyoxalase system (Glo). Glo1 activity prevents the accumulation of MG, thereby suppressing cellular damage. MG concentration is increased 2 to 5-fold in plasma of diabetic patients. Emerging evidence suggest that MG contributes to endothelial dysfunction and vascular complications, but the underlying mechanisms remain to be clarified. This work aims at elucidating the MG effect on the angiogenic ability of endothelial cells and the molecular mechanisms involved.
Materials and methods: Proliferation, migration and invasion were evaluated by cell-growth curves and transwell assays in mouse aortic endothelial cells isolated from Glo1 knockdown mice (GloKD MAEC) and their wild type littermates (WT MAEC). Glo1, HoxA5 and NFκB mRNA levels were measured by Real Time PCR, while MG intracellular concentrations by HPLC. HoxA5 expression was silenced by the use of specific siRNAs. NFκB protein levels and activation were evaluated by western blot of protein lysate from cytosol/nuclear fractionation. Binding of NFκB on HoxA5 promoter was then analyzed by Chromatin Immuno Precipitation assay.
Results: GloKD MAEC show a 50% reduction of Glo1 mRNA levels and a 5-fold increase of MG intracellular concentrations compared to WT MAEC ($p=0.001$). GloKD MAEC show a slower cell growth (GloKD MAEC 106833±15610 vs WT MAEC 202733±62699 cells at 24h, $p=0.002$), a reduced migration (GloKD MAEC 0.06±0.01 vs WT MAEC 0.09±0.02 OD, preliminary data) and invasion ability (GloKD MAEC 0.9±0.5 vs WT MAEC 5.9±2.7 fold increase of cell number over basal, $p=0.04$) compared to WT MAEC. mRNA levels of the anti-angiogenic gene HoxA5 are 2-fold increased in GloKD MAEC compared to WT MAEC ($p=0.01$), and its silencing improves the invasion ability of GloKD MAEC (GloKD MAEC HoxA5 siRNA 5.60±3.20 vs GkloKD MAEC scramble siRNA 0.85±0.2 fold increase of cell number over basal, $p=0.04$). Moreover, GloKD MAEC show a ~2-fold increase of NFκB mRNA ($p<0.001$) and protein levels both in the cytosol and in the nucleus, compared to WT MAEC ($p\leq0.05$). Interestingly, the binding of NFκB to the HoxA5 promoter is 1.4-fold higher in GloKD MAEC compared to WT MAEC ($p=0.02$).
Conclusion: These results suggest that MG accumulation impairs the angiogenic ability of GloKD MAEC. HoxA5 may be, at least in part, responsible of this effect, and its overexpression is likely due to a higher binding of NFκB to HoxA5 promoter. Further investigation of the molecular mechanisms by which MG impairs the angiogenic process will highlights new targets for the prevention and treatment of diabetes vascular disease.
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Disclosure: **A. Leone:** None.

1098

Glyoxalase 1 mRNA levels in whole blood are higher in individuals with type 2 diabetes and associated with plasma markers of endothelial activation

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Background and aims: Methylglyoxal (MGO) has been identified as the most reactive precursor in the formation of Advanced glycation endproducts (AGEs). Glyoxalase 1 (GLO1) is the rate-limiting enzyme in the concersion of MGO into D-lactate. In animal models of diabetes, GLO1 overexpression has been shown to be protective against complications. Although GLO1 may be a key enzyme to prevent diabetic complications, it remains unclear whether general GLO1 expression is changed in type 2 diabetes (T2DM).
Materials and methods: We measured levels of circulating GLO1 mRNA in whole blood of 474 individuals with normal glucose metabolism (NGM, n=191; women 37.7%; age 64.7 ± 7.3), impaired glucose metabolism (IGM, n=116; 42.28%; 66.9 ± 6.9) or T2DM (n=167;3 7.1%; 67.5 ± 6.3)) derived from the Cohort on Diabetes and Atherosclerosis Maastricht (CODAM) study. GLO1 mRNA levels were normalised for reference genes (HRPT1 and WHKY1), and ln- transformed prior to linear regression analysis. Markers of endothelial activation (soluble vascular adhesion molecule, soluble intercellular adhesion molecule 1, van Von Willebrand factor and soluble E-selectin) were measured in plasma and combined in a Z-score. Macrovascular disease was characterised by measuring carotid intima thickness (cIMT), ankle-brachial index (ABI) and by a recorded history of cardiovascular disease (CVD). All analyses were adjusted for age, sex, IGM and T2DM, waist circumference, smoking, systolic blood pressure, blood lipids, estimated glomerular filtration rate, prior CVD and medication use.
Results: Compaired to normal glucose metabolism, T2DM was associated with higher GLO1 mRNA levels (β: 0.485 SD; 95%CI: 0.169 to 0.800; P=0.003). Additional adjustment for fasting plasma glucose (β: 0.455 SD; 95%CI: 0.087 to 0.824; P=0.016) and Hba1c (β: 0.450 SD; 95% CI: 0.083 to 0.816; P=0.016) did not change the results. Additional adjustment of these analyses for use of insulin-, metformin-, sulfanyl urea derivates as well ARBs and ACE-inhibitors did not change the results (β: 0.488 SD; 95%CI: 0.170 to 0.805; P=0.003). IGM was not associated with GLO1 mRNA levels (β: -0.128 SD; 95%CI: -0.361 to 0.105; P=0.280). Furthermore, higher GLO1 mRNA levels were associated with higher levels of endothelial activation markers (β: 0.142 SD; 0.056 to 0.227; P=0.001), but no associations were found with markers of macrovascular disease (cIMT, ABI and prior CVD).
Conclusion: Circulating GLO1 expression is higher in T2DM, independently of conventional cardiovascular risk factors, glycaemic control, as well as glucose-lowering and anti-hypertensive treatments previously linked to increased GLO1 activity. These changes in GLO1 expression may reflect a defensive mechanism in response to increased MGO levels in diabetes and endothelial activation.
Supported by: CTMM
Disclosure: **N.M.J. Hanssen:** None.

1099

Acute hypoglycaemia increases pro-inflammatory cytokine production of peripheral mononuclear white blood cells

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Background and aims: Severe hypoglycemia is associated with cardiovascular events in patients with diabetes. This may be explained by an acute increase in circulating pro-inflammatory cytokines and pro-atherothrombotic factors induced by hypoglycemia, but little is known about the underlying mechanisms, nor about the effect of hypoglycemia on the composition and inflammatory function of immune cells. We investigated these effects using ex vivo stimulations of peripheral blood mononuclear cells (PBMCs) obtained during experimentally induced hypoglycemia in healthy subjects, patients with type 1 diabetes (T1DM) and T1DM patients with impaired awareness of hypoglycemia (IAH), who inherently to IAH, had extensive previous exposure to hypoglycemia.
Materials and methods: We performed a two-step hyperinsulinemic (60 mU m^2 m^{-1}) euglycemic (5.0 mmol/L)-hypoglycemic (2.6 mmol/L) clamp in 11 healthy controls (HC), 10 T1DM, and 10 T1DM-IAH. Counterregulatory hormones, peripheral total white blood cell (WBC) and differential count were determined at regular intervals. PBMCs were isolated at euglycemia and after 1 h of hypoglycemia. Subsequently, equal numbers of PBMCs were stimulated for 24 h with the following stimuli: E. coli-derived lipopolysaccharide (LPS), TLR-2 agonist Pam3Cys, a lysate of M. tuberculosis (MTB) strain H37Rv, or heat-killed C. albicans, all in medium containing 5.5 mmol/L glucose. Cytokines (TNF-α, IL-6 and IL-1β) in cell supernatants were measured by ELISA.
Results: Hypoglycemia induced a strong adrenaline response, which was 4-fold higher in HC and T1DM than in T1DM-IAH ($p<0.001$). Hypoglycemia increased WBC count in HC (mean difference: 2.1 ± 1.5 x10^9/L) and T1DM (1.6 ± 1.0 x10^9/L), but not in T1DM-IAH (0.2 ± 0.4 x10^9/L). The increased WBC count was mainly due to an increased number of lymphocytes ($p<0.01$) and correlated positively with the adrenaline response to hypoglycemia ($R^2 = 0.70$, $p<0.001$). Interestingly, PBMCs from HC and T1DM isolated during hypoglycemia showed a stronger inflammatory response to LPS, Pam3Cys and MTB, reflected by an elevated production of the pro-inflammatory cytokines IL-6, TNF-α and IL-1β, than PBMCs isolated during euglycemia. This effect was attenuated in T1DM-IAH, in whom hypoglycemia only increased the TNF-α response to MTB and C. albicans.
Conclusion: Hypoglycemia increases WBC count and the inflammatory responses of PBMCs in HC and T1DM, most likely due to adrenergic recruitment of WBCs from the marginal pool. Increased inflammatory responses of immune cells in response to hypoglycemia could promote a chronic inflammatory state in T1DM and thereby aggravate vascular complications associated with diabetes. However, pro-inflammatory effects of hypoglycemia are less pronounced in T1DM patients with extensive previous exposure to hypoglycemia.
Clinical Trial Registration Number: NCT02308293
Supported by: EFSD/GSK, Dutch Diabetes Research Foundation
Disclosure: **J.M. Ratter:** None.

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Dicarbonyl stress induces glyoxalase 1 copy number increase in mouse embryonic stem cells in vitro and escapes mutation lethality in gene trapping

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Background and aims: Glyoxalase 1 (Glo1) is part of the glyoxalase metabolic pathway which catalyses the metabolism of the reactive metabolite and glycating agent, methylglyoxal (MG), and thereby prevents formation of advanced glycation endproducts (AGEs). Glo1 deficiency in the kidney, retina and nerve in diabetes is associated with development of diabetic nephropathy, retinopathy and neuropathy and Glo1 deficiency is a driver of cardiovascular disease. The GLO1 gene is a hotspot for duplication with 2% prevalence in the human population. Homozygous inheritance of a rare frameshift mutation in GLO1 was embryonically lethal. It was surprising that a putative Glo1 knockout mice produced by the International Mouse Knockout Consortium (IMKC) by gene trapping had a phenotype for putative homozygous mutants similar to wild-type.

The aim of this study was to examine MG metabolism in the IMKC putative Glo1 knockout mouse and the basis for the healthy phenotype.
Materials and methods: Glo1 mutant mice produced by the IMKC were obtained from the European Mutant Mouse Archive (Heidelberg, Germany). Wild-type control siblings of heterozygote C57BL/6 mice were produced. Control mouse liver samples of strains C57BL/6J (Glo1 copy number = 2) and DBA/1J (Glo1 copy number = 4) were from Jackson Laboratories (Bar Harbor, USA). Mouse embryonic stem cells (mESCs) of C57BL/6 mouse strain were cultured in vitro with and without 200 micromolar MG, added each day for 12 days. Glo1 copy number assays were performed by the TaqMan® Copy Number Assays, with reference genes Tfrc. A Glo1 paralogue ratio tests assay was developed for corroboration. mRNA expression analysis was performed using the Taqman gene expression assay protocol, with Rn 18s was internal reference gene. Glo1 activity and MG concentration was assayed by conventional spectrophotometric and liquid chromatography-tandem mass spectrometry techniques.
Results: Analysis of tissues activities, protein and mRNA of Glo1 in the putative knockout mouse revealed normal, wild-type levels. A normal low level concentration of MG was also found. The Glo1 mutant mice had 1 or 2 copies of the mutated Glo1 gene but retained 2 copies of the functional wild-type gene produced by copy number increase in all tissues analysed. Neither the precursor stem cells nor C57BL/6J genetic background had endogenous increased Glo1 gene copy number. Inheritance patterns of the Glo1 alleles indicated that a wild-type allele was inserted on the same chromosome 17 homologue as the mutant allele. Accordingly, it was not possible to produce progeny with < 2 copies of wild-type Glo1 alleles and Glo1 expression and activity deficiency. We reasoned that dicarbonyl stress induced by Glo1 mutation during gene trapping had induced wild-type Glo1 allele copy number increase. This could be modelled by incubation of mouse embryonic stem cells with exogenous MG where we found low level induced increase in Glo1 copy number. High intensity genome-wide DNA microarray analysis revealed that the Glo1 copy number alteration was specific for the Glo1 genetic locus.
Conclusion: Gene trapping mutation by the IMKC was successful but it induced concomitant Glo1 gene duplication focussed on and selective for the Glo1 genomic domain. Glo1 duplication was functional, thereby maintaining wild-type levels of Glo1 expression and activity and sustained the healthy phenotype.
Supported by: A.S. PhD studentship from Ministry of Education, Government of Saudi Arabia
Disclosure: **A. Shafie:** Grants; Yes.

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Complement C3 and hypofibrinolysis in diabetes: role of protein glycation and development of a new methodology to reduce thrombosis risk

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Background and aims: Diabetes is associated with increased cardiovascular disease due to clustering of risk factors along with thrombotic abnormalities. Complement C3, a central component of the complement pathway, prolongs fibrin clot lysis, an effect that is enhanced in diabetes and thereby represents a potential diabetes-specific therapeutic target. Our aims were to i) study the fibrinolytic properties of C3 from diabetes patients and explore potential post-translational modifications ii) elucidate potential binding sites between fibrinogen and C3 and iii) modulate fibrin clot lysis by targeted interference of fibrinogen-C3 interactions.
Materials and methods: Complement C3 and fibrinogen were purified from type 1 diabetes patients and healthy controls (n=6 in each group). Turbidimetric analysis was employed to study fibrinolysis whereas mass spectrometry, microarray screening and molecular modelling evaluated C3 glycation and interaction sites with fibrinogen. Moreover, a novel, non-antibody binding protein (Adhiron) was used to interfere with fibrinogen-C3 interaction, as a tool to modulate thrombosis risk.
Results: C3 from diabetes patients resulted in excessive prolongation of clot lysis time, compared with controls (522±166 seconds vs 195±105 seconds, respectively; p=0.04). This prolongation showed wide inter-individual variability ranging from 144 to 1476 seconds. Mass spectrometry identified *in-vivo* glycation of lysine residues 502, 685, 1139, 1203, 1209 and 1526 within diabetic C3 but no glycation was detected within control protein. A novel, non-antibody binding protein (Adhiron) which binds to fibrinogen and interacts with C3, resulted in complete abolition of C3-induced prolongation of lysis, reducing lysis time from 728±25.1 to 632±23.7 seconds (p=0.005). Peptide microarray screening identified 2 peptide motifs within the β chain of fibrinogen (residues 424-433, 435-445) that bound to C3, which were similar to Adhiron binding sites as predicted by molecular modelling.
Conclusion: Glycation of certain lysine residues within C3 may result in variable interaction with fibrinogen during clot formation and thus affect incorporation into the clot with a subsequent variation in clot lysis prolongation. Inhibition of fibrinogen-C3 interaction using a novel method of identifying binding proteins could lead to a diabetes specific therapeutic target for reducing atherothrombotic risk, the main cause of mortality in this population.
Supported by: Sir Jules Thorn Charitable Trust
Disclosure: **R.J. King:** None.

1102

Inflammatory CD14++CD16+ monocytes contribute to atherosclerosis in type 2 diabetes

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Background and aims: Mononuclear cell lineages are believed to be core players of atherosclerosis. Recent studies have shown that circulating mononuclear cells are heterogeneous and involved in chronic inflammatory disease such as coronary heart disease. Monocytes can be functionary divided into three different phenotypes based on the differential expression of CD14 and CD16: CD14++CD16- monocytes, CD14++CD16+ monocytes, and CD14+CD16++ monocytes. In these subsets, CD14++CD16+ monocytes are elevated in chronic inflammatory disease. And more, recent studies reported that CD14++CD16+ monocytes increase with Type 2 diabetes and CVD events. In precritical stages of atherosclerosis, increase of CD14++CD16+ monocytes has been indicated to be associated with pulse wave velocity (PWV) in CKD patients. However, little is known about the association between polarized CD14++CD16+ monocytes and eary stage of atherosclerosis in Type 2 diabetes. In this study, we cross-sectionally investigated the association between CD14++CD16+ monocytes and atherosclerosis indexes in Type 2 diabetes.
Materials and methods: The subjects in this cross-sectional study were 105 Type 2 diabetes (59 males and 46 females, age: 64 ± 11 years, duration of diabetes: 14.5 (6-21) years,) who were admitted in our diabetes center. Patients with CKD stage 5, infection, steroids therapy and cancer were excluded. Circulating leukocytes were collected from the patients and then fluorescently labeled with CD16 and CD14 antibodies. Monocyte population was gated by foward scatters and side scatters. Distribution of monocyte subsets were identified by flow cytometry. As indexes of atherosclerosis, flow-mediated dilatation (FMD), pulse wave velocity (PWV), ankle branchial pressure index (ABI) and intima-media thickness (IMT) were measured using a novel ultrasound device

(UNEXEF18G), a PWV meter (model PWV-200) and high-resolution B-mode ultrasonography (Prosound F75 echo tracking system).
Results: The mean monocyte count was 531 ± 253 cells/μl. The percentage of CD14++CD16- monocytes was 78.3 ± 6.7%, CD14++CD16+ monocytes: 6.7 ± 2.9%, CD14+CD16++ monocytes: 5.7 ± 2.9%, respectively. Mean PWV was 1160 ± 296 cm/sec. Mean IMT displayed 1.03 ± 0.30 mm. And mean ABI was 1.09 ± 0.14. Simple linear regression analysis showed that CD14++CD16+ monocytes was significantly associated with PWV (r=0.280, p=0.005). On the other hand, CD14++CD16 monocytes did not reach significant correlation with IMT (r=0.159, p=0.108), FMD (r=-0.080, p=0.426) and ABI (r=0.037, p=0.711). On multiple regression analysis including systolic blood pressure, HbA1c, HDL-C, LDL-C, eGFR, duration of diabetes, smoking history, BMI and CD14++CD16+ monocytes as independent factors, significant independent dontributors to PWV (R^2=0.406), P=<0.001) were CD14++CD16+ monocytes (β=0.204, 0.035), duration of diabetes (β=0.333, p=0.001), BMI (β=-0.217, p=0.023) and eGFR (β=-0.241, p=0.016). While, CD14++CD16+ monocytes was not related to IMT, FMD and ABI.
Conclusion: CD14++CD16+ monocytes are associated with the index of arterial sclerotic change in Type 2 diabetes.
Disclosure: **M. Nakamura:** None.

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Anti-inflammatory lipoxin A4 attenuates atherosclerosis progression in diabetic ApoE-/- mice

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Background and aims: Patients with diabetes have mortality from cardiovascular disease that is over twice that observed in the general population, resulting in atherosclerotic plaque formation. The diabetic plaque is characterised by abnormalities in both endothelial and vascular smooth muscle cell function. Lipoxins (LXs) are endogenous small molecule lipid mediators with the potential to treat multiple inflammatory diseases. Here we investigated the potency of endogenous (LXA4) and stable LX analogues (Benzo-LX) in attenuating atherosclerosis in diabetic ApoE-/- mice.
Materials and methods: 6-week-old apolipoprotein E knockout (ApoE-/-) mice were randomly divided into control and diabetes groups. Diabetes was induced with low-dose streptozotocin. Mice in both groups were randomly divided into vehicle, endogenous LX, and stable LX analogue groups, and followed for 10-20 weeks. Primary mouse aortic endothelial cells and vascular smooth muscle cells were stimulated with PDGF (5ng/ml) or TNF-alpha (1ng/ml) with or without lipoxin A4 (0.1nM), and markers of inflammation and fibrosis were assessed.
Results: LXs significantly attenuated aortic plaque progression in diabetic ApoE-/- mice (P<0.05), and also inhibited expression of markers of inflammation, including vascular cell adhesion molecule-1 (VCAM), monocyte chemoattractant protein 1 (MCP1) and pro-inflammatory interleukin 6 (IL-6) in aortic tissue. LXA4 significantly attenuated both PDGF and TNF-alpha signalling in smooth muscle and endothelial cells through inhibition of expression of PDGF-receptor (PDGFRβ1), TNF-receptor (TNFR1), VCAM, MCP1 and IL-6. Lipoxin A4 suppressed PDGF-mediated migration and proliferation, and TNF-alpha mediated NF-kB activity in smooth muscle cells. Furthermore, lipoxin A4 significantly attenuated TNF-alpha mediated monocyte adhesion to aortic endothelial cells.
Conclusion: Taken together, our data suggests that lipoxin A4 may be used as a novel therapeutic in diabetes-associated atherosclerosis, through suppression of atherosclerotic plaque formation, and inhibition of PDGF and TNF-alpha signalling pathways in vascular cells.

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Disclosure: **E. Brennan:** None.

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Increased serum levels of interleukin-1 receptor antagonist (IL-1ra) precede coronary heart disease: meta-analysis of 4 prospective cohort studies

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Background and aims: Interleukin-1β (IL-1β) represents a key proinflammatory cytokine in the development of coronary heart disease (CHD). The activity of IL-1β is counter-regulated by interleukin-1 receptor antagonist (IL-1ra), an endogenous inhibitor. Data on circulating IL-1ra levels and cardiovascular risk are controversial. Therefore, this study aimed (i) to examine the association between serum IL-1ra and incident CHD in the MONICA/KORA (MONItoring of trends and determinants in CArdiovascular disease/Cooperative Health Research in the Region of Augsburg) Augsburg case-cohort study, (ii) to identify further population-based studies in a systematic review for meta-analysis and (iii) to test whether the association between IL-1ra and incident CHD is explained by other inflammation-related biomarkers.
Materials and methods: The analysis in the MONICA/KORA study is based on data from 803 CHD cases and 1942 non-cases with a follow-up time of 16.0±5.8 years. We performed a systematic literature review to identify further studies that described the association between circulating levels of IL-1ra and cardiovascular outcomes (fatal and non-fatal myocardial infarction, sudden cardiac death and/or cardiovascular mortality). The association between IL-1ra and incident CHD was quantified using an inverse variance weighting approach in a fixed effects meta-analysis. Data on serum levels of 15 inflammation-related biomarkers available in the MONICA/KORA study were used to assess their impact on the association between IL-1ra and incident CHD in Cox proportional hazards analyses.
Results: Four population-based prospective cohort studies (MONICA/KORA Augsburg case-cohort, FINRISK 1997, Rotterdam and Belfast PRIME Men cohort studies) were included in this analysis. Mean follow-up times ranged between 10 and 16 years. The meta-analysis is based on a total of 1,700 CHD cases and 14,050 noncases. The pooled hazard ratio (95% CI) for incident CHD was 1.15 (1.08; 1.22) per SD of log-transformed IL-1ra serum levels after adjustment for age, sex, anthropometric, metabolic and lifestyle factors (p<0.0001). There was no heterogeneity in effect sizes (I2 (95% CI) 0 (0; 67)%, p=0.64). The excess risk for CHD was attenuated by ≥10% after additional adjustment for serum levels of high-sensitivity C-reactive protein (hsCRP), interleukin (IL)-6, myeloperoxidase (MPO), soluble E-selectin or soluble intercellular adhesion molecule-1 (sICAM-1) in the MONICA/KORA study.
Conclusion: Serum IL-1ra levels are positively associated with increased risk of CHD after adjustment for multiple confounders in a meta-analysis of four European cohorts. This association may at least partially reflect a counter-regulation to subclinical inflammation, oxidative stress and endothelial activation.
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PS 116 Statins and beyond

1105

Statin worsens glycaemic control of type 2 diabetic patients in target LDL-C level and LDL-C reduction dependent manners: a meta-analysis

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Background and aims: Recent studies have demonstrated that a lower target low density lipoprotein cholesterol (LDL-c) level, higher LDL-c reduction and higher dose of statin therapy increased risk of incident diabetes. However, the effects of statin therapy on glycemic control in diabetic patients remain unclear.
Materials and methods: MEDLINE, EMBASE and Cochrane were searched for RCTs which compared statins with placebo and compared high-dose with low-dose statin therapy in type 2 diabetes (T2DM). Trials with target LDL-c levels ≤2.59mmol/L or LDL-c reduction ≥30% were analysed. We then calculated mean differences in HbA1c and FBG by stratified target LDL-c level and relative LDL-c reduction. Meta-regression was used to investigate potential sources of heterogeneity between trials.
Results: Meta-analysis showed that intensive LDL-c lowering statin therapy increases the level of HbA1c compared to placebo (SMD 0.10%, 95% CI 0.01, 0.20). Stratified analyses revealed that the effects were more significant as target LDL-c level lowers (≤2.6 mmol/L) and LDL-c reduction increases (≥30%), respectively. High-dose statin was proved to elevate HbA1c (SMD 0.32%, 95% CI 0.16, 0.48) and FBG (SMD 0.29%, 95% CI 0.13, 0.45) comparing to low-dose statin. Meta-regression analyses showed that lower baseline LDL-c (p=0.009) and endpoint LDL-c levels (p=0.003) were risk factors involving in increasing HbA1c level of T2DM during statin therapy.
Conclusion: Statin therapy worsens glycemic control of T2DM in target LDL-c level and LDL-c reduction dependent manners. Further study is needed to elucidate other risk factors (e.g. statin dose) of statin induced poor glycemic control.
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Long-term efficacy of evolocumab in reducing lipids in EU subjects with and without type 2 diabetes: an analysis from the open-label extension OSLER studies

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Background and aims: A recent meta-analysis of 12-week randomised clinical trials with the PCSK9 inhibitor evolocumab (EvoMab) in patients with hyperlipidemia showed marked reductions from baseline (BL) in lipids in subjects with (n=413) and without (n=2119) type 2 diabetes mellitus (T2DM). A 1-year pooled analysis resulted in marked reduction in lipids in subjects with and without T2DM. Here we further evaluate the long-term efficacy of EvoMab vs standard of care (SoC) on 1-year lipids level change in EU subjects with and without T2DM enrolled in the open-label extension (OLE) studies OSLER 1 and OSLER 2.
Materials and methods: A population-based 1-year interim OSLER 1 and 2 integrated analysis was performed on subjects enrolled at EU study sites. Demographic data were collected. Subjects were pooled according to the assigned OLE re-randomised 2:1 treatment (EvoMab + SoC or SoC) with the choice of 420 mg monthly or 140 mg every 2 weeks. Both EvoMab dosing regimens were combined as they have been shown to be clinically equivalent. Changes in lipids were evaluated at week 48 in subjects with and without T2DM.
Results: A total of 1802 subjects were randomised from EU study sites to EvoMab + SoC or SoC, 190 with and 1612 without T2DM. T2DM subjects represented 10% of EU subjects analysis and generally presented with more cardiovascular risk factors: hypertension 83% (vs 49% all EU), coronary artery disease 34% (vs 19% all EU), and cerebrovascular/peripheral arterial disease 19% (vs 11% all EU). Efficacy data are shown in the Table. At week 48, reduction in lipid levels were seen in subjects with and without T2DM who received EvoMab + SoC: LDL-C (-54.1% and -48.9%), non-HDL-C (-41.6% and -40.4%), TC (-30.0% and -27.7%), Lp(a) (median % change from BL: -25.0% and -22.4%), and TG (median % change from BL: -6.8% and -3.6%). Mean increases in HDL-C were comparable in EU subjects with and without T2DM (8.2% vs 7.9%, respectively).
Conclusion: In OSLER 1 and 2 OLE EU subjects that comprised 10% of T2DM subjects, 1-year EvoMab + SoC treatment yielded significant reduction in lipid levels notably in LDL-C and Lp(a) as compared with SoC alone, with similar reductions in patients with and without T2DM. Overall, these results were consistent with previous data from 1-year pooled analysis. EvoMab as an add-on therapy to statins allows T2DM subjects to reach LDL-C treatment goals. Table. Evolocumab 48-week efficacy data in EU subjects with and without T2DM

Efficacy	EvoMab + SoC				SoC			
	Subjects with T2DM		Subjects without T2DM		Subjects with T2DM		Subjects without T2DM	
	Mean parent study baseline (SD), [n]	Mean % change from baseline at week 48 (SE), [n]	Mean parent study baseline (SD), [n]	Mean % change from baseline at week 48 (SE), [n]	Mean parent study baseline (SD), [n]	Mean % change from baseline at week 48 (SE), [n]	Mean parent study baseline (SD), [n]	Mean % change from baseline at week 48 (SE), [n]
Calculated LDL-C (mmol/L)	2.86 (0.95), [121]	-54.1 (3.2), [106]	3.38 (1.32), [1084]	-48.9 (1.0), [1025]	2.83 (1.09), [69]	4.9 (4.5), [64]	3.42 (1.38), [528]	8.6 (1.8), [484]
Non-HDL-C (mmol/L)	3.70 (1.08), [121]	-41.6 (3.0), [108]	4.04 (1.42), [1084]	-40.4 (0.9), [1034]	3.69 (1.23), [69]	4.3 (4.2), [65]	4.11 (1.48), [528]	7.8 (1.5), [490]
Total Cholesterol (mmol/L)	4.93 (1.09), [121]	-30.0 (2.2), [108]	5.56 (1.42), [1084]	-27.7 (0.7), [1034]	4.96 (1.22), [69]	1.6 (2.9), [65]	5.62 (1.49), [528]	4.3 (1.0), [490]
Triglycerides (mmol/L) median, (Q1,Q3), [n]	1.69 (1.27, 2.34), [121]	-6.8 (-29.2, 20.2), [108]	1.28 (0.95, 1.75), [1084]	-3.6 (-23.8, 20.0), [1034]	1.65 (1.18, 2.32), [69]	5.4 (-20.4, 38.8), [65]	1.25 (0.98, 1.82), [528]	5.1 (-17.5, 27.8), [490]
Lp(a) (nmol/L) median, (Q1,Q3), [n]	26.0 (11.0, 133.0), [118]	-25.0 (-45.5, -2.2), [108]	34.0 (11.0,138.5) , [1076]	-22.4 (-40.9,-3.2), [1038]	39.0 (15.0, 147.0), [69]	-1.2 (-16.7, 15.5), [66]	30.0 (9.0, 135.0), [525]	0.0 (-16.1, 14.1), [496]
HDL-C (mmol/L)	1.23 (0.35), [121]	8.2 (1.6), [108]	1.52 (0.47), [1084]	7.9 (0.5), [1034]	1.28 (0.38), [69]	0.0 (2.2), [65]	1.51 (0.48), [528]	1.2 (0.7), [491]

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MEDI4166: a PCSK9 Ab-GLP-1 fusion molecule that elicits robust antidiabetic and antihyperlipidaemic effects in rodents and non-human primates

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Background and aims: Type 2 diabetes (T2D) is a chronic metabolic disease characterized by hyperglycemia and increased cardiovascular (CV) risk. Achieving robust glycemic control and significantly reduced

CV risk are twin goals that are the cornerstone of therapy in type 2 diabetic subjects. MEDI4166 is an antibody-peptide genetic fusion molecule comprised of an anti-proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody that is linked to a GLP-1 analog. MEDI4166 has been engineered to provide optimal glycemic control and PCSK9 suppression in one molecule.

Materials and methods: Chinese hamster ovary cells stably expressing GLP-1 receptors were used to evaluate the effect of MEDI4166 on cAMP production. HepG2 hepatoma cell line was used to evaluate the effect of MEDI4166 on LDL uptake. C57Bl/6 mice fed a 60% high-fat diet and cynomolgus monkeys were used to evaluate the metabolic effects of subcutaneously injected MEDI4166.

Results: MEDI4166 fully activated GLP-1 receptors expressed in CHO cells relative to native GLP-1, and was able to restore LDL uptake by HepG2 cells treated with recombinant PCSK9. MEDI4166 (1 or 10 mg/kg) administered subcutaneously (sc) into diet-induced obese (DIO) mice significantly reduced blood glucose excursion in an ipGTT conducted 7 days following a single injection as compared to vehicle-treated mice. The effects of the higher dose on fasted glucose levels lasted up to 14 days following the single sc injection. Repeated once-weekly (QW) dosing with MEDI4166 (3, 10 or 30 mg/kg, sc) in DIO mice for 26 days dose-dependently reduced body weight and fat mass as compared to vehicle-treated mice. In addition, MEDI4166 significantly reduced blood glucose at all doses investigated. Repeated QW administration of MEDI4166 (3, 10 or 30 mg/kg, sc) for 4 weeks in diabetic db/db mice dose-dependently reduced 4 h-fasted blood glucose levels. In addition, a single dose of MEDI4166 (10 mg/kg, sc) administered to cynomolgus monkeys produced a decrease in LDL-C that correlated with a MEDI4166-mediated decrease in free PCSK9 levels in serum.

Conclusion: MEDI4166 provides robust glucose control and weight loss in rodent models of T2D and obesity. In addition, MEDI4166 reduces LDL-C levels in cynomolgus monkeys. MEDI4166 is being developed for the treatment of T2D in patients who require control of blood glucose and LDL-C.

Disclosure: **A. Konkar:** None.

1108

Relationship between triglyceride-rich lipoprotein cholesterol concentrations and non-HDL cholesterol goal attainment in ODYSSEY phase 3 trials

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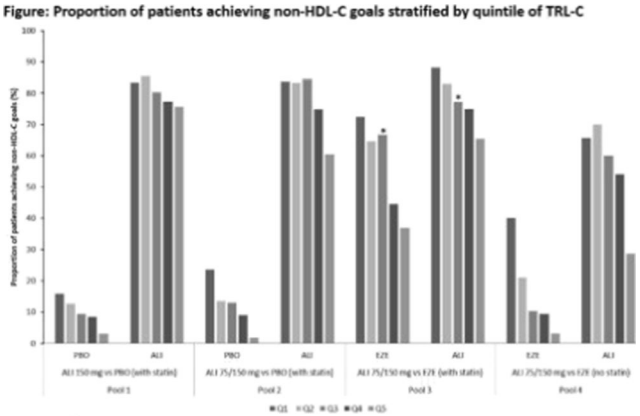
Background and aims: Non-high-density lipoprotein cholesterol (non-HDL-C) is more strongly associated with cardiovascular (CV) events than low-density lipoprotein cholesterol (LDL-C). Many guidelines recommend its use as a secondary target for lipid-lowering therapy, especially in patients with diabetes, obesity or elevated triglycerides (TG). TG levels and hence TG-rich lipoprotein cholesterol (TRL-C) levels (defined as [non-HDL-C] - [LDL-C]) are inversely correlated with attainment of non-HDL-C targets among patients receiving statins. We assessed the impact of TRL-C on non-HDL-C goal attainment using data from 4883 patients receiving placebo (PBO), ezetimibe (EZE) or alirocumab (ALI) in 10 ODYSSEY clinical trials.

Materials and methods: Studies were pooled as follows: Pool 1, ALI 150 mg every 2 weeks (Q2W) vs PBO (with background statins); Pool 2, ALI 75/150 mg Q2W vs PBO (with statins); Pool 3, ALI 75/150 mg Q2W vs EZE (with statins); Pool 4, ALI 75/150 mg Q2W vs EZE (no statins). Within each pool we assessed the impact of ALI, EZE and PBO on attainment of non-HDL-C goals (<100 and <130 mg/dL in patients at very high and moderate/high CV risk, respectively) at Week 24 among quintiles of baseline TRL-C levels (Q1-Q5).

Results: Among patients treated with PBO or EZE, increasing quintiles of TRL-C were associated with a lower proportion of patients achieving non-HDL-C goals (from Q1 to Q5, Pool 1: 15.9%-3.1%, Pool 2: 23.7%-1.7%, Pool 3: 72.4%-37.0%, Pool 4: 40.0%-3.3%). Corresponding figures for ALI showed a less marked trend and proportions of patients reaching non-HDL-C goals were higher vs control regardless of quintile (from Q1 to Q5, Pool 1: 83.4%-75.6%, Pool 2: 83.7%-60.4%, Pool 3: 88.2%-65.4%, Pool 4: 65.7%-28.6%; Figure). There was no evidence of effect modification across Q1-Q5 (p-value for homogeneity of ORs between 0.2-0.4).

Conclusion: In contrast to patients treated with statin, EZE or statin plus EZE, baseline TRL-C levels had a more modest impact on non-HDL-C goal attainment for ALI-treated patients. Among the TRL-C quintiles and in all patient pools, ALI treatment resulted in greater non-HDL-C goal attainment. Whether this translates into reductions in CV events among high-risk patients with elevated TRL-C will be assessed in the ongoing ODYSSEY OUTCOMES trial.

Figure: Proportion of patients achieving non-HDL-C goals stratified by quintile of TRL-C



ALI, alirocumab; EZE, ezetimibe; PBO, placebo. Within each pool, quintile by quintile comparisons of treatments (alirocumab vs. control) each had a p<0.05, unless denoted by *.

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Efficacy and safety of alirocumab in patients with multiple manifestations of cardiovascular disease: a post-hoc analysis of the ODYSSEY programme

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Background and aims: Patients with multiple manifestations of cardiovascular disease (CVD) are at the greatest risk for CV events. These patients, if not at LDL cholesterol (LDL-C) target, may benefit from additional LDL-C lowering therapy with alirocumab, an inhibitor of proprotein convertase subtilisin/kexin type 9. We assessed the efficacy and safety of alirocumab versus placebo on a background of maximally tolerated dose of statins ± other lipid-lowering therapies in patients with multiple manifestations of CVD.

Materials and methods: Two pooled cohorts, based on alirocumab dose regimen, were considered: Pool 1: alirocumab 75 mg every 2 weeks (Q2W) increasing to 150 mg Q2W at Week 12 if LDL-C goals were ≥70 mg/dL at Week 8 (ODYSSEY FH I, FH II, COMBO I); Pool 2: alirocumab 150 mg Q2W (ODYSSEY LONG TERM, HIGH FH). Multiple manifestations of CVD were defined as patients with a history of multiple (≥2) CV events in a single vascular bed (separated ≥30 days) or patients with >1 affected vascular bed

Results: A total of 720 patients met the criteria for multiple manifestations of CVD. Baseline characteristics were comparable across treatment groups. There was a significant difference between the alirocumab and placebo groups in percent change in LDL-C level from baseline to Week 24 (least-squares mean difference vs placebo, Pool 1: -56.3% [95% confidence interval (CI) -65.5 to -47.0] P<0.0001; Pool 2: -61.4% [95% CI -66.0 to -56.8] P<0.0001) (Table). These data are consistent with overall findings from ODYSSEY. Pooled safety was similar for alirocumab versus placebo (Table).

Conclusion: Patients with multiple manifestations of CVD experienced a substantial LDL-C reduction with alirocumab, consistent with the overall findings from ODYSSEY. Alirocumab was well tolerated. These data support the addition of alirocumab therapy in patients with multiple manifestations of CVD who require additional LDL-C lowering.

Table. Summary of results

	Pool 1 ODYSSEY FH I, FH II, COMBO I		Pool 2 ODYSSEY LONG TERM, HIGH FH	
Characteristic	**Alirocumab 75/150 mg Q2W**	**Placebo**	**Alirocumab 150 mg Q2W**	**Placebo**
Sample size*	89	44	370	217
Age, mean (SD) years	62.9 (10.3)	61.6 (9.5)	62.1 (9.6)	63.3 (9.5)
Male, n (%)	56 (62.9)	30 (68.2)	279 (75.4)	160 (73.7)
Type 1 diabetes, n (%)	—	—	1 (0.3)	2 (0.9)
Type 2 diabetes, n (%)	19 (21.3)	8 (18.2)	101 (27.3)	55 (25.3)
Baseline LDL-C, mean, mg/dL	119.0	129.3	117.9	117.4
% change in LDL-C at Week 24, least squares mean	-51.5	4.8	-61.7	-0.3
Any treatment-emergent adverse event, n (%)	73 (82.0)	32 (72.7)	304 (82.2)	188 (86.6)
Any treatment-emergent serious adverse event, n (%)	15 (16.9)	7 (15.9)	85 (23.0)	43 (19.8)

*May vary in efficacy analyses

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Plasma PCSK9 is associated with the severity of coronary artery lesions in patients with acute coronary syndrome

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Background and aims: PCSK9 acts as an endogenous natural inhibitor of the LDL receptor (LDLR) pathway, by targeting the receptor to lysosomes for degradation. Plasma PCSK9 concentrations have been shown to be positively associated with LDL-cholesterol (LDL-C). Whether PCSK9 is associated with coronary angiographic lesions remains unknown. The primary goal was to assess the correlation between plasma concentration of PCSK9 and coronary damage severity in patients with acute coronary syndrome (ACS).

Materials and methods: In this monocentric, observational, prospective study, coronary lesions were measured using the SYNTAX score at the admission in cardiac ICU (Day (D) 0). Plasma PCSK9 concentration were measured using ELISA assay at D0, 1, 2, 3 & 4. Spearman's correlations were used to determine the association between PCSK9 levels, SYNTAX score and metabolic parameters.

Results: One hundred seventy-four patients with ACS (119 were without statins and 55 under statins at D0) were included. The mean age of the patients was 59±14 years, 79% were male, 43% were current smokers 13% had diabetes, 42% hypertension and 46% dyslipidemia. The mean value of SYNTAX score was 10.8±7.9 (n=173). After admission, PCSK9 concentrations reached a maximum level at D2 and remained stable until D4 (D0: 304±122 ng/ml, D1: 349±138 ng/ml, D2: 398±166 ng/ml; D3: 398±157 ng/ml; D4: 390±172 ng/ml; p<0.001). In patients without statins, PCSK9 at D0 was associated with LDL-C (rho=0.226, p=0.017) and ApoB (rho=0.282, p=0.005), but also triglycerides (rho=0.202, p=0.03). PCSK9 at D0 was positively associated with SYNTAX (rho=0.154, p=0.04) in the whole population. This association was stronger in patients without statins (rho=0.239, p=0.009) and was maintained after adjusting for LDL-C (P=0.014).

Conclusion: This study demonstrates for the first time that plasma PCSK9 level is positively associated with the severity of coronary artery lesions, at least partly independently of LDL-C concentrations. This reinforces the potential interest of PCSK9 inhibition for treating cardiovascular diseases.

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Disclosure: **B. Cariou:** None.

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Alirocumab efficacy and safety in patients with pre-diabetes versus normoglycaemia

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Background and aims: Persons with pre-diabetes (PD) are at increased risk of developing cardiovascular disease as well as diabetes, but statins may worsen their glycaemia and/or increase the risk of developing diabetes. We examined low-density lipoprotein cholesterol (LDL-C) reductions with the proprotein convertase subtilisin/kexin type 9 (PCSK9) antibody alirocumab (ALI) in patients with hypercholesterolemia and with either PD or normoglycaemia (NG) at baseline, using data from 10 Phase 3 trials.

Materials and methods: Patients received ALI or control (placebo/ezetimibe) for 24-104 weeks, with maximally tolerated statin in most cases. In eight trials, ALI 75 mg every 2 weeks (Q2W) was increased to 150 mg Q2W at Week 12 based on Week 8 LDL-C; the other two trials used 150 mg Q2W throughout. PD (n=1860) was defined at baseline as HbA1c ≥5.7% and <6.5% or two fasting plasma glucose (FPG) values ≥100 mg/dL (but ≤1 value ≥126 mg/dL), or as specific terms reported in medical history (Custom Medical Dictionary of Regulatory Activities Query "impaired glucose control" included preferred terms "glucose tolerance impaired", "impaired fasting glucose", "insulin resistance", and

"insulin resistance syndrome"). Those with a clinical diagnosis of diabetes were excluded from analysis and the remaining patients were classified as NG (n=1481).

Results: LDL-C reductions from baseline to Week 24 with ALI were 44.0-61.8% in patients with PD, and 45.8-59.5% in those with NG (Table). LDL-C reductions were generally similar in those with and without baseline triglycerides (TG) ≥150 mg/dL in those with PD (Table) and in those with NG. The baseline HbA1c values were 5.8% in patients with PD and 5.3-5.4% in those with NG. FPG ranged from 102.1-105.3 mg/dL and 92.3-94.2 mg/dL in patients with PD and NG, respectively. ALI had no effect on HbA1c or FPG in either patient population. Adverse event rates were generally similar in those with and without PD. Nasopharyngitis occurred in ≥5% patients in all ALI and control subgroups.

Conclusion: ALI reduced LDL-C versus controls similarly in those with PD versus NG, with no effect on glycaemia, and was well-tolerated up to 104 weeks.

Table. LDL-C reductions in patients with pre-diabetes or normoglycaemia at baseline

Efficacy analysis (ITT): LS mean (SE) % change from baseline to Week 24 in calculated LDL-C	PD				NG				Interaction *P*-value PD vs NG
	ALI		Control		ALI		Control		
	n	% change	n	% change	n	% change	n	% change	
ALI 150 mg Q2W vs PBO (+ statin)	586	-61.8 (1.2)	290	2.1 (1.6)	431	-59.5 (1.4)	225	-0.1 (1.9)	0.1431
ALI 75/150 mg Q2W vs PBO (+ statin)†	269	-52.4 (1.7)	127	3.3 (2.4)	282	-46.1 (1.6)	140	8.9 (2.3)	0.8541
ALI 75/150 mg Q2W vs EZE (+ statin)†	263	-51.7 (2.2)	169	-16.1 (2.6)	148	-45.8 (2.8)	96	-21.8 (3.6)	0.0428
ALI 75/150 mg Q2W vs EZE (no statin)†	64	-44.0 (2.9)	71	-16.0 (2.7)	71	-46.3 (2.7)	76	-13.7 (2.6)	0.4073
Efficacy analysis according to baseline TG levels in patients with PD at baseline	**PD with TG <150 mg/dL**				**PD with TG ≥150 mg/dL**				
ALI 150 mg Q2W vs PBO (+ statin)	377	-59.4 (1.4)	172	0.2 (2.1)	209	-66.3 (1.9)	118	5.1 (2.6)	
ALI 75/150 mg Q2W vs PBO (+ statin)†	174	-52.8 (2.1)	93	3.0 (2.8)	95	-51.8 (2.9)	33	4.4 (4.7)	
ALI 75/150 mg Q2W vs EZE (+ statin)†	157	-50.6 (2.8)	100	-16.4 (3.5)	106	-53.4 (3.4)	69	-15.8 (4.2)	
ALI 75/150 mg Q2W vs EZE (no statin)†	31	-43.8 (4.1)	40	-20.0 (3.6)	33	-44.3 (4.0)	31	-10.8 (4.2)	

ALI, alirocumab; EZE, ezetimibe; ITT, intent-to-treat; LDL-C, low-density lipoprotein cholesterol; LS, least-squares; NG, normoglycaemia; PBO, placebo; PD, pre-diabetes; Q2W, every 2 weeks; SE, standard error; TG, triglycerides. 10 Phase 3 trials in four pools according to study design. †Alirocumab dose was increased from 75 to 150 mg Q2W in 17.3–35.1% and 19.6–50.8% of patients with PD and NG, respectively, at Week 12, depending on Week 8 LDL-C levels.

Clinical Trial Registration Number: NCT01644188; NCT01730040; NCT01730053; NCT01644175; NCT01623115; NCT01709500; NCT01507831; NCT01617655; NCT01709513; NCT01644474

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1112

Effects of K-877, a novel selective PPARα modulator (SPPARMα), on lipid and glucose metabolism in fasting and postprandial states in type 2 diabetic patients with dyslipidaemia

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Background and aims: Type 2 diabetes mellitus (T2DM) is frequently associated with dyslipidemia, which contributes to the increased risk for developing atherosclerosis. Previous studies showed that K-877 (pemafibrate), a novel selective PPARα modulator (SPPARMα), potently lowered plasma TG level and increased HDL-C in patients with dyslipidemia, and it exhibited fewer adverse events such as elevated levels of serum liver enzymes and creatinine than fenofibrate. To investigate whether K-877 improves fasting and postprandial lipoprotein profiles as well as glycemic control in patients with T2DM and dyslipidemia, we performed a randomized, double-blind, placebo-controlled phase 3 trial.

Materials and methods: Patients with T2DM and dyslipidemia (HbA1c ≧ 6.2%, TG ≧ 150 and < 1000 mg/dL) were randomized to receive placebo (P), K-877 0.2 mg/day (L) or K-877 0.4 mg/day (H) for 24 weeks. At baseline and week 24, plasma lipids and glucose were sequentially measured over 6.5 hours after ingestion of Meal Test C (592 kcal, fat 28.5 g, carbohydrate 75.0 g, protein 8.0 g) in some facilities. An ANCOVA model with baseline level as a covariate was used for efficacy analyses.

Results: A total of 167 patients (Age, 60.5 ± 10.5 years; BMI, 25.9 ± 3.5 kg/m^2; HbA1c, 6.96 ± 0.44%; TG, 262.1 ± 104.1 mg/dL) were randomized to P, L and H groups (n = 57, 54 and 56, respectively). The meal tolerance test was performed in 18, 22 and 22 patients, respectively. K-877 significantly reduced fasting TG (-10.8%, -44.3% and -45.1%, p < 0.001 vs P) and remnant lipoprotein cholesterol (RemL-C) (1.1%, -49.1% and -48.3%, p < 0.001 vs P). The percentages of patients reaching TG < 150 mg/dL were 15.8%, 81.5% and 70.9%. HbA1c did not change compared to placebo (0.06%, 0.18% and 0.16%, p = 0.208 for L and p = 0.297 for H vs P). K-877 significantly reduced fasting plasma glucose (2.8, -2.6 and -2.4 mg/dL, p < 0.05 vs P), fasting plasma insulin (0.51, -1.32 and -1.46 μU/mL, p < 0.01 vs P) and HOMA-IR (0.30, -0.57 and -0.44, p < 0.05 vs P) as of on-treatment levels during 24 weeks assessed by repeated measures ANCOVA (post hoc analysis). K-877 significantly ameliorated postprandial hyperlipidemia without affecting postprandial plasma glucose and insulin: AUC0-6.5h of TG (-7.5%, -48.0% and -45.2%, p < 0.001 vs P) and that of RemL-C (-9.6%, -55.0% and -55.2%, p < 0.001 vs P). The incidence rates of adverse events/adverse drug reactions were not different between placebo and K-877 groups (71.9%/12.3%, 66.7%/11.1% and 60.0%/16.4%, respectively).

Conclusion: K-877 significantly ameliorated dyslipidemia in both fasting and postprandial states in patients with T2DM. K-877 may also improve insulin sensitivity. K-877 could be a promising therapy for atherogenic dyslipidemia in patients with T2DM.

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Disclosure: **E. Araki:** Grants; Nippon Boehringer Ingelheim Co., Ltd., Novo Nordisk Pharma Ltd., ONO PHARMACEUTICAL CO., LTD., Sanofi KK, Mitsubishi Tanabe Pharma Corporation, Novartis Pharma K.K., Kowa Pharmaceutical Co. Ltd., Astellas Pharma Inc., AstraZeneca K.K., Takeda Pharmaceutical Company Ltd., Taisho Toyama Pharmaceutical Co. Ltd, Pfizer, DAIICHI SANKYO COMPANY Ltd.. Lecture/other fees; Kowa Company Ltd., MSD, Nippon Boehringer Ingelheim Co., Ltd., Novo Nordisk Pharma Ltd., ONO PHARMACEUTICAL CO., LTD., Sanofi KK, Mitsubishi Tanabe Pharma Corporation, Novartis Pharma K.K., Kowa Pharmaceutical Co. Ltd., Astellas Pharma Inc., AstraZeneca K.K., Takeda Pharmaceutical Company Ltd., Eli Lilly Japan K.K., DAIICHI SANKYO COMPANY Ltd.

PS 117 Heart failure is not an option

1113

Glycosylated haemoglobin predicts mortality in patients with heart failure and unknown diabetes: insights from the Swedish Heart Failure registry (SwedeHF)

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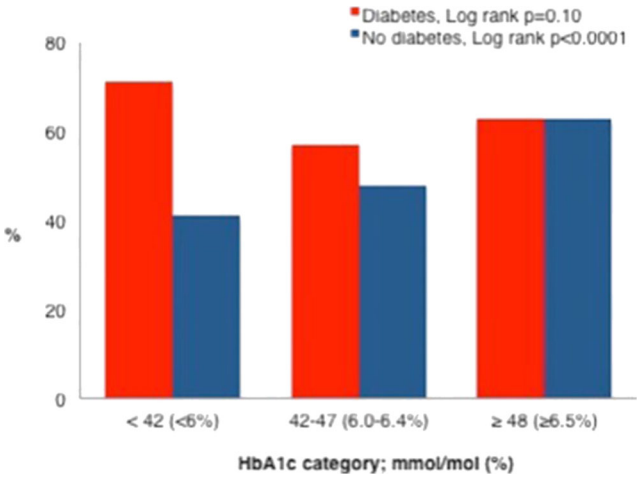
Background and aims: Dysglycaemia often coexists with heart failure and compromises long-term prognosis. Glycosylated haemoglobin (HbA1c) can be used as a marker for the glycaemic state especially in stressful situations. The aim of the current study was to investigate the prognostic implications of increasing HbA1c in patients with heart failure, with and without a reported diabetes diagnosis.

Materials and methods: Patients with and without previously known diabetes included in the Swedish national heart failure registry (SwedeHF) between 2003-2011 with a reported HbA1c (n=2181) were followed for all cause mortality until December 31, 2014. Mortality was analysed by glycaemic state as defined by the International Diabetes Expert Committee (IDEC) in 2009: normal: HbA1c IFFC <42 mmol/mol (DCCT <6.0%); high risk for diabetes 42-47 mmol/mol (6.0-6.4%); and diabetes: ≥48 mmol/mol (≥6.5%) and by a history of diabetes. Hazard ratios (HR) of mortality were estimated in a Cox regression model adjusting for age, gender, kidney function and level of heart failure care (hospitalisation or out patient clinic-visit).

Results: The number of patients without reported diabetes was 921 (71 ±12 years, 66% men, 14% EF ≥50%) of whom 15% (n=136) had an HbA1c ≥48 mmol/mol and 30% (n=273) 42-47 mmol/mol. Among the 1260 patients with previously known diabetes (72±11 years, 66% men, 18% EF ≥50%), 8% had an HbA1c <42mmol/mol, 16% 42-47mmol/mol and 76% ≥48 mmol/mol. The median follow-up time was 4.4 (IQR 2-6) years. Absolute mortality by HbA1c category is depicted in Figure 1. Mortality increased with higher HbA1c in the group without previously known diabetes (log-rank p<0.0001) but not in those with known diabetes (log-rank p=0.10). In the group without previously known diabetes the adjusted HR for mortality with HbA1c ≥48 vs. <42 mmol/mol was 1.44 (95% CI 1.11-1.87). The corresponding HR for those with known diabetes was 0.97 (95% CI 0.76-1.25).

Conclusion: According to criteria based on HbA1c, 15% of the patients with heart failure had previously undetected and further 30% were at high risk for diabetes. In these patients, long-term mortality deteriorated with increasing HbA1c. In contrast HbA1c did not predict mortality in patients with known diabetes. This emphasizes the importance of screening for dysglycaemia in populations with heart failure and favours the assumption that glucose control should be initiated early in the development of dysglycaemia.

Figure. Long-term mortality (%) by HbA1c in patients with and without diabetes



Disclosure: **I. Johansson:** None.

1114

The effect of metformin on cardiac function and selected metabolic parameters in patients with type 2 diabetes/prediabetes and chronic heart failure

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Background and aims: Metformin is currently the first choice treatment for patients with type 2 diabetes (DM). Mechanisms of cardiovascular effects of metformin are not fully understood and there are no clearly designed intervention studies testing the metabolic and cardioprotective effects of metformin in patients with DM and chronic heart failure (HF).The aim of this study was to evaluate the effects of metformin on myocardial functions and selected metabolic indicators in patients with DM/prediabetes and chronic HF with reduced ejection fraction.

Materials and methods: A randomized, double-blind, placebo-controlled, crossover study in a total time of six months testing the effect of 3- month usage of metformin vs. placebo. 31 treatment naive diabetic patients with chronic HF were randomized to metformin (2 g/day) or to placebo group. After the three months of treatment the medication was changed and the treatment continued for next 3 months. At the beginning and the end of the intervention period (3 times in total) the panel metabolic and cardiovascular tests was done. It included common clinical measurements, meal test, testing of immunoreactive insulin (IRI) and selected gastrointestinal peptides, DEXA, echocardiography and spiroergometry.

Results: 26 patients completed the protocol. The results are shown in table. Metformin in comparison with placebo decreased the level of HbA1c (p<0.003), reduced the blood glucose area under the curve (glucose AUC; p<0.001) and IRI (p <0.01) during the meal test. The use of metformin was associated with increased levels of GLP-1 after a meal test stimulation (p<0.01). Metformin compared to placebo did not affect the weight and waist circumference, while both regiments decreased fat in visceral area (measured by DEXA). Cardiac structure and function remained unchanged after both treatment periods.

Conclusion: Our results demonstrate the efficacy and safety of metformin in diabetic patients with systolic heart failure. Additionally it shows that one of the pathways behind the effect of metformin on glucoregulation is increase of stimulated levels of GLP-1.

Table: The effect of metformin on selected metabolic indicators

	Baseline value		MET		PL		ΔMET-PL		p
Total number/women/men)	26/ 4/22								
Age (years)	59.08	±6.21							
Weight (kg)	100.2	±18.9	98.2	±19.6	99.2	±19,8	1,03	±3,2	ns.
BMI (kg/m^2)	33.1	±4.6	32.4	±4.8	32.7	±4.8	0.3	±1.07	ns.
Waist circumference (cm)	112.2	±12.8	111	±12.7	112	±13.7	1,07	±4,1	ns.
HbA_{1C} (mmol/mol)	45.8	±7.9	44.4	±6.2	48.2	±10.2	3.8	±6,06	0.003
glu AUC	1409	±329	1250	±244	1427	±448	177	±281	0.00
IRI AUC	10424	±6 887	8219	±4 185	10271	±4 710	1672	±4 874	0.01
GLP180 (ng/ml)	15.4	±11.2	33.9	±21.9	19.4	±17.3	-9.4	±19.3	0.01

Abbreviations: MET – value measured after metformin treatment; PL – value measured after placebo; ΔMET-PL – the difference metformin or placebo . induced change; P – significance of differences after metformin versus placebo evaluated by paired t-test

Clinical Trial Registration Nubmer: NCT01690091
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Disclosure: **E. Stoláriková:** Grants; Supported by MZCR 00023001.

1115

Effect of dpp-4 inhibitors therapy on left atrial volume index in patients with type 2 diabetes

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Background and aims: Recent large scale clinical trials have shown that treatment with dipeptidyl-peptidase-4 (DPP-4) inhibitors has a neutral effect on cardiovascular outcomes. Left atrial volume index (LAVI) is part of cardiac remodelling in a variety of cardiovascular diseases and a strong predictor of cardiovascular morbidity and mortality. Therefore, the aim of the present study was to estimate the effect of DDP-4 inhibitors treatment to LAVI in patients with type 2 diabetes (T2D).
Materials and methods: 95 patients (55 males) with T2D, mean age (±SD) 65.1±9.1 years, HbA1c 6.4±0.8%, body-mass index (BMI) 29.21±5.4 Kg/m2, duration of diabetes 8.1±4.9 years receiving anti-diabetic treatment with metformin (40 patients, group A) or metformin plus DPP-4 inhibitors (55 patients, group B) for at least 6 months without known cardiovascular disease were enrolled into the study. All study patients underwent fully clinical examination and ultrasound examination of the heart while a blood sample was taken at fasting state. Patients were divided according to left atrial volume index (LAVI) ≥32 ml/m2.
Results: LAVI ≥32 ml/m2 was found in 14 patients (17.3%). LAVI did not differ between the two study groups (group A:25.1±6.0 vs. group B:25.9±7.1 ml/m2, P=0.58). Of the study participants; 63.8% had arterial hypertension, 78.9% dyslipidemia, 10.6% retinopathy, 17.0% neuropathy while 13.7% were current smokers. Multivariate regression analysis (backward), after controlling for age, sex, BMI, duration of T2D, HbA1c, smoking status, dyslipidemia, neuropathy, retinopathy, DPP-4 inhibitors and metformin therapy, C-reactive protein, creatinine clearance, uric acid, LDL- and HDL-cholesterol levels, showed that LAVI was positive related only with arterial hypertension (beta=0.39, p=0.008), white blood cells count (beta=0.262, p=0.09), and triglycerides level (beta=0.42, p=0.07). No significant association between LAVI and DPP-4 inhibitors therapy was found. Furthermore, regarding the well-established indexes of left ventricular (LV) diastolic dysfunction no significant association between LAVI and LV ejection fraction, LV mass index and E/e' ratio was found.
Conclusion: The results of the present study showed that treatment with DDP-4 inhibitors has a neutral effect on LAVI. On the contrary, presence of hypertension, white blood cells count and a triglycerides level were the only determinants of LAVI.
Disclosure: **A. Papazafiropoulou:** None.

1116

The effect of metformin on serum levels of Trimethylamine-N-oxide in patients with type 2 diabetes/prediabetes and chronic heart failure

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Background and aims: Trimethylamine-N-oxide (TMAO) is a product of gut microbiota. The high plasma levels are associated with the progression of atherosclerosis and increased risk of fatal cardiovascular events. The serum levels of TMAO are increased in obese patients with type 2 diabetes. However, the influence of pharmacological intervention on TMAO levels was not investigated till now. The aim of the study was to evaluate the effect of metformin on serum levels of TMAO and to explore its possible association to insulin resistance and diabetes control in patients with chronic heart failure and type 2 diabetes or prediabetes (DM+HF).
Materials and methods: The six-month randomized, interventional, double-blind, placebo-controlled, cross-over study, testing the effect of 3-month metformin intake (2g/day) vs. placebo on selected metabolic parameters, including TMAO levels. 31 patients with DM + HF were enrolled. 26 patients ended the study. At the baseline and at the end of each intervention period (3 times in total) the panel metabolic and cardiovascular tests was done. It included the routine clinical laboratory tests, measurement of the serum levels of TMAO and the hyperinsulinemic euglycemic clamp (3 hrs) for assessment of insulin sensitivity.
Results: The metformin intake significantly reduced HbA1c levels compared to placebo ($p<0.003$). However, the serum levels of TMAO were not significantly changed by metformin, as well as the insulin sensitivity, measured as a metabolic clearance of glucose (MCR) during the clamp. There were no significant correlations between the TMAO levels (baseline and after interventions) and HbA1c, serum lipids, BMI or MCR.
Conclusion: The metformin treatment does not significantly influence the TMAO levels. Our results do not support the relationship between the TMAO levels and the metabolic control of diabetes or insulin resistance in patients with type-2 diabetes and heart failure.

Tab.1: **Clinical and laboratory characteristics – baseline, after metformin (MET) and placebo (PL) intake and the changes during interventions (Δ)**

	TMAO (ug.ml-1)	MCR(ml.kg-1.min)	BMI	HbA1c (mmol/mol)
Baseline	1.03 ±1.29	3.17±1.40	33.11 ±4.60	45.89 ±8.00
MET	0.97±0.69	4.05±1.66	32.44 ±4.83	44.41 ±6.28
PL	0.84±0.95	3.65±1.62	32.78±4.89	48.26 ±10.23
Δ MET-PL	0.13±1.1	-0.26±1.07	0.34±1.08	3.85±6,06

Data are expressed as average ± SE. Differences were not statistically significant (paired t-test).

Clinical Trial Registration Number: NCT01690091
Supported by: Institutional Support MZCR 00023001 (IKEM, Prague, Czech Republic).
Disclosure: **K. Velebova:** None.

1117

Influence of type 2 diabetes on five-year survival for acute decompensated heart failure patients

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Background and aims: To evaluate the impact of type 2 diabetes mellitus (T2DM) on the course and prognosis of chronic heart failure (CHF)
Materials and methods: The study of the hospital register of acute decompensated heart failure (ADHF) was performed. The register contains 735 consecutively admitted ADHF patients for 2010-2011. Median follow-up was 1790 days.

Results: 254 patients (35% of the cohort) suffered from T2DM. Diabetic patients' average age was 70 ± 9 years, nondiabetic ADHF patients age was 68 ± 12 (p = 0.04, Student's t-test). Women accounted for 66% of the T2DM patients vs. 45% of nondiabetic ADHF patients (p <0.001 Pearson Chi-square). In the T2DM group atrial fibrillation was less common (37% vs. 51%, p <0.001, Pearson Chi-square), chronic obstructive pulmonary disease was also less common (23% vs. 34%, p = 0,003, Pearson Chi-square). T2DM patients were characterized by more frequent hypertension (89% vs 79%, p <0.001, Pearson Chi-square) and obesity (50% vs. 27%, p <0.001, Pearson Chi-square). The initial creatinine level was higher in the T2DM group (114 ± 67 vs. 102 ± 51 mmol / l, p = 0.009, Student's t-test). Insulin was administered to 34% of diabetic patients. The other T2DM patients received oral hypoglycemic therapy. Hospital ADHF mortality rate in T2DM group was 10.2% (26 patients) versus 6.0% (29 patients), p = 0.04 (Pearson Chi-square). Multivariate analysis was performed: the presence of type 2 diabetes increased the risk of death during the index hospitalization due to ADHF 2.0-fold (OR 2.0, 95% CI 1.1 - 3.6, p = 0.03, logistic regression). Re-hospitalization due to ADHF over the next 18 months was higher in T2DM patients: 22% (51 cases) vs. 16% (74 cases), p = 0.06 (Pearson Chi-square). 18-month T2DM patients survival rate was 0.69 vs. 0.77 (p = 0.03, Kaplan-Meier). The presence of T2DM increased the risk of death within 18 months 1.4-fold (p = 0.04, Cox regression). 165 (65%) T2DM patients died over 5 years vs. 278 (58%) nondiabetic patients, the survival curves differed significantly (p = 0.03, Kaplan-Meier). In the Cox model, the presence of type 2 diabetes increased the risk of death within 5 years 1.2-fold (p = 0.03). The study of the causes of death (total 443 cases) revealed that in T2DM group 52% of outcomes happened due to progression of heart failure. In the absence of diabetes, only 41% of outcomes related to the progression of heart failure (p = 0.03, Pearson Chi-square).

Conclusion: Diabetes can be considered the most common comorbidity in acute decompensated heart failure patients (up to 35% of cases). Diabetes increased demand of re-hospitalization due to heart failure over 18 months. T2DM is an independent risk factor for death during the index hospitalization and over the next 18 months and 5 years (increasing the risk of death 1.2 - 2.0-fold)

Disclosure: **I. Pochinka:** None.

1118

Effect of empagliflozin on heart failure outcomes in subgroups by age: results from EMPA-REG OUTCOME

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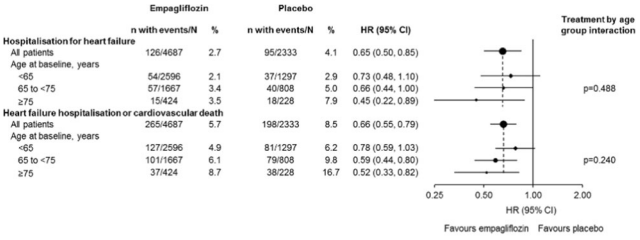
Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin (EMPA) added to standard of care significantly reduced 3-point major adverse cardiovascular (CV) events, CV death, hospitalisation for heart failure, and heart failure hospitalisation or CV death (composite) in patients with type 2 diabetes (T2DM) and high CV risk. We investigated the effect of age on reduction in hospitalisation for heart failure and heart failure hospitalisation or CV death with EMPA.

Materials and methods: Patients in EMPA-REG OUTCOME were randomised to receive EMPA 10 mg, EMPA 25 mg or placebo (PBO). We analysed hospitalisation for heart failure and heart failure hospitalisation or CV death in the pooled EMPA group versus PBO in subgroups by baseline age (<65, 65 to <75, ≥75 years).

Results: A total of 7020 patients were treated. Median observation time was 3.1 years. Mean (SD) age at baseline was 63.2 (8.8) years in the PBO group and 63.1 (8.6) years in the EMPA group. Reductions in hospitalisation for heart failure and the composite of heart failure hospitalisation or CV death with EMPA vs PBO were consistent across age categories (Figure). Across age subgroups, reported adverse events were consistent with the known safety profile of EMPA.

Conclusion: EMPA, added to standard of care, reduced the risk of hospitalisation for heart failure and heart failure hospitalisation or CV death in patients with T2DM and high CV risk irrespective of age.



Clinical Trial Registration Number: NCT01131676

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.

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1119

Heart failure hospitalisation or cardiovascular death with empagliflozin in patients with type 2 diabetes and high cardiovascular risk: analysis over time

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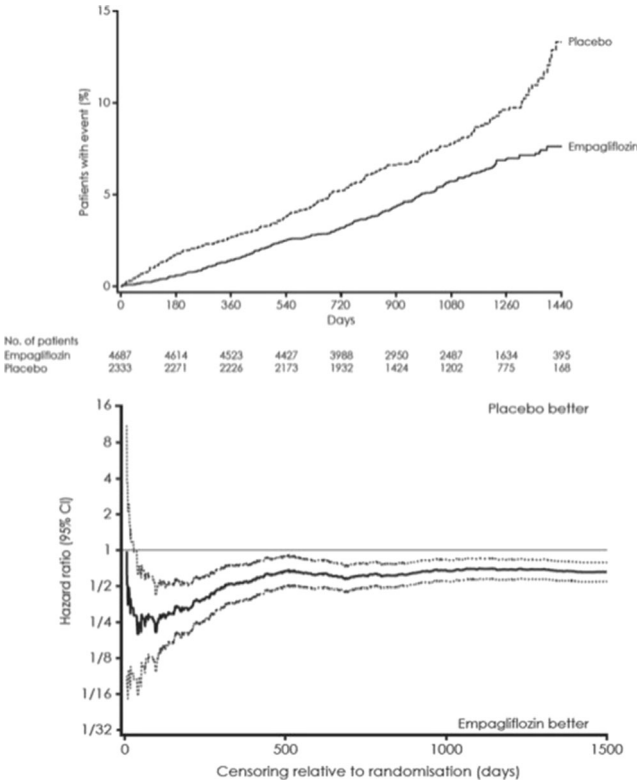
Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin (EMPA) given in addition to standard of care significantly reduced 3-point major adverse cardiovascular (CV) events (3-point MACE; composite of CV death, non-fatal myocardial infarction, non-fatal stroke) in patients with type 2 diabetes (T2DM) and high CV risk. This reduction was driven by a 38% reduction in CV death vs placebo (PBO). EMPA also reduced hospitalisation for heart failure by 35% and the composite of heart failure hospitalisation or CV death by 34%. We investigated hazard ratios with EMPA vs PBO for the composite of heart failure hospitalisation or CV death at time points following randomisation.

Materials and methods: Patients were randomised to receive EMPA 10 mg, EMPA 25 mg, or PBO in addition to standard of care. The cumulative probabilities of experiencing the composite of heart failure hospitalisation or CV death were analysed for pooled EMPA versus PBO in patients treated with ≥1 dose of study drug including all events from randomisation until study end. Hazard ratios and 95% confidence intervals (obtained from Cox regression analyses) were derived at each time point following randomisation until the last observation of the last patient. All events until the respective day were considered and patients without events were censored at the respective day.

Results: Reductions in the risk of heart failure hospitalisation or CV death with EMPA vs PBO were observed by the first month and persisted for the duration of the trial (Figure). Hazard ratio stabilised as the number of patients with events increased over time.

Conclusion: In patients with T2DM and high CV risk, an early reduction in the risk of heart failure hospitalisation or CV death was observed in patients treated with EMPA. This reduction in risk persisted throughout the duration of the trial.



Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **P. van de Borne:** Employment/Consultancy; Erasme Hospital. Grants; AstraZeneca, Biotronic, Actelion. Honorarium; AstraZeneca, Amgen, MSD. Non-financial support; Amgen, Sanofi.

1120

Arterial stiffness is related to myocardial impairment assessed with advanced echocardiography in type 1 diabetes patients with normal left ventricular ejection fraction

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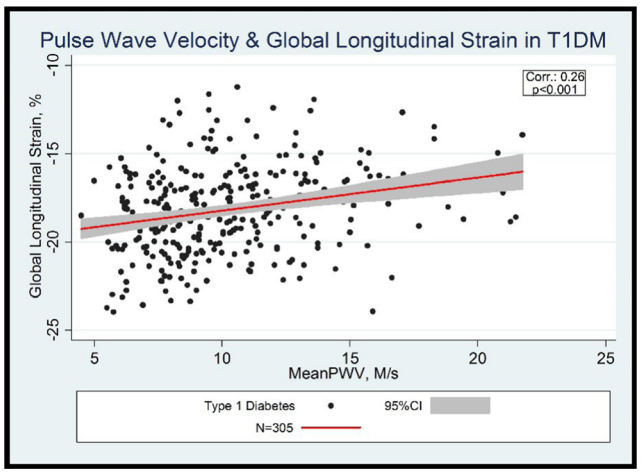
Background and aims: Diabetes is associated with higher arterial stiffness, which is considered an early marker of cardiovascular disease. The coupling between arterial stiffness and myocardial function is still unresolved. We investigated if higher arterial stiffness is associated with early myocardial impairment assessed with advanced echocardiography.
Materials and methods: In 305 type 1 diabetes patients without known heart disease and with normal left ventricular ejection fraction (LVEF) (biplane LVEF >45%), but varying degrees of albuminuria, we measured pulse wave velocity (PWV) as a measure of arterial stiffness, performed conventional and speckle-tracking echocardiography to assess global longitudinal strain (GLS). Associations between PWV and myocardial function were reported as standardized beta-values from adjusted regression models including age, gender, mean arterial pressure, body mass index, HbA1c, diabetes duration, eGFR, degree of albuminuria, total cholesterol, heart rate and smoking.

Results: Patients were 54 ± 12 years (mean ± SD), 152 (50%) males, mean diabetes duration 31 ± 16 years, HbA1c 65 ± 12 mmol/ mol, LVEF 58 ± 5%, GLS -18.2 ± 2.6%, and PWV 10.2 ± 3.4 m/s. There was no association between PWV and LVEF (p=0.93). Conversely, there was a highly significant association between PWV and GLS in both crude (stand. β-coef: 0.25, p<0.001) and multivariable models (stand. β-coef: 0.16, p=0.036). Also, diastolic function measured as E/e' was highly associated with PWV in both crude (stand. β-coef: 0.43, p<0.001) and multivariable models (stand. β-coef: 0.17, p=0.016).
Conclusion: In type 1 diabetes patients with normal LVEF and without known heart disease, higher arterial stiffness is independently associated with early systolic and diastolic myocardial impairment detectable by advanced echocardiography.



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Supported by: The Danish Heart Foundation
Disclosure: **S. Theilade:** None.

PS 118 Predictive potential of dyslipidaemia

1121

Increased risk for progression of coronary artery calcification in subjects with high baseline Lp(a) levels: the Kangbuk Samsung health study

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Background and aims: The results from previous studies support the association of lipoprotein(a) [Lp(a)] levels and coronary artery disease risk. In this study, we analyzed the association between baseline Lp(a) levels and future progression of coronary artery calcification (CAC) in apparently healthy Korean adults.
Materials and methods: In 2,611 participants (Mean age 41 years, 92% men) in whom health check-up program was repetitively performed in 4 years apart (2010 and 2014), coronary artery calcium score (CACS) were measured by multi-detector computed tomography. Baseline Lp(a) was measured by high-sensitivity immunoturbidimetric assay in all subjects. Progression of CAC was defined with change of CACS between 4 years higher than 0.
Results: In bivariate correlation analyses with baseline Lp(a) with other metabolic parameters, age, total cholesterol, HDL-C, LDL-C and CACS showed positive correlation, and body weight, fasting glucose level, blood pressure and triglyceride level showed negative correlation with baseline Lp(a) level. After 4 years, 635 subjects (24.3%) had CAC progression. The participants who had CAC progression were older, composed of more men, were obeser, higher fasting glucose levels and had worse baseline lipid profiles compared as those who did not have CAC progression during 4 years of follow-up. Mean serum Lp(a) level was significantly higher in subjects who had CAC progression compared as those who did not have CAC progression (32.5 vs. 28.9 mg/dL, $p<0.01$). When the risk for CAC progression according to baseline Lp(a) was calculated, those with Lp(a) level $\geq$ 50 mg/dL had odds ratio of 1.366 (95% CI 1.053~1.771) for CAC progression compared as those with Lp(a) < 50 mg/dL after adjustment for confounding factors.
Conclusion: In this study, the subjects who had higher Lp(a) showed significantly higher risk for CAC progression after 4 years of follow-up, suggesting the role of high Lp(a) in CAC progression.
Disclosure: **D. Lee:** None.

1122

Type 1 diabetes: specific plasma lipoprotein subclass profiles associate with insulin resistance and arterial stiffness

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Background and aims: Although type 1 diabetes (T1DM) is characterized by insulin deficiency, insulin resistance (IR) is a common finding. In fact, the combination of T1DM and features of IR and type 2 diabetes is known as double diabetes and carries an elevated risk of cardiovascular disease (CVD). To gain some insight into the pathophysiology of CVD in T1DM, the current study evaluated the potential relationships among plasma lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy (LSP-NMR), IR and arterial stiffness (AS) (as a measure of subclinical atherosclerosis).
Materials and methods: Eighty-four patients (35-65 years old) with T1DM of >10-year duration and without established CVD were consecutively evaluated for: 1) age, sex, diabetes duration, physical activity, smoking, alcohol intake, BMI, blood pressure, fasting plasma glucose, HbA1c and conventional lipid profile, 2) insulin sensitivity (estimate of glucose disposal rate -eGDR-) calculated with the following validated equation: eGDR=24.31-12.22*(WHR)-3.29*(Hypertension 0=No1=Yes)-0.57*(HbA1), 3) microvascular complications, 4) AS (aortic pulse-wave velocity -aPWV- by applanation tonometry) and 5) LSP-NMR (Liposcale®, Biosfer Teslab). Those subjects with an eGDR less than the median were classified as having IR and the rest as not having IR.
Results: T1DM patients with IR, compared with those without IR, were more hypertensive ($p<0.001$), had higher BMI ($p=0.009$), HbA_{1c} ($p=0.003$), aPWV ($p<0.001$) and lower HDL-cholesterol concentrations ($p=0.025$). They also had higher VLDL-triglycerides content ($p=0.029$) and total number of VLDL-particles ($p=0.027$) with lower HDL-cholesterol content ($p=0.038$) and HDL cholesterol/triglycerides ratio ($p=0.006$) as compared with T1DM without IR. In addition, aPWV associated positively with VLDL-triglycerides content (ß=0.238; $p=0.003$), total number of VLDL-particles (ß=0.173; $p=0.037$) and large VLDL-particles (ß=0.168; $p=0.042$), independently of classical cardiovascular risk factors (including conventional lipid profile), statin treatment and eGDR.
Conclusion: LSP-NMR identifies lipid abnormalities associated with AS in T1DM that are not reflected by the conventional lipid profile. Thus, the LSP-NMR may help in identifying patients with T1DM at highest risk for CVD.
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Disclosure: **G. Llauradó:** None.

1123

Evaluation of three triglyceride metrics in relation to cardiovascular outcomes in 87,648 UK type 2 diabetes subjects

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Background and aims: Patients with type 2 diabetes mellitus (T2DM) often experience a combination of dyslipidemia with increased triglycerides and low-density lipoprotein (LDL) cholesterol as defining features. Hypertriglyceridaemia (HTG) is an important risk factor for cardiovascular (CV) disease with prevalence estimates approximating 50% in T2DM and it is often unresponsive to statins. The CV benefit of LDL cholesterol lowering through statin treatment is well established. Despite 68 years of evidence establishing the relation between triglycerides (TG) and cardiovascular disease, HTG continues to be a controversial risk factor and target for therapy. Previous studies have not proven a significant positive relation between HTG and cardiovascular mortality; however, recent meta-analyses have found otherwise. It remains unclear whether interventions to lower TG among patients with HTG in combination with low LDL, impacts CV outcomes. It is also questionable whether controlling HTG in a clinical setting including blood pressure control and LDL cholesterol lowering drugs may prevent CV outcomes among diabetics. Adding to the complexity is the selection of the most appropriate TG metric when evaluating repeated measures of TG (in addition to baseline TG) to estimate time to CV outcomes. The aim of this study was to evaluate HTG in relation to CV

events (myocardial infarction, heart failure, and ischaemic stroke) in a contemporary cohort of incident T2DM patients.

Materials and methods: Primary health care data, Clinical Practice Research Data Link (CPRD) was used to identify a representative T2DM population (01/01/1998 to 31/12/2014). Date of diagnosis was defined as first recorded diagnosis or use of T2DM medication. Patient's ≥18 to ≤40 yrs were included. Potential confounders between repeated measures of TG and incident CV outcomes included: gender, age, BMI, blood pressure (BP), smoking status and HbA1c. Follow-up time was defined as one year after diagnosis date to incident CV outcomes, death, lost to follow-up, or end of study 31/12/2015. Proportional hazard models compared the association between 3 TG variables and CV outcomes: baseline, updated mean and updated latest. Baseline TG was determined as the average of all TG recordings during the baseline period of one year following diabetes diagnosis. Updated latest TG and updated mean TG are time-varying variables which are recalculated each time a new TG measurement is recorded.

Results: There was a significant association between TG and CV events. The estimated overall risk increase per 1 unit (mmol/L) increase in TG ranged from 5% for latest TG to 8% for the updated mean TG. When categorized by TG, the updated latest variable showed a J-shaped increased risk for the lowest TG category of <1.12 (mmol/L) compared to the referent category (ref=1.12-1.48) with HR of 1.16 (1.03-1.18), and updated mean showed a similar tendency, TG HRs 1.07 (0.99-1.15).

Conclusion: Hypertriglyceridemia remains a significant risk factor for CV events in persons with diabetes. Both, current TG levels at diagnosis and the overall estimates support this association and TG metrics differed only slightly.

Disclosure: **S. Skrtic:** Employment/Consultancy; Employee of AstraZeneca including AstraZeneca share holding.

1124

High LDLC levels despite statin therapy predicts higher 30 day mortality after acute myocardial infarction in Korean patients with type 2 diabetes

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Background and aims: Recently, it is emphasized that the intensity of statin therapy than the target goal of low-density lipoprotein cholesterol (LDL-C) in the treatment of dyslipidemia. However, there is controversy about this, especially in Asian populations. We aimed to evaluate the relation between LDL-C levels with statin therapy at admission and 30-day mortality in patient with acute myocardial infarction (AMI) in Korean patients with type 2 diabetes mellitus.

Materials and methods: We analyzed 2,570 type 2 diabetic patients who underwent percutaneous coronary intervention with AMI from Korea Acute Myocardial Infarction Registry (KAMIR) and Korea Working Group on Myocardial Infarction (KorMI). The patients were divided into four groups according to LDL-C levels and status of statin therapy at admission: group A, <100 mg/dL without statin treatment; group B, <100 mg/dL with statin treatment ; group C, ≥100 mg/dL without treatment ; group D, ≥100 mg/dL with treatment.

Results: In a Cox proportional hazards regression analysis, group D showed the highest 30-day mortality rate after adjusting for age, gender and other cardiovascular risk factors (HR 3.24, 95% CI 1.20 to 8.79, p=0.02). Also, in previously statin-treated patients, high LDL-C at admission was significant predictor of 30-day mortality rate after adjusting for multiple factors.

Conclusion: In this study, high LDL-C levels despite statin therapy at admission was an independent prognostic factor in early mortality after AMI. These data suggest that the achievement of LDL-C target goal with optimal statin therapy predict better clinical outcome in AMI in Korean type 2 diabetic patients.

Disclosure: **S. Lee:** None.

1125

Cardiovascular (CV) outcomes according to LDL cholesterol (LDL-C) levels in EMPA-REG OUTCOME

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Background and aims: Evidence suggests a dose-dependency between LDL-C levels and CV risk. We analysed the effects of empagliflozin (EMPA) compared to placebo on CV outcomes according to different LDL-C levels.

Materials and methods: In EMPA-REG OUTCOME, patients with T2DM and high CV risk received EMPA 10 mg or 25 mg or placebo in addition to standard of care. All CV outcomes were independently adjudicated. We investigated the time to (first) 3P-MACE, CV death, hospitalisation for heart failure (HHF) and total mortality for EMPA vs placebo between baseline LDL-C categories: < 1.81, 1.81- <2.20, 2.20-<2.59, 2.59 - 2.98, and > 2.98 mmol/L by a Cox regression including the interaction of baseline LDL-C category and treatment.

Results: Of the 7020 patients randomised and treated, 81.0% of patients received lipid lowering therapy (77.0% statins) and the mean (SD) LDL-C was 2.22 (0.92) mmol/L. Baseline characteristics by categories of LDL-C, indicated differences in statin use, diabetes duration, blood pressure, use of antihypertensives, and proportion with albuminuria. The CV incidence rates varied according to LDL-C levels, however, the impact of empagliflozin on these CV outcomes were consistent with the overall trial results (interaction p-values: 0.278 3P-MACE, 0.0852 CV death, 0.5005 HHF and 0.1563 total mortality) (table).

Conclusion: The modulating effects of EMPA on CV outcomes did not differ between the categories of baseline LDL-C levels.

Table. Cardiovascular outcomes according to LDL cholesterol levels and by treatment with either empagliflozin or placebo.

	N	Statin use	Mean (SD) LDL-C mmol/L	3P-MACE HR (95% CI)/ incidence rate EMPA/PBO per 1,000 yrs at risk	CV death HR (95% CI)/ incidence rate EMPA/PBO per 1,000 yrs at risk	HHF HR (95% CI)/ incidence rate EMPA/PBO per 1,000 yrs at risk	Total mortality HR (95% CI)/ incidence rate EMPA/PBO per 1,000 yrs at risk
Overall population	*7020*	*77.0%*	*2.22 (0.92)*	*0.86 (0.74, 0.99)/ 37.4/43.9*	*0.62 (0.49, 0.77)/ 12.4/20.2*	*0.65 (0.50, 0.85)/ 9.4/14.5*	*0.68 (0.57, 0.82)/ 19.4/28.6*
LDL-C < 1.81 mmol/L	2669	91.1%	1.39 (0.31)	0.74 (0.58, 0.94)/ 33.5/44.6	0.48 (0.33, 0.71)/ 9.7/19.9	0.52 (0.34, 0.80)/ 8.2/15.3	0.66 (0.48, 0.91)/ 16.2/24.4
LDL-C 1.81-<2.20 mmol/L	1294	87.9%	2.00 (0.10)	0.94 (0.66, 1.35)/ 34.3/37.8	0.69 (0.39, 1.21)/ 11.2/16.9	0.57 (0.31, 1.06)/ 8.2/15.9	0.77 (0.49, 1.20)/ 18.8/25.4
LDL-C 2.20-<2.59 mmol/L	986	83.9%	2.38 (0.11)	1.25 (0.82, 1.91)/ 40.4/32.7	1.42 (0.71, 2.83)/ 15.9/11.4	0.88 (0.42, 1.86)/ 10.0/11.7	1.04 (0.61, 1.77)/ 21.6/20.8
LDL-C 2.59-2.98 mmol/L	736	75.1%	2.77 (0.11)	0.82 (0.53, 1.27)/ 42.1/50.8	0.52 (0.26, 1.02)/ 12.6/23.9	1.06 (0.47, 2.40)/ 14.5/13.2	0.41 (0.24, 0.69)/ 18.5/44.6
LDL-C > 2.98 mmol/L	1247	73.5%	3.74 (0.72)	0.77 (0.56, 1.07)/ 42.4/54.9	0.51 (0.32, 0.80)/ 15.6/30.3	0.71 (0.36, 1.39)/ 9.3/12.9	0.61 (0.41, 0.89)/ 25.1/41.0
p-value for Interaction (treatment by baseline LDL-C cat.)	*N/A*	*N/A*	*N/A*	***0.2785***	***0.0852***	***0.5005***	***0.1563***
LDL-C analysed in mg/dL and converted to mmol/L at x0.0259							

Clinical Trial Registration Number: NCT01131676

Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.

Disclosure: **G. Langslet:** Employment/Consultancy; Amgen, Sanofi, Boehringer Ingelheim, Janssen. Lecture/other fees; Amgen, Sanofi, Boehringer Ingelheim.

1126

Carbamylation of HDL in type 2 diabetes

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Background and aims: Carbamylation is a post-translational modification of proteins due to the spontaneous non-enzymatic reaction between isocyanic acid and specific free functional groups of proteins. Carbamylation of proteins is previously considered only quantitatively important in uremic conditions, but recent studies have shown that carbamylation of protein can occur even in the absence of uremia by an alternative mechanism of cyanate formation and protein carbamylation mediated by myeloperoxidase. Apolipoproteins can be carbamylated in vitro and it has been suggested that lipoproteins are subjected to carbamylation in the circulation. We aim to evaluate whether HDL is carbamylated in patients with type 2 diabetes without nephropathy and the effect on the anti-oxidative property of HDL.
Materials and methods: 65 type 2 diabetic patients and 70 healthy controls were recruited. Subjects with nephropathy and impaired renal function were excluded. Plasma carbamylated HDL (cHDL) concentration was measured by an in-house sandwich ELISA using polyclonal rabbit anti-human cHDL antibody. The capacity of plasma total HDL to inhibit LDL oxidation ex vivo was determined by incubating HDL from each subject with a reference LDL in the presence of dichlorofluorescein which fluoresced upon interaction with lipid oxidation products.
Results: Diabetic patients had significantly lower HDL-cholesterol than controls (1.21 ± 0.32 mmol/L vs 1.44 ± 0.37 respectively, p<0.01) but their plasma cHDL levels were significantly increased [11.9 ug/ml (6.4 - 20.9) vs 9.3 (5.7 - 15.3) respectively, median (interquartile range), p<0.05]. These differences remained significant even after adjusting for age, gender, body mass index, smoking and the use of lipid lowering agents. The ability of HDL from diabetic patients in protecting LDL against oxidation was impaired with more lipid peroxide formed during the incubation than control subjects (3430 ± 354 fluorescence intensity vs 3047 ± 342 respectively, p<0.05). In the diabetic patients, there was a significant correlation between plasma cHDL and the degree of impairment of anti-oxidative capacity of HDL (r=0.41, p<0.01).
Conclusion: Diabetic patients have increased concentration of cHDL even in the absence of nephropathy and renal impairment, and plasma cHDL level was associated with impairment of the anti-oxidative property of HDL. Hence, our data suggest that protein carbamylation may render HDL dysfunctional in type 2 diabetes.
Disclosure: **K.C.B. Tan:** None.

1127

Serum malondialdehyde-modified LDL levels are increased in type 2 diabetic patients with nephropathy

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Background and aims: Although recent studies have shown that increased urinary albumin excretion (UAE) and a decreased estimated glomerular filtration rate (eGFR) are associated with the development of cardiovascular disease (CVD) in diabetes, the underlying mechanisms have not been fully elucidated. Oxidized LDL (oxLDL) has a pivotal role in the initiation and progression of atherosclerosis. Moreover, oxLDL has been reported to contribute to the progression of diabetic nephropathy. Malondialdehyde-modified LDL (MDA-LDL) is one type of oxLDL. MDA-LDL and the MDA-LDL-to-LDL-cholesterol ratio (MDA-LDL/LDL) are positively associated with CVD. We investigated the association between serum MDA-LDL related variables and diabetic nephropathy.
Materials and methods: A cross-sectional study was performed in 365 patients with type 2 diabetes (T2D). Serum MDA-LDL levels were measured using ELISA.
Results: Table 1 shows that mean serum MDA and MDA-LDL/LDL levels could be stratified by UAE or eGFR as determined by Kruskal-Wallis test for differences between groups and Jonckheere-Terpstra test for trend. Serum MDA-LDL levels were significantly correlated with age ($r = -0.180$, $p = 0.001$), HbA1c ($r = 0.162$, $p = 0.002$), (ln) C-reactive protein (CRP) ($r = 0.169$, $p = 0.001$), (ln) UAE ($r = 0.186$, $p = 0.000$), (ln) triglyceride (TG) ($r = 0.422$, $p = 0.000$), (ln) HDL-cholesterol (HDL-C) ($r = -0.151$, $p = 0.004$), and LDL-cholesterol (LDL-C) ($r = 0.586$, $p = 0.000$). Serum MDA-LDL/LDL levels were significantly correlated with eGFR ($r = -0.163$, $p = 0.002$), (ln) CRP ($r = 0.111$, $p = 0.034$), (ln) UAE ($r = 0.173$, $p = 0.001$), (ln) TG ($r = 0.443$, $p = 0.000$), (ln) HDL-C ($r = -0.260$, $p = 0.000$), and LDL-C ($r = -0.193$, $p = 0.000$). Stepwise multiple regression analyses showed that (ln) TG and LDL-C were independent determinants of MDA-LDL ($r^2 = 0.270$) and (ln) TG, LDL-C, BMI, and eGFR were independent determinants of MDA-LDL/LDL ($r^2 = 0.287$).
Conclusion: Serum MDA-LDL and MDA-LDL/LDL levels were significantly increased with an increasing severity of diabetic nephropathy. Serum TG and LDL levels were independent determinants of serum MDA-LDL and MDA-LDL/LDL levels. In addition, eGFR levels were independently associated with serum MDA-LDL/LDL levels. The significant association between eGFR and MDA-LDL/LDL suggests that having a decreased eGFR augments oxidative stress, or vice versa, in patients with T2D.

Table 1. Mean serum MDA-LDL and MDA-LDL/LDL levels stratified by UAE and eGFR

	UAE (mg/day)				p	p for trend
	< 30	> 30 < 300	> 300			
n	240	91	34			
MDA-LDL (U/L)	101.3 ± 40.6	104.6 ± 45.6	128.7 ± 69.0		0.024	0.070
MDA-LDL/LDL (U/mg)	0.088 ± 0.032	0.094 ± 0.031	0.104 ± 0.035		0.009	0.003
	eGFR (ml/min/1.73 m²)				p	p for trend
	≥ 90	< 90 > 60	< 60 > 30	< 30		
n	160	138	55	12		
MDA-LDL (U/L)	106.6 ± 46.3	101.8 ± 39.8	98.7 ± 44.0	138.8 ± 86.0	0.212	0.461
MDA-LDL/LDL (U/mg)	0.087 ± 0.031	0.090 ± 0.027	0.098 ± 0.031	0.128 ± 0.070	0.012	0.003

Supported by: KAKENHI
Disclosure: **H. Suzuki:** Grants; KAKENHI.

1128

Association of PPARG2 Pro12Ala, APOE E2/E3/E4, TNF-α polymorphisms with macrovascular disease, lipid-lowering and endothelial response on atorvastatin in type 2 diabetes

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Background and aims: The clinical background of atherosclerosis (AS) and glomerulosclerosis is endothelial dysfunction (ED), which may be an early marker of macrovascular diseases (MVD). Statin therapy improving macrovascular outcomes shows interindividual variability. The aim of the study was to assess the potential genetic markers of MVD, the lipid-lowering and endothelial response to atorvastatin therapy in patients with type 2 diabetes (T2D)
Materials and methods: The trial included the cross-sectional study of 233 patients with T2D divided due to the presence of MVD (defined as and/or coronary heart disease, myocardial infarction, stroke, AS of the lower extremities and/or carotid arteries) and chronic kidney disease (CKD) into groups: MVD+,CKD+ [n=22], MVD+,CKD- [n=114], MVD-,CKD- [n=97]; and prospective open study of atorvastatin response in 97 T2D statin-naïve patients (10/20 mg: n=49/48; male/female %: 23/77, mean age 64 years). Before and after 52 weeks of statin therapy,

patients had fasting lipid profile and ED parameters performed by peripheral arterial tonometry with reactive hyperemia. All patients were genotyped for a complex of candidate genes modulating the vascular damage due to hyperinsulinemia, inflammation and lipid metabolism: PPARG2 Pro12Ala, TNF-α G(-308)A and G(-238)A, ACE I/D, APOE E2/E3/E4, SLCO1B Val174Ala and LIPC C(-514)T, using polymerase chain reaction in real time with the TaqMan probes. Statistic analysis performed using χ2, the Mann-Whitney, Kruskal-Wallis and Wilcoxon tests, p <0,05
Results: We found significant differences in genotypes distribution of TNF-α G(-308)A between MVD-CKD- and MVD+CVD+ groups showed the protective effect of GG genotype excess in the dominant model of inheritance GG vs GA+AA [OR=0,29; 95%CI 0,10-0,89, p=0.02]. There was no difference in basal levels of total cholesterol (TC), LDL, triglycerides (TG) and ED parameters between the genotypes. The lipid-lowering effect of atorvastatin associated with genotypes distribution of PPARG2 gene: TC -20.74% vs -4.6% vs -5.61%, p = 0.04 for Pro/Pro vs. Pro/Ala vs Ala/Ala; LDL -26.00% vs -6.11% vs -7.32%, respectively, p = 0.029; and APOE gene: TC -46,25% for E4/E4 vs. +33,33% for E4/E2, +5,73% for E3/E2, +11,80% for E3/E4, -10,92% for E3/E3, p=0,01; TG -56,52% vs. +24,43%, +19,63%, +8,05%, -20,00%, respectively, p=0,04. The endothelial response - the amplitude of postocclusive wave on atorvastatin was greater in GG vs GA carries of TNFα G(238)A: +8,16% vs. -0,93%, p=0,04; and GA vs. GG of TNFα G(308)A: +44% vs. -4.4%, p=0,004. There was no statistically significant association with ED, MVD, CKD and atorvastatin response and other studied markers.
Conclusion: Significant association of TNF-α gene polymorphism with combined risk of MVD and CKD as well as ED parameters suggests an important role of inflammation in the genesis of vascular complications in T2D. The variability of lipid-lowering and endothelial response on statin therapy might be genetically determined and associated with PPARG2 Pro12Ala, APOE E2/E3/E4 and TNF-α polymorphisms account for interindividual sensitivity of atorvastatin admission
Disclosure: **N.O. Lebedeva:** None.

PS 119 The heart of the matter: clinical

1129

Serum levels of angiogenic regulators in type 2 diabetic patients: the relationships with cardiovascular complications

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Background and aims: The impaired angiogenesis is considered as a feature of diabetic macrovascular complications. It was demonstrated that enhanced glucose oscillations can suppress angiogenic response to hypoxia. The aim of the study was to assess the relationships between serum levels of principal regulators of angiogenesis, cardiovascular disease and glucose variability (GV) in type 2 diabetic patients.
Materials and methods: We observed 196 patients with type 2 diabetes, 43 M/153 F, 43-70 years of age. Coronary artery disease (CAD) was verified in 67 patients by treadmill test and/or coronary angiography. The levels of vascular endothelial growth factor A, C and D (VEGF-A, VEGF-C, VEGF-D), placental growth factor (PLGF), angiopoietin-2, epidermal growth factor (EGF), heparin-bound EGF-like growth factor (HB-EGF), endoglin, soluble Fas ligand (sFASL), insulin-like growth factor-binding protein-1 (IGF-BP1), transforming growth factor α (TGF-α), and urokinase plasminogen activator (uPA), were assessed by Multiplex assay and compared to control (24 healthy subjects matched by age and sex). Serum NT-proBNP level was determined by ELISA. The parameters of glucose variability (GV), including standard deviation, mean amplitude of glucose excursions, continuous overlapping net glycemic action, lability index, M-value, and mean absolute glucose, were derived from continuous glucose monitoring recordings.
Results: Diabetic subjects, when compared to control, had decreased serum levels of VEGF-A (p=0.03), VEGF-C (p=0.006), PLGF (p=0.04), endoglin (p=0.01), IGF-BP1 (p=0.03), and uPA (p=0.02). Concentrations of other regulators demonstrated no significant differences between two groups. There was an increase in the levels of VEGF-A, angiopoietin-2 and TGF-α in diabetic patients with CAD, as compared to those without (p=0.04, p=0.02 and p=0.02 respectively). The elevation of NT-proBNP level was associated with increment of sFASL (p=0.02) and VEGF-A (p=0.03). Notwithstanding, in subgroups of diabetic patients with CAD or chronic heart failure (CHF) none of the regulators exceeded control significantly. The concentration of angiogenic peptides demonstrated no relationships with HbA1c level, mean monitored glucose and all estimated GV parameters.
Conclusion: The results indicate the depletion in principal regulators of angiogenesis in patients with type 2 diabetes. Nevertheless, the presence of CAD and/or CHF can increase serum levels of angiogenic peptides in this cohort. The parameters of GV demonstrate no relationships with serum levels of angiogenic factors.
Supported by: RSF (14-15-00082)
Disclosure: **V.V. Klimontov:** None.

1130

Effect of empagliflozin on cardiovascular death in subgroups by age: results from EMPA-REG OUTCOME

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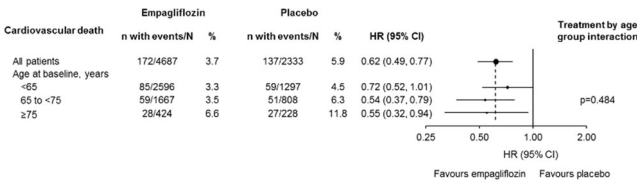
Background and aims: In the EMPA-REG OUTCOME trial, empagliflozin (EMPA) given in addition to standard of care significantly reduced 3-point major adverse cardiovascular (CV) events (composite of CV death, non-fatal myocardial infarction, non-fatal stroke), CV death and all-cause mortality vs placebo (PBO) in patients with type 2 diabetes (T2DM) and high CV risk. Here we investigate the effect of age on the reduction in CV death with EMPA.
Materials and methods: Patients in EMPA-REG OUTCOME were randomised to receive EMPA 10 mg, EMPA 25 mg or PBO. CV death was analysed in the pooled EMPA group vs PBO in subgroups by baseline age (<65, 65 to <75, ≥75 years).
Results: A total of 7020 patients were treated. Median observation time was 3.1 years. Mean (SD) age at baseline was 63.2 (8.8) years in the PBO group and 63.1 (8.6) years in the EMPA group. The benefit of EMPA vs PBO on CV death was consistent across age categories (Figure). Across age subgroups, reported adverse events were consistent with the known safety profile of EMPA.
Conclusion: EMPA, in addition to standard of care, reduced the risk of CV death in patients with T2DM and high CV risk irrespective of age.



Cox regression model including sex, baseline body mass index, baseline HbA_{1c}, baseline estimated glomerular filtration rate, region, treatment, age group and treatment by age group interaction, in patients treated with ≥1 dose of study drug.

Clinical Trial Registration Number: NCT01131676
Supported by: Boehringer Ingelheim & Eli Lilly and Company Diabetes Alliance.
Disclosure: **E. Toural:** Grants; Boehringer Ingelheim.

1131

Prevalence of ECG abnormalities in a population based diabetes cohort. The diabetes care system cohort study

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Background and aims: Certain guidelines recommend routine ECGs for those with T2DM. Few studies have assessed the prevalence and determinants of ECG abnormalities in people with T2DM. Information regarding the prevalence of ECG abnormalities, a non-invasive and low-cost screening modality, could help guide efforts to develop risk stratification tools to identify those with high risk of vascular complications . We therefore examined he prevalence of ECG abnormalities and its associations with glycemic control among people with T2DM.
Materials and methods: The Diabetes Care System cohort is a large 'open' prospective extensively phenotyped population based cohort of over 11.000 people with T2DM with annual repeated measurements. Clinical measures are collected annually as part of routine diabetes care and data have a high internal validity due to the centrally organized standardized examinations. Standard 12-lead ECGs were digitally recorded and coded using standard Minnesota ECG Classification in 9099 people with T2DM. ECG abnormalities were classified in 9099 participants as major (Minnesota codes: 1-1 and 1-2, 4-1, 5-1, 5-2, 7-1-1), minor (Minnesota codes: 1-3, 4-3, 5-3), and atrial fibrillation or atrial flutter (Minnesota codes: 8-3-1, 8-3-2). For this study we report the results of ECG abnormalities over the years 2013-2015. The last available ECG of each patient was taken for Minnesota coding. Sex-specific prevalence rates of ECG abnormalities were calculated and logistic regression models were fitted to examine cross-sectional associations between participant characteristics and ECG abnormalities. Multivariate analyses were performed to investigate the association between HbA1c and major and/or minor ECG abnormalities.
Results: Participants had a mean age of 67.2 years and were 45.8% women8.8% had at least one major ECG abnormality; 6.3% had one minor ECG abnormality only. Men had a higher prevalence of ECG abnormalities than women (16.4% men vs. 13.6% women). In univariate analysis, gender, age, HbA1c , former smoking, and LDL were statistically significant associated with major and/or minor ECG abnormalities, while HDL and BMI were inversed statistically significant related with major and/or minor ECG abnormalities. In multivariate analysis Hba1c (in %) was significantly associated with the prevalence of major (OR: 1.16 (95% CI: 1.09 to 1.24) and major or minor ECG abnormalities (OR: 1.12 (95% CI: 1.06 to 1.18) after adjustment for confounding factors.
Conclusion: The prevalence of ECG abnormalities was relatively high in this cohort of people with T2DM. HbA1c levels were independently associated with ECG abnormalities. Longitudinal associations between risk factors and (changes in) ECG abnormalities require further investigation to incorporate ECG abnormalities in a risk stratification tool to identify those at increased cardiovascular risk.
Disclosure: **G. Nijpels:** None.

1132

Risk for cardiovascular diseases based on relationship between postload and fasting plasma glucose levels in the normal range

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Background and aims: Individuals whose 2h plasma glucose (2hPG) levels did not return to their fasting plasma glucose (FPG) level (2hPG > FPG), increased risk of cardiovascular diseases than those whose 2hPG did (2hPG <=FPG) during an oral glucose tolerance test in Europeans. However, the risk for cardiovascular risk was not well known in Chinese. We will assess the risk for and prevalence of cardiovascular diseases (CVD) in relation to FPG and 2hPG levels within normoglycemic range in Chinese population.
Materials and methods: Data from Qingdao Diabetes Prevention Program comprising 1687 men and 2568 women aged 35-74 years who had FPG < 6.1 mmol/L and 2hPG FPG as compared with those whose 2hPG<=FPG, controlling for age, BMI, total cholesterol, uric acid, triglycerides, smoking status, drinking status, frequency of vegetable and fruit consumptions.
Results: A total of 829 (986) individuals was classified as CVD in men (women). The prevalence of CVD was significantly higher in the Group II than in the Group I (P <0.01). The individuals from the Group II was older and had higher BMI, diastolic blood pressure, total cholesterol, triglycerides, uric acid and insulin resistance than those in the Group I (P <0.01 for all comparisons). The multivariable adjusted ORs (95%CIs) for prevalence of CVD was 1.23 (1.06-1.42), 1.07(1.06-1.08), 1.17(1.14-1.19) and 1.43(1.16-1.77)for Group II vs. Group I, age, BMI and drinking status, respectively. The ORs (95%CIs) for prevalence of CVD was also significant difference after additional adjustment for insulin resistance, vegetable and fruit intake in a subgroup of individuals.

Conclusion: In individuals with both FPG and 2hPG within normoglycemic range, high 2hPG was significantly associated with insulin resistance and increased risk of CVD. Studies are warranted to evaluate the causal relevance of these findings.
Clinical Trial Registration Number: NCT01053195
Supported by: Qingdao Outstanding Health Professional Development Fund
Disclosure: **Y. Wang:** None.

1133

Effects of serum bilirubin on the risk of cardiovascular disease

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Background and aims: We tested the hypothesis that higher levels of bilirubin, a bile pigment with antioxidant properties, are associated with a decreased risk of cardiovascular disease(CVD).
Materials and methods: This study analyzed data from the Korean Health and Genome Study to examine the impact of serum total bilirubin (TB) on CVD and CVD death. Serum TB was measured in a total of 8,844 subjects (4,196 males and 4,648 females) and evaluated for the development of new onset CVD from 2001 to 2012 (mean 8.1 years of follow-up).
Results: During the follow-up period, 689 cases of incident CVD (7.8%) were identified, and the prevalence of metabolic syndrome (MetS) at baseline was 26.1%. The incidence of MetS decreased across bilirubin tertile categories. In addition to MetS itself, individual components of MetS significantly decreased with increased bilirubin tertiles. Moreover, the incidence of CVD decreased across bilirubin tertile categories. The hazard ratios (HRs) for developing coronary heart disease (CHD) and CVD death was significantly lower in the highest tertile group (>0.63 mg/dL) in comparison to the lowest tertile group (<0.44 mg/dL) after adjusting for all confounding variables.
Conclusion: Our study clearly shows that mildly elevated serum bilirubin levels provide protection against incident CHD and CVD death.
Disclosure: **S. Park:** None.

1134

The level of phospholipid acyltransferase 1 in diabetic coronary heart disease patients' myocardial cell and analysis of the influence factors

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Background and aims: To investigate the mRNA and protein expression of Phospholipid Acyltransferase 1 (ALCAT1) in myocardial cell of coronary heart disease with Type 2 Diabetes Mellitus (T2DM), and then analyze the influence factors.
Materials and methods: Divide cardiac surgery patients that conducted in the hospital into 4 groups, Rheumatic heart disease (Group A); Rheumatic heart disease with T2DM (Group B); Coronary heart disease (Group C); Coronary heart disease with T2DM (Group D). Take Grain size organization from right auricle to analyze during surgery, RT-PCR and Western-blot were employed to test the mRNA and protein levels of ALCAT1.
Results: (1) The level of systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol (TC), low density lipoprotein (LDL-C), high density lipoprotein(HDL-C), triglyceride(TG), fasting blood-glucose (FBG), HbA1c, serum creatinine (Scr) and ejection fraction (EF) showed significant difference among those groups (P<0.05). (2) The mRNA levels of ALCAT1 in A, B, C, D groups were 0.029±0.005,0.041 ±0.004,0.036±0.006 and 0.053±0.004, which statistically revealed remarkable difference (P<0.05); And the protein level of ALCAT1 showed similar trend. (3) The mRNA level of ALCAT1 had positive correlation with SBP, DBP, TC, LDL-C, HDL-C, TG, FBG, HbA1c and Scr(P<0.05), which had negative correlation with EF(P<0.05).
Conclusion: The mRNA and protein levels of ALCAT1 in coronary heart disease patients' myocardial cell were higher than patients with rheumatic heart disease, which were higher for the patients complicated with diabetes than those non-diabetes. The results above indirectly hinted that coronary heart disease and diabetes can aggravate the cardiolipin's pathological reconstruction in myocardial cell, and caused mitochondrial dysfunction.
Supported by: NSFC U1204805
Disclosure: **J. Yang:** None.

1135

Adding novel biomarkers to current cardiovascular risk scores for people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study (ET2DS)

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Background and aims: Increasing evidence suggests novel biomarkers may improve cardiovascular (CV) risk prediction in the general population. Whether they could improve current CV risk scores in people with Type 2 diabetes is uncertain.
Materials and methods: Conventional CV risk factors and novel biomarkers were measured in 1066 adults (48.7% female) aged 60-74 years with Type 2 diabetes participating in the population-based ET2DS. Seven year follow-up for incident CV events used clinical examination, hospital admission record and death certificate linkage. Predictors in the QRISK2 cardiovascular risk score (age, sex, smoking, atrial fibrillation, chronic kidney disease, rheumatoid arthritis, hypertension, BMI, sBP and total:HDL cholesterol) were considered for the basic model, which was also adjusted for prevalent CV disease and lipid-lowering drugs. The following novel biomarkers were then added to this starting model individually to assess their added value to the model: Ankle Brachial Index (ABI), N-terminal pro brain natriuretic peptide (NT-proBNP), troponin, gamma-glutamyl transpeptidase (Gamma-GT) and an inflammatory factor (*g*) combining C-reactive protein, interleukin-6, tumor necrosis factor alpha and fibrinogen using principal components analysis.
Results: 208 (19.5%) subjects had an incident CV event (first fatal or non-fatal myocardial infarction, new onset angina, fatal or non-fatal stroke, transient ischaemic attack or other fatal ischaemic hear disease). Results showed baseline ABI, NT-proBNP and troponin were significantly associated with risk of CV events over-and-above QRISK2 (odds ratios for 1 standard deviation increase in biomarker 0.81 (95% CI 0.69, 0.96), 1.24 (1.02, 1.52) and 1.48 (1.22, 1.80) respectively). No significant association was found for Gamma-GT and *g*. C statistics improved from 0.729 (basic model) to 0.735, 0.731, 0.745 and 0.730 for ABI, NT-proBNP, troponin and Gamma-GT respectively and all models had good calibration, assessed using the Hosmer-Lemeshow goodness-of-fit test (p-values all > 0.05). Only troponin provided a net improvement in correctly reclassifying people who both did and did not experience a CV event (net reclassification improvement for people who did suffer an event was 1.5% and net reclassification improvement for people who did not suffer an event was 3.8%).

Conclusion: These preliminary results suggest moderate potential for selected novel biomarkers to add value to current CV risk scores. Further investigation will be carried out on the biomarkers in combination to discover the panel of biomarkers which best predict CV disease.

Table 1: adding novel biomarkers to the basic model, with model summary measures

	Basic model	+ ABI	+ NT-pro BNP	+ Trop-onin	+ Gamma -GT	+ *g*
OR for a 1 SD increase in biomarker (95% CI)	-	0.81 (0.69, 0.96)	1.24 (1.02, 1.52)	1.48 (1.22, 1.80)	1.18 (0.99, 1.39)	1.07 (0.89, 1.29)
Model summary measures:						
C statistic	0.729	0.735	0.731	0.745	0.730	0.725
Net reclassification improvement – event (%)	-	-1.0	-1.5	1.5	-0.5	1.0
Net reclassification improvement – no event (%)	-	2.7	0.6	3.8	1.6	-0.6
Hosmer-Lemeshow p-value	0.878	0.636	0.974	0.269	0.070	0.334

Supported by: MRC
Disclosure: **A.H. Price:** None.

1136

The prognostic role of high sensitivity CRP and triglycerides on the new onset of left ventricular diastolic dysfunction in type 2 diabetes patients: a 4-year prospective study

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Background and aims: Diabetes Mellitus is a major risk factor for heart failure through the path of diabetic cardiomyopathy and diastolic dysfunction of left ventricle (LVDD). This prospective observational study focuses on the biomarkers and the echocardiographic findings that could predict the new onset of LVDD on type 2 diabetes patients.
Materials and methods: We enrolled 48 volunteers (26 males) with type 2 diabetes of mean age 55.4±10.0 years, mean HbA1c 7.5±1.5%, mean BMI 29.4±5.1Kg/m2 and mean diabetes duration 2.8±0.8 years with normal both systolic and diastolic cardiac function. We collected demographic, clinical and laboratory data and performed echocardiographic evaluation to each participant. Fasting plasma was drawn for BNP, sST2 and hs-CRP measurements. This procedure was repeated every 12 months for a 48-month time period.
Results: Findings at baseline demonstrated that 41.7% of recruits had arterial hypertension, 45.8% dyslipidemia, 45.8% smoking. On metformin therapy was the 91.7% while 27.1 on SU, 8.3% on DPP-4 inhibitors, 8.3% on glinides and 4.2% on insulin therapy. Among the anti-hypertensives, 18.8% was on ACE-inhibitors, 16.7% ARB's, 4.2% HCT and 4.2% b-blockers. At the end of the study 28 subjects had LVDD. Univariate analysis showed that the presence of LVDD was related with: BMI [odds ratio (OR): 1.14, 95% confidence interval (CI): 0.99-1.29, p=0.05], therapy with ARB's (OR: 0.17, 95% CI: 0.03-1.01, p=0.05), hs-CRP (OR: 1.29, 95% CI: 1.04-1.59, p=0.02), HDL-C (OR:0.95, 95% CI: 0.91-0.99, p=0.03), fasting triglycerides (OR: 1.02, 95% CI: 1.00-1.04, p=0.003) and Left Ventricular Myocardial Index (LVMI) (OR: 1.05, 95% CI: 1.00-1.08, p=0.03). No correlation was observed between LVDD and sex, age, diabetes duration, history of hypertension, dyslipidemia, smoking habits, oral antihyperglycemic agents, ESR, fibrinogen, total cholesterol, LDL-C, uric acid, BNP and sST2. Logarithmic regression analysis revealed that the new onset of LVDD was positively correlated with hs-CRP levels (OR: 1.12, 95%CI:1.08-1.51, p=0.02) and fasting triglycerides (OR:1.07, 95%CI:1.01-1.14, p=0.02) and negatively with the therapy with ARB's (OR:0.14, 95%CI:0.05-0.43, p=0.05).
Conclusion: Higher hs-CRP levels and fasting triglycerides may predict the new onset of LVDD - the echocardiographic depiction of diabetic cardiomyopathy - in type 2 diabetes patients with normal cardiac function, while therapy with ARB's may play a protective role in this 4-year prospective study.
Disclosure: **E. Fousteris:** None.

PS 120 My sweet heart

1137

Estimated 10-year benefit for diabetes complication rates by 1% HbA_{1c} decrements in people with type 2 diabetes and cardiovascular disease

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Background and aims: Randomised controlled trials and meta-analyses of glucose lowering in type 2 diabetes mellitus (T2DM) demonstrate reductions in microvascular outcomes and modest reductions in macrovascular complications. International guidelines recommend individualisation of HbA1c targets; however, few data are available on the potential benefits that different targets might achieve. We have estimated 10-year risks for micro- and macrovascular T2DM complications when targeting HbA1c levels between 10% and 6% to quantify the likely incremental benefits.

Materials and methods: We used UKPDS Outcomes Model v2 (OM2) to estimate 10-year event rates for myocardial infarction (MI), stroke, blindness and amputation for a contemporaneous population with T2DM and cardiovascular disease enrolled in the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS). Complete baseline risk factor values for age, sex, ethnicity, systolic BP, HDL, LDL, weight, heart rate, haemoglobin, smoking status, presence of albuminuria, atrial fibrillation and history of micro- and macrovascular events, were available for 5766 of 14724 enrolled patients. Complication rates were estimated with HbA1c levels held constant at 10%, 9%, 8%, 7% and 6% for each individual whilst maintaining their risk factors at their baseline values. Point estimates of event rates (PEER) were compared using ANOVA, and relative risk reductions (RRR) at each 1% HbA1c decrement compared using Tukey pairwise comparisons, with $p<0.05$ as significant.

Results: Patients were mean (SD) age 66.2 (7.8) years, T2DM duration 10.9 (8.2) years, systolic BP 134 (17) mmHg, LDL 2.3 (0.9) mmol/l, HDL 1.1 (0.3) mmol/l, with 27.5% women, 84.6% White ethnicity and 10.9% current smokers. PEERs decreased significantly for each HbA1c decrement from 10% to 6% for all simulated outcomes; p for trend <0.001 (Table). RRRs increased to a similar extent for each 1% HbA1c decrement, but numerically were greater for micro- than macrovascular complications. RRR estimates when targeting an HbA1c of 7.0% (current guideline target) from a baseline of 10% were 12.9%, 14.8%, 43.1% and 61.8% for MI, stroke, blindness and amputation respectively.

Conclusion: As expected, greater estimated risk reductions were seen with HbA1c lowering for micro- than macrovascular complications. These simulated outcomes provide patients and clinicians a guide to the potential glucose-lowering benefit possible when targeting progressively lower HbA1c values from a baseline of 10%. Running OM2 for individual patients could give personalised risk reduction estimates to help inform diabetes management.

	HbA1c (%)	10.0	9.0	8.0	7.0	6.0
Macrovascular Complications	MI	24.2 (20.7-27.6)	23.0 (19.7-26.3)	21.9 (18.7-25.0)	20.9 (17.8–24.0)	19.9 (16.9–23.1)
	Cumulative RRR	Reference	4.3	8.5	12.9	17.3
	Stroke	14.0 (10.9 - 17.2)	13.3 (10.4-16.1)	12.6 (9.9–15.3)	11.9 (9.3–14.5)	11.3 (8.7–13.8)
	Cumulative RRR	Reference	4.7	8.7	14.8	21.1
Microvascular Complications	Blindness	7.4 (4.4-10.3)	6.3 (3.8–8.9)	5.4 (3.2–7.7)	4.6 (2.7–6.6)	3.9 (2.3–5.7)
	Cumulative RRR	Reference	14.4	28.7	43.1	57.5
	Amputation	5.5 (4.3–7.2)	4.5 (3.6–5.8)	3.72 (2.9–4.8)	3.1 (2.4–3.9)	2.6 (1.9–3.4)
	Cumulative RRR	Reference	21.0	41.6	61.8	81.8

Data presented as PEER (%) with 95% CI; Cumulative RRR are % and compared to an HbA1c of 10% (reference).

Disclosure: **S.A. Mostafa:** None.

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Metabolic subtypes and all-cause and cardiovascular mortality in patients with type 1 diabetes

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Background and aims: Identification of type 1 diabetes metabolic subtypes from the complex and heterogeneous dataset by self-organizing map (SOM) may contain significant prognostic information on mortality and burden of complications. Therefore, we revisit our original subtypes modelling from over seven years ago, and investigate what has happened to the five gender-specific subgroups of the patients with respect to all-cause and cardiovascular (CVD) mortality, against the expected sex- and age-specific mortality in Finland.

Materials and methods: The study included type 1 diabetes patients (2,059 men, 1,924 women) from the Finnish Diabetic Nephropathy Study (FinnDiane), who were involved in the original subtype modelling and had follow-up data on vital status and causes of death. Mortality data were obtained from the national Causes of Death Register and from Statistics Finland. Standardized all-cause and cardiovascular mortality ratios (SMRs) were weighted by the follow-up times and matched for age and sex, and statistics were estimated by bootstrapping. The metabolic subtypes (derived from 14 biochemical measures) were: good glycaemic control (subtype A), high HDL cholesterol (B), advanced kidney disease (C), metabolic syndrome (D) and low cholesterol (E).

Results: A total of 388 deaths occurred in men (10-year mortality rate 14.4%) and 244 deaths in women (10-year mortality rate 9.4%) during the median follow-up of 14.0 (IQR 11.7 - 16.0) years. For subtypes A (good glycemic control) and E (low cholesterol), the relative risk of all-cause mortality did not differ from the background population ($P > 0.1$). The highest risk was observed both in women (9-fold) and men (4-fold) for advanced kidney disease (subtype C, $P < 0.0001$). The metabolic syndrome (subtype D) increased the risk 5-fold in women and three-fold in men ($P < 0.0001$). Overall CVD mortality was similar between men and women, but SMRs were sex-specific (13.4 for women vs. 5.6 for men, $P < 0.0001$) and the same phenomenon was observed across the subtypes. The highest CVD mortality risk was observed in women (46-fold, $P < 0.0001$) and men (12-fold, $P < 0.0001$) for subtype C (advanced kidney disease). Notably, women with good glycemic control (A) had four times higher CVD mortality risk ($P=0.01$), while in men there was no added risk compared to the background population ($P=0.2$).

Conclusion: Men and women with type 1 diabetes and favorable metabolic phenotypes have similar risk of all-cause mortality compared to the background population. However, women have higher relative risk of CVD mortality than men, suggesting that specific attention to diabetes management is warranted in women. Advanced kidney disease is the leading contributor to excess all-cause and CVD mortality in patients with type 1 diabetes.

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Disclosure: **R. Lithovius:** None.

1139

Interrelationship between treatment choice, glycaemic control and event incidence: optimal management of glycaemia in older patients with type 2 diabetes

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Background and aims: There are limited data characterising the relationship between treatment choice, HbA1c control and incidence of vascular complications among older patients with type 2 diabetes (T2D).

Materials and methods: A retrospective observational study in patients >=65 years that failed metformin (M) monotherapy and escalated to second-line treatment (switch to or addition of a sulfonylurea (SU), dipeptidyl peptidase-4 inhibitor (DPP4) or thiazolidinedione (TZD)) was conducted using the UK Clinical Practice Research Datalink primary care database between 01-01-2008 and 31-12-2014. Baseline patient characteristics were assessed using descriptive summary statistics. Incidence of T2D-related micro- and macrovascular complications and event rates per 1,000 person years were estimated.

Results: A total of 514,734 patients were identified as having type T2D; 10,484 patients were eligible for inclusion. At baseline, patients had a mean age of 73 years, HbA1c of 8.3%, duration of diabetes of 6 years, and a body weight of 87Kg. The SU monotherapy cohort had the highest event rate per 1,000 person years (118.07), compared with an overall event rate of 109.55. The lowest event rate was observed in the M+DPP4 group (101.88). In patients achieving a HbA1c reduction of 0.5% or greater over 12 months the start of second-line treatment, event rates were lowest for M+DPP4 (96.27), M following +TZD (91.09) and TZD monotherapy (91.47), compared with 108.81 for M+SU.

Conclusion: This analysis showed that older patients prescribed M+DPP4 had the lowest overall event rate with SU-based regimens associated with the highest overall event incidence; in patients achieving a HbA1c reduction>=0.5%, all regimens had lower event rates compared with M+SU. There was an apparent relationship between treatment choice and event risk, which may be mediated via HbA1c control.

Supported by: Takeda Development Centre Europe Ltd.

Disclosure: **J. Puelles:** None.

1140

Glycaemic targets and cardiovascular events: comparing diabetic population of more or less than 70 years old. Escarval Risk study

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Background and aims: To analyze the relationship between glycosylated hemoglobin level and the incidence of cardiovascular events (CVE) and compare among patients with diabetes mellitus (DM) under and over 70 years.

Materials and methods: Prospective cohort study of 17573 patients with diabetes, free of CVE in 2007 followed by 5 years (2007-2012): 11284 were under 70 years old and 6289 were over 70. CVE: hospitalization by ischemic heart disease or stroke or death. Setting: Primary Care. Source: Electronic medical records Abucasis and hospitalization electronic records. Participation of 954 clinical doctors and nurses from Primary Care. Variables: age, sex, fasting glucose, glycosylated hemoglobin A1c (A1c), systolic blood pressure (SBP) and diastolic (DBP), body mass index (BMI), total cholesterol (TC), HDL, LDL and triglycerides (TG). Odds Ratio (OR) Crude and adjusted for different variables were calculated.

Results: The incidence of cardiovascular events was respectively 6.3% vs 11.9% (p = 0.000). The table shows the comparative results between patients younger and older than 70 years. See fig 1. OR = Odds Ratio; 1 = Crude OR. 2 = OR Adjusted for blood pressure, lipid profile, age and sex.

Conclusion: There is an association between levels of A1c and cardiovascular events. In patients younger than 70 years, the risk of CVE starts from an A1c of 6.5% A1c. In patients older than 70 years, the risk increases weakly from 7-7.5% and clearly from 8%.

		CV Events (%)	OR1	p	OR2	p
< 70 years old	A1c<=6,5% vs > 6,5%	5.3 vs 7.1	1,35 (1,16-1,57)	0,000	1,23 (1,03-1,48)	0,022
	A1c<=7% vs > 7%	5,5 vs 7,4	1,38 (1,19-1,60)	0,000	1,26 (1,05-1,51)	0,012
	A1c<=7,5% vs > 7,5%	5,5 vs 7,4	1,38 (1,19-1,60)	0,000	1,40 (1,15-1,70)	0,001
	A1c<=8% vs > 8%	5,8 vs 8,2	1,45 (1,22-1,71)	0,000	1,41 (1,13-1,74)	0,002
>= 70 years old	A1c<=6,5% vs > 6,5%	11,2 vs 12,5	1,13 (0,98-1,31)	0,085	1,09 (0,93-1,27)	0.249
	A1c<=7% vs > 7%	11,2 vs 13,2	1,21 (1,04-1,40)	0,000	1,04 (1,00-1,07)	0,045
	A1c<=7,5% vs > 7,5%	11,3 vs 13,9	1,26 (1,07-1,49)	0,006	1,20 (0,99-1,44)	0,051
	A1c<=8% vs > 8%	11,4 vs 14,5	1,31 (1,08-1,59)	0,006	1,25 (1,01-1,55)	0,034

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Disclosure: **A. Cebrian-Cuenca:** None.

1141

Sex differences in the Trial Evaluating Cardiovascular Outcomes with Sitagliptin (TECOS)

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Background and aims: TECOS was an international, randomized, double-blind, placebo-controlled trial assessing the impact of sitagliptin added to usual care on cardiovascular (CV) outcomes in patients with type 2 diabetes (T2DM) and atherosclerotic CV disease. Although women without diabetes have lower risk for CV events than men, this advantage is reportedly lost in women with T2DM. We examined sex differences in baseline CV disease burden, risk factor management, and outcomes in the TECOS population.

Materials and methods: Cox proportional hazards models were used to analyze the association between sex and key CV endpoints and secondary outcomes, as well as the interaction between sex and treatment effect, controlling for baseline characteristics.

Results: A total of 4,297 women and 10,374 men in the intention-to-treat population were followed for a median of 3.0 years. At baseline, women were slightly older than men (66 vs 65 years) but had similar median duration of diabetes (10 years). More women had cerebrovascular disease (31.2% vs 21.7%), peripheral arterial disease (20.6% vs 14.9%) and heart failure (21.8% vs 16.4%) while more men had coronary heart disease (79.3% vs 61.3%). Women had higher BMI (30.5 vs 29.2 kg/m^2), systolic BP (135 vs 132 mmHg), and LDL cholesterol levels (2.4 vs 2.1 mmol/L); worse renal function (eGFR 68.0 vs 74.0 mL/min/1.73m^2); but lower rates of smoking (8.5% vs 12.6%) (p<0.0001 for all). At baseline women were less likely to use aspirin (72.5% vs 81.0%) or a statin (73.0% vs 82.7%) than men (both p<0.0001). During the trial, the primary composite outcome of CV death, myocardial infarction (MI), stroke, or hospitalization for unstable angina (UA) occurred in 418 (9.7%) women and 1,272 (12.3%) men across treatment groups, corresponding to 3.48 vs. 4.38 events/100 patient-years (crude HR 0.79; 95% CI 0.71, 0.89). This association was strengthened after adjustment for baseline characteristics (HR 0.71; 95% CI 0.60, 0.83, p< 0.0001). Women also had significantly lower risks of secondary endpoints including a composite of CV death, MI, or stroke as well as the individual endpoints of CV death, all-cause death, MI, or stroke (Table). There were no interactions between sex and the effect of sitagliptin treatment upon CV outcomes (p>0.10 for all).
Conclusion: In this large prospective trial of patients with T2DM and CVD, women had worse CV risk factor profiles and less extensive use of indicated medications than men. Despite this, women had significantly lower risk for new major CV events. These data suggest that the cardioprotective effect of female sex extends to populations with T2DM.

Table: Association of Sex and Endpoints

Endpoint	Total events (W)	Events per 100 pt-yrs (W)	Total events (M)	Events per 100 pt-yrs (M)	HR (95% CI) for female v. male	Adjusted HR (95% CI) for female v. male	Adjusted p
CV death, MI, Stroke, or Hospitalization for UA	418	3.48	1272	4.38	0.79 (0.71, 0.89)	0.71 (0.60, 0.83)	<0.0001
CV death, MI, Stroke	371	3.06	1120	3.82	0.80 (0.71, 0.90)	0.69 (0.58, 0.81)	<0.0001
CV death	199	1.55	547	1.76	0.82 (0.70, 0.97)	0.64 (0.50, 0.81)	0.0003
Myocardial infarction	136	1.11	480	1.61	0.73 (0.60, 0.88)	0.70 (0.54, 0.91)	0.0081
Stroke	96	0.78	265	0.88	0.86 (0.68, 1.09)	0.66 (0.47, 0.92)	0.0151
Hospitalization for UA	58	0.47	187	0.62	0.78 (0.58, 1.05)	0.87 (0.56, 1.33)	0.5159
All Cause Death	279	2.17	805	2.58	0.80 (0.69, 0.91)	0.68 (0.56, 0.82)	<0.0001
Hospitalization for HF	115	0.93	342	1.14	0.83 (0.67, 1.03)	0.84 (0.64, 1.12)	0.2414

Cox proportional hazards regression models are used. Data are censored at the last day the patient is known to be free of the event. Models are repeated with adjustment for duration of diabetes and baseline variables determined to differ.
W = women; M = men; MI = myocardial infarction; UA = unstable angina; HF = heart failure

Clinical Trial Registration Number: NCT00790205
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The haemoglobin glycation index does not predict the risk for complications in patients treated with aleglitazar: a post hoc analysis from the AleCardio trial

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Background and aims: The haemoglobin glycation index (HGI) quantifies the interindividual variation in the propensity for glycation and is a predictor of diabetes complications in type 1 diabetes. In the ACCORD trial, a subgroup of type 2 diabetes patients with the highest HGI did not benefit from intensive treatment and had greater risk for major cardiac adverse events (MACE) and mortality. We determined whether HGI predicts outcomes of treatment with aleglitazar in the AleCardio trial.
Materials and methods: In the AleCardio trial 7,226 type 2 diabetes patients, hospitalized for an acute coronary syndrome, were randomized to aleglitazar or placebo. We included 6,458 patients with available baseline HbA_{1c} and fasting plasma glucose (FPG). A linear regression equation, HbA_{1c} (%) = 5.45 + 0.0158 * FPG (mg/dL), was used to calculate predicted HbA_{1c} and derive HGI (= observed HbA_{1c} - predicted HbA_{1c}). HGI ranged from -4.67% to 6.85% with a median of -0.30%. Patients were divided into tertiles, with HGI cut-off points of ≤ -0.75% and ≥ 0.33%. We determined treatment effect on primary outcome MACE (cardiovascular death, nonfatal myocardial infarction or stroke), total mortality and hypoglycaemia by Cox proportional hazard regression.
Results: Compared to low and intermediate, the high HGI subgroup consisted of more non-Caucasians, and patients in this group were significantly younger, had a longer duration of diabetes and used more often insulin (P < 0.001). Baseline retinopathy occurred significantly more often in the high HGI subgroup (P < 0.001). There was no interaction between HGI and treatment with aleglitazar on MACE (P = 0.97), with equal effect in the high (HR 0.99; 95% CI 0.76-1.1.28, P = 0.92), intermediate (HR 0.94; 95% CI 0.71-1.24; P = 0.66) and low subgroup (HR 0.96; 95% CI 0.73-1.27, P = 0.79). Treatment with aleglitazar had no effect on the risk for total mortality (HR 1.08; 95% CI 0.84-1.38, P = 0.55). This result did not differ per subgroup (P = 0.68). Use of aleglitazar was associated with hypoglycaemia in all subgroups (overall HR 1.69; 95% CI 1.48-1.94, P < 0.001). Hypoglycaemia occurred less often in the low HGI subgroup as compared to the intermediate subgroup (11% vs. 14%, unadjusted HR 0.77; 95% CI 0.65-0.91, P= 0.002) and high subgroup (11% vs. 17%, unadjusted HR 0.62; 95% CI 0.50-0.75, P < 0.001). However, this difference disappeared after adjustment, in particular for duration of diabetes, insulin and sulfonylurea use.
Conclusion: The effect of aleglitazar on MACE, total mortality and hypoglycaemia did not differ between HGI subgroups.
Disclosure: **S.C.J. van Steen:** None.

1143

The effect of continuous subcutaneous insulin infusion treatment on lipid monitoring in type 1 diabetes patients: one-year follow up

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Background and aims: Type 1 Diabetes Mellitus (T1DM) is a chronic disease that affects blood glucose levels. Patients should be assessed for atherosclerotic cardiovascular disease (ASCVD) risk factors, including dyslipidemia. Quantitative lipid abnormalities are observed in patients with poorly controlled T1DM (increased triglyceride and LDL levels). Patients with optimally controlled T1DM show normal or slightly decreased triglycerides and LDL levels and sometimes increased HDL levels. Several studies have shown that patients with higher HbA1c had much more lipid quantitative disorders than patients with optimal HbA1c control. Continuous Subcutaneous Insulin Infusion (CSII) is often used in order to improve the glycemic control of diabetic patients. The aim of this study was to investigate the outcome of CSII in monitoring total cholesterol, HDL, LDL and triglycerides in antilipidemic-naive T1DM patients.
Materials and methods: A retrospective cohort including 250 T1DM patients treated with CSII who were followed in the Diabetes Center of

our Department between 2008 and 2015. None of the patients had received treatment with an antilipidemic agent. Paired samples T-test was used to calculate total cholesterol, HDL, LDL and triglyceride levels, as well as HbA1c before the initiation of CSII treatment (baseline) vs. at one-year follow up. Statistical significance was defined when $p<0.05$.

Results: The patients' age ranged from 20 to 69 years old; diabetes duration since diagnosis ranged from 4 to 49 years. The mean body weight (BW) was not significantly different at baseline (mean BW 72,66±14,74 kg) vs. one year after the initiation of CSII (mean BW 73,71±15,47 kg). At baseline, the mean total cholesterol value was 186,96±37,86 mg/dl, mean HDL 58,50±21,75 mg/dl, mean LDL 120,29±30,99 mg/dl and mean triglycerides 96,05±50,90 mg/dl. At one-year follow up, mean total cholesterol value was 186,80±36,20 mg/dl, mean HDL 63,34±17,57 mg/dl, mean LDL 114,61±32,28 mg/dl and mean triglycerides 85,37±42,62 mg/dl. No statistically significant reduction was found in total cholesterol, LDL and triglyceride levels and there was a trend of increasing HDL after one year of CSII treatment (Table 1). The mean HbA1c was 8,33±2,03% at baseline vs. 7,49±1,38% at one-year follow up, indicating a significant improvement in the patients' glycemic control ($p<0,01$).

Conclusion: Our study included 250 T1DM patients who were started on CSII treatment, resulting in a significant reduction in HbA1c. One year after CSII initiation, no improvement was demonstrated in the lipid profile of these T1DM patients. Further studies are needed to evaluate the effect of CSII administration on lipoprotein metabolism.

Table 1	Baseline	1-year follow up	P (sign)
Body weight (kg)	72,66±14,74	73,71±15,47	0,27
HbA1c (%)	8,33±2,03	7,49±1,38	<0,01
Total Cholesterol (mg/dl)	186,96±37,86	186,80±36,20	0,98
HDL (mg/dl)	58,50±21,75	63,34±17,57	0,09
LDL (mg/dl)	120,29±30,99	114,61±32,28	0,47
Triglycerides (mg/dl)	96,05±50,90	85,37±42,62	0,30

Disclosure: **K. Chantziara:** None.

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Estimation of cardiovascular risk in diabetes

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Background and aims: To evaluate the effect of glycemic parameters in patients with type 2 diabetes and coronary heart disease on cardiovascular system.

Materials and methods: The study involved 85 patients with type 2 diabetes aged 43 to 79 years in conjunction with coronary heart disease and a hypertensive disease. All patients underwent simultaneous monitoring of blood glucose levels and the cardiovascular system. Blood glucose monitoring is carried out for 1-3 days this used CGMS (Continuous Glucose Monitoring System) device. At the same time conducted monitoring of the cardiovascular system using monitors Holter ECG and blood pressure dayly monitoring . Holter monitoring is carried out for 1 day.

Results: In assessing the impact of daily glycemia on the occurrence of ventricular arrhythmias it has been shown that an increase in hypoglycemia and glycemic variability increases the risk of arrhythmia. We had found a significant association between the frequency of ventricular arrhythmias and hypoglycemia index ($r = 0,38$ $p = 0,03$). Was found direct significant correlation between SD ($r = 0,53$, $p = 0,05$) , mean amplitude of glycemic fluctuations MAGE ($r = 0,52$, $p = 0,02$) and number of PVCs (ventricular arrhythmias). In assessing the impact of short-term fluctuations of glycemia on heart rate variability it has been found that the effect depends from the average blood glucose level , on which drop occurs. When blood glucose less than 10 mmol \ l was detected increase parasympathetic component and heart rate reduction. Can talk about the formation of mechanisms that protect against the fall of heart rate variability, and, respectively, and the occurrence of arrhythmias in reply the drop glucose when normoglycemia. While when more than 15 mmol\ l found increase in heart rate in response to increasing glucose fluctuation amplitude with decrease of the parasympathetic component, ($r = -0,71$, $p = 0,01$). When analyzing the effect glycemic parameter on the myocardial ischemia, it was revealed that the only chronic glucose toxicity affects the occurrence of ischemia. In patients with episodes of ischemia had significantly higher glycated hemoglobin 7.88 ± 2,15 (in patients without ischemia) versus 8,79 ± 2,11 (in patients with ischemia) ($p = 0,006$). While the average level of blood glucose, glycemic variability indices, the index of hypoglycemia, ie diurnal glycemic indices, did not affect the incidence and duration of ischemia.

Conclusion: 1. Found glycemic thresholds (less than 4.2 mmol / l and above 15 mmol / l), under which increases the likelihood of developing cardiac arrhythmias that need to be considered when determining the target values of glycemia from the position of the stratification of sudden cardiac death risk in patients with diabetes type 2 diabetes and coronary heart disease. 2. High glycemic variability (MAGE more than 5 mmol / L) is associated with an increased risk of dangerous ventricular arrhythmias. 3. It was confirmed that the predictors of myocardial ischemia are the level of glycosylated hemoglobin, increased systolic blood pressure, high pulse pressure, violation of circadian blood pressure profile

Disclosure: **E.A. Ermakova:** None.

PS 121 The early years

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Gender differences in insulin resistance during adolescence: a longitudinal study (EarlyBird)

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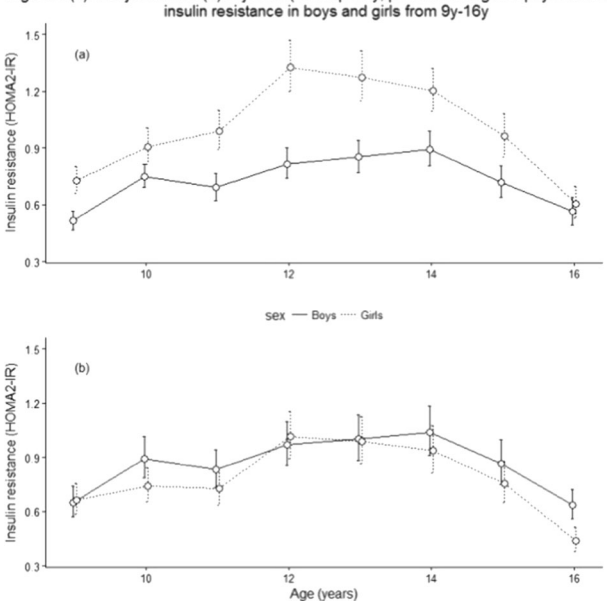
Background and aims: The risk of type 2 diabetes is increasing significantly in teenage girls and this may be explained by their greater insulin resistance (IR). Cross-sectional studies reveal that IR is higher in prepubertal girls than boys, although adult males have higher IR and diabetes risk. Longitudinal studies into this gender difference (GD) are scarce. Our hypothesis was that the intrinsic gender difference in IR would persist from childhood through adolescence.
Materials and methods: Longitudinal cohort of 292 children (147 boys) studied annually from 9-16y (EarlyBird Study). Measures: IR (Homeostasis model assessment-2), % body fat (dual energy x-ray absorptiometry), pubertal stage (age at peak height velocity; APHV), physical activity (accelerometry). Cross-sectional univariate analyses and multi-level modelling were used to establish influence of covariates and age-related trends in IR.
Results: Unadjusted age-related trends in IR are shown in Fig 1a. Girls were 21-63% more insulin resistant than boys between 9 and 15y (mean IR in boys ranged from 0.51 (CI 0.47-0.57) to 0.89 (0.81-0.98), girls from 0.73 (0.66-0.80) to 1.33 (1.20-1.47), $p<0.005$). At 16y the GD was not significant (boys 0.56 (0.49-0.64), girls 0.60 (0.53-0.69), $p=0.45$). Girls had greater % fat than boys at each age ($p<0.001$) and earlier APHV (11.6y v 13.3y, $p<0.001$) while the boys were significantly more active than girls throughout ($p<0.001$). Adjusting for each covariate separately attenuated the GD in IR significantly, with % fat having the greatest effect. In the final model (Fig 1b), adjusted for all covariates, there was no significant GD in IR from 9-15y: IR boys ranged from 0.65 (0.57-0.74) to 1.04 (0.91-1.18) and girls from 0.66 (0.59-0.75) to 1.01 (0.89-1.15), $p>0.07$). At 16y, boys were 30% more insulin resistant than girls (boys 0.63 (0.56-0.72), girls 0.44 (0.38-0.51), $p=0.001$).
Conclusion: The adolescent gender difference in IR is predominantly explained by adiposity, with smaller effects of pubertal timing and activity, thus we reject our hypothesis. A divergence occurred around the age of 16 years when males became more insulin resistant than females. These findings may explain the changing female to male predisposition to type 2 diabetes between childhood and adulthood.

Figure 1 (a) unadjusted and (b) adjusted (for adiposity, pubertal timing and physical activity) insulin resistance in boys and girls from 9y-16y



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Disclosure: **S.C. Jeffery:** None.

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Wrist circumference is associated with left ventricular dysfunction in obese children

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Background and aims: In an attempt to identify an easy-to-detect clinical marker of insulin resistance in obese children we have previously demonstrated, by Nuclear Magnetic Resonance Imaging, an association between the circumference of the wrist, in particular its bone component, and insulin levels and homeostasis model assessment of insulin resistance. In the light of evidence that insulin resistance in childhood is associated with increased left ventricular mass, the aim of the present study was to assess the association of the wrist circumference with structural and functional changes of the left ventricle parameters in a population of obese children and adolescents.
Materials and methods: One hundred and six (55 male and 51 female, mean age 9.8±2.9 years) obese children and adolescents were consecutively enrolled. In all subjects body weight, height, wrist circumference, waist circumference and BMIz-score, fasting glucose, fasting insulin and lipid profiles were evaluated at entry. All subjects underwent echocardiographic assessment and the following parameters were evaluated: LV dimension at end diastole (LVDd) and systole (LVDs), LV posterior wall thickness at end diastole (LVPWd), and systole (LVPWs), interventricular septal thickness at end diastole (IVSd),and systole (IVSs) and epicardial adipose tissue (EAT). LV ejection fraction (LVEF), LV fractional shortening (LVFS) and LV mass were obtained according to the American Society of Echocardiography. Regarding LV function were evaluated: early (E) and late (A) mitral velocity; E-to-A ratio. TDI echocardiography of the septal and lateral mitral anulus was used to measure the early (e') and late (a') anular diastolic and systolic (s') tissue velocities. E-to-e' and e'-to-a' ratios were also calculated. Statistical analysis was performed using SAS v.9.4.
Results: Wrist circumference correlated with all parameters of left ventricular dimensions (LVDd, LVPWd, IVSd, LVDs, LVPWs and IVSs),

LV mass and EAT ($p<0.0001$ for all comparisons). The strongest correlations were observed between wrist circumference and LVDd and LVDs ($r=0.73$ and $r=0.68$ respectively). Regarding LV function, we did not observe any statistical significant correlations except for an inversely correlation between wrist circumference and E peak ($r=-0.26$, $p<0.01$). Results of the multivariate regression analysis adjusted for gender and tanner stage, using left ventricular dimensions as the dependent variables, showed that wrist circumference explained 40% of the variance of LV mass, 53% of LVDd and 45% of LVDs ($p<0.001$ for all variables) Moreover, wrist circumference was also a significant contributor to the variance of EAT (beta=0.07 ± 0.02, $p<0.0001$) explaining the 16% of its variance. In relation to parameters of LV function, we observed that the only significant association was between wrist circumference and early E peak. In particular wrist circumference explained the 7% of the variance of E peak and showed an inverse association (beta=- 3.08 ± 1.28, $p=0.02$).
Conclusion: The results of the present study suggest that wrist circumference could be used as a marker of cardiovascular risk in obese children and adolescents, opening new perspectives in the prediction of cardiovascular diseases.
Disclosure: **S. Zampetti:** None.

1147

Type 2 diabetes in adolescents with positive specific autoantibodies

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Background and aims: To study autoimmunity in adolescents with DM, clinically interpreted as T2D.
Materials and methods: We examined 80 adolescents with T2D, diagnosed on basis of glycemia according to WHO criteria of DM, presence of hyperinsulinemia and insulin resistance (IR) and/or absence of insulin requirement three years and more. Our patient was divided in two groups, with positive and negative antibodies. The control group is 23 adolescents with T1D, comparable on age and severity of obesity. The duration of observation for patients is 3 years. The control group in the study of HLA-DRB1, DQ genes is 599 children with T1D.The antibodies to ICA, IAA, GAD, IA2 measured. The secretion of C-peptide assessed on standard carbohydrate breakfast test (50 g. of carbohydrate).
Results: The positive antibodies detected in 15.2% of T2D patients: ICA in 9.1%, IAA in 6.1%, GAD and IA2 were negative. The antibodies titer was low, below 20 U/ml. Among patients with T1D detected all type of antibodies (21.7%, 21.7%, 39.1%, 56.5% respectively). The HbA1c level during diabetes manifestation did not differ for patients with T2D, however, in "AT-" group was significantly lower, than for patients with T1D. The high risk HLA frequency for T1D was significantly higher in "AT+ "group in comparison with "AT-" and comparable with frequency for T1D. The high risk HLA genotypes for children with T2D detected significantly rarely than for T1D. The C-peptide secretion in "AT+" group was middle between secretions in "AT-" and T1D groups. In three years, the "AT+" group had not decrease of secretion in comparison with T1D. Conversely, C-peptide secretion in "AT+" increased significantly and did not differ from "AT-" group. The therapeutic tactics in two groups of T2D was similar, only one patient in "AT+" group with duration of disease more 3 years received insulin, whereas in T1D groups it received all patients.
Conclusion: The good C-peptide secretion and absence of insulin requirement in 3 years after DM manifestation, absence of specific antibodies in "AT+" group, allow to interpret it as T2D. However, high risk occurrence of high risk HLA for T1D manifestation and relatively small duration of follow-up does not allow to exclude the latent autoimmune diabetes of young (LADY).
Disclosure: **A.O. Emelyanov:** None.

1148

Impact of autoantibody values on severity of type 1 diabetes manifestation in children

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Background and aims: It is known that autoantibodies against pancreatic beta cells are destructing factors leading to insulin insufficiency and clinical manifestation of type 1 diabetes mellitus (DM). Their values are informative in making diagnosis of type 1 DM and evaluating the autoagressive attacking process. Though, there is no evidence to assume that these antibodies predict the severity of the clinical manifestation. The aim of this work is to reveal the potential impact of antibodies absolute values on severity of clinical manifestation of type 1 DM.
Materials and methods: 60 children with the clinical manifestation of type 1 DM aged from 2 to 19 years were included in the investigation with average age of 6,31 years, male/female ratio was 34/26. Clinical examination and anti-GAD, anti-IA, and anti-ICA autoantibodies, C-peptide, ketone bodies, HbA1c levels were evaluated. Statistical analyzes were performed to determine the significance of findings. In all cases null hypothesis was rejected if $p<0.05$.
Results: 15% of examined patients had normal C-peptide and high levels of HbA1c and antibodies. No any correlation has been found between absolute levels of antibodies and C-peptide ($r<0.02$), as well as between antibodies and BMI absolute values ($r<0.03$). 86.7% of patients had been admitted with severe clinical features and ketoacidosis, 94,2% from which were precomatose or comatose in different grades of unconsciousness. Interestingly 95,9% (N=47) of precomatose and comatose patients found to have thirtyfold higher levels of antibodies versus to 4,1% with slightly increased antibodies ($p<0.05$). Ketoacidosis was positively associated with highly increased titters antibodies levels ($p<0.05$) without sex predominance.
Conclusion: Highly increased (>thirtyfold) anti-beta-cell antibodies' titters are connected with severity of clinical manifestation of type 1 DM and are mostly associated with ketoacidotic precoma and coma. We assume that as highly are increased anti-beta-cell antibodies, as aggressive the destruction process and as severe the clinical features of type 1 DM manifestation in children. Further investigations should be done to evaluate the relationships between antibodies' levels at the manifestation of type 1 DM and severity and lability of its duration, and later complications.
Disclosure: **L.V. Navasardyan:** None.

1149

Preliminary observations of gastric emptying in diabetic ketoacidosis

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Background and aims: Diabetic ketoacidosis (DKA) is frequently associated with upper gastrointestinal symptoms, including anorexia, nausea and vomiting. Although marked gastric stasis has been reported anecdotally, gastric emptying in the acute phase of DKA has not been formally studied. However, the assumption that gastric emptying is often markedly delayed in patients with DKA, even in the absence of prominent gastrointestinal symptoms, has led to a conservative approach to the introduction of oral intake in this patient group. Our study, which employed the 'gold standard' technique of scintigraphy to quantify gastric emptying, was designed to determine: 1) whether gastric emptying is delayed in the acute recovery phase of DKA and 2) whether any abnormalities in gastric emptying during the acute phase are reversible.
Materials and methods: Patients with type 1 diabetes presenting with DKA were eligible. Those with a past history of significant gastrointestinal

disease, or a requirement for medication known to affect gastrointestinal function, were excluded. DKA was managed according to a standard hospital protocol and antiemetics were not administered within 5 half-lives of the first gastric emptying measurement. Thus far 5 patients with T1DM [3 male; age 29 (12) years; body mass index 23 (3) kg/m2; HbA1c 11.3 (1.9) %; duration of diabetes 10 (11) years] have been studied on two occasions. The initial gastric emptying measurement was performed when oral intake was deemed to be clinically appropriate by the treating endocrinologist; the second measurement was performed following discharge [16 (21) days] when the patient was clinically well. Gastric emptying (scintigraphy) and blood glucose were monitored for 2 hours following ingestion of a standardised meal: 100-g ground beef (270 kcal, 25 g protein, 21 g fat) labelled with 20 MBq 99mTc-sulfur colloid, followed by 150 ml of 10% dextrose labelled with 7 MBq 67Ga-EDTA. Solid (% intragastric retention at 100 min, T100) and liquid (50% emptying time, T50) gastric emptying were determined, and compared to an established control range (T100: 6-61%; T50: 4-31 min). Data are presented as median (interquartile range).
Results: In the acute setting, solid gastric emptying (T100) was delayed in one patient, while liquid gastric emptying (T50) was delayed in three. In all cases the magnitude of the delay in gastric emptying was modest. In the follow up study, the rate of solid gastric emptying was normal in all patients, while liquid gastric emptying remained delayed in those patients in whom liquid emptying was delayed at baseline. Solid and liquid gastric emptying tended to be slower at the initial visit (solid T100: 47 (20) vs. 38 (33) %; P=0.27; liquid T50: 37 (25) min vs. 35 (15), P=0.28). There was no difference in baseline blood glucose concentrations or incremental area under the glucose curve (iAUC 0-120 min) across study days (9.6 (0.3) mmol/L vs. 6.5 (3.9) mmol/L; P=0.11; 166.5 (387.8) mmol/L*min vs. 280.5 (474) mmol/L*min; P=0.50).
Conclusion: In a small cohort of type 1 patients, gastric emptying of solids and liquids was not markedly delayed in the acute recovery phase of DKA, nor was there significant change in gastric emptying on follow-up when patients were clinically well.
Disclosure: **L.K. Phillips:** None.

1150

DKA at diagnosis of type 1 diabetes predicts poor long-term glycaemic control

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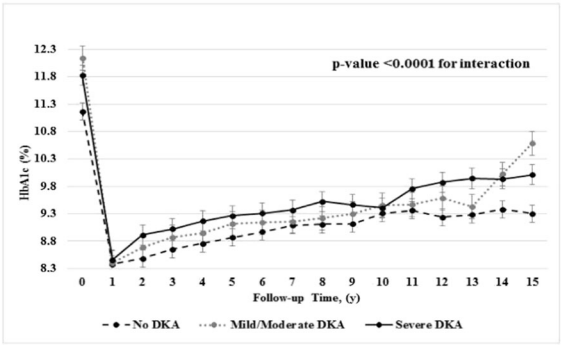
Background and aims: The diagnosis of type 1 diabetes in children is often associated with diabetic ketoacidosis (DKA). We tested the hypothesis that DKA at diagnosis is an independent predictor of poor glycemic control later in the course of the disease.
Materials and methods: A cohort of 3439 Colorado residents diagnosed with type 1 diabetes before the age of 18 years, between 1998-2012, was followed at the Barbara Davis Center for Childhood Diabetes for up to 15 years. Of those, 39% had DKA at diagnosis. DKA was further classified as severe (pH <7.10 or bicarbonate <5mEq/L) or moderate/mild (pH 7.10-7.29 or bicarbonate 5-14mEq/L). HbA1c was measured using the DCA 2000 Analyzer at each visit (3.6/y, on average; median 19 times/patient). Individual average HbA1c was calculated annually, starting 60 days after diagnosis. The effect of DKA on long-term HbA1c was analyzed in an adjusted linear mixed model (Figure).
Results: Long-term HbA1c was significantly higher in children diagnosed in DKA, especially those in severe DKA (p<0.0001 for the interaction). The difference in HbA1c between the DKA versus no-DKA groups appeared to increase over time, from initially 0.3% to 0.9% at 15 years of follow-up.
Conclusion: DKA at diagnosis of type 1 diabetes in children predicts chronic worse glycemic control, independent of demographic factors, insulin pump treatment and access to diabetes care.

Figure: Least-square means of HbA1c by severity of DKA from the repeated-measurement mixed model adjusting for age, race, sex, insurance status, family history of diabetes, insulin pump use, and baseline HbA1c



Disclosure: **A. Rewers:** None.

1151

The gestational diabetes placental pericyte: unique angiogenic profile from South India

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Background and aims: The role of the Placental pericyte in maintaining structural stability of foetal blood vessels is poorly understood in healthy and diabetic patients. In India 20-70% of Gestational Diabetes Mellitus mothers and new borns develop Type 2 Diabetes. The aims of the study was to 1. Phenotypically characterise the hitherto unknown healthy placental and GDM pericyte and 2. evaluate whether an angiogenic response of blood vessels in GDM placentas is responsible for abnormal function of blood vessels in vivo.
Materials and methods: 15 cases each of healthy and GDM placentas from cohorts from South India were included in this study. We have optimized protocols for the isolation, characterization and expansion of foetal placental EPCs and pericytes cultured from the placental disc and umbilical cord blood. Placental samples were subjected to histological examination, including immunohistochemistry, transmission electron microscopy, gene expression and in vivo transplantation into CB17/Icr-Prkdcscid/IcrIcoCrl Severe Combined Immune Deficient (SCID) mice.
Results: A distinctive "GDM placental pericyte phenotype" has been noted in South Indian placentas that is CD146 positive, Desmin positive, Alpha Smooth Muscle Actin positive, NG2 negative and PDGFR-Beta negative. Up regulation of angiogenic marker profiles (VEGF-1, VEGF Receptor 1, VEGF Receptor 2, , Angpt1 and 2, CXCR4, SDF-1 Alpha, HIF-1 Alpha) reveal abnormalities of blood vessels of GDM vascular progenitor cells ($p \leq 0.001$). Paradoxically, while the histological lesions in GDM showed marked angiogenic response such as chorangiosis and increased capillary diameter of blood vessels in the GDM vasculature ($p \leq 0.001$; in vitro and in vivo, the angiogenic response is muted. We have demonstrated by transmission electron microscopy that the placental pericyte in GDM shows pericyte ghosts, increased microvessel density and thickening of capillary basement membranes reminiscent of adult Type 2 Proliferative Diabetic Retinopathy that was not seen in healthy placentas ($p \leq 0.001$). Endothelial cell irregularity was noted in 76% GDM vs. 10.4% healthy placentas ($p \leq 0.001$). Pre pregnancy BMI, placental size, sex of the baby and HbA1C did not infuence the findings. Non-Parametric, Mann Whitney was done.
Conclusion: PDGFR-Beta negative placental pericytes in vivo in GDM are leaky and non- functional.Despite several animal models of disease to

study T2D, none of them consistently reproduce adult human Type 2 proliferative retinopathy. We have detected a striking resemblance of placental foetal vascular progenitor cells exposed to short duration hyperglycemia from GDM in a cohort from South India that resembles adult Type 2 proliferative diabetic retinal cells. This could point to the role of the intrauterine environment influencing the development of Type 2 Diabetic microvascular disease in adult hood. Targeting molecules such as the PDGFR-Beta or Angpt 1 and 2 pathways might help our understanding of abnormal blood vessel flow in GDM placental vasculature, and in adult T2 Diabetic retinopathy.

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Disclosure: **R. Samuel:** None.

PS 122 Markers of vascular disease

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Are recommended goals for secondary prevention of myocardial infarction or stroke achieved in real life? A German/Austrian DPV analysis of 29,325 patients with type 2 diabetes

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Background and aims: Studies investigating adherence to current secondary prevention guidelines of myocardial infarction (MI), or stroke in the high-risk group of subjects with type 2 diabetes (T2D) are lacking. We therefore analyzed whether medical care is in accordance with guideline recommendations for secondary prevention of MI or stroke in T2D subjects from Germany and Austria.

Materials and methods: 29,325 patients (≥20 years of age) with T2D and MI, or stroke, documented between the years 2006 and 2015 were selected from the "Diabetes-Patienten-Verlaufsdokumentation" (DPV) database. According to national guidelines, we analyzed in patients with MI: HbA1C (<7.5%), blood pressure (<130/80mmHg), LDL-cholesterol (<100mg/dl), body mass index (BMI) (<27kg/m2), medical treatment (inhibitors of platelet aggregation (IPA), statins, beta blockers, ACE inhibitors, and any type of antihypertensives), smoking (yes/no), and physical activity (yes/no). In patients with stroke, we analyzed HbA1C (<7.5%), blood pressure (systolic 120-<140mmHg / diastolic 70-<90mmHg), LDL-cholesterol (<100mg/dl), medical treatment (IPA, statins, ACE inhibitors, and any type of antihypertensives), smoking (yes/no), and physical activity (yes/no).

Results: HbA1C <7.5% was achieved in 64.9% (MI), and in 61.1% (stroke) of patients. LDL <100mg/dl was documented in 56.2% (MI), and in 42.2% (stroke). Non-smoking was reported in 92.0% (MI), and in 93.1% (stroke), physical activity in 9.6% (MI), and 5.5% (stroke). Target values of blood pressure were reached in 67.0% (MI), and in 89.9% (stroke). IPA was prescribed in 50.7% (MI), and 31.7% (stroke). 57.0% (MI), and 40.1% (stroke) used statins, 65.1% (MI), and 65.8% (stroke) used any type of antihypertensives, and ACE inhibitors were prescribed in 49.7% (MI), and 41.3% (stroke). BMI <27kg/m2 was documented in 32.0%, and the use of beta blockers in 59.5% of the subjects with MI.

Conclusion: Medication and lifestyle changes according to guideline recommendations for secondary prevention in subjects with T2D who already experienced MI or stroke are suboptimal. This analysis also confirms the need to improve secondary prevention of CVD risk factors. There remains great potential to reduce the risk of repeated macrovascular events and premature death, as well as to increase patients' quality of life.

Supported by: EFSD supported by AstraZeneca; BMBF

Disclosure: **B. Bohn:** None.

1153

High glycated haemoglobin level is associated with increased risk for recurrence of ischaemic non-cardioembolic stroke in diabetic patients

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Background and aims: Stroke is expected to be the second more frequent cause of mortality worldwide by 2020, while the prevalence of diabetes for all age-groups worldwide is estimated to be 4.4% in 2030. Clinical and experimental evidence suggest that both diabetes and insulin resistance cause a combination of endothelial dysfunctions, which may diminish the anti-atherogenic role of the vascular endothelium. The role of chronic hyperglycemia in the development of diabetic microvascular complications and in neuropathy has been clearly established. However, the biochemical or cellular links between elevated blood glucose levels, and the vascular lesions remain incompletely understood. Glycated hemoglobin (HbA1c) level reflects the mean glucose control range for the previous 2 to 3 months in patients with or without diabetes mellitus. HbA1c level is widely recommended as the therapeutic guideline for the prevention of cardiovascular complications in patients with diabetes.
Materials and methods: 125 patients (65 men and 60 women) who suffered from a first-ever ischemic non-embolic stroke were included in the study. The diagnosis of stroke was set either clinically and /or had proper imaging findings. They were all under medical supervision in our Lipid, Diabetes and Metabolism Unit every three months for at least two years. Patients with Atrial Fibrillation, Coronary Artery Disease, Heart Failure (NYHA>3) and smokers were excluded. BMI, Glycated Hemoglubin, Total Cholesterol, LDL and Triglycerides were regularly tested and interventions were made accordingly. There was no intervention in the patients' anti-coagulant therapy. Subjects were divided into 4 groups according to the quartiles of HbA1c level for the assessment of risk for ischemic stroke: 1st quartile, HbA1c≤5.3%; 2nd quartile, 5.3<HbA1c≤5.6%; 3rd quartile, 5.66.0%. Finally, we checked who of these patients had a second event in a two-year period after the first one.
Results: Among the diabetic men, 82.9% had high LDL, 51.4% had high TG and only 14% had a normal BMI. After two years of therapy 79.4% achieved the LDL goal, 95.2% the TG goal, 40% had desirable HBA1c, yet 22.9% increased their BMI. Among the diabetic women, 90% had high LDL, 30% had high TG and only 6.7% had a normal BMI. After two years of therapy 81% achieved the LDL goal, 100% the TG goal, 32.3% had desirable HBA1c, yet 19.7% increased their BMI. 14.4% of the patients (15.3% of men and 13.3% of women) had already had a second cerebral event by the end of the two-year period of supervision. Statistically it seems that patients with HbA1c>6.0% have a really increased risk of recurrence of ischemic stroke compared to those with HbA1c≤5.3%.
Conclusion: Diabetes has been clearly established as a risk factor for first stroke but not as one for recurrent stroke. Yet there is evidence that it may be a predictor for stroke recurrence. Thus, additional studies should be performed.
Disclosure: **A. Bourdakis:** None.

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High overall cardiovascular risk and mortality in patients with atrial fibrillation suffering from diabetes: a nationwide report

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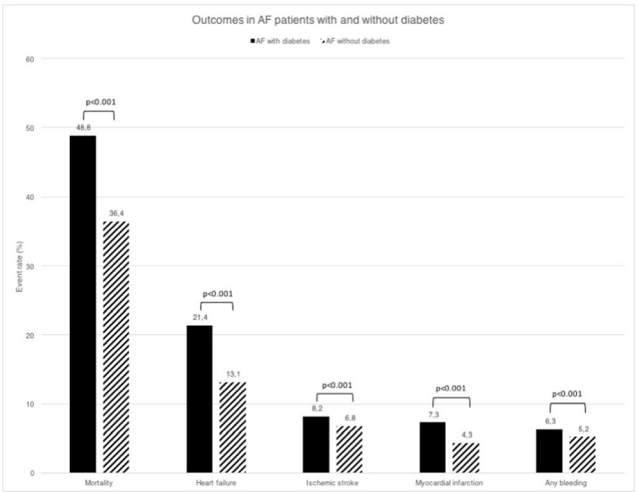
Background and aims: People with diabetes are at increased risk for cardiovascular disease. The prognostic implications of diabetes in patients with atrial fibrillation, beyond stroke, are not previously studied from a nationwide perspective. We aimed to describe mortality and morbidity patterns of patients with atrial fibrillation with and without diabetes in Sweden.
Materials and methods: Information on mortality, disease and pharmacological therapy were extracted from the Swedish Prescribed Drug Register, Cause of Death Register, and Swedish National Patient Register (NPR). All 326 832 persons with a diagnosis of atrial fibrillation between January 1, 2006 and December 31, 2012, who survived for at least 30 days, were followed for mortality and combined event (first of mortality, heart failure, ischemic stroke or myocardial infarction) until 31 December 2013. Patients ≤20 years old and those with valvular atrial fibrillation were excluded. Hazard ratios were calculated using a Cox proportional hazard regression model adjusting for comorbidities, medication and socioeconomic factors.
Results: Diabetes was present in 17.7% (n=57 953). Mean follow up time was 3.7 years (0.9 to 8 years or ≈1 200 000 person-years). The most frequent events were mortality (48.8% vs. 36.4%; p<0.001), heart failure (21.4% vs. 13.1%; p<0.001), ischemic stroke (8.2 vs. 6.8%; p<0.001), myocardial infarction (7.3 vs. 4.3%; p<0.001) and any bleeding (6.3 vs. 5.2%; p<0.001) in those with and without diabetes, respectively. Diabetes predicted mortality (HR;95% CI; 1.28; 1.25-1.31, ages < 65 years 1.59;1.43-1.77), combined event (1.22;1.20-1.25, < 65 years 1.41;1.31-1.53) and bleeding (1.12; 1.06-1.19). The standardized mortality ratio compared to the general population for patients with atrial fibrillation but without diabetes was 133% (95% CI; 131-135%) and for those with atrial fibrillation and diabetes 206% (95% CI; 200-212%).
Conclusion: Patients with atrial fibrillation and diabetes have high event rates for mortality and heart failure, with levels far exceeding event rates for stroke. Diabetes is an especially strong risk factor at ages < 65 years. Increased awareness of the overall cardiovascular risk in these patients and improved treatment strategies are needed.



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Disclosure: **S. Karayiannides:** Grants; Grant from Swedish Heart and Lung-foundation, R&D funding from Department of Internal Medicine, Danderyd Hospital.

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Characteristics and cardiovascular complications of a large cohort of adults diagnosed with type 2 diabetes < 45 years

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Background and aims: We are confronted with an increasing prevalence of type 2 diabetes (T2DM) in younger people. More research is necessary

to better understand the natural history of T2DM in younger adults. Our aim was to evaluate the characteristics, the microvascular -and cardiovascular complications in a large Belgian cohort of adults diagnosed with T2DM < 45 years.

Materials and methods: Retrospective analysis of the medical files of the last visit of patients diagnosed with T2DM < 45 years attending our university hospital between 01-01-2004 and 30-06-2015. Differences between men and women and between Caucasians and ethnic minorities (EM) were analyzed with independent sample T test for continuous data and with chi square tests for categorical data. Multivariable logistic regression was used to adjust for confounders such as smoking, HbA1c, BMI, LDL cholesterol, blood pressure, diabetes duration, age and microalbuminuria.

Results: A cohort of 886 adults was identified with an age at diagnosis of 37.3±6.4 years, 44.1% were women and 12.1% were from an EM background. Age of patients at the last visit was 57.3 ±12.5 years with a diabetes duration of 20.5±11.8 years, 32.4% were overweight and 56.8% were obese. Mean HbA1c was 7.3% ± 1.3 with 45.5% of patients reaching a HbA1c target < 7.0% and 81.9% received insulin. Of all patients 49.9% was hypertensive and 34.1% did not reach the LDL cholesterol target in primary or secondary prevention. Diabetic retinopathy was present in 40.2% and microalbuminuria in 34.4% of patients. Of all patients 0.9% received carotid surgery, 6.9% received vascular surgery of the lower limbs, 7.2% had a cerebrovascular accident (CVA) and 20.1% had a myocardial infarction or cardiac surgery. Over a mean follow up of 8.4 ± 4.9 years, 2.6% of patients underwent a second surgery of the lower limbs, 1.0% had a new CVA and 4.1% had a new myocardial infarction or cardiac intervention. Compared to women, men had a shorter diabetes duration (19.3 ±11.0 vs. 22.0±12.7, p=0.001), a higher HbA1c (7.3%±1.4 vs. 7.1%±1.2, p=0.021), lower rates of obesity (51.2% vs. 64.1%, p<0.0001), higher rates of hypertension (53.4% vs. 45.3%, p=0.019), smoked more often(20.2% vs. 12.0%, p=0.001), had a lower LDL-cholesterol (74.1mg/dl ±30.1 vs 81.7mg/dl ±31.2, p<0.0001) and higher rates of microalbuminuria (40.6% vs. 26.2%, p=0.001). After adjustment for confounders, rates of myocardial infarction and cardiac surgery remained significantly higher in men compared to women (24.3% vs. 14.8%, p=0.010). Compared to Caucasians, EM patients were younger at diagnosis (35.4±6.8 years vs. 37.6±6.2 years, p=0.001), had a shorter diabetes duration at last visit (15.8±10.6 years vs. 21.2±11.9 years, p=0.002) and were less often obese (43.3% vs. 55.6%, p=0.007). After adjustment for confounders, rates of myocardial infarction and cardiac surgery were not significantly different between EM and Caucasians (10.4% vs. 21.6%,p=0.960).

Conclusion: These data highlight the heavy toll a diagnosis of T2DM <45 years brings with it. These individuals have high rates of microvascular complications and cardiovascular events at a relatively young age. In our population, men are at particularly high risk for myocardial infarctions.

Disclosure: **B. Deconinck:** None.

1156

TSP-2 levels are higher in patients with type 2 diabetes, but do not increase with severity of PAD

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Background and aims: Atherosclerotic complications are a major contributor to increased morbidity and mortality in diabetic patients. Yet, underlying mechanisms on accelerated atherosclerotic lesion progression in both macro- and microvascular complications cannot be solely explained by typical risk factors. Thrombospondin-2 (TSP2), as a member of the thrombospondin family, has antiangiogenic effects. Experimental bench and animal data linked increased TSP-2 to suppressed angiogenesis in diabetic conditions. Translation to the clinical significance of these findings is missing. The aim of this study is to evaluate latter described effects in a cohort of patients with peripheral arterial disease (PAD) with or without type 2 diabetes mellitus.

Materials and methods: 353 patients with PAD got enrolled at the outpatient department of the Division of Angiology at General Hospital Vienna. Blood was collected after an overnight fast, centrifuged and frozen by -80°C until TSP2 (ng/ml) was measured by a magnetic bead assay (Luminex, R&D Systems, Minneapolis, MN). PAD was diagnosed by Fontaine classification, ABI measurement, which was performed by experienced personnel and Oscillometry. A standarised OGTT was additionally performed. Exclusion criteria were as follows: Fontaine stage > II; malignant disease, serum creatinine >3mg/dl and type 1 diabetes mellitus. Type 2 Diabetes mellitus (T2DM; n=127) was diagnosed by fasting glucose >126mg/dl, HbA1c >6.5%, glucose >200mg/dl after 2 hours or antidiabetic treatment at study entry. Normal glucose tolerance (NGT; n=47) was defined as fasting glucose <100mg/dl, HbA1c <5.7%, glucose <140mg/dl after 2 hours. Patients with values in between were counted to the prediabetes cohort (PRE; n=179). To enlighten difference between patients with normal insulin sensitivity and patients with pathologic conditions T2DM and PRE were consolidated to disturbed glucose tolerance (DGT). Statistical analysis included Mann-Whitney-U test and binary logistic regression analysis.

Results: Patients of the NGT cohort had significant lower median TSP2 values than those of the DGT cohort (296.29 (IQR: 217.46-409.27) vs. 372.85 (265.33-507.48); p=0.018) following Mann-Whitney-U analysis. In contrast, TSP2 values were not different between patients with PAD stage I vs. stage II (351.06 (253.53-473.10) vs. 379.13 (264.19-503.25); p=0.261). Binary logistic regression was performed for NGT vs. DGT for further evaluation of this association. The unadjusted Odds ratio (OR) was 1.47 (1.11-1.96) for every increase of 1 standard deviation (SD) of TSP2. In a next step age and gender were added to the model and increased OR for every increase of 1 SD to 1.52 (1.14-2.02). Further adjustment for HDL-C, Triglycerides, BMI, systolic blood pressure and smoking habits slightly decreased OR for every increase of 1 SD to 1.44 (1.08-1.93). OR remained stable by 1.45 (1.07-1.95) in the last step after adjustment for CRP and serum creatinine.

Conclusion: This study uncovers for the first time an association between TSP2 and prevalent diabetic conditions in humans. This finding remained stable after adjustment for several typical risk factors. Since TSP2 values did not increase with severity of PAD, but with coexistence of DGT in a PAD cohort, TSP2 could be a novel marker for augmented vascular complications by inhibition of vessel formation in this major subgroup of cardiovascular patients.

Disclosure: **B. Zierfuss:** None.

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Plasma levels of asymmetric dimethyl arginine and endothelial dysfunction in diabetic subjects with neuropathic foot ulcer

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Background and aims: Asymmetric dimethyl arginine (p-ADMA) is an amino acid that acts as an endogenous competitive inhibitor of Nitric oxide synthase, leading to endothelial dysfunction (ED). The aim of this study was to evaluate the relationship between p-ADMA level and ED in diabetic subjects with neuropathic foot ulcer (NFU), and study the possible predictors of p-ADMA level.

Materials and methods: 80 diabetic subjects of matched age, sex and BMI were included; 40 with NFU(G1), 20 with peripheral nerve dysfunction (PND) (G2) and 20 without PND (G3), plus 20 matched healthy subjects (G4). Subjects with renal or hepatic impairment, ischemic heart disease, smoking or using statins were excluded. Flow-mediated-dilatation (FMD) of brachial artery and Carotid-intima-media-thickness (CIMT)

were measured using high-resolution ultrasound to evaluate ED and subclinical atherosclerosis, respectively. p-ADMA levels were assayed by ELISA kits supplied by EAGLE-BIOSCIENCES, INC (Germany)
Results: G1&2 had a significantly lower FMD than G3&4 [-5.09(-22.5-22.92), 4.67(-15-23.91) vs. 15.74(8.33-36.59) and 20.1(10.0-46.15) %, respectively] (p< 0.001), and higher CIMT [0.9(0.6-1.5), 0.9(0.6-1.3) vs. 0.6(0.5-0.8) and 0.7(0.5-0.9) cm, respectively] (p<0.001). However, there was no significant change in p-ADMA between the study groups [704.5(508-3611), 687(286-2863), 678(506-874), 642(383-797) ng/L, respectively] (p=0.126). p-ADMA was positively correlated with diabetes duration, systolic blood-pressure, serum total cholesterol, triglycerides and CIMT (r=0.299, p=0.007, r=0.298, p=0.007, r=0.390, p< 0.001, r=0.237, p=0.034, r=0.330, p=0.003, respectively), with no significant correlation with FMD (r=-0.176, p=0.118). FMD was inversely and strongly related to CIMT (r=-0.520, p<0.001). p-ADMA levels were significantly higher in uncontrolled hypertensive patients in comparison to controlled and normotensive subjects [717(286-3611) vs. 648(335-874) and 686(526-857) ng/L, respectively] (p=0.026). Metformin users and hypertensive subjects on ACEIs or ARBs had the lowest p-ADMA levels than the non users (p<0.001, p=0.007, respectively).
Conclusion: The remarkable ED in diabetic subjects with NFU is unlikely to be due to alteration in p-ADMA. Further studies are needed in order to conclude a causal association between p-ADMA and ED in this group of patients.
Disclosure: **F.A. Kyrillos:** None.

1158

Clinical neuropathy and proinsulin positive mononuclear cells in type 2 diabetes with neuropathic and neuroischaemic lesions. Italian leukaemia association Treviso project

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Background and aims: Diabetic neuropathy is the leading cause of non traumatic limb amputations; In a streptozotocin and ob/ob mouse model of diabetic neuropathy, proinsulin positive (PI+) bone marrow derived monocyte cells could have a predominant role. These cells have fusion and apoptosis capacity for hyperglycemic neural tissue and induced elevated TNFα cytokine values. Neuropathic and neuroischemic patients with type 2 diabetes (T2D) and foot lesions would represent a good neuropathy human model to investigate PI+ monocite cells (PIMNC)
Materials and methods: To evaluate peripheral PIMNC by flow citometry FACSCanto Diva Software and clinical neuropathy by electromyography (EMG) Metronic KeyPoint describing conduction velocity (CV) and mean amplitude potential (MAP) in bilateral sensory and motor nerves: superficial (SP) and deep peroneous (DP) nerves, sural (S) and tibial (T) nerves, femoral and saphenous nerves. TNFα was measured with ELISA (RayBiotech, USA). We studied T2D patients without complications (10D), with neuropathy (10N), and with neuropathic foot lesions without (9N1) or with (12N2) critical limb ischemia (pO2 <30 mm Hg). 5 healthy younger subjects were compared as controls (C)
Results: EMG CV and MAP values were significantly reduced in N1 and N2 versus D and N (p<0,01); as for sural and tibial nerves, superficial and deep peroneous nerves are early damaged in N (mean MAP 7,5±1,2 mV) versus D (10±2 p <0,03). TNFα significantly increased from C to N2 (275 ±6; 319±22; 425±31; 431±28; 579±25 pg/ml). PIMNC were present only in diabetic population (0,5±0,1 vs 0,007±0,0% Ficoll treated samples p=0,006), significantly inversely correlated with CV and MAP impairment of all evaluated nerves in the diabetic population (p<0,013 R2= -0,4) and positively correlated with inflammatory cytochine TNFα (p<0,004 R2= 0,7) and heavly with diabetes duration (p<0,01 R2= 0,15) independently of median metabolic control (ns with cumulative on time HbA1c). Motor nerve DP and T more correlated with PIMNC (R2= 0,7).
Conclusion: For the first time, we demonstrated proinsulin expression in circulating mononuclear cells of T2D patients and a clear involvement of this proinflammatory cellular pattern in diabetic neuropathic and neuroischemic lower limb complications. Classical and non classical EMG parameters for diabetic neuropathy describe the progression of the diabetic foot desease. We hypothyze that human bone marrow derived PIMNC could migrate along territorial innervation explaining the EMG decribed early damage of the nerves passing by leg bones and identifying hyperglicemic-dependent direct and indirect leading cause of foot lesions.
Supported by: AIL Treviso Section
Disclosure: **M. Sambataro:** None.

1159

ProBNP strongly predicts future vascular events in peripheral arterial disease patients with as well as in those without the metabolic syndrome

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Background and aims: Pro B-type natriuretic peptide (proBNP) is an established prognostic biomarker in patients with heart failure. Its power to predict cardiovascular endpoints in peripheral arterial disease (PAD) patients with the metabolic syndrome (MetS) is unclear and is addressed in the present study.
Materials and methods: We prospectively recorded cardiovascular events over a mean follow-up period of 4.9±1.7 years in a consecutive series of 319 patients with sonographically proven PAD, including 144 subjects with the MetS and 175 without the MetS. Presence of the MetS was defined according to the current harmonized consensus definition.
Results: At baseline, proBNP did not differ significantly between PAD patients with the MetS (n=144) and those who did not have the MetS (1037 ±3386 pg/ml vs. 1027±3864 pg/ml; p=0.759). During follow-up, the incidence of cardiovascular events was 57.7% among PAD patients with the MetS and 46.2% among PAD subjects without the MetS (p=0.042). Serum proBNP significantly predicted the incidence of cardiovascular events after adjustment for age, gender, BMI, smoking, systolic and diastolic blood pressure, LDL cholesterol, HDL cholesterol and the eGFR both in patients with the MetS (standardized adjusted HR 1.68 [1.30-2.17]; p<0.001) and in subjects without the MetS (HR 1.40 [1.17-1.67]; p<0.001).
Conclusion: We conclude that proBNP strongly and independently from conventional risk factors predicts future vascular events in PAD patients with the MetS as well as in PAD patients without the MetS.
Disclosure: **P. Rein:** None.

PS 123 Liver in diabetes

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γ-Glutamyltransferase concentrations and obstructive sleep apnea syndrome: effect of bariatric surgery

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Background and aims: Obstructive Sleep Apnea Syndrome (OSAS) is associated with increased prevalence of type 2 diabetes (T2DM) and non-alcoholic fatty liver disease (NAFLD). While it is known that after bariatric surgery OSAS, T2DM and NAFLD all improve, the link between liver enzymes and OSAS in morbid obesity has not been examined.
Materials and methods: We studied 164 morbidly obese men and women (116 with normal glucose tolerance and 48 with T2DM) undergoing Roux-en-Y-gastric bypass (RYGB) surgery. Before the operation, all subjects received a 3-hour OGTT, from which insulin sensitivity was estimated as the OGIS index. OSAS was diagnosed by polysomnography (using the Apnea-Hypopnea Index (AHI) = mild 5-15, moderate 15-30, severe >30). Six months after surgery, a subgroup of subjects (33 NGT and 11 T2DM) was re-evaluated for all parameters.
Results: Age and BMI were significantly higher in the OSAS group (n=83, p=0.001) and the prevalence of T2DM was 73% compared to 38% in non-OSAS group (n=81, p<0.0001). Both fasting plasma glucose (111±48 vs 92±25 mg/dL) and HbA1c (7.3±4.5 vs 5.9±1%) were higher in OSAS group regardless of AHI, while insulin sensitivity was lower (346 ±71 vs 390±69, p=0.03). Before surgery, the OSAS group had higher serum ALT (33 [18] vs 19 [20] U/L, median [IQR]) and γGT levels (36 [30] vs 21 [16]) than the non-OSAS group. In multivariate logistic analysis, HbA1c (p=0.015), BMI (p=0.026) and γGT (p=0.033) were independently associated with the presence of OSAS. In a similar model, the severity of OSAS and HbA1c were explanatory variables of γGT concentrations. Six months after surgery, BMI and HbA1c decreased similarly in both groups, while fasting glycaemia declined more in OSAS than non-OSAS subjects. Furthermore, AST, ALT and γGT decreased in a greater degree in the former than the latter (AST -21% vs 0.6%, ALT -60% vs -16% and γGT -53% vs -45%, p=0.03, p=0.01 and p=0.018, respectively). In logistic regression, surgery-induced changes in γGT were positively associated with baseline HbA1c (p=0.026) and presence of OSAS (p=0.05).
Conclusion: In morbidly obese subjects, the presence of OSAS is associated with higher γGT concentrations independently of obesity and HbA1c. After surgery, however, serum γGT concentrations improve to a greater extent in patients with OSAS at baseline.
Supported by: EMIF grant (IMI JU GA 115372-2).
Disclosure: **D. Guarino:** None.

1161

γ-Glutamyltranspeptidase fractions in subjects with type 2 diabetes: relation to insulin sensitivity

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Background and aims: Elevated serum γ-glutamyltranspeptidase (γGT) levels have been reported to be an independent risk factor for the development of type 2 diabetes (T2DM). Recently, four γGT fractions have been identified in the serum, but their separate pathophysiological role has not been assessed. We investigated the relationship between the serum γGT profile and the mechanisms underlying T2DM in morbidly obese subjects before and after bariatric surgery.
Materials and methods: Twenty-nine T2DM subjects (22 women and 7 men), wait-listed for Roux-en-Y gastric bypass (RYGB) (n=21) or laparoscopic sleeve gastrectomy (LSG) (n=8), received a 5-hour OGTT before, 15 days and one year after surgery. Insulin sensitivity was assessed by the OGIS-index and ß-cell function by modelling analysis of the C-peptide response to glucose. Total and fractional γGT (b-, s-, m- and f-γGT) activity was performed using a fast-protein liquid chromatography system fitted out with a gel-filtration column and a fluorescent detector.
Results: Before surgery all but 5 patients had total γGT values within the gender-specific reference ranges (median and 95th percentile: 25.6 U/L and 60.5 U/L for men; 14.4 U/L and 30.9 U/L for women). At 15 days post-surgery, total γGT activity was ~25% increased after RYGB (due to increases in b- and s-γGT fractions only), but 40% decreased after LSG (p=0.009 for the surgery*time interaction). One-year post-surgery, all patients showed a ~50% reduction in total and fractional γGT compared to baseline. In particular, b-γGT declined 60% while the other fractions were ~27% reduced (p<0.002 for all) equally in RYGB and LSG. In patients with biopsy-proven steatohepatitis (10 of 29), pre-surgery total, and b-, s- and m-γGT fractions were significant higher than in patients with low-grade steatosis (p=0.01, p=0.002 and p=0.02, respectively); one-year post-surgery, however, there was no difference in total or fractional γGT activity between these subgroups. b-γGT was positively associated with fasting glucose (r=0.49) and insulin (r=0.56) and HbA1c (r=0.57), and negatively with β-cell glucose sensitivity (r=-0.33) and OGIS (r=-0.55). In a multiple regression model, b-γGT was the only fraction related to insulin sensititvity (p=0.02) independently of BMI, fasting glucose and triglycerides.
Conclusion: Total γGT and its b-, s-, and m-γGT fractions are higher in steatohepatitis than simple steatosis; b-γGT is independently associated with insulin resistance. Shortly post-surgery, total γGT and fractions increase in RYGB subjects; one year later, however, both total γGT and fractions are markedly reduced regardless of kind of surgery.
Supported by: EMIF grant (IMI JU GA 115372-2).
Disclosure: **M. Nannipieri:** None.

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Chronic insulin deficiency and hyperglycaemia in INSC94Y transgenic pigs causes liver transcriptome changes in pathways of amino acid and lipid metabolism

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Background and aims: The liver is able to take up and release glucose, acting as a key regulator of glucose homeostasis. Insulin inhibits both hepatic glycogenolysis and gluconeogenesis. Consequently, insulin deficiency results in increased hepatic glucose production. Transgenic pigs expressing mutant insulin C94Y exhibit impaired insulin secretion and increased beta-cell apoptosis. A cohort of five female INSC94Y transgenic (TG) pigs was maintained with limited insulin treatment for two years. Five female wild-type (WT) littermates served as controls. A comprehensive biobank of body fluids and tissues (Munich MIDY Pig biobank) was established using standardized sampling protocols. In the present study, transcriptome profiling of liver samples was performed to address effects of chronic insulin deficiency and hyperglycaemia.
Materials and methods: RNA was isolated by a TRIzol-based procedure. Sequencing libraries were constructed using the Encore Complete RNA-Seq library system (NuGEN) and sequenced on an Illumina HiSeq 1500 as 100 b single reads. Reads were mapped to the porcine reference genome sequence (assembly Sscrofa10.2) and transcript abundances were calculated. DESeq2 was performed to find differentially abundant transcripts. Gene Set Enrichment Analysis (GSEA) was used to determine

whether gene sets from KEGG pathways show statistically significant, concordant differences between liver samples from TG and WT pigs.
Results: Principal component analysis on the transcriptome data set revealed that samples were separated according to the genotype (TG vs. WT) as PC1, explaining 35% of the variance. Transcripts of 11,200 genes were captured in the liver RNA, but only 14 genes were identified as differentially expressed between TG and WT. To study whether certain pathways were affected by chronic diabetes, Gene Set Enrichment Analyses (GSEA) were performed. Transcripts from 21 KEGG pathway gene sets (www.genome.jp/kegg/) with a false discovery rate lower than 0.25 were found to be significantly enriched in TG liver samples. The majority were related to amino acid metabolism, with highest ($p<0.01$) normalised enrichment scores (NES) for the pathways cysteine and methionine metabolism (NES 1.87), arginine and proline metabolism (NES 1.75), and glycine, serine and threonine metabolism (NES 1.69). Another gene set enriched in TG liver samples was related to peroxisome (NES 1.67) with PEX11A, a gene involved in regulation of peroxisome maintenance and proliferation, having the highest rank. In addition, gene sets of KEGG pathways fatty acid metabolism and biosynthesis, glycolysis/gluconeogenesis, tricarboxylic acid cycle, pentose phosphate pathway, and PPAR signalling were enriched in TG liver samples, while gene sets related to immune functions were enriched in WT liver samples.
Conclusion: Chronic insulin deficiency in a clinically relevant pig model affected metabolic pathways of glucogenic amino acids in the liver, which may contribute to increased hepatic glucose production by stimulated gluconeogenesis. Increased abundance of transcripts of the peroxisome pathway is in line with altered lipid metabolism and fatty acid beta-oxidation in chronic diabetes mellitus.
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Disclosure: **M. Backmann:** None.

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Exogenous DLK1 treatment ameliorates hepatic steatosis and fibrosis by AMPK activation

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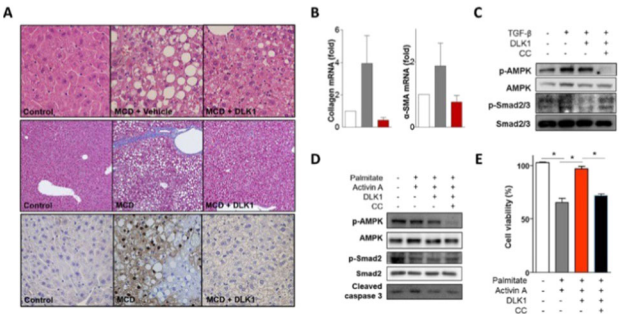
Background and aims: Notch signaling activation is involved in development and progression of lipogenesis and fibrosis in the liver resulted in various spectrums of non-alcoholic fatty liver disease (NAFLD). One of the endogenous inhibitors of Notch signaling, Delta-like 1 homolog (DLK1), is widely expressed in developing tissues. Previously, we showed that DLK1 treatment reduced hepatic steatosis and hyperglycemia via AMPK activation in the liver using db/db mice. This animal model, however, has a limitation that it represents only hepatic lipid accumulation, not severe inflammation and cirrhosis as shown in advanced form of NAFLD in human. Therefore, we investigated the effect of exogenous DLK1 in vitro and in vivo on the development or progression of NAFLD.
Materials and methods: A soluble DLK1 peptide was generated with fusion between a human Fc fragment and extracellular domain of DLK1. For in vivo study, 7-week-old C57BL/6J mice were divided into three groups: (1) fed with chow diet and vehicle treated; (2) fed with a diet completely devoid of methionine and choline (MCD) and vehicle treated; (3) fed with a MCD diet and DLK1 treated for a period of 4 weeks. DLK1 (15mg/kg) was injected intraperitoneally twice weekly. HepG2 cells and LX-2 cells were used for in vitro study.
Results: DLK1 treated MCD-fed mice showed significantly lower degree of steatosis and fibrosis, with decreased hepatocyte apoptosis compared with the vehicle-treated group in histologic analysis. Hepatic triglyceride content, hydroxyproline content, as well as serum levels of liver enzymes were markedly decreased with DLK1 treatment. Next, to stimulate hepatic stellate cells, we used 5ng/mL of in LX-2 cells. In activated LX-2 cells, pretreatment with DLK1 reduced fibrogenic gene expression such as α-SMA and collagen. Also, DLK1 treatment increased AMPK activation and reduced Smad2/3 phosphorylation induced by TGF-β. However, compound C treatment blocked this effect on TGF-β/Smad signaling. Lastly, we tested whether DLK1 reduces TGF-β-induced apoptosis in steatotic hepatocyte and whether the mechanism is attained by AMPK activation. Treatment with palmitate and TGF-β decreased AMPK phosphorylation, increased Smad2 phosphorylation, and increased apoptosis assessed by cleaved caspase 3 expression in HepG2 cells. DLK1 pretreatment increased AMPK activation and decreased apoptosis, furthermore, compound C treatment interrupted this anti-apoptotic effect. We confirmed this effect in MTT assay.
Conclusion: These results show that exogenous administration of DLK1 reduced hepatic steatosis, fibrosis, and apoptosis. These effects of DLK1 were attained by activation of AMPK. Our study suggests that DLK1 might be a novel therapeutic approach for treating various stages of NAFLD.

Figure 1. Exogenous DLK1 ameliorated hepatic steatosis, fibrosis, and apoptosis in MCD-fed mice (A), inhibited the activaiton of LX-2 cells via TGF-β/Smad2 signaling pathway (B,C), and reduced hepatocyte apoptosis in HepG2 cells by AMPK activation (D,E).



Disclosure: **Y. Shin:** None.

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Non-alcoholic fatty liver disease in coronary artery disease patients: association with impaired glucose metabolism and with future cardiovascular event risk

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Background and aims: Data on non-alcoholic fatty liver disease (NAFLD) in patients with the combination of both impaired glucose metabolism and established cardiovascular disease are scarce. We therefore aimed at investigating its association with the glycemic state as well as its impact on cardiovascular event risk.
Materials and methods: We enrolled a large series of 1791 patients with established cardiovascular disease (1472 patients with angiographically proven coronary artery disease and 319 patients with sonographically proven peripheral arterial disease) using the validated fatty liver index to diagnose NAFLD.
Results: At baseline, 42.5%, 36.5%, and 19.8% of our patients had normal fasting glucose (NFG), impaired fasting glucose (IFG), and type 2 diabetes (T2DM), respectively. The prevalence of NAFLD significantly increased from 34.2% over 52.2% to 62.7% through these categories of the glycemic state ($p<0.001$). Prospectively, we recorded 701 cardiovascular events over a mean follow-up period of 5.6± 3.3 years. Cardiovascular event risk significantly ($p<0.001$) increased from 30.7% in patients with NFG over 33.3% in patients with IFG to 46.5% in patients with T2DM. NAFLD significantly predicted cardiovascular event risk both univariately and in

age- and gender adjusted analyses (HRs 1.23 [1.05-1.45]; p=0.012 and 1.27 [1.08-1.50]; p=0.005, respectively), but not after additional adjustment for the glycemic state (HR 1.15 [0.97 - 1.37]; p=0.098).
Conclusion: We conclude that the prevalence of NAFLD in CAD patients is high and gradually increases with a worsening glycemic state; however, it does not predict cardiovascular events independently from impaired glucose metabolism in this patient population.
Disclosure: **C.H. Saely:** None.

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Pentraxin 3 level in serum is associated with LDL-C and apo C3 in patients with type 2 diabetes and NAFLD

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Background and aims: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury in many countries around the world. NAFLD is present in up to 80% of type 2 diabetic (t2DM) patients. NAFLD is associated with insulin resistance, obesity, hypertension and atherogenic dyslipidemia. NAFLD includes a variety of histopathological findings ranging from simple steatosis to nonalcoholic steatohepatitis, which can only be differentiated by liver biopsy. There is gathering evidence that plasma pentraxin 3 (PTX3) level is a novel marker for nonalcoholic steatohepatitis and severity of liver fibrosis. Advanced fibrosis is the most significant predictor of mortality in NAFLD patients. Pentraxin 3 is an essential component of innate immunity and a member of the long pentraxin superfamily, which are soluble proteins induced by various inflammatory stimuli. PTX3 is independently associated with the risk of developing vascular events. PTX3, a marker of inflammation, is produced locally in relevant cells such as endothelial cells, macrophages and granulocytes. There are some data, that PTX3 also possess anti-microbial, anti-inflammatory and cardioprotective properties. In this study we aimed to determine factors associated with PTX 3 serum concentrations in patients with DM 2 and NAFLD.
Materials and methods: Material included consecutive patients with DM2 from outpatient diabetic clinic. In each patients standardized questionnaire, anthropometric measurements, fasting serum lipids, glucose, glycated hemoglobin HbA1c were performed. Serum lipids concentrations were determined by enzymatic methods, using Roche reagents, HbA1c by high pressure liquid chromatography, cytokeratin 18 fragment (CK-18), a marker of NAFLD and PTX 3 were determined by ELISA method using PEVIVA and Cloud-Clone corp. reagents respectively. NAFLD was diagnosed by ultrasonography. Pearson and Spearman correlation coefficients were calculated.
Results: We examined 79 patients with DM2 and NAFLD. Mean age was 57.3+/-10.6 years, mean diabetes duration 8.0 +/-5.9 years, HbA1c 8.54+/-2.2%, BMI 33.4 +/-5.2kg/m2. 91,9% of patients had arterial hypertension and 82.1% had dyslipidemia. Coronary artery disease (cad) was present in 36.7% of patients and 16.5% of patients had diabetic kidney disease. Mean (SD) values of PTX 3 in NAFLD patients were 4.30 (1.768) ng/ml, in patients with cad 4.58 (2,05) and in patients with diabetic kidney disease 5.05 (1.68) ng/ml. Median ((Interquartile range IQR) of CK-18 levels were 225.7 (207.3) U/L. PTX 3 concentrations correlated significantly positively with total cholesterol (r=0.47, p= 0.0000) and LDL-cholesterol (r=0.39, p =0.0009), triglycerides (r = 0.39, p =0.0004) and apolipoprotein C3 (r = 0.55, p= 0.0001) levels. We did not find any correlations with glycemic control, transaminases, GGTP, obesity parameters or CK-18, a marker of NAFLD.
Conclusion: PTX3, a marker of clinically more advanced liver disease and fibrosis is associated strongly with apo C3 and lipid cardiovascular risk factors (total cholesterol, LDL cholesterol, and triglycerides). The results of our study suggest that modification of blood lipid values could potentially be of importance in modulation of progression of NAFLD in patients with type 2 diabetes.

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Disclosure: **M. Waluś:** None.

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Risk score to predict advanced liver fibrosis in patients with type 2 diabetes

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Background and aims: Non-alcoholic fatty liver disease (NAFLD) in patients with type 2 diabetes (T2D) is much more prevalent than the general population. In particular, the prevalence of liver fibrosis, the more severe form of NAFLD, is higher in T2D. We aimed to construct a simple risk score to identify T2D who are at high risk to develop advanced liver disease using demographic and laboratory data readily available in routine tests at the diabetes clinic.
Materials and methods: A consecutive cohort of 1918 Hong Kong Chinese patients with T2D who had attended their regular diabetic complications screening and had liver assessment done by transient elastography were recruited between March 2013 and May 2014. The cohort was divided into training set and validation set randomly with a ratio of 2:1 for the construction and validation of the risk model respectively. A 10-fold cross validation in the whole cohort was performed in addition to validation in the validation set. The performance of the score in identifying advanced liver fibrosis and hepatocellular carcinoma (HCC) was tested by a retrospective cohort which had documentation of baseline characteristics and clinical outcomes. In both cohorts, patients with secondary causes for liver steatosis, such as viral hepatitis, steatogenic medication and excessive alcoholic consumption, were excluded. Advanced liver fibrosis was defined as liver stiffness measurement (LSM) score ≥9.6 kPa with M probe and ≥ 9.3 kPa with XL probe respectively. In the retrospective cohort, liver diseases were identified using hospital discharge diagnoses with International Classification of Disease, Ninth Revision (ICD-9) codes: 155 (malignant neoplasm of liver primary) and 571.5(cirrhosis of liver without alcohol).
Results: Using multivariate backward stepwise logistic regression, 7 variables (age, gender, body mass index, HDL-cholesterol, platelet count, alanine aminotransferase and albuminuria) were identified. A score based on the odds ratios in the logistic regression was constructed. The areas under receiver operating characteristic (ROC) curve for the training set and validation set were 0.78 [95% confidence interval (CI): 0.74-0.81)and 0.76 (95% CI: 0.71-0.80) respectively. Area under ROC for the cross-validation of the entire cohort was 0.76 (95%CI: 0.67-0.85). Using cutoff value of 4, the score had a 89.7% sensitivity in identifying patients with HCC or cirrhosis.
Conclusion: The risk score is potentially useful for clinicians in identifying patients with T2D at risk to develop liver complications for more in-depth evaluations.
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Inflammation-dependent inactivation of hepatic GABP results in dyslipidaemia and promotes atherogenesis

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Background and aims: Diabetes and resulting complications are closely intertwined with inflammatory processes. Elevated circulating reactive metabolites and proinflammatory cytokines such as Tumor necrosis factor-alpha (TNF-α) affect metabolic tissues by impairing physiologic hormone and nutrient signaling. Until today, the molecular mechanisms linking cytokine signaling and metabolic dysfunction in the liver are only marginally understood.
Materials and methods: - High throughput Mammalian 1-hybrid screen - Mammalian 2-hybrid assay - Co-Immunoprecipitation, immunoblots - Mouse studies in wt, db/db and LDLRKO mice - AAV-mediated hepatocyte specific KD - qRT-PCR - Transcriptomics analysis
Results: Using a high throughput screen we could identify the transcription factor subunit GA-binding protein alpha (GAbpα) as a negative target of TNF-α signaling. Furthermore, our results suggest that the TNF-dependent reduction in activity of GAbp is due to redox-sensitive dissociation of the two subunits GAbpα and GAbpβ. Hepatocyte-specific ablation of GAbp activity in mice triggered severe hypercholesterolemia and furthermore proved to promote early atherogenesis in LDLRko mice.
Conclusion: Our data on the TNF-α-dependent ROS-mediated repression of GAbp activity provides an elegant novel link between inflammation and diabetic complications such as macroangiopathy. Reconstitution of functional GAbp in the liver might hold promise for future innovative therapy approaches in the field inflammation-associated dyslipidemia and atherosclerosis.
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