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Role of fasting duration and weekday in incretin and glucose regulation

Kim K B Clemmensen[1], Jonas S Quist[1], Dorte Vistisen[1], Daniel R Witte[2,3], Anna Jonsson[4], Oluf Pedersen[4], Torben Hansen[4], Jens J Holst[4,5], Torsten Lauritzen[6], Marit E Jørgensen[1,7], Signe Torekov[4,5] and **Kristine Færch[1]**

[1]Department of Clinical Epidemiology, Steno Diabetes Center Copenhagen, Gentofte, Denmark
[2]Department of Public Health, Aarhus University, Aarhus, Denmark
[3]Danish Diabetes Academy, Odense, Denmark
[4]NNF Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark
[5]Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark
[6]Section for General Practice, Department of Public Health, Aarhus University, Aarhus, Denmark
[7]National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark

Correspondence should be addressed to K K Clemmensen: **kim.katrine.bjerring.clemmensen.01@regionh.dk**

Abstract

Fasting duration has been associated with lower fasting blood glucose levels, but higher 2-h post-load levels, and research has indicated an adverse effect of 'weekend behavior' on human metabolism. We investigated associations of fasting duration and weekday of examination with glucose, insulin, glucagon and incretin responses to an oral glucose tolerance test (OGTT). This cross-sectional study is based on data from the ADDITION-PRO study, where 2082 individuals attended a health examination including an OGTT. Linear regression analysis was applied to study the associations of overnight fasting duration and day of the week with glucose, insulin, glucagon, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) responses to an OGTT. We found that a 1 h longer fasting duration was associated with 1.7% (95% CI: 0.8,2.5) higher 2-h glucose levels, as well as a 3.0% (95% CI: 1.3,4.7) higher GIP and 2.3% (95% CI: 0.3,4.4) higher GLP-1 response. Fasting insulin levels were 20.6% (95% CI: 11.2,30.7) higher on Mondays compared to the other weekdays, with similar fasting glucose levels (1.7%, 95% CI: 0.0,3.4). In this study, longer overnight fasting duration was associated with a worsening of glucose tolerance and increased incretin response to oral glucose. We found higher fasting insulin levels on Mondays compared to the other days of the week, potentially indicating a worsened glucose regulation after the weekend.

Introduction

Glucose metabolism exhibits circadian rhythmicity (1, 2). Longer fasting duration before an oral glucose tolerance test (OGTT) has been associated with lower fasting glucose but higher post-load glucose levels (3). Additionally, the time of the day of an OGTT seems to influence the glucose response with higher post-load blood glucose levels in the afternoon and evening compared to the morning (1, 3). The incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) account for a substantial part of the insulin secreted after ingestion of glucose (4, 5, 6). It has been shown that the incretin effect is impaired in type 2 diabetes and obesity (5, 7, 8). Also, the secretion of both GLP-1 and GIP has been shown to exhibit a circadian pattern, with lower levels in the morning and higher levels in the late afternoon (9, 10).

The timing of sleep and meals shows variation over the week, with other activity patterns in the weekend

compared to the weekdays, a term called social jetlag (11, 12). A large observational study has reported higher energy intake and poorer diet quality in weekends compared to weekdays (13). Also, studies on the effect of weekday on triglyceride levels showed higher triglyceride levels on Mondays compared to Fridays (14, 15), indicating an acute adverse effect of 'weekend behavior' on metabolism. 'Weekend behavior' is the combination of changes in dietary intake as well as a shift in sleep schedule in the weekends compared to the daily rhythm in the weekdays (11, 12, 13). However, knowledge on the role of weekend behavior on glucose metabolism and incretin hormone response is lacking.

The objectives of the present cross-sectional study were to investigate associations of fasting duration and weekday of examination with glucose, insulin, glucagon, GIP and GLP-1 responses to an OGTT. We hypothesized that longer overnight fasting duration was associated with greater incretin response and lower fasting levels of glucose and insulin. Moreover, we anticipated that glucose regulation on Mondays would be worse than on the other weekdays because of less healthy 'weekend behavior'.

Materials and methods

This cross-sectional study was based on data from the Danish ADDITION-PRO study. The ADDITION-PRO study is nested in the ADDITION-Denmark study, a longitudinal risk-stratified cohort study of individuals at high risk of type 2 diabetes conducted from 2001 to 2006 (16). A subset of the participants from the ADDITION-Denmark study was invited to a follow-up health examination conducted from 2009 to 2011. The subset included both participants with diabetes, impaired glucose tolerance and participants at low to high risk of developing type 2 diabetes assessed by glycaemic status and score on a slightly modified version of the Danish diabetes risk questionnaire (16, 17). Of the 4188 individuals invited, 2082 (50%) attended the follow-up examination. The ADDITION-PRO study is described in detail elsewhere (16). Of the 2082 participants in the ADDITION-PRO study, we excluded 336 with known diabetes and 20 who had fasted for less than 8 h (minimum required fasting duration), leaving 1726 participants for analysis.

The ADDITION-PRO study was conducted in accordance with the Helsinki Declaration, and participants gave written informed consent before testing. The study was approved by the Ethical Committee of the Central Denmark Region (No. 20080229).

Biochemical measurements

Blood samples for the measurements of glucose, insulin, glucagon, GIP and GLP-1 were drawn in the fasting state and 30- and 120-min post-load during a standard 75 g OGTT. The participants were asked to fast for a minimum of 8 h prior to the test. The timing of their last meal before the test was recorded as well as the date and time of the test. All OGTTs were performed on weekdays (Monday–Friday), with 199 participants on Mondays, 914 on Tuesdays, 126 on Wednesdays, 318 on Thursdays and 166 on Fridays. Three participants had missing information on weekday of examination and were omitted from the weekday analyses.

Glucose and insulin were analysed in the central laboratory at the Steno Diabetes Center Copenhagen, Gentofte, Denmark. For the glucose measurements, the plasma was prepared immediately after collection in fluoride-heparin coated tubes, placed on ice and thereafter centrifuged at 2200 g for 10 min at 4°C (16). From 2009 to 2010, glucose was measured using the Hitachi 912 system (Roche Diagnostics), but gradually in 2010 this was changed to the Vitros 5600 Integrated System (Ortho Clinical Diagnostics, Illkirch Cedex, France) (16). For the insulin measurements, whole blood was incubated for 0.5–1.5 h at room temperature, centrifuged for 10 min at 2200 g and serum samples were analysed with an immunoassay (AutoDELFIA, Perkin Elmer) (16). The intraassay coefficient of variation was 0.011, and the interassay coefficient of variation was 0.036 with serum insulin levels of 129 pmol/L (18).

Samples for measurement of glucagon, GIP and GLP-1 were first stored in a biobank and were after completion of the study analysed within 2 months using identical quality controls and batches for all reagents (16, 19). Plasma stored in the biobank was from blood sampled in chilled EDTA coated tubes, put on ice immediately and centrifuged within 30 min after collection at 2200 g for 10 min at 4°C and stored at −80°C (16). Glucagon was measured using radioimmuno assays using a COOH terminus which measures intact glucagon; for details see Færch *et al.* (19). Total plasma GIP and GLP-1 concentrations (comprising both intact GIP and GLP-1 and the metabolites GIP(3–42) and GLP-1(9–36)amide, respectively) were measured by radioimmuno assays as previously described (19). The analytical detection limit for GIP and glucagon was 1 pmol/L, and the intraassay coefficient of variation was 6.0% and the interassay coefficient of variation was 15% (19, 20). The analytical detection limit for GLP-1 was 1 pmol/L, the intraassay

coefficient of variation was 6.0% and the interassay coefficient of variation was 1.5% (19).

Calculations

Early and total responses to the OGTT in plasma glucose, serum insulin, plasma glucagon, plasma GIP and plasma GLP-1 were calculated as total areas under the curves (tAUCs) using the trapezoid rule from the basal state to 30 min (early response, $tAUC_{0-30}$) and 120 min (total response, $tAUC_{0-120}$). Relative early response ($rAUC_{0-30}$) was calculated as the ratio $tAUC_{0-30}/$(fasting concentration × 30 min) and relative total response ($rAUC_{0-120}$) as $tAUC_{0-120}/$(fasting concentration × 120 min). Thus, the rAUC reflects the change in the concentrations of glucose and the different hormones relative to the basal (fasting) level. The rAUC is always positive and can therefore be logarithmically transformed.

The overnight fasting duration was calculated as the time between the last meal and the start of the OGTT. Weekdays were grouped into Monday (yes/no). Smoking status was dichotomized as current smoker vs non-smoker. Physical activity energy expenditure was calculated from combined accelerometry and heart rate monitoring for 7 days. A step test was used to assess cardiorespiratory fitness (16).

Statistical analysis

Differences in characteristics between day of examination were compared with chi-square or *t*-test. Linear regression analysis was used to study the associations of fasting duration and weekday of examination with plasma levels at 0, 30 and 120 min as well as with early and total relative responses of glucose, insulin, glucagon, GIP and GLP-1. The analyses were adjusted for age, sex, BMI and smoking status as well as weekday or fasting duration, respectively. The outcome variables were logarithmically transformed before analysis to obtain normally distributed model residuals.

R version 3.6.0 was used for the statistical analyses. A two-sided α-level of 0.05 was used.

Results

Participant characteristics

Participant characteristics, stratified by weekday of examination, are presented in Table 1.

Participants examined on Mondays were slightly healthier than those examined on the other weekdays with lower age and HbA_{1c} levels as well as lower BMI and waist circumference. The OGTT started between 07:00 and 10:00 h in the morning for more than 99% of the participants, with a median (quartile 1, quartile 3) starting time at 08:25 h (07:50 h,08:51 h).

There was a slightly higher proportion of men among those who attended (53%) than among non-attendees (43%; *P*<0.001). Sixty-eight percent of attendees had no relatives with diabetes compared to 74% of the non-attendees (*P*<0.001), and a lower proportion of the attendees than the non-attendees were normal weight (36% vs 40%; *P*=0.006). There were no differences in age, hypertension and physical inactivity between attendees and non-attendees (16).

Fasting duration

The crude and adjusted estimates from the linear regression analyses of overnight fasting duration with glucose and hormone levels are shown in Table 2. In the adjusted analyses, fasting glucose levels were not associated with the length of the overnight fast (Table 2, Fig. 1). However, a 1 h longer fasting duration was associated with 1.7% (95% CI: 0.8,2.5) higher 2-h glucose levels and 3.6% (95% CI: 1.4,5.8) higher 2-h insulin levels (Table 2). No association between any of the glucagon measures and fasting duration was observed (Fig. 1 and Table 2).

With each additional hour of overnight fast, the mean fasting levels of GIP were 2.4% (95% CI: −4.1,−0.7) lower and fasting GLP-1 levels were 2.9% (95% CI: −5.1,−0.6) lower (Fig. 2 and Table 2). Early GIP ($rAUC_{0-30\ min}$) and total ($rAUC_{0-120\ min}$) GIP and GLP-1 responses were positively associated with fasting duration (Fig. 2 and Table 2).

Weekdays

The differences in fasting levels, $rAUC_{0-30\ min}$ and $rAUC_{0-120\ min}$ for glucose, insulin, glucagon, GIP and GLP-1 between Mondays and the other days of the week combined are shown in Table 3. Fasting glucose levels were almost similar on Mondays compared to the other days of the week but the $rAUC_{0-30\ min}$ and $rAUC_{0-120\ min}$ for glucose were lower on Mondays (Table 3). Fasting insulin levels were 20.6% (95% CI: 11.2,30.7) higher and 30-min insulin levels were 13.1% (95% CI: 2.8,24.4) higher on Mondays. $rAUC_{0-120\ min}$ of insulin was lower (−8.4%, 95% CI:−14.7,−1.5, *P*=0.017) on Mondays compared with the other week days (Table 3). The measures for glucagon,

Table 1 Characteristics of the ADDITION-PRO study population by weekday of examination.

	Monday	Tuesday–Friday	*P*-value
n	199	1524	
Men (*n* (%))	100 (50)	821 (54)	0.375
Age (years)	64 (7)	66 (7)	<0.001
Height (cm)	170 (9)	170 (9)	0.945
BMI (kg/m^2)	26.5 (4.1)	27.3 (4.6)	0.020
Waist circumference (cm)	94.2 (13.1)	95.5 (13.0)	0.200
Hip circumference (cm)	100.6 (8.2)	101.7 (9.9)	0.132
Fat percentage	32.0 (7.6)	32.0 (8.4)	0.988
HbA$_{1c}$ (%)	5.6 (0.4)	5.7 (0.4)	0.004
HbA$_{1c}$ (mmol/mol)	38 (5)	39 (5)	0.004
Glucose tolerance status (*n* (%))			0.944
i-IFG	41 (20.6)	293 (19.2)	–
i-IGT	17 (8.5)	117 (7.7)	–
IFG&IGT	19 (9.5)	143 (9.4)	–
Screen detected type 2 diabetes	22 (11.1)	161 (10.6)	–
Overnight fasting (h)	11.8 (10.6,12.9)	11.5 (10.3,13.0)	0.450
Time of fasting sample (h:min)	08:06 (07:42,08:48)	08:24 (08:00,08:54)	<0.001
PAEE (kJ/kg/day)	34.3 (15.7)	32.3 (15.8)	0.203
CRF (mL O$_2$/kg/min)	30.1 (5.3)	29.9 (5.4)	0.589
Fasting plasma glucose (mmol/L)	6.1 (0.7)	6.0 (0.8)	0.395
Fasting plasma insulin (pmol/L)	52.2 (47.3)	45.4 (32.5)	0.009
Fasting plasma glucagon (pmol/L)	11.8 (8.0)	11.3 (8.1)	0.394
Fasting plasma GLP-1 (pmol/L)	11.8 (6.8)	12.1 (5.9)	0.479
Fasting plasma GIP (pmol/L)	9.7 (5.9)	9.7 (6.1)	0.952

Data are shown as means (s.d.) or median (quartile 1, quartile 3).
CRF, cardiorespiratory fitness; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; IFG, impaired fasting glucose; i-IFG, isolated IFG; IGT, impaired glucose tolerance; i-IGT, isolated IGT; PAEE, physical activity energy expenditure.

GIP and GLP-1 did not differ between Mondays and the other weekdays.

Discussion

We found slightly higher 2-h glucose and insulin levels in participants who had a longer overnight fasting duration prior to their OGTT. For GIP and GLP-1, 2–3% higher response to oral glucose (rAUC$_{0–120}$) with 1 h longer overnight fasting durations were observed. We found similar levels of fasting glucose on Mondays compared to the other days of the week combined, while fasting insulin was found to be 20.6% higher on Mondays, indicating a worsened glucose regulation after the weekend.

Comparison with other studies

Our findings partly support previous findings from the large British population-based Whitehall II cohort study (3), where longer fasting duration was associated with higher 2-h post-load glucose levels. That longer fasting duration is associated with higher 2-h post-glucose load circulating glucose and insulin concentrations is not surprising, as the body with prolonged fasting can be expected to become more insulin resistant (21). However, we did not find an association between fasting duration and fasting glucose levels as was found in the Whitehall II study (3). One possible explanation for the discrepancy is that the Whitehall II study included the time of day of the OGTT in their analysis, which attenuated the association of fasting duration with fasting glucose levels (3). In the present study, the time range of the starting time of the OGTT was too short to add information to the analysis. In a large primary care-based study of more than 25,000 individuals, fasting glucose levels were stable when the fasting duration exceeded approximately 3 h (22), which is in line with our results. Contrary to our findings, a study including 1006 women found 4% lower 2-h glucose levels for each 3-h increment in fasting duration (23). However, in that study, the overnight fasting duration was determined by a telephone 24-h dietary recall conducted 3–10 days after the OGTT (23), which may have been associated with risk of recall bias. In our study, a 3 h longer fasting duration was associated with a 5.1% higher 2-h glucose, which is substantial; that is, the 2-h glucose would increase from, for example, 6.4 mmol/L (median 2-h post-load glucose level) to 6.7 mmol/L. There is a lack

Table 2 Back transformed (from log scale) estimates in percent difference for associations between overnight fasting duration and glucose, insulin, glucagon, GIP and GLP-1.

	Unadjusted			Adjusted for age, sex, BMI, smoking status and weekday		
	n	Estimate (%) 95% CI	P	n	Estimate (%) 95%CI	P
Plasma glucose						
Fasting	1717	0.0 (−0.4,0.3)	0.844	1706	−0.2 (−0.5,0.1)	0.267
30 min	1702	0.4 (−0.2,0.9)	0.159	1691	0.2 (−0.3,0.7)	0.488
120 min	1710	2.0 (1.1,3.9)	<0.001	1699	1.7 (0.8,2.5)	<0.001
$rAUC_{0-30}$	1702	0.2 (0.0,0.5)	0.068	1691	0.2 (0.0,0.5)	0.109
$rAUC_{0-120}$	1698	0.9 (0.5,1.3)	<0.001	1687	0.8 (0.4,1.2)	<0.001
Plasma insulin						
Fasting	1716	1.6 (−0.3,3.4)	0.094	1705	0.7 (−0.9,2.2)	0.400
30 min	1699	1.3 (−0.6,3.3)	0.172	1688	0.7 (−1.2,2.5)	0.481
120 min	1708	4.8 (2.3,7.2)	<0.001	1697	3.6 (1.4,5.8)	0.001
$rAUC_{0-30}$	1698	0.0 (−1.5,1.4)	0.957	1687	0.2 (−1.2,1.6)	0.805
$rAUC_{0-120}$	1694	1.2 (−0.2,2.7)	0.084	1683	1.3 (−0.1,2.7)	0.066
Plasma glucagon						
Fasting	1466	0.5 (−1.4,2.5)	0.601	1455	0.3 (−1.8,1.9)	0.967
30 min	1453	2.0 (0.0,4.0)	0.048	1442	1.0 (−0.8,2.9)	0.278
120 min	1457	−0.4 (−3.0,2.3)	0.783	1446	−0.8 (−3.4,1.9)	0.553
$rAUC_{0-30}$	1450	0.2 (−0.5,1.0)	0.579	1439	0.0 (−0.7,0.8)	0.903
$rAUC_{0-120}$	1442	0.2 (−0.9,1.4)	0.720	1431	0.0 (−1.1,1.2)	0.972
Plasma GIP						
Fasting	1402	−2.6 (−4.3,−0.9)	0.003	1391	−2.4 (−4.1,−0.7)	0.006
30 min	1390	0.5 (−0.9,1.9)	0.511	1380	0.5 (−0.9,1.9)	0.468
120 min	1394	0.4 (−1.0,1.8)	0.552	1383	0.7 (−0.7,2.0)	0.328
$rAUC_{0-30}$	1386	2.7 (1.2,4.3)	<0.001	1376	2.6 (1.1,4.1)	0.001
$rAUC_{0-120}$	1377	3.0 (1.4,4.7)	<0.001	1367	3.0 (1.3,4.7)	0.001
Plasma GLP-1						
Fasting	1464	−2.3 (−4.6,0.0)	0.046	1453	−2.9 (−5.1,−0.6)	0.013
30 min	1453	−1.5 (−2.5,1.5)	0.601	1442	−0.9 (−2.8,1.0)	0.358
120 min	1457	−0.8 (−2.4,0.9)	0.380	1446	−0.7 (−2.3,0.9)	0.388
$rAUC_{0-30}$	1448	1.4 (−0.5,3.2)	0.149	1437	1.6 (−0.3,3.4)	0.093
$rAUC_{0-120}$	1440	1.9 (−0.2,4.0)	0.073	1429	2.3 (0.3,4.4)	0.026

Estimates are per 1 h increase in fasting duration. Linear regression models.
GIP glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; $rAUC_{0-30}$, relative early response; $rAUC_{0-120}$, relative total response.

of studies investigating associations of overnight fasting duration with glucagon, GIP and GLP-1, so our findings of lower GIP and GLP-1 levels in response to longer fasting duration are novel and need confirmation in other populations.

Participants examined on Mondays in the present study were slightly younger and had slightly lower BMI and HbA_{1c} than those examined on other weekdays, so these characteristics are unlikely to explain our findings of a slightly worse glucose regulation in those examined on Mondays. To our knowledge, no previous studies have investigated associations between weekday of examination and glucose levels during an OGTT. A study on triglyceride levels based on 1.8 million blood samples found 5% higher levels on Mondays compared to Fridays among out-patients and 1.9% among in-patients, suggesting that the effect of weekend behavior is likely to be attenuated when hospitalized (14). In a study of 807 Danish children with up to three different visit days,

a marker of insulin resistance in the fasting state was found to be 35% higher on Mondays compared to Fridays (15). These findings are in agreement with our observation of 20.6% higher fasting insulin levels and similar fasting glucose levels on Mondays. The higher fasting insulin levels on Mondays could possibly be due to 'weekend behavior', but we did not find higher glucose levels as we had also hypothesized.

No previous population-based studies have investigated associations between weekday of examination and GIP and GLP-1 levels. We did not find any differences in levels of GIP and GLP-1 on Mondays compared to the other days of the week. In general, fasting levels of gut hormones including GIP and GLP-1 are not very well defined, because the levels are usually close to the detection limit of the assays and likely to be influenced by non-specific factors, whereas the measured changes after glucose or meal ingestion are more likely to represent real changes in hormone concentration (24).
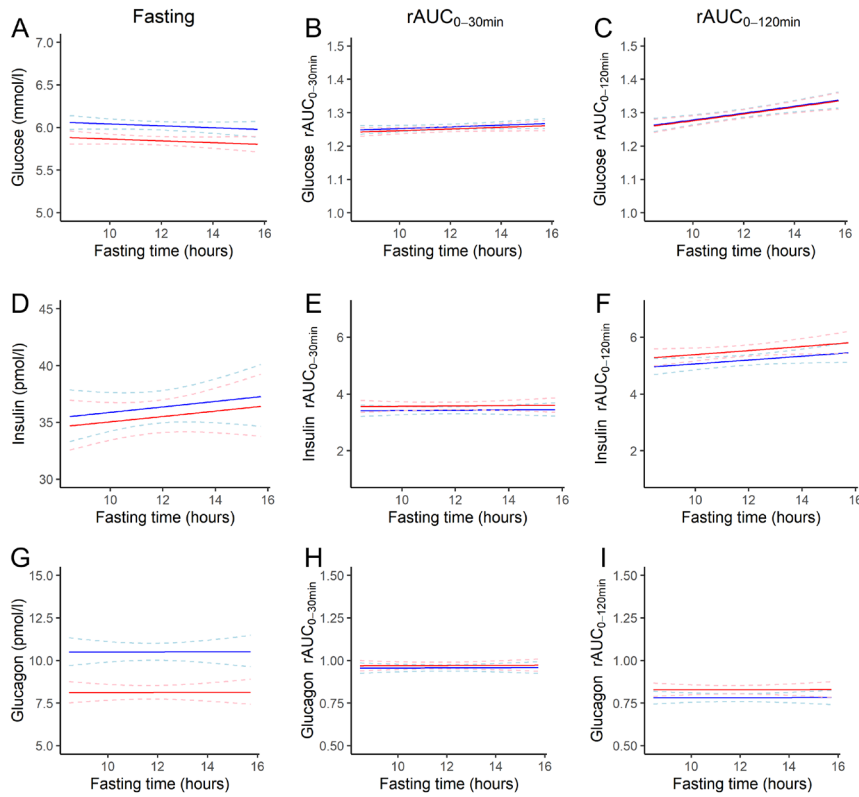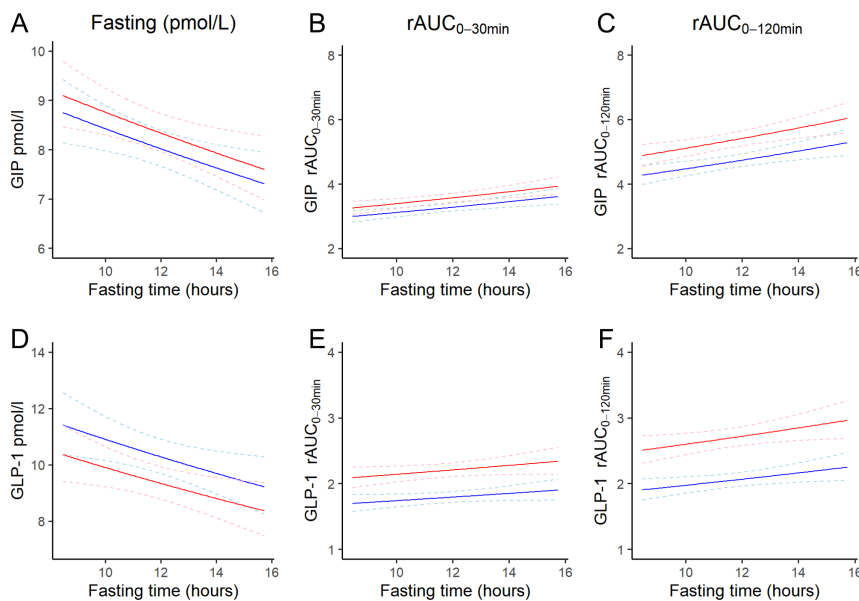
Figure 1

Relationship between fasting duration and fasting levels, $rAUC_{0-30}$ and $rAUC_{0-120}$ for glucose (A, B and C), insulin (D, E and F) and glucagon (G, H and I), respectively, for a hypothetical man (blue lines) and woman (red lines) aged 66 years (population mean), BMI 27 kg/m^2 (population mean), non-smoker (most frequent smoking status), not on a Monday. Dashed lines show the 95% confidence intervals.

Strengths and limitations

We had a unique opportunity to assess associations of fasting duration and weekday of examination with different markers of glucose metabolism in the ADDITION-PRO cohort, which included more than 1700 individuals with data from standardized three-point OGTTs with measurements of glucose, insulin and glucagon as well as the incretins GIP and GLP-1. A limitation of the study was that – as is standard for OGTT – the participants were instructed to fast for at least 8 h prior to the test. Hence, we were not able to investigate overnight fasting durations shorter than 8 h. The fasting duration interquartile range only spanned a few hours in our study population (10.4–13.0 h) which limited our ability to conclude anything beyond this fasting

Figure 2

Relationship between fasting duration and fasting levels, $rAUC_{0-30}$ and $rAUC_{0-120}$ for GLP-1 (A, B and C) and GIP (D, E and F), respectively, for a hypothetical man (blue lines) and woman (red lines) aged 66 years (population mean), BMI 27 kg/m^2 (population mean), non-smoker (most frequent smoking status), not on a Monday. Dashed lines show the 95% confidence intervals.

Table 3 Back transformed (from log scale) estimates in percent difference between Mondays vs the other weekdays for glucose, insulin, glucagon, GIP and GLP-1.

	Unadjusted			**Adjusted for age, sex, BMI, smoking status and fasting duration**		
	n	Estimate (%) 95% CI	*P*	*n*	Estimate (%) 95% CI	*P*
Plasma glucose						
Fasting	1718	0.9 (−0.9,2.7)	0.316	1706	1.7 (0.0,3.4)	0.051
30 min	1703	−2.1 (−4.8,0.7)	0.134	1691	−0.9 (−3.5,1.8)	0.508
120 min	1711	−5.1 (−9.5,−0.6)	0.028	1699	−2.9 (−7.2,1.5)	0.187
$rAUC_{0-30}$	1703	−1.8 (−3.1,−0.5)	0.007	1691	−1.5 (−2.8,−0.2)	0.020
$rAUC_{0-120}$	1699	−3.6 (−5.6,−1.6)	<0.001	1687	−2.9 (−4.8,−0.9)	0.004
Plasma insulin						
Fasting	1717	14.8 (4.4,26.3)	0.005	1705	20.6 (11.2,30.7)	<0.001
30 min	1700	8.7 (−1.7,20.1)	0.104	1688	13.1 (2.8,24.4)	0.012
120 min	1709	1.8 (−9.9,15.1)	0.774	1697	9.1 (−2.5,22.1)	0.128
$rAUC_{0-30}$	1699	−5.5 (−12.4,2.0)	0.145	1687	−6.4 (−13.1,0.9)	0.084
$rAUC_{0-120}$	1695	−8.4 (−14.9,−1.5)	0.019	1683	−8.4 (−14.7,−1.5)	0.017
Plasma glucagon						
Fasting	1467	3.8 (−5.7,14.2)	0.445	1455	6.1 (−3.2,16.2)	0.207
30 min	1454	−0.8 (−10.1,9.3)	0.864	1442	3.1 (−6.0,13.1)	0.514
120 min	1458	2.7 (−9.9,17.0)	0.693	1446	5.7 (−7.2,20.5)	0.405
$rAUC_{0-30}$	1451	−2.7 (−6.2,1.1)	0.158	1439	−2.0 (−5.6,1.8)	0.299
$rAUC_{0-120}$	1443	−1.8 (−7.2,3.9)	0.537	1431	−0.9 (−6.3,4.9)	0.765
Plasma GIP						
Fasting	1403	−1.3 (−9.5,7.8)	0.777	1391	−2.1 (−10.3,6.8)	0.626
30 min	1391	3.4 (−3.7,11.0)	0.354	1380	4.0 (−3.0,11.6)	0.270
120 min	1395	3.8 (−3.1,11.2)	0.292	1383	3.6 (−3.0,10.7)	0.296
$rAUC_{0-30}$	1387	4.7 (−2.7,12.7)	0.221	1376	5.8 (−1.6,13.9)	0.129
$rAUC_{-120}$	1378	5.1 (−3.1,14.1)	0.228	1367	6.3 (−2.0,15.2)	0.139
Plasma GLP-1						
Fasting	1465	−7.4 (−17.4,3.9)	0.193	1453	−3.7 (−14.1,7.9)	0.518
30 min	1454	−2.3 (−11.6,7.9)	0.646	1442	1.1 (−8.2,11.4)	0.826
120 min	1458	0.5 (−7.6,9.3)	0.911	1446	2.2 (−5.6,10.6)	0.595
$rAUC_{0-30}$	1449	3.4 (−5.5,13.2)	0.464	1437	2.8 (−6.0,12.4)	0.549
$rAUC_{0-120}$	1441	6.2 (−4.0,17.6)	0.243	1429	4.7 (−5.1,15.6)	0.359

Linear regression models.
GIP glucose-dependent insulinotropic polypeptide; GLP-1, and glucagon-like peptide 1; $rAUC_{0-30}$, relative early response; $rAUC_{0-120}$, relative total response.

duration. However, this is likely to reflect real-life lengths of overnight fasting (12), and we believe the results are both generalizable and relevant to real-life situations. As this is a cross-sectional study, we do not know whether the fasting duration on the day of examination reflects the habitual overnight fasting durations. The study population was sampled specifically to include both individuals with low as well as high risk of diabetes; this was done to ensure that participants with different levels of diabetes risk were included. It consisted only of middle-aged and older individuals, and only half of the invited individuals participated. The attendees were more likely to be men, overweight and to have a family history of diabetes (16). We cannot rule out that there are inter-individual differences in the participants that attended ADDITION-PRO on different days of the week, which was not explained by the confounders adjusted for

in the analysis. Nevertheless, differences in clinical characteristics are not likely to explain the findings; if anything, the health status of those attending examination on a Monday was slightly better than those examined on the other weekdays. The reason for this remains unknown.

A previous study found that the mean age in those with a social jetlag of <1 h was 64 years compared to 57 years in those with a social jetlag ≥1 h (25). Also, the increase in total energy intake from weekdays to the weekends seems to be more pronounced among those aged 18–64 years compared to those aged ≥65 years (13). Accordingly, the relatively high mean age of the participants in the present study might translate into less pronounced differences between weekday and weekend behavior than would be expected in a younger population.

Implications

The basis for prevention of type 2 diabetes among overweight individuals at high risk of developing the disease is weight loss and increased physical activity (26). Since maintaining the initial weight-loss after participating in a weight loss program is difficult (27), it is important to have multiple tools at one's disposal when recommending lifestyle changes to individuals at high risk of diabetes. Restricting the time of eating during the day to a shorter time interval has shown potential as a weight loss strategy (12). Restricting the daily eating window (so-called 'time-restricted eating') has been shown to lead to reductions in energy intake (12, 28, 29). Despite this fact, a randomized cross-over study found that insulin sensitivity was improved in response to 'early time-restricted eating' (eating duration: 6 h/day, dinner before 15:00 h) despite no reduction in energy intake and body weight (30). The study found no differences in glucose concentrations during an OGTT between the groups (30). However, recent short-term cross-over studies have suggested that 'early time-restricted eating' with concomitant longer fasting duration is associated with improved glucose tolerance during a mixed meal test and reductions in fasting glucose concentrations (31), as well as in 24-h mean glucose and glycaemic variability (32) during continuous glucose monitoring despite similar energy intake (32). As we did not see lower fasting plasma glucose levels among those with longer fasting duration, this can also be used to reassure people that their body can maintain a normal blood glucose level even after having a longer overnight fast. Late night food consumption has been proposed as a risk factor for type 2 diabetes and cardiometabolic health (33), which may possibly be explained by a shorter overnight fasting duration and excess energy intake. Also, experimental circadian misalignment has been shown to lead to decreased glucose tolerance (34, 35). Less pronounced cases of circadian misalignment could be the result of shorter overnight fasting durations.

The present study points to an association between weekday of examination and glucose metabolism. This finding may be due to different weekend behavior, for example, different sleeping and eating patterns, compared to weekdays (25). Therefore, examining the participants on the same day of the week in studies with repeated measurements seems prudent when logistically possible; however, studies with randomised day of examination are needed to confirm the results.

Conclusion

We found that longer fasting duration is associated with slightly higher 2-h glucose and insulin levels and greater GIP and GLP-1 response after glucose ingestion. Additionally, we found higher fasting insulin levels and similar fasting glucose levels on Mondays compared to the other days of the week pointing to a worse glucose regulation after the weekend.

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