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THE ROLE OF HYPERGLYCEMIA, HYPERINSULINEMIA AND ELEVATED FREE FATTY ACIDS FOR CARDIAC FUNCTION IN PATIENTS WITH TYPE 2 DIABETES - THE HYPERCARD2 STUDY

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ABSTRACT

Introduction: Type 2 diabetes (T2D) is characterised by elevated plasma glucose, free fatty acid (FFA) and insulin concentrations, and this metabolic profile is linked to diabetic cardiomyopathy, a diastolic dysfunction at first, and increased cardiovascular disease (CVD) risk. Shifting cardiac metabolism towards glucose utilisation has been suggested to improve cardiovascular function and CVD risk, but insulin treatment increases overall glucose oxidation and lowers lipid oxidation, without reducing CVD risk, whereas SGLT-2 inhibitors (SGLT-2i) increase FFA, ketone body concentrations and lipid oxidation while decreasing insulin concentrations and CVD risk. The aim of the present study is to elucidate the importance of different metabolic profiles obtained during treatment with an SGLT2i versus insulin for myocardial function in patients with T2D.

Methods and analyses: Randomised, cross-over study, where 20 patients with T2D and BMI >28 kg/m2 receive 25 mg empagliflozin qd or NPH insulin bid first for 5 weeks followed by a 3-week washout before crossing over to the remaining treatment. Insulin treatment is titrated to achieve similar glycaemic control as with empagliflozin. In those randomised to insulin first, glycaemia during an initial empagliflozin run-in period prior to randomisation serves as target glucose. Metabolic and cardiac evaluation is performed before and at the end of each treatment period.

The primary endpoint is change (treatment – washout) in left ventricular peak filling rate, as assessed by cardiac MR (CMR) with and without acute lowering of plasma FFAs with acipimox. Secondary and explorative endpoints are changes in left atrial passive emptying fraction, left ventricular ejection fraction, central blood volume and metabolic parameters.

Ethics and dissemination: This study is approved by the Danish Medicines Agency, the Danish Data Protection Agency and the Capital Region Ethics Committee. The trial is conducted in accordance with ICH-GCP guidelines and the Helsinki Declaration. Trial registration number: EudraCT: 2017-002101

STRENGTHS AND LIMITATIONS

- Comparison with NPH Insulin, which has opposite metabolic effects to empagliflozin, provides a strong basis for detecting metabolic effects on cardiac function.
- Repeated CMR, during depletion of plasma FFAs with acipimox during treatments and washouts allows for dissection of the individual roles of hyperglycaemia, hyperinsulinemia and elevated free fatty acids on cardiac function in T2D.
- Cross-over over design is more difficult to perform, but provides greater statistical power
- Effects of metabolic changes on cardiac function are limited to the 5-week intervention period, which excludes effects arising from longer-term treatment.

INTRODUCTION

Type 2 diabetes (T2D) is characterised by hyperglycaemia, hyperinsulinemia, increased free fatty acids and impaired tissue glucose uptake and oxidation [1]. T2D is associated with an increased cardiovascular morbidity, and the more dysregulated the metabolic state, the greater the cardiovascular risk [2–5]. T2D develops when insulin secretion can no longer compensate for the ambient insulin resistance, and therefore previous treatments has focused on increasing insulin signalling by either exogenous insulin administration, stimulation of endogenous insulin secretion or enhancing insulin sensitivity [1].

Diabetic cardiomyopathy (DCM), is an early "silent" complication to T2D, independent of hypertension and/or coronary heart disease. It is characterised by left ventricular (LV) hypertrophy and diastolic dysfunction [6] and has been linked to the increased cardiovascular risk in T2D [7]. DCM may be accurately described by measuring left ventricular peak filling rate (LVPFR) and left ventricular ejection fraction (LVEF) using cardiac magnetic resonance imaging (CMR) [8,9]. Both diastole and systole are energy requiring processes and sensitive to changes in energy availability [10,11]. Interestingly, cardiac metabolism in patients with T2D is altered and depends more on lipid oxidation and less on glucose oxidation compared to non-diabetic controls [12,13]. It has been argued that glucose oxidation is a better source of energy for the heart than lipid oxidation, especially during stress such as myocardial ischemia, because this yields more ATP pr. unit oxygen [14]. However, manipulating cardiac metabolism towards glucose oxidation, by administering glucose-insulin (-potassium) infusions in patients with hyperglycaemia and myocardial infarction has been attempted, but did not improve survival in neither diabetic nor non-diabetic patients [15– 17]. In intensive care unit patients, strict glycaemic control using insulin has been associated with increased mortality [18], and in patients with T2D and increased CVD risk, intensive glycaemic control has not reduced CVD risk compared to conventional glycaemic control [19–22] and in the ACCORD study, which involved aggressive insulin treatment resulted in excess mortality [23]. Thus, insulin treatment does not prevent cardiovascular events in patients with T2D nor improve prognosis when such occur [24].

SGLT2-inhibition (SGLT2i), on the other hand, is a newer treatment principle in T2D, which has proven effective in attenuating the risk of myocardial infarctions, worsening of heart failure, cardiovascular mortality and all-cause mortality in patients with T2D [25–27]

SGLT2i increases renal glucose excretion thereby lowering plasma glucose and insulin levels and increasing glucagon release, lipolysis and ketogenesis [28,29]. Additionally, tissue glucose uptake and oxidation is reduced and hepatic glucose production increased [30]. The exact cardioprotective mechanisms of SGLT2i are not yet understood, but has been proposed to be linked to improved haemodynamics [31], inhibition of myocardial Na⁺/H⁺ exchange [32,33] or reductions in inflammatory activity [34,35].

An early and interesting hypothesis proposed that changes in cardiac metabolism may be responsible for the cardioprotective effect of SGLT2i. The lowered glucose and insulin

concentrations, persistent hyperketonaemia and elevated free fatty acids, caused by SGLT2i treatment, leads to reduced glucose uptake, increased ketone body uptake and oxidation and unchanged uptake of free fatty acids in the heart while overall lipid oxidation is increased [36,37]. This altered energy metabolism may improve myocardial function, especially during myocardial stress [38–40]. The SGLT2i induced myocardial fuel switch from glucose to fatty acids and ketone bodies, has been suggested to ameliorate adverse cardiac remodelling and heart failure in nondiabetic porcine models [41], and it is noteworthy that eliminating the availability of free fatty acids to insulin resistant hearts can lead to cardiac dysfunction in rodents and in humans, suggesting an important role for lipid metabolism in cardiac function [42–45]. Cardiovascular endpoint trials with SGLT2 inhibitors have shown effects within weeks after initiation of treatment, coinciding with the metabolic effects of the treatment [25,37]

Altogether, SGLT2 inhibitors "amplify" some components of the dysmetabolic profile of T2D and works opposite the metabolic effects of insulin. This raises the question of how cardiac function in patients with T2D depends on lipid and glucose oxidation in the resting state and during stress, and how increasing or lowering blood glucose, free fatty acids, ketone bodies and insulin concentrations influence cardiac function.

Objective

The primary objective of the present study is to evaluate myocardial function in patients with T2D and high risk of CV events using advanced cardiac magnetic resonance imaging (CMR) scans during rest, chronotropic stress and under depletion of plasma free fatty acids before and after 5 weeks of empagliflozin treatment (high free fatty acid and ketone body concentrations, high lipid oxidation and low insulin concentrations) and before and after 5 weeks of human insulin treatment titrated to yield glycaemic control similar to the empagliflozin treatment period (low free fatty acid and ketone body concentrations, high insulin concentrations and glucose oxidation).

Hypothesis

We hypothesise that hyperinsulinemia and hyperglycaemia are conditions that negatively affect cardiac function in T2D, while the availability of free fatty acids and ketone bodies and switching metabolism towards lipid oxidation improves cardiac diastolic and systolic function. Thus, we expect that lowering plasma glucose insulin-independently, and increasing fatty acid concentrations, lipid oxidation and ketone body availability with empagliflozin treatment, improves myocardial function in patients with T2D, and that depleting plasma of free fatty acids during empagliflozin treatment will impair cardiac function.

METHODS AND ANALYSES

Design

This is a 20-week prospective, investigator-initiated, comparator controlled, open label, 2-arm cross-over, human study where subjects are randomised in blocks of 3-5 to NPH insulin or Empagliflozin treatment (25 mg once daily) for 5 ± 1 weeks, followed by 3 ± 1 weeks wash-out and

cross-over of treatment for 5±1 weeks (figure 1). For 7 weeks preceding randomisation, but after inclusion, patients undergo a program of 2 weeks of washout of pre-existing antiglycaemic treatment (except metformin), 2 weeks of empagliflozin run-in (used for glycaemic target and titration of treatment in participants randomised to insulin first, see below) followed by 3 weeks of wash-out. During run-in and treatment periods, participants measure blood glucose twice daily (fasting and before evening meal), and during washouts patients measure fasting blood glucose. After the screening visit (V0) there are four study visits (V1-4) – before and at the end of each treatment period. Each visit consists of three study days – a metabolic study day (MET) and two CMR study days. Randomisation is performed at V1 after the metabolic study day.

Participants

Twenty subjects older than 18 years diagnosed with T2D, a BMI $\geq 28 \text{ kg/m}^2$, HbA1c $\leq 9\%$, fasting C-peptide >500 pmol/L and unchanged antiglycaemic treatment for 12 weeks prior to screening, and who are at a risk of cardiovascular disease (CVD), are eligible for the study. High CVD risk is modified from the EMPA-REG protocol [46]. Inclusion, exclusion and withdrawal criteria are listed in Box 1.

Recruitment

Participants are recruited from the Department of Endocrinology and Cardiology at Hvidovre Hospital and are identified by reviewing laboratory results and patient files. Potential participants will be contacted by means of a recruitment letter, in which they are informed of the opportunity to participate in a scientific research project. We also will advertise for participant in local newspapers and on the internet as well as social media (e.g. www.forsøgsperson.dk; www.sundhed.dk and www.facebook.com).

Outcomes

The primary outcome is change in myocardial diastolic function. This was chosen because firstly, diastole is a highly energy requiring process [10,11], and secondly, because diastolic dysfunction (with or without LV hypertrophy) is the notable early manifestation of diabetic cardiomyopathy [6]. Thus, if changes in overall energy metabolism are to affect cardiac function in patients with T2D, it may well occur in diastole at the earliest. Diastolic cardiac function can be accurately assessed using CMR by measuring left ventricular peak filling rate (LVPFR) and left atrial passive emptying fraction (LAPEF) [47]. Our primary outcome measure is change (LVPFR_{treatment} – LVPFR_{wash-out}) in LVPFR (Δ LVPFR). All endpoints are listed in Box 2.

Randomisation and intervention

Participants are randomised consecutively by lottery in blocks of 3-5 to treatment with either subcutaneous NPH insulin (Insulatard®) twice daily or oral empagliflozin (Jardiance®) 25 mg once daily first. All patient will receive both treatments during the trial. Randomisation is performed at V1. NPH insulin is initiated at a dose of 0.2 IU/kg body weight/day and is titrated daily over phone (phone contacts, figure 1) by 0.05 IU/kg body weight/day until average blood glucose over three consecutive days is within ± 1 mmol/L of the individual glycaemic target. In participants

randomised to insulin first, the glycaemic target is average fasting and evening glucose concentrations during the second week of empagliflozin run-in. In patients randomised to insulin second, the glycaemic target is average fasting and pre-prandial evening BG values of week 3 and 4 during the first (empagliflozin) treatment period.

As previously discussed, insulin and empagliflozin represents two metabolically opposing methods for lowering plasma glucose concentrations. By titrating insulin treatment to match the glycaemic control found with empagliflozin in the same participants, the result is two distinct metabolic phenotypes: one with hyperinsulinemia and suppressed levels of FFAs (NPH insulin treatment), and one with reduced insulin levels and increased levels of FFAs (empagliflozin treatment) - but both with the same levels of glycaemic control (Figure 2). NPH Insulin has been chosen over more modern human insulin analogues, as it is not albumin bound and can be measured in an ordinary insulin assay.

Safety considerations

 During washout periods blood glucose concentrations will increase – that is a separate point of the study, but severely dysregulated diabetes is an exclusion criterium to ensure participant safety. The risk of severe hyperglycaemia is reduced in several ways in the study:

- Existing metformin treatment is continued throughout the whole study as background antiglycaemic treatment.
- In case of fasting BG concentrations of more than 13 mmol/L, patients are instructed to contact study personnel.
- Phone contacts by study investigator are planned in the second week of washout periods to follow up on the patient and enquire to hyperglycaemic events or other adverse events.
- As soon as the final day (CMR with acipimox) of a washout visit (visit 1 or 3) is completed, antiglycaemic treatment according to study drug sequence is commenced to minimise time spent in hyperglycaemia.

In case of fasting BG > 13 mmol/L, the patient will be contacted daily for two additional days. If average fasting BG over the 3 days > 13 mmol/L that triggers an extra safety visit, where fasting plasma glucose (PG) is measured. If PG > 13 mmol/L on the day of the extra visit, then the patient is withdrawn from the study and antihyperglycaemic treatment is initiated.

Screening visit (V0)

Once oral and written informed consent is obtained by the study investigator, the screening procedure follows. Medical history is recorded, screening blood samples drawn, and an ECG, recording of blood pressure, pulse rate and registration of anthropometric data are performed, and patients are screened according to in- and exclusion criteria. A standard transthoracic echocardiography is performed, and VO2max is estimated (Box 3).

Study visits

All study visits consist of three study days – a metabolic study day and two CMR study days (Box 4).

The metabolic study day

The metabolic study is conducted at the Department of Endocrinology, Hvidovre Hospital, to document the metabolic effects of each study drug.

Participants meet in the morning after an overnight fast. Anthropometric data, blood pressure, pulse rate and an ECG are recorded, and two catheters, one in each arm are inserted for infusion of tracers and for repeated drawing of arterialised blood samples respectively. Baseline and safety blood samples are taken (Box 5), the participant empties bladder and the investigational drug (V2, V4) and the participants usual medications are administered at 0800h. Body composition is determined by Dual energy x-ray absorptiometry scan (DXA).

Basal metabolism

Primed infusions of stable glucose ($[6,6-D_2]$ -glucose) and glycerol ($[1,1,2,3,3-D_5]$ -glycerol) tracers are initiated (T=-180 min). Blood is sampled at -30, -15 and -2 min to characterise glucose, lipid and amino acid metabolism. The patient empties bladder, urine is weighed, and samples are taken for determination of tracer concentrations and urinary nitrogen excretion, and the 5h-OGTT is initiated.

5h-OGTT

The patient ingests anhydrous glucose (72 g) with added [U-13C6]-glucose tracer (3 g) dissolved in 250 mL of water over 5 minutes (T=0 min). Intravenous tracer infusions continue unchanged. Blood is sampled regularly for 5 hours for characterisation of postprandial glucose, lipid and amino acid metabolism (Box 4 and 5). The patient empties bladder regularly during and at the end of the OGTT. Urine is sampled for nitrogen excretion and tracers/tracees.

Fat and muscle biopsies: Biopsies are obtained during the basal (T=-60 min) and the maximally insulin stimulated (T=60 min) state. Muscle biopsies are considered proxies for cardiomyocyte metabolic status. 30 min ventilated hood indirect calorimetry (Vyaire Vyntus® CPX) is performed during the basal period (prior to biopsies) t= -90 min and postprandially at t=60 min for determination of fasting and postprandial energy expenditure and respiratory quotient.

Exercise test (50% VO2max)

At T=300 min, the participant is exercised at 60 W for 4 minutes after which work load is increased until oxygen consumption is 50% of estimated VO2max. Pulse rate is recorded with a chest mounted pulse rate monitor and oxygen consumption with a mask mounted indirect calorimeter. Blood is sampled to characterise glucose, lipid and amino acid metabolism (Box 4 and 5). After 30 min, VO2max is estimated by increasing workload by 50W until a pulse rate increase of 30. When pulse is steady for 2 min, oxygen consumption and pulse rate are recorded, and the test is stopped. VO2max is estimated by linear extrapolation to the theoretical maximum pulse rate (220-age).

Ad libitum meal test

SGLT2 inhibition is associated with a lower weight reduction than predicted from the urinary energy loss. SGLT2 inhibition does not change resting energy expenditure or blunt the thermogenic

effect of feeding, suggesting that energy intake is increased [37]. Therefore, the metabolic study day is ended with an ad *libitum meal*, consisting of thoroughly mixed pasta bolognese (fixed nutrient composition and energy content). Patients are placed in a quiet corner and instructed to eat until full. Two glasses of water (total 300 mL) are allowed with the meal. The meal is weighed before and after serving and ad libitum meal intake defined as the difference. Throughout the day patients are asked to score their hunger, satiety and sensation of fullness on a visual analogue scale [48].

Cardiac evaluation

Two CMR days are performed during each visit (V1-V4). In addition, diurnal blood pressure and Holter monitoring are performed. CMR is conducted at the Department of Cardiology, Rigshospitalet, Copenhagen, whereas Holter monitoring and diurnal blood pressure monitoring are performed at the Department of Cardiology, Hvidovre Hospital.

Participants meet fasting and morning medication, including investigational medicinal product (V2 and V4), is administered. Anthropometric data are recorded, and two intravenous catheters are inserted into an antecubital and the contralateral dorsal hand vein for infusion of adenosine, gadolinium contrast and glycopyrrolate and for blood sampling respectively (Box 5). Prior to CMR a transthoracic echocardiography is performed (Box 3)

CMR is performed on a 1.5 Tesla scanner (Siemens Aera; Siemens; Erlangen; Germany) with the patient lying supine on the back, using an 18-channel cardiac coil with continuous ECG gating.

Cine 2-, 3- and 4-chamber images, complete transverse and short axis cine stacks covering the whole heart are acquired. All images are obtained during end-expiratory breath-holds. Myocardial perfusion images during rest and stress are obtained at the basal, mid-ventricular and apical cardiac short-axis level. Rest perfusion images of the myocardium is acquired using an intravenous bolus of gadolinium contrast (Gadovist®, Bayer AG, Germany) 0.075 mmol/kg bodyweight. The time of gadolinium contrast entry into the right and the left ventricle is accurately determined, and this transit time of gadovist multiplied by cardiac output is used to calculate the pulmonary and central (pulmonary + cardiac) blood volume.

Myocardial stress perfusion images are obtained with an i.v dose of 0.075 mmol/kg of gadovist during and 10 min after an intravenous adenosine (140 ug/min) administrated for maximum 4 minutes). This is followed by evaluation of cardiac function during chronotropic stress, where short axis cine stack will be reacquired 10 minutes after the administration of intravenous glycopyrrolate (4 μ g/kg, max. 400 μ g, given as a bolus). This approach has been shown to unmasque subclinical diastolic dysfunction as has been demonstrated in normal healthy elderly [47].

Cardiac MRI, Acipimox

CMR scans will follow the same procedure as described above, but participants are instructed to ingest 250 mg acipimox p.o. twice, 4 hours before and right before the scan, to determine

myocardial function. This repeated administration of acipimox is required for adequate suppression of hormone sensitive lipase activity and depletion of plasma FFAs (28). This has been shown to gradually impair cardiac function [49], and is done to disclose any coupling between FFA availability and cardiac function.

CMR image analysis

Is performed using Circle42 (Circle Cardiovascular Imaging Inc., Calgary Canada, v5.5.1). LV volumes, LV mass, LV ejection fraction (LVEF) and LV peak filling rate are determined by tracing of the endo- and epicardial contours in end-diastolic and end-systolic phases. The papillary muscles are excluded from the myocardium. On native and post-contrast T1-mappings, endocardial and epicardial borders are traced, and the mean extra cellular volume (ECV) is calculated from areas outside late gadolinium enhancement (LGE) lesions. For determination of the ECV within an LGE lesion, myocardium without LGE in the segment is excluded. Myocardial perfusion scans are inspected for perfusion defects. Regions with infarctions, sub-endocardial perfusion defects or dark-rim artefacts will be excluded. Blinded to clinical data, the analyses will be reviewed and finalised by two CMR specialist.

Diurnal blood pressure and Holter monitoring

Between study days during each visit, diurnal blood pressure is recorded (ScottCare, ABP 320, Cleveland, OH)) for 24 hours with 15 minutes intervals between 6.00 to 22.00- and 60-min intervals during night-time. Cardiac rhythm is evaluated with Holter monitoring (SCOTTCARE, CHROMA, model RZ153C, Cleveland, OH) for 48 hours.

Lien

Patient and public involvement No patient involved.

ANALYSES

Blood and tissue samples

Subcutaneous fat- and muscle biopsies: Local analgesia is applied before sampling with a Bergströms cannula. Samples are immediately frozen in liquid nitrogen and stored at -80°C. Blood and urine samples: Samples are spun, aliquoted and stored at -20°C (GLP-1, PYY, Glucagon) or – 80°C for later analysis. Bedside plasma glucose measurements are performed using the glucose oxidase technique (YSI model 2300 STAT Plus; YSI,Yellow Springs, OH). Home blood glucose measurements are carried out on Contour XT (Ascensia Diabetes Care Holdings AG, Basel, Switzerland). Safety blood- and urine samples are analysed on the same day at the Department of Clinical Biochemistry, Hvidovre Hospital.

Statistical methods

Sample size calculation

Measures of myocardial function are highly reproducible when assessed using CMR, and interstudy and cohort coefficients of variation are in the range of 3-5% [50–52]

Using the same CMR protocol as the present, Ahtarovski et al found a mean difference of 92 ml/s in Left Ventricular Peak Filling Rate between healthy young (585±62 ml/s) and healthy elderly

subjects (493±55 ml/s) [52]. We assume that T2D patients have LVPFR corresponding to healthy elderly subjects, and we assume that empagliflozin treatment improves LVPFR by 30 ml/s (Δ LVPFR=30 ml/s) from baseline and that insulin treatment does not improve LVPFR (Δ LVPFR=0 ml/s).

Conservatively setting the standard deviation of between treatment differences of Δ LVPFR at 30 ml/s, a number of 20 patients would be adequate to determine a 30 ml/s difference between the two treatments with a power of 93% and a two-sided significance level of 0.01, when evaluating data with the paired student's t-test.

Statistical analysis plan

 The primary and secondary endpoints are analysed assuming no period effect or treatment-period interaction. This assumption is reasonable, given results from similar studies, where no such interactions or effects have been reported [53]. Normally distributed data are presented using standard descriptive statistics, and reported as mean (SD) for normally distributed and median (Q1;Q3) for non-normally distributed data. Likewise, comparisons of normally distributed data is done using the paired Student's t-test for all completers, whereas Wilcoxon's paired signed rank test will be used if data is non-normally distributed.

Ethics and dissemination

The study is conducted according to ICH GCP guidelines E6 (R2) and registered with the Danish Medicines Agency (EudraCT no. 2017-002101-35, The Capital Region Ethical Committee (H-17018846) and the Danish Data Protection Agency (2012-58-0004; AHH-2017-093, I-Suite nr.: 06012). Our results, regardless of outcome, will be published in relevant scientific journals. In addition, we will seek to disseminate results through presentations at scientific meetings. Publication will take place as soon as scientifically feasible. No later than 12 months after unregistering of the study, will results be made available at www.clinicalregister.eu.

Timeframe

Screenings are performed from January 2018. Last patient, last visit is expected second half of 2021 after which the study will be unregistered with the Danish Medicines Agency and the Capital Region Municipal Ethical Committee within 90 days. Data analyses are expected to be completed by Winter 2022.

DISCUSSION

The profound and swift benefits of SGLT2i on cardiovascular risk in T2D have inspired the discussion of metabolism and its importance for cardiac function in patients with T2D [54–58]. Especially since, SGLT2 inhibitors have metabolic effects that by and large are opposite to those of insulin treatment. Thus, insulin treatment is associated with increased tissue glucose uptake and utilisation, but suppression of lipid mobilisation and oxidation as well as lowering of plasma concentrations of ketone bodies [59]. SGLT2 inhibitors increase lipid mobilisation and oxidation, increase plasma ketone body concentrations and reduce tissue glucose uptake [37,60]. Both treatments lower plasma glucose, but insulin treatment increases whereas SGLT2i treatment decreases plasma insulin concentrations. Whether such changes in metabolism affect cardiac function, is still unsettled, but forcing cardiac glucose uptake and utilisation through insulin treatment has been suggested by some to benefit and by others to impair cardiac function [45,61–

64], while yet others have suggested increased lipid and ketone body oxidation to be important for proper cardiac function in T2D [44,55,64]. Studies on SGLT2i treatments and the effects on cardiac function are beginning to emerge. In a recent study, 42 patients with T2D were randomised to 12 weeks of empagliflozin 10 mg or placebo once daily. SGLT2 inhibition was shown to rapidly improve diastolic cardiac function as evaluated with echocardiography [65]. In a placebo controlled cross-over design, after 4 weeks of empagliflozin treatment in patients with T2D, myocardial glucose uptake was reduced and fatty acid oxidation unaltered, but this did not significantly change myocardial oxygen consumption or cardiac efficiency, nor any measure of cardiac function [66]. In a Swedish study, 6 weeks dapagliflozin treatment showed unchanged cardiac fatty acid uptake, a trend toward reduced left atrial maximal volume, and reduced LV oxygen consumption and external work compared to placebo [67], and in the only study found, where an active comparator was used, 10 mg empagliflozin once daily for 12 weeks did not change cardiac lipid accumulation (as measured by MR spectrometry), cardiac function or cardiac metabolism compared to sitagliptin 50 mg daily [68].

In conclusion, existing studies in humans have shown divergent results regarding changes in cardiac diastolic function with little changes in cardiac metabolism. However, most studies have compared cardiac effects of SGLT2i to placebo, thus not accounting for the circumstances that characterised the EMPA-REG trial, where anti glycaemic treatment was intensified in the placebo group concurrently [25]. Thus, the CVD risk benefits of the study may have arisen from unfavourable metabolic consequences of the treatment in the placebo arm. In the one study with an active comparator empagliflozin was compared to sitagliptin, which not only affects the incretin system but also has less specific metabolic effect [69]. Therefore, to date our study, is the one to most directly pursue the coupling between metabolism and cardiac function, by choosing insulin as the comparator, and by including the effects of acute lowering of free fatty acid concentrations in plasma on cardiac function.

Disclosure summary

All authors have completed the ICMJE uniform disclosure form and declare: no support from any organisations for the submitted work; SM and NBJ have received research grants from Boehringer Ingelheim and JJH serves on advisory boards for Novo Nordisk, no other relationships or activities that could appear to have influenced the submitted work.

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Contributions Author contributions

	: Eligibility criteria ion criteria
Age >	18 years
	$\geq 28 \text{ kg/m}^2$
	$c \le 9\%$ ($\le 10\%$ in diet or metformin treated only)
	g C-peptide \geq 500 pmol/L
	inged glycaemic treatment for 3 months prior to inclusion
	cardiovascular risk as one of the following:
•	Previous myocardial infarction, stroke or peripheral arterial disease more than 2 mont prior to informed consent
•	Evidence of multi-vessel coronary arterial disease (CAD) but without prior myocardia infarction, if more than 50% stenosis is present, if revascularised (CABG or PCI) mor than 2 months prior or if 1 vessel is vascularised and the other with 50% stenosis. Single vessel CAD without prior myocardial infarction if more than 50% stenosis is present, not revascularised and positive stress test for ischemia.
Exclu	sion criteria
Insuli	n treatment within 3 months from informed consent
	l diabetes
• •	iatric disorder or mental retardation
2	or alcohol abuse within 3 months from informed consent
	ompliance
	nia (Hb < 6.4 mmol/L) or other blood dyscrasias causing haemolysis or unstable
	ocytes.
Indica	tion of liver disease (ALT or Alkaline phosphatase elevation above 3x upper normal lir red renal function (eGFR<45 ml/min/1.73 m ²)
Anti-c	besity medication within 3 months from informed consent
Syster	nic steroid treatment within 6 weeks from informed consent.
-	ncontrolled endocrine disorder except T2D
Bariat absorp	ric surgery or other gastrointestinal conditions that may compromise gastrointestinal otion
Peptic	ulcer – verified endoscopically
-	orm of surgery within 3 months of informed consent
Acute conser	myocardial infarction, stroke or peripheral arterial disease within 2 months of informed at.
Persist	tent or permanent atrial fibrillation
Inabili	ity to undergo experimental procedures including exclusion criteria for CMR scanning: ntable cardioverter defibrillator/pacemaker
	nagnetic clips
Impla	
Implan Ferror	ronhohia
Implan Ferror	rophobia.

Known closed-angle glaucoma	
known severe prostate hyperplasia	
Tachycardia (HR > 100 at rest)	
Known bladder atony	
Cardia insufficiency or non-conger	nital pylorus stenosis -verified endoscopically
Known gastroparesis	
Contraindications to adenosine:	
2nd or 3rd degree atrioventricular b	block
Severe hypotension (BP \leq 90/50 m	mHg)
Long QT syndrome	
Unstable angina pectoris	
Decompensated heart failure	
Sinus node dysfunction	
Chronic obstructive pulmonary dis-	ease or asthma bronchiale (FEV1 \leq 50% of expected)
Allergy towards any of the drugs of	r diagnostics used in the protocol (insulin, empagliflozin,
acipimox, glycopyrrolate, adenosin	
Any condition which in the opinion	n of the investigator may jeopardize subject safety or
compliance with the protocol.	
Withdrawal criteria	
Subjects may withdraw from the st	
Pregnancy discovered during the ex-	-
-	reactions associated with the planned experiments, includir
severe glycaemic dysregulation dur	ring washout periods.
Box 2: Endpoints	
Primary end point	Change in left ventricular peak filling rate

Primary end point	Change in left ventricular peak filling rate							
	(Δ LVPFR)							
Secondary endpoints	Change in left atrial passive emptying fraction							
	(ALAPEF)							
	Change in left ventricular ejection fraction							
	(Δ LVEF)							
Explorative endpoints include	Cardiovascular:							
	Change in VO2max and exercise tolerance test							
	variables							
	Change in central blood volume and							
	haematocrite							
	Change in heart rate variability							
	Change in left ventricular volume							
	Metabolic:							

Basal and postprandial AUC Free Fa and glycerol turnover Endogenous glucose production and glucose disposal (metabolic clearance glucose) Fasting and postprandial energy expe and respiratory quotient	
Endogenous glucose production and glucose disposal (metabolic clearance glucose) Fasting and postprandial energy expe	tty Acids
glucose disposal (metabolic clearance glucose) Fasting and postprandial energy expe	
glucose) Fasting and postprandial energy expe	tissue
Fasting and postprandial energy expe	e of
and respiratory quotient	enditure
Glucagon-Insulin ratio	
Insulin sensitivity (AUC glucose met	tabolic
clearance / AUC insulin concentratio	on)
Beta-cell function (prehepatic insulin	secretion
rate, correlated to ambient glucose)	

	BMJ Open
	 Basal and postprandial AUC Free Fatty Acids and glycerol turnover Endogenous glucose production and tissue glucose disposal (metabolic clearance of glucose) Fasting and postprandial energy expenditure and respiratory quotient Glucagon-Insulin ratio Insulin sensitivity (AUC glucose metabolic clearance / AUC insulin concentration) Beta-cell function (prehepatic insulin secretion rate, correlated to ambient glucose)
Box 3: Screening procedures Blood samples	Haematology (haemoglobin, thrombocytes, haematocrit, leukocytes), liver and renal function tests (creatinine, eGFR (Cockroft- Gault formula), alkaline phosphatases, alanine aminotransferases, lactate dehydrogenase, bilirubin, amylase, sodium, potassium), fasting P-glucose, C-peptide, HbA1c, TSH, Urinary Albumin/creatinine mass ratio, and in fertile
Echocardiography	women, U-hCG. Parasternal long axis view, parasternal short axis view at aortic, mitral and apex levels, apical 4-chamber view, LVEF, E/E', E', LVEDV/BSA.
Estimation of VO2max	Maximum oxygen uptake is estimated using Åstrøm's two-point test performed on a cycle ergometer during indirect calorimetry. From measurements of VO2 at two sub-maximal pulse rates VO2max is estimated by linear extrapolation to the theoretical maximal pulse rate (220-age) [70].

Box 4: Visit overview		
Metabolic study day	Cardiac MR	Cardiac MR, Acipimox
- DXA-scan and fasting safety	- Fasting blood samples,	Same protocol as Cardiac I
and efficacy blood samples	before and after CMR.	day, but during
- Determination of 3-hour	- Echocardiography	pharmacological suppressi
basal metabolism.	- CMR Rest	of hormone sensitive lipase
Infusion of glucose and	Without enhancement	activity and depletion of
glycerol tracers	With enhancement and	plasma free fatty acids.
Basal muscle and fat	adenosine infusion	- 48h Holter monitoring.
biopsies	- CMR Stress	
Basal energy expenditure	Unenhanced repeated during	3
and determination of	pharmacological chronotropic	
respiratory quotient	stress with glycopyrrolate	
- 5-hour OGTT	infusion.	
with oral glucose tracer and	- 24h ambulant blood pressure	
continued intravenous		
glucose and glycerol tracer.		
Fat- and muscle biopsies at maximum insulin stimulation		
- Exercise test and		
determination of VO2max		
- Ad libitum meal.		
Box 5: Blood samples on metab	olic and CMR study days inclu	le
Metabolic study day		s: glucose, insulin, C-peptide,
		As, triglycerides, total amino
		one bodies, tracers/tracees, gu
	hormones.	, , , , , , , , , , , , , , , , , , , ,
	HbA1c, urate	, blood urea nitrogen, cortisol,
	sampled at ba	-
Cardiac MRI days	Markers of ca	rdiac function, including pro-
	ANP and pro	BNP, glucose, insulin, C-pept
		As, triglycerides, ketone bodie
	hamataarita	re drawn before and after CM

Box 5: Blood samples on metabolic and CMR study days include									
Metabolic study day	Blood samples: glucose, insulin, C-peptide,								
	glucagon, FFAs, triglycerides, total amino								
	acids and ketone bodies, tracers/tracees, gut								
	hormones.								
	HbA1c, urate, blood urea nitrogen, cortisol, is								
	sampled at baseline.								
Cardiac MRI days	Markers of cardiac function, including pro-								
	ANP and pro-BNP, glucose, insulin, C-peptide,								
	glucagon, FFAs, triglycerides, ketone bodies,								
	haematocrit are drawn before and after CMR.								

Figure legends

Figure 1. Study outline. Included patients undergo a 7 week program of washout of pre-existing antiglycemic treatment (except metformin) and run-in of empagliflozin. Herafter they are randomized to treatment for 5 ± 1 weeks, followed by 3 ± 1 weeks wash-out and cross-over to 5 ± 1 weeks treatment with the remaining study drug.

Figure 2. Metabolic profile of the two study drugs. Schematic representation of the metabolic changes expected with the two study drug treatments in a patient randomized to insulin first. Insulin treatment is characterized by low glucose, low FFAs and high insulin concentrations; empagliflozin treatment by low glucose, high FFAs and low insulin.

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References:

6		
7	1	Defronzo RA. From the Triumvirate to the Ominous Octet : A New Paradigm for the
8		Treatment of Type 2 Diabetes Mellitus. ::773–95. doi:10.2337/db09-9028
9	2	De Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and
10		cardiovascular mortality in the Hoorn population: The Hoorn study. <i>Diabetologia</i>
11		1999; 42 :926–31. doi:10.1007/s001250051249
12	3	Pyorala M, Miettinen H, Laakso M, <i>et al.</i> Plasma insulin and all-cause, cardiovascular, and
12	5	noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study.
13		Diabetes Care 2000;23:1097–102. doi:10.2337/diacare.23.8.1097
	4	Lakka HM, Lakka TA, Tuomilehto J, <i>et al.</i> Hyperinsulinemia and the risk of cardiovascular
15	т	death and acute coronary and cerebrovascular events in men: The Kuopio Ischaemic Heart
16		Disease Risk Factor Study. Arch Intern Med 2000;160:1160–8.
17		doi:10.1001/archinte.160.8.1160
18	5	Selvin E, Marinopoulos S, Berkenblit G, <i>et al.</i> Meta-analysis: Glycosylated hemoglobin and
19	5	
20		cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141. doi:10.7326/0003-
21	6	4819-141-6-200409210-00007
22	6	Li N, Zhou H. Sglt2 inhibitors: A novel player in the treatment and prevention of diabetic
23	-	cardiomyopathy. Drug Des Devel Ther 2020;14:4775-88. doi:10.2147/DDDT.S269514
24	7	Roos A De, Radder JK. Diastolic Dysfunction Is Associated With Altered Myocardial
25		Metabolism in Asymptomatic Normotensive Patients With Well-Controlled Type 2 Diabetes
26	_	Mellitus. 2003;42. doi:10.1016/S0735-1097(03)00625-9
27	8	Tarquini R, Lazzeri C, Pala L, et al. The diabetic cardiomyopathy. Acta Diabetol
28		2011; 48 :173–81. doi:10.1007/s00592-010-0180-x
29	9	Komi S, Inoue Y, Hata H, et al. Cardiovascular magnetic resonance evaluation of left
30		ventricular peak filling rate using steady-state free precession and phase contrast sequences.
31		Springerplus 2016;5. doi:10.1186/s40064-016-2878-x
32	10	Diamant M, Lamb HJ, Groeneveld Y, et al. Diastolic dysfunction is associated with altered
33		myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2
34		diabetes mellitus. J Am Coll Cardiol 2003;42:328-35. doi:10.1016/S0735-1097(03)00625-9
35	11	Rosano G, Coats A. Modulation of Cardiac Metabolism in Heart Failure. Int Cardiovasc
36		Forum J 2019;17:99–103. doi:10.17987/icfj.v17i0.597
37	12	Ungar I, Gilbert M, Siegel A, et al. Studies on myocardial metabolism. IV. Myocardial
38		metabolism in diabetes. Am J Med 1955; 18 :385–96. doi:10.1016/0002-9343(55)90218-7
39	13	Rijzewijk LJ, van der Meer RW, Lamb HJ, <i>et al.</i> Altered myocardial substrate metabolism
40	10	and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies
40 41		with cardiac positron emission tomography and magnetic resonance imaging. J Am Coll
		<i>Cardiol</i> 2009; 54 :1524–32. doi:10.1016/j.jacc.2009.04.074
42	14	Stanley WC, Lopaschuk GD, Hall JL, <i>et al.</i> Regulation of myocardial carbohydrate
43	17	metabolism under normal and ischaemic conditions. Potential for pharmacological
44		interventions. Cardiovasc Res 1997; 33 :243–57. doi:10.1016/S0008-6363(96)00245-3
45	15	
46	15	Mehta SR, Yusuf S, Díaz R, <i>et al.</i> Effect of glucose-insulin-potassium infusion on mortality
47		in patients with acute ST-segment elevation myocardial infarction: The CREATE-ECLA
48	17	randomized controlled trial. J Am Med Assoc 2005;293:437–46. doi:10.1001/jama.293.4.437
49	16	Malmberg K, Rydén L, Wedel H, et al. FASTTRACK intense metabolic control by means of
50		insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2):
51		Effects on mortality and morbidity. <i>Eur Heart J</i> 2005; 26 :650–61.
52	. –	doi:10.1093/eurheartj/ehi199
53	17	Malmberg K, Rydén L, Efendic S, et al. Randomized trial of insulin-glucose infusion
54		followed by subcutaneous insulin treatment in diabetic patients with acute myocardial
55		infarction (DIGAMI study): Effects on mortality at 1 year. J Am Coll Cardiol 1995;26:57–
56		65. doi:10.1016/0735-1097(95)00126-K
57	18	NICE-SUGAR Study Investigators, Finfer S, Chittock DR, et al. Intensive versus
58		conventional glucose control in critically ill patients. <i>N Engl J Med</i> 2009; 360 :1283–97.
59		doi:10.1056/NEJMoa0810625
60		
00		

5

6

7

8

9

19 Hemmingsen B, Lund SS, Gluud C, et al. Intensive glycaemic control for patients with type 2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised clinical trials. BMJ 2011;343:d6898.http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3223424&tool= pmcentrez&rendertype=abstract (accessed 9 Sep 2015). 20 Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in 10 veterans with type 2 diabetes. N Engl J Med 2009;360:129-39. 11 doi:10.1056/NEJMoa0808431 12 ADVANCE Collaborative Group, Patel A, MacMahon S, et al. Intensive blood glucose 21 13 control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 14 2008;358:2560-72. doi:10.1056/NEJMoa0802987 15 22 Basal Insulin and Cardiovascular and Other Outcomes in Dysglycemia. N Engl J Med 16 2012;367:319-28. doi:10.1056/nejmoa1203858 17 23 Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, et 18 al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-19 59. doi:10.1056/NEJMoa0802743 20 Sciences HH, Miller ME, Byington RP, et al. Effects of Intensive Glucose Lowering in Type 24 21 2 Diabetes. N Engl J Med 2008;358:2545-59. doi:10.1056/nejmoa0802743 22 Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and 25 23 Mortality in Type 2 Diabetes. N Engl J Med 2015;373:2117–28. 24 doi:10.1056/NEJMoa1504720 25 26 McMurray JJV, Solomon SD, Inzucchi SE, et al. Dapagliflozin in Patients with Heart Failure 26 and Reduced Ejection Fraction. N Engl J Med 2019;381:1995-2008. 27 doi:10.1056/nejmoa1911303 28 Zou CY, Liu XK, Sang YQ, et al. Effects of SGLT2 inhibitors on cardiovascular outcomes 27 29 and mortality in type 2 diabetes: A meta-analysis. Med (United States) 2019;98. 30 doi:10.1097/MD.000000000018245 31 Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose 28 32 cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest 2014;124:499–508. 33 doi:10.1172/JCI72227 34 29 Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin 35 sensitivity but enhances endogenous glucose production. J Clin Invest 2014;124:509–14. 36 doi:10.1172/JCI70704 37 30 Ferrannini E, Baldi S, Frascerra S, et al. Renal Handling of Ketones in Response to Sodium -38 Glucose Cotransporter 2 Inhibition in Patients With Type 2 Diabetes. 2017;40:771–6. 39 doi:10.2337/dc16-2724 40 31 Chilton R, Tikkanen I, Cannon CP, et al. Effects of empagliflozin on blood pressure and 41 markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. Diabetes 42 Obes Metab 2015;17:1180–93. doi:10.1111/dom.12572 43 32 Trum M, Riechel J, Lebek S, et al. Empagli fl ozin inhibits Na + / H + exchanger activity in 44 human atrial cardiomyocytes. 2020;:4429-37. doi:10.1002/ehf2.13024 45 Baartscheer A, Schumacher CA, Wüst RCI, et al. Empagliflozin decreases myocardial 33 46 cytoplasmic Na + through inhibition of the cardiac Na + / H + exchanger in rats and rabbits. 47 2017;:568-73. doi:10.1007/s00125-016-4134-x 48 34 Garvey WT, Gaal L Van, Leiter LA, et al. Effects of canagli fl ozin versus glimepiride on 49 adipokines and in fl ammatory biomarkers in type 2 diabetes \Rightarrow . 2018;85:32–7. 50 doi:10.1016/j.metabol.2018.02.002 51 Heerspink HJL, Perco P, Mulder S, et al. Canagliflozin reduces inflammation and fibrosis 35 52 biomarkers : a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabetic kidney disease. 2019;:1154–66. Lauritsen KM, Nielsen BRR, Tolbod LP, *et al.* SGLT2 Inhibition Does Not Affect 53 54 36 55 Myocardial Fatty Acid Oxidation or Uptake, But Reduces Myocardial Glucose Uptake and 56 Blood Flow in Individuals With Type 2 Diabetes- a Randomized Double-Blind, Placebo-57 Controlled Crossover Trial. Diabetes 2020;:db200921. doi:10.2337/db20-0921 58 37 Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose 59 60

 cotransporter 2 inhibition in type 2 diabetic patients. <i>J Clin Invest</i> 2014;124:499–508. doi:10.1172/JCI72227 Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: Glucose excretion and lipid mobilization – not storage – saves lives. <i>J Diabetes Complications</i> 2016;30:753. doi:10.1016/j.diacomp.2016.02.015 Fkanayake P, Hupfeld C, Mudaliar S, Sodium-Glucose Cotransporter Type 2 (SGI.T-2) Inhibitors and Ketogenesis: the Good and the Bad. <i>Curr Diab Rep</i> 2020;20. doi:10.1007/s11892-020-01359-z Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis: <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 Santos-Gallego CG, Requena-Danez JA, San Antonio R, <i>et al.</i> Empgifildzin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Mycocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:19114–44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krsak M, <i>et al.</i> Suppression of plasma free fatty acids reduces mycocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130–7. doi:10.1106/Gil.3224914 Harmancey R, Vasquez HG, Gulrhie PH, <i>et al.</i> Decreased long-chain fatty acid volation impairs postischermic recovery of the insulin-resistant rat heart. <i>FASEB</i> J 2013;27:3966–78. doi:10.1096/j.13-224914 Harmancey R, Jam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed tat heart. <i>FASEB</i> J 2012;26:3118–26. doi:10.1096/j.12- 208991 Zimman B, Inzucchi SE, Lachin JM, <i>et al.</i> Retrinolad, design, and baseline characteristics of a randomized, Japaeebo-controlled ecardiovascular outcome trial of mengifiborain (EMPA-REG OUT	2		
 doi:10.1172/JCIT2227 Jorgensen NB, Pedersen J, Vaag AA, EMPA-REG: Glucose exerction and lipid mobilization – not storage – saves lives. <i>J Diabetes Complications</i> 2016;30:753. doi:10.1016/j.idiacomp.2016.02.015 Ekanayake P, Hupfeld C, Mudaliar S, Sodium-Glucose Cotransporter Type 2 (SGLT-2) Inhibitors and Ketogenesis: the Good and the Bad. <i>Curr Diab Rep</i> 2020;20. doi:10.1078/11892-020-01359-z Ferrannin F, Mark M, Mayoux F. CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, et al. Empaghlfonzin Amcliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Num Metab Cardiovase Dis</i> 2016;26:387–92. doi:10.1016/j.jnumec. Circulation 2006;11:42130-7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmaneey R, Vasquez HG, Guthrie PH, et al. Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/jf.12-234914 Harmaneey R, Lam TN, Labrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/jf.12-208991 Zimman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of emageliflozin (EMPA-REG OUTCOME^{EM}). <i>Cardiovasc Diabetol</i> 2014;31:102. doi:10.1186/1475-24801-3-1002 Zimman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled eardi	3		
 Jargensen NB, Pedersen J, Vaag AA, EMPA-REG: Glucose excretion and lipid mobilization – not storage – saves lives. <i>J Diabetes Controllecations</i> 2016;30:753. doi:10.1016/j.jdiacomp.2016.02.015 Ekanayake P, Hupfeld C, Mudaliar S. Sodium-Glucose Cotransporter Type 2 (SGLT-2) Inhibitors and Ketogenesis: the Good and the Bad. <i>Curr Diab Rep</i> 2020;20. doi:10.1007/s11892-020-01359-z Ferrannin E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/de16-0330 Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, et al. Empagliflozin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.106/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces carliac work and efficiency in eradiomyopathic heart failure. <i>Circulation</i> 2006;114:2130–7. doi:10.106/Gif1.3-234914 Harmancey R, Vasquez HG, Guthric PH, et al. Decreased long-chain fatty acid oxidation impars postischemic recovery of the insulin-resistance improves metabolic and contracitic efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:318–26. doi:10.1096/fj.12-2 208991 Zimman B, Inzuechi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empaglifiozin (EMPA-REG OUTCOMETM). <i>Cardiowase Diabeol</i> 2014;21:302. doi:1			
 and storage – saves lives. <i>J Diabetes Complications</i> 2016;30:253. doi:10.1016/j.jdiacomp.2016.02.015 Fkanayake P, Hupfeld C, Mudaliar S, Sodium-Glucose Cotransporter Type 2 (SGLT-2) Inhibitors and Ketogenesis: the Good and the Bad. <i>Curr Diab Rep</i> 2020;20. doi:10.1075/s11892-200-10359-z Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;30:1108-14. doi:10.2337/dc15-0330 Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, <i>et al.</i> Empagliflorin Amcliorates Adverse 1 eff Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.1016/j.jdice.2019.01.056 Wolf P, Winhofer Y, Krsak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.1016/j.jdice.2019.01.056 Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130–7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12-208991 Zinman B, Inzuechi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empaglifiozin (EMPA-REG OUTCOME¹⁶). <i>Cardiovasc Diabeol</i> 2014;31:02. doi:10.1169/15.202 Zinman B, Inzuechi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of visual analogue		20	
 Tot storage - stress fitses and the second stress of the second storage of		38	
 39 Bernstein (1997) (Bindenfingenetics) (Bindenfingen			
 Inhibifors and Kelogenesis: the Good and the Bad. <i>Curr Diab Rep</i> 2020;20. doi:10.1007/s11892-020-01359-2 Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;39:1108-14. doi:10.2337/dc16-0330 Santos-Gallego CG, Requena-Ibanca JA, San Antonio R, <i>et al</i>. Empagiiflozin Amoliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Inerceptics. <i>J Am Coll Cardiol</i> 2019;73:1931-44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, <i>et al</i>. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387-92. doi:10.1016/j.jnumecd.2016.03.012 Tuunanen H, Engblom E, Naum A, <i>et al</i>. Free fatty acid depletion acutely decreases cardiae work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130-7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guttrin FH, <i>et al</i>. Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966-78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, <i>et al</i>. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118-26. doi:10.1096/fj.12-208991 Zinman B, Inzuechi SE, Lachin JM, <i>et al</i>. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagifilozin (EMPA-REG OUTCOME^{FM}). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K A, Iversen KK, Labong JT, <i>et al</i>. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>An J Physiol Neurol</i> 70, 27403. Grothues F, Smith GC, Moon JCC, <i>et al</i>. Comp		39	
 doi:10.1007/s11892-020-01359-z Ferrannini E, Mark M, Mayoux E, CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, <i>et al.</i> Empagilifozin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.mmeed.2016.03.012 Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiae work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130–7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain faity acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12-208991 Zimman B, Inzuechi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled ardiovascular outcome trial of empagilitozin (EMPA-REG OUTCOME^{FM}). <i>Cardiovasc Diabetol</i> 2014;31:2012, doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lonborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Hear Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visua	10	57	
 40 Ferrannini E, Mark M, Mayoux E, CV protection in the EMPA-REG OUTCOME trial: A thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;39:1108-14. doi:10.2337/dc16-0330 41 Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, <i>et al.</i> Empagliflozin Ameliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Inergetics. <i>J Am Coll Cardiol</i> 2019;73:1931-44. doi:10.1016/j.jacc.2019.01.056 42 Wolf P, Winhofer Y, Krsak M, <i>et al.</i> Suppression of plasma free fatty acid sreduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovase Dis</i> 2016;26:387-92. doi:10.1016/j.numecd.2016.03.012 43 Tuunanon H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiae work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130-7. doi:10.1016/j.CIRCULATIONAHA.106.045184 44 Harmaneey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966-78. doi:10.1096/fj.13-234914 45 Harmaneey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118-26. doi:10.1096/fj.12-208991 46 Zimman B, Inzucchi SF, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOME^{1M}). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 47 Ahtarovski K a, Iversen KK, Lamborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolat stress in healthy young and clderly as evaluated by cardiae magnetic resonance. <i>An J Physiol Hear Circ Physiol</i> 2012;303:1H469-73. doi:10.1152/ajpheart.00365.2012 48 Flint A, Rabon A, Blundell JE, <i>et al.</i> Reproducibility, powr and validity of visual analogue scales			
 thrifty substrate hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 Santos-Gallego CG, Requena-Boancz JA, San Antonio R, <i>et al.</i> Empagliflozin Amcliorates Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. <i>J Am Coll Cardiol</i> 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numced.2016.03.012 Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130–7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation impairs postischenic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12- 208991 Zimman B, Inzucchi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REIG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;31:012. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lonborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajphcart.00365.2012 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single fest meal studies. <i>Im J Obes Relat Metab Disord</i> 2000		40	
 41 Santös-Gallego CG, Requena-Ibanez JA, San Antonio R, et al. Empagilfozin Ameliorates Adverse Left Ventrualar Remodeling in Nondiabetic Heart Failure by Enhancing Myocardial Energetics. J Am Coll Cardiol 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056 42 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. Nutr Metab Cardiovasc Dis 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 43 Tuunanen H, Engblom E, Naum A, et al. Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. Circulation 2006;114:2130–7. doi:10.1161/CIRCULATIONAHA.106.645184 44 Harmancey R, Vasquez HG, Guthrie PH, et al. Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. FASEB J 2013;27:3966–78. doi:10.1096/fj.13-234914 45 Harmancey R, Lam TN, Lubrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. FASEB J 2012;26:3118–26. doi:10.1096/fj.12- 208891 46 Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagilflozin (EMPA-REG OUTCOMETM). Cardiovasc Diabetol 2014;13:102. doi:10.1186/1475-2840-13-102 47 Ahtarovski K a, Iversen KK, Lamborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. Am J Physiol Heart Circ Physiol 2012;303:11469–73. doi:10.1152/ajplaerl.00365.2012 48 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 49 Wolf P, Winhofer Y, Krssak M, et al.		-	
 Myocardial Energetics. J Am Coll Cardiol 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. Nutr Metab Cardiovasc Dis 2016;26:387–92. doi:10.1016/j.numed.2016.03.012 Tuunanen H, Engblom E, Naum A, et al. Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. Circulation 2006;114:2130–7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guthrie PH, et al. Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. FASEB J 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Iam TN, Lubrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. FASEB J 2012;26:3118–26. doi:10.1096/fj.12-208991 Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placeboc-controlled cardiovascular outcome trial of empaglifloxin (EMPA-REG OUTCOMETM). Cardiovasc Diabetol 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lonborg JT, et al. Left atrial and ventricular function during dobutamine and glycopytrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. Am J Physiol Heart Circ Physiol 2012;303:11469-73. doi:10.1152/ajheart.00365.2012 Flint a, Raben a, Blundell JF, et al. Reproducibility, power and validity of visual analogue scales in patienst my stolic function in type 2 diabetes. Nutr Metab Cardiovasc Dis 2016;26:387–92. doi:10.1016/j.numced.2016.03.012 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol 20		41	
 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numed.2016.03.012 Tuunanen H, Engblom E, Naum A, et al. Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130–7. doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guthrie PH, et al. Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12-208991 Zimman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lonborg JT, et al. Left atrial and ventricular function during dobutamine and glycopytrolate stress in healthy young and elderly as evaluated by cardiae magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajphcart.00365.2012 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Nut Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numed.2016.30.12 Gotthues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocatiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/j.S002-9149(02)0231-0 Morton G, Jogiy			
 42 Woll Y, Winker Y, Bagherson D, Pasan V, et al. Suppression of plasma charge actualy ac			
 Portection and systement of the formation of the systement of		42	
 43 Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiac work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130-7. doi:10.1161/CIRCULATIONAHA.106.645184 44 Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966-78. doi:10.1096/fj.13-234914 45 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118-26. doi:10.1096/fj.12- 208991 46 Zinman B, Inzucchi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 47 Ahtarovski K a, Iversen KK, Lonborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 48 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:387-92. doi:10.1016/j.numed.2016.03.012 49 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387-92. doi:10.1016/j.numed.2016.03.012 50 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echoeardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29-34. doi:10.1016/j.			
 45 Finding Production (2006) 142:130-7. 46 Work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006;114:2130-7. 47 Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966-78. 48 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118-26. doi:10.1096/fj.12-208991 49 Zinman B, Inzucchi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflorin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 40 Ahtarovski K a, Iversen KK, Lonborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. 41 Goitou J, Sabard J, Sa		42	
 doi:10.1161/CIRCULATIONAHA.106.645184 Harmancey R, Vasquez HG, Guthrie PH, et al. Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB</i> J 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB</i> J 2012;26:3118–26. doi:10.1096/fj.12- 208991 Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lonborg JT, et al. Left attrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajheart.00365.2012 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab</i> <i>Disord</i> 2000;24:38-48. http://www.ncbi.nlm.nih.gov/pubmcd/10702749 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numed.2016.03.012 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29-34. doi:10.1016/S0029-9149(02)02381-0 Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance exit Mvo-dimensional echocardiography in normal subjects and in patients with heart fa		43	
 Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12- 208991 Zinman B, Inzucchi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab</i> <i>Disord</i> 2000;24:38–48.http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numed.2016.03.012 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29-34. doi:10.1016/j.0020-2149(02)02381-0 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1193/ajpheart.003			
 impairs postischemic recovery of the insulin-resistant rat heart. <i>FASEB J</i> 2013;27:3966–78. doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12-208991 Zinman B, Inzucchi SE, Lachin JM, <i>et al.</i> Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lonborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numeed.2016.03.012 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardial 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/chjci/jcs103 <l< td=""><td></td><td>11</td><td></td></l<>		11	
 doi:10.1096/fj.13-234914 Harmancey R, Lam TN, Lubrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12- 208991 Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38-48.http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krsak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numed.2016.03.012 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascul magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopytrolate stress in healthy young and elderly as evaluated by cardiac magneti	24		
 Harmancey R, Lam TN, Lubrano GM, et al. Insulin resistance improves metabolic and contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12- 208991 Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopytrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance effusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1193/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear </i>	25		
 contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012;26:3118–26. doi:10.1096/fj.12-208991 di Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krsak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 Kum		45	
 208991 Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a randomized, placebo-controlled cardiovascular outcome trial of empaliflozin (EMPA-REG OUTCOMETM). Cardiovasc Diabetol 2014;13:102. doi:10.1186/1475-2840-13-102 Ahtarovski K a, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopytrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. Am J Physiol Heart Circ Physiol 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord 2000;24:38-48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. Nutr Metab Cardiovasc Dis 2016;26:387-92. doi:10.1016/j.numecd.2016.03.012 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol 2002;90:29-34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. Eur Heart J Cardiovascular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. Am J Physiol - Hear Circ Physiol 2012;303:1469-73. doi:10.1152/ajpheart.00365.2012 Ahtarovski KA, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. Am J Physiol - Hear Circ Physiol 2012;303:1			
 ³⁰ randomized, placebo-controlled cardiovascular outcome trial of activation (EMPA-REG OUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-2840-13-102 ³¹ Ahtarovski K a, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopytrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 ³² 48 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 ³⁴ 49 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 ³⁵ 50 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 ³⁶ 51 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/epii/jeil/s013 ³⁶ 52 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 ³⁶ 54 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, pl			
 GUTCOMETM). <i>Cardiovasc Diabetol</i> 2014;13:102. doi:10.1186/1475-12840-13-102 Ahtarovski K a, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab</i> <i>Disord</i> 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29-34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovasc Imaging 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis. <i>Diabetes Care</i>		46	Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a
 47 Ahtarovski K a, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012;303:H1469-73. doi:10.1152/ajpheart.00365.2012 48 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab</i> <i>Disord</i> 2000;24:38–48.http://www.ncbi.nlm.nih.gov/pubmed/10702749 49 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 50 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 51 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 52 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 53 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes</i> <i>Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 54 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty			
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 doi:10.1152/ajpheart.00365.2012 48 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38–48.http://www.ncbi.nlm.nih.gov/pubmed/10702749 49 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 50 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 51 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovasc <i>Imaging</i> 2012;13:954–60. doi:10.103/ehjci/jes103 52 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;30:1469–73. doi:10.1152/ajpheart.00365.2012 53 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability. <i>X</i> andomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 54 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & quot;Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 			
 48 Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab</i> <i>Disord</i> 2000;24:38–48.http://www.ncbi.nlm.nih.gov/pubmed/10702749 49 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 50 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 51 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 52 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 53 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 54 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 			
 Finit a, Raden a, Dinteri JJ, <i>et al.</i> Reproductionity, power latit valuation of visual analogae scales in assessment of appetite sensations in single test meal studies. <i>Int J Obes Relat Metab Disord</i> 2000;24:38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749 Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & quot;Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 		10	
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 myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016;26:387–92. doi:10.1016/j.numecd.2016.03.012 Grothues F, Smith GC, Moon JCC, <i>et al.</i> Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & quot;Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 	39	49	
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 Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. Eur Heart J Cardiovasc Imaging 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. Am J Physiol - Hear Circ Physiol 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 Kumarathurai P, Anholm C, Larsen BS, et al. Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. Diabetes Care 2017;40:117–24. doi:10.2337/dc16-1580 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & quot;Thrifty Substrate" Hypothesis. Diabetes Care 2016;39:1108–14. doi:10.2337/dc16-0330 			
 cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. <i>Am J Cardiol</i> 2002;90:29–34. doi:10.1016/S0002-9149(02)02381-0 Morton G, Jogiya R, Plein S, <i>et al.</i> Quantitative cardiovascular magnetic resonance perfusion imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012;13:954–60. doi:10.1093/ehjci/jes103 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes</i> <i>Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & quot;Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 		50	
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 Ahtarovski KA, Iversen KK, Lønborg JT, <i>et al.</i> Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 53 53 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 54 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & "Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 			
 Antarovski RA, iversen RK, Eginoorg 51, et al. Effect and ventredia infection during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. <i>Am J Physiol - Hear Circ Physiol</i> 2012;303:1469–73. doi:10.1152/ajpheart.00365.2012 53 Kumarathurai P, Anholm C, Larsen BS, <i>et al.</i> Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. <i>Diabetes Care</i> 2017;40:117–24. doi:10.2337/dc16-1580 54 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A & "Thrifty Substrate" Hypothesis. <i>Diabetes Care</i> 2016;39:1108–14. doi:10.2337/dc16-0330 		50	
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58 doi:10.2337/dc16-0330			
	60		

- Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: Glucose excretion and lipid mobilization - not storage - saves lives. J Diabetes Complications 2016;30:753. doi:10.1016/j.jdiacomp.2016.02.015 The Lancet Diabetes Endocrinology. Getting to the heart of the matter in type 2 diabetes. lancet Diabetes Endocrinol 2015; 3:827. doi:10.1016/S2213-8587(15)00384-8 DeFronzo RA. The EMPA-REG study: What has it told us? A diabetologist's perspective. J Diabetes Complications 2016;30:1-2. doi:10.1016/j.jdiacomp.2015.10.013 Nirengi S, Peres Valgas da Silva C, Stanford KI. Disruption of energy utilization in diabetic cardiomyopathy; a mini review. Curr. Opin. Pharmacol. 2020;54:82-90. doi:10.1016/j.coph.2020.08.015 DeFronzo RA. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. Diabetes 2009;58:773-95. doi:10.2337/db09-Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest 2014;124:509-14. doi:10.1172/JCI70704 Stanley WC, Lopaschuk GD, McCormack JG, Regulation of energy substrate metabolism in the diabetic heart. Cardiovasc Res 1997;34:25-33.http://www.ncbi.nlm.nih.gov/pubmed/9217869 (accessed 3 Feb 2016). Nolan CJ, Ruderman NB, Kahn SE, et al. Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. Diabetes 2015;64:673-86. doi:10.2337/db14-0694 Taegtmeyer H, Beauloye C, Harmancey R, et al. Comment on Nolan et al. Insulin Resistance as a Physiological Defense Against Metabolic Stress: Implications for the Management of Subsets of Type 2 Diabetes. Diabetes 2015;64:673-686. *Diabetes* 2015;64:e37. doi:10.2337/db15-0655 Taegtmeyer H, Beauloye C, Harmancey R, et al. Insulin resistance protects the heart from fuel overload in dysregulated metabolic states. Am J Physiol Heart Circ Physiol 2013;**305**:H1693-7. doi:10.1152/ajpheart.00854.2012 Rau M, Thiele K, Hartmann NUK, et al. Empagliflozin does not change cardiac index nor systemic vascular resistance but rapidly improves left ventricular filling pressure in patients with type 2 diabetes: a randomized controlled study. Cardiovasc Diabetol 2021;20. doi:10.1186/s12933-020-01175-5 Lauritsen KM, Nielsen BRR, Tolbod LP, et al. SGLT2 Inhibition Does Not Affect Myocardial Fatty Acid Oxidation or Uptake, But Reduces Myocardial Glucose Uptake and Blood Flow in Individuals With Type 2 Diabetes- a Randomized Double-Blind, Placebo-Controlled Crossover Trial. Diabetes 2020;70:db200921. doi:10.2337/db20-0921 Oldgren J, Laurila S, åkerblom A, et al. Effects of 6 weeks treatment with dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, on myocardial function and metabolism in patients with type 2 diabetes: a randomized placebo-controlled exploratory study. *Diabetes, Obes* Metab 2021;:dom.14363. doi:10.1111/dom.14363 Hiruma S, Shigiyama F, Hisatake S, et al. A prospective randomized study comparing effects of empagliflozin to sitagliptin on cardiac fat accumulation, cardiac function, and cardiac metabolism in patients with early-stage type 2 diabetes: the ASSET study. Cardiovasc
 - Diabetol 2021;20. doi:10.1186/s12933-021-01228-3 Deacon CF. A review of dipeptidyl peptidase-4 inhibitors. Hot topics from randomized controlled trials. Diabetes, Obes. Metab. 2018;20:34–46. doi:10.1111/dom.13135

For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

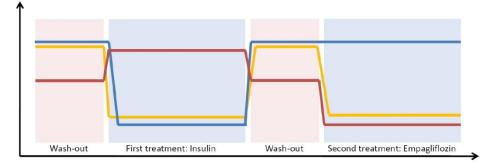
- Nordsborg N, Timmerman M. Testmanual - patientinterview og konditionstest. 2.0. København: : Sundhedsstyrelsen 2006.

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	w	wk ash out	ru	wk In in mpa		3 we wash			Random		5 weeks empagliflozin 25 mg x 1			-	3 week wash out		-	→ 5 weeks NPH Insulin					
Time ± 1 (weeks)	0	2		4		6	7			8		10		12		14	15		16		18		20
Visits	V 0						V 1							V 2			V 3						V 4
Phone contacts (PC)		PC 1		PC 2		PC 3				PC 4		PC 5				PC 6			PC 7		PC 8		

Figure 1. Study outline

169x61mm (220 x 220 DPI)

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407x150mm (96 x 96 DPI)

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THE ROLE OF HYPERGLYCEMIA, HYPERINSULINEMIA AND ELEVATED FREE FATTY ACIDS FOR CARDIAC FUNCTION IN PATIENTS WITH TYPE 2 DIABETES - THE HYPERCARD2 STUDY

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ABSTRACT

Introduction: Type 2 diabetes (T2D) is characterised by elevated plasma glucose, free fatty acid (FFA) and insulin concentrations, and this metabolic profile is linked to diabetic cardiomyopathy, a diastolic dysfunction at first, and increased cardiovascular disease (CVD) risk. Shifting cardiac metabolism towards glucose utilisation has been suggested to improve cardiovascular function and CVD risk, but insulin treatment increases overall glucose oxidation and lowers lipid oxidation, without reducing CVD risk, whereas SGLT-2 inhibitors (SGLT-2i) increase FFA, ketone body concentrations and lipid oxidation while decreasing insulin concentrations and CVD risk. The aim of the present study is to elucidate the importance of different metabolic profiles obtained during treatment with a SGLT2i versus insulin for myocardial function in patients with T2D.

Methods and analyses: Randomised, cross-over study, where 20 patients with T2D and BMI >28 kg/m2 receive 25 mg empagliflozin qd or NPH insulin bid first for 5 weeks followed by a 3-week washout before crossing over to the remaining treatment. Insulin treatment is titrated to achieve similar glycaemic control as with empagliflozin. In those randomised to insulin first, glycaemia during an initial empagliflozin run-in period prior to randomisation serves as target glucose. Metabolic and cardiac evaluation is performed before and at the end of each treatment period.

The primary endpoint is change (treatment – washout) in left ventricular peak filling rate, as assessed by cardiac MR (CMR) with and without acute lowering of plasma FFAs with acipimox. Secondary and explorative endpoints are changes in left atrial passive emptying fraction, left ventricular ejection fraction, central blood volume and metabolic parameters.

Ethics and dissemination: This study is approved by the Danish Medicines Agency, the Danish Data Protection Agency and the Capital Region Ethics Committee. The trial is conducted in accordance with ICH-GCP guidelines and the Helsinki Declaration. Trial registration number: EudraCT: 2017-002101.

STRENGTHS AND LIMITATIONS

- Comparison with NPH Insulin, which has opposite metabolic effects to empagliflozin, provides a strong basis for detecting metabolic effects on cardiac function.
- Repeated cardiac MR, during depletion of plasma FFAs with acipimox during treatments and washouts allows for dissection of the individual roles of hyperglycaemia, hyperinsulinemia and elevated free fatty acids on cardiac function in T2D.
- A cross-over over design is vulnerable to dropout, but provides greater statistical power
- Effects of metabolic changes on cardiac function are limited to the 5-week intervention period, which excludes effects arising from longer-term treatment.

INTRODUCTION

Type 2 diabetes (T2D) is characterised by hyperglycaemia, hyperinsulinemia, increased free fatty acids and impaired tissue glucose uptake and oxidation [1]. T2D is associated with an increased cardiovascular morbidity, and the more dysregulated the metabolic state, the greater the cardiovascular risk [2–5]. T2D develops when insulin secretion can no longer compensate for the ambient insulin resistance, and therefore previous treatments has focused on increasing insulin signalling by either exogenous insulin administration, stimulation of endogenous insulin secretion or enhancing insulin sensitivity [1].

Diabetic cardiomyopathy (DCM), is an early "silent" complication to T2D, independent of hypertension and/or coronary heart disease. It is characterised by left ventricular (LV) hypertrophy and diastolic dysfunction [6,7] and has been linked to the increased cardiovascular risk in T2D [8]. DCM may be accurately described by measuring left ventricular peak filling rate (LVPFR) and left ventricular ejection fraction (LVEF) using cardiac magnetic resonance imaging (CMR) [9,10]. Both diastole and systole are energy requiring processes and sensitive to changes in energy availability [11,12]. Interestingly, cardiac metabolism in patients with T2D is altered and depends more on lipid oxidation and less on glucose oxidation compared to non-diabetic controls [13,14]. It has been argued that glucose oxidation is a better source of energy for the heart than lipid oxidation, especially during stress such as myocardial ischemia, because this yields more ATP pr. unit oxygen [15]. However, manipulating cardiac metabolism towards glucose oxidation, by administering glucose-insulin (-potassium) infusions in patients with hyperglycaemia and myocardial infarction has been attempted, but did not improve survival in neither diabetic nor non-diabetic patients [16– 18]. In intensive care unit patients, strict glycaemic control using insulin has been associated with increased mortality [19], and in patients with T2D and increased CVD risk, intensive glycaemic control has not reduced CVD risk compared to conventional glycaemic control [20–23] and in the ACCORD study, which involved aggressive insulin treatment resulted in excess mortality [24]. Thus, insulin treatment does not prevent cardiovascular events in patients with T2D nor improve prognosis when such occur [25].

SGLT2-inhibition (SGLT2i), on the other hand, is a newer treatment principle in T2D, which has proven effective in attenuating the risk of myocardial infarctions, worsening of heart failure, cardiovascular mortality and all-cause mortality in patients with T2D [26–28]

SGLT2i increases renal glucose excretion thereby lowering plasma glucose and insulin levels and increasing glucagon release, lipolysis and ketogenesis [29,30]. Additionally, tissue glucose uptake and oxidation is reduced and hepatic glucose production increased [31]. The exact cardioprotective mechanisms of SGLT2i are not yet understood, but has been proposed to be linked to improved haemodynamics [32], inhibition of myocardial Na⁺/H⁺ exchange [33,34] or reductions in inflammatory activity [35,36].

An early and interesting hypothesis proposed that changes in cardiac metabolism may be responsible for the cardioprotective effect of SGLT2i. The lowered glucose and insulin

concentrations, persistent hyperketonaemia and elevated free fatty acids, caused by SGLT2i treatment, leads to reduced glucose uptake, increased ketone body uptake and oxidation and unchanged uptake of free fatty acids in the heart while overall lipid oxidation is increased [37,38]. This altered energy metabolism may rapidly improve myocardial function, especially during myocardial stress [39–42]. The SGLT2i induced myocardial fuel switch from glucose to fatty acids and ketone bodies, has been suggested to ameliorate adverse cardiac remodelling and heart failure in nondiabetic porcine models [43], and it is noteworthy that eliminating the availability of free fatty acids to insulin resistant hearts can lead to cardiac dysfunction in rodents and in humans, suggesting an important role for lipid metabolism in cardiac function [44–47]. Cardiovascular endpoint trials with SGLT2 inhibitors have shown effects within weeks after initiation of treatment, coinciding with the metabolic effects of the treatment [26,38]

Altogether, SGLT2 inhibitors "amplify" some components of the dysmetabolic profile of T2D and works opposite the metabolic effects of insulin. This raises the question of how cardiac function in patients with T2D depends on lipid and glucose oxidation in the resting state and during stress, and how increasing or lowering blood glucose, free fatty acids, ketone bodies and insulin concentrations influence cardiac function.

Objective

The primary objective of the present study is to evaluate myocardial function in patients with T2D and high risk of CV events using advanced cardiac magnetic resonance imaging (CMR) scans during rest, chronotropic stress and under depletion of plasma free fatty acids before and after 5 weeks of empagliflozin treatment (high free fatty acid and ketone body concentrations, high lipid oxidation and low insulin concentrations) and before and after 5 weeks of human insulin treatment titrated to yield glycaemic control similar to the empagliflozin treatment period (low free fatty acid and ketone body concentrations, high insulin concentrations and glucose oxidation).

Hypothesis

We hypothesise that hyperinsulinemia and hyperglycaemia are conditions that negatively affect cardiac function in T2D, while the availability of free fatty acids and ketone bodies and switching metabolism towards lipid oxidation improves cardiac diastolic and systolic function. Thus, we expect that lowering plasma glucose insulin-independently, and increasing fatty acid concentrations, lipid oxidation and ketone body availability with empagliflozin treatment, improves myocardial function in patients with T2D, and that depleting plasma of free fatty acids during empagliflozin treatment will impair cardiac function.

METHODS AND ANALYSES

Design

This is a 20-week prospective, investigator-initiated, comparator controlled, open label, 2-arm cross-over, human study where subjects are randomised in blocks of 3-5 to NPH insulin or Empagliflozin treatment (25 mg once daily) for 5±1 weeks, followed by 3±1 weeks wash-out and

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 cross-over of treatment for 5±1 weeks (figure 1). For 7 weeks preceding randomisation, but after inclusion, patients undergo a program of 2 weeks of washout of pre-existing antiglycaemic treatment (except metformin), 2 weeks of empagliflozin run-in (used for glycaemic target and titration of treatment in participants randomised to insulin first, see below) followed by 3 weeks of wash-out. During run-in and treatment periods, participants measure blood glucose twice daily (fasting and before evening meal), and during washouts patients measure fasting blood glucose. After the screening visit (V0) there are four study visits (V1-4) – before and at the end of each treatment period. Each visit consists of three study days – a metabolic study day (MET) and two CMR study days. Randomisation is performed at V1 after the metabolic study day.

Participants

Twenty subjects older than 18 years diagnosed with T2D, a BMI \geq 28 kg/m², HbA1c \leq 9%, fasting C-peptide >500 pmol/L and unchanged antiglycaemic treatment for 12 weeks prior to screening, and who are at a risk of cardiovascular disease (CVD), are eligible for the study. High CVD risk is modified from the EMPA-REG protocol [48]. Inclusion, exclusion and withdrawal criteria are listed in Box 1.

Recruitment

Participants are recruited from the Department of Endocrinology and Cardiology at Hvidovre Hospital and are identified by reviewing laboratory results and patient files. Potential participants will be contacted by means of a recruitment letter, in which they are informed of the opportunity to participate in a scientific research project. We also will advertise for participant in local newspapers and on the internet as well as social media (e.g. www.forsøgsperson.dk; www.sundhed.dk and www.facebook.com).

Outcomes

The primary outcome is change in myocardial diastolic function. This was chosen because firstly, diastole is a highly energy requiring process [11,12], and secondly, because diastolic dysfunction (with or without LV hypertrophy) is the notable early manifestation of diabetic cardiomyopathy [49]. Thus, if changes in overall energy metabolism are to affect cardiac function in patients with T2D, it may well occur in diastole at the earliest. Diastolic cardiac function can be accurately assessed using CMR by measuring left ventricular peak filling rate (LVPFR) and left atrial passive emptying fraction (LAPEF) [50]. Our primary outcome measure is change (LVPFR_{treatment} – LVPFR_{wash-out}) in LVPFR (ΔLVPFR). All endpoints are listed in Box 2.

Randomisation and intervention

Participants are randomised consecutively by lottery in blocks of 3-5 to treatment with either subcutaneous NPH insulin (Insulatard®) twice daily or oral empagliflozin (Jardiance®) 25 mg once daily first. All patient will receive both treatments during the trial. Randomisation is performed at V1. NPH insulin is initiated at a dose of 0.2 IU/kg body weight/day and is titrated daily over phone (phone contacts, figure 1) by 0.05 IU/kg body weight/day until average blood glucose over three consecutive days is within ± 1 mmol/L of the individual glycaemic target. In participants randomised to insulin first, the glycaemic target is average fasting and evening glucose concentrations during the second week of empagliflozin run-in. In patients randomised to insulin second, the glycaemic target is average fasting and pre-prandial evening BG values of week 3 and 4 during the first (empagliflozin) treatment period.

As previously discussed, insulin and empagliflozin represents two metabolically opposing methods for lowering plasma glucose concentrations. By titrating insulin treatment to match the glycaemic control found with empagliflozin in the same participants, the result is two distinct metabolic phenotypes: one with hyperinsulinemia and suppressed levels of FFAs (NPH insulin treatment), and one with reduced insulin levels and increased levels of FFAs (empagliflozin treatment) - but both with the same levels of glycaemic control (Figure 2). NPH Insulin has been chosen over more modern human insulin analogues, as it is not albumin bound and can be measured in an ordinary insulin assay.

Safety considerations

 During washout periods blood glucose concentrations will increase – that is a separate point of the study, but severely dysregulated diabetes is an exclusion criterium to ensure participant safety. The risk of severe hyperglycaemia is reduced in several ways in the study:

- Existing metformin treatment is continued throughout the whole study as background antiglycaemic treatment.
- In case of fasting BG concentrations of more than 13 mmol/L, patients are instructed to contact study personnel.
- Phone contacts by study investigator are planned in the second week of washout periods to follow up on the patient and enquire to hyperglycaemic events or other adverse events.
- As soon as the final day (CMR with acipimox) of a washout visit (visit 1 or 3) is completed, antiglycaemic treatment according to study drug sequence is commenced to minimise time spent in hyperglycaemia.

In case of fasting BG > 13 mmol/L, the patient will be contacted daily for two additional days. If average fasting BG over the 3 days > 13 mmol/L that triggers an extra safety visit, where fasting plasma glucose (PG) is measured. If PG > 13 mmol/L on the day of the extra visit, then the patient is withdrawn from the study and antihyperglycaemic treatment is initiated.

Screening visit (V0)

Once oral and written informed consent is obtained by the study investigator, the screening procedure follows. Medical history is recorded, screening blood samples drawn, and an ECG, recording of blood pressure, pulse rate and registration of anthropometric data are performed, and patients are screened according to in- and exclusion criteria. A standard transthoracic echocardiography is performed, and VO2max is estimated (Box 3).

Study visits

All study visits consist of three study days – a metabolic study day and two CMR study days (Box 4).

The metabolic study day

The metabolic study is conducted at the Department of Endocrinology, Hvidovre Hospital, to document the metabolic effects of each study drug.

Participants meet in the morning after an overnight fast. Anthropometric data, blood pressure, pulse rate and an ECG are recorded, and two catheters, one in each arm are inserted for infusion of tracers and for repeated drawing of arterialised blood samples respectively. Baseline and safety blood samples are taken (Box 5), the participant empties bladder and the investigational drug (V2, V4) and the participants usual medications are administered at 0800h. Body composition is determined by Dual energy x-ray absorptiometry scan (DXA).

Basal metabolism

Primed infusions of stable glucose ($[6,6-D_2]$ -glucose) and glycerol ($[1,1,2,3,3-D_5]$ -glycerol) tracers are initiated (T=-180 min). Blood is sampled at -30, -15 and -2 min to characterise glucose, lipid and amino acid metabolism. The patient empties bladder, urine is weighed, and samples are taken for determination of tracer concentrations and urinary nitrogen excretion, and the 5h-OGTT is initiated.

5h-OGTT

The patient ingests anhydrous glucose (72 g) with added [U-13C6]-glucose tracer (3 g) dissolved in 250 mL of water over 5 minutes (T=0 min). Intravenous tracer infusions continue unchanged. Blood is sampled regularly for 5 hours for characterisation of postprandial glucose, lipid and amino acid metabolism (Box 4 and 5). The patient empties bladder regularly during and at the end of the OGTT. Urine is sampled for nitrogen excretion and tracers/tracees.

Fat and muscle biopsies: Biopsies are obtained during the basal (T=-60 min) and the maximally insulin stimulated (T=60 min) state. Muscle biopsies are considered proxies for cardiomyocyte metabolic status. 30 min ventilated hood indirect calorimetry (Vyaire Vyntus® CPX) is performed during the basal period (prior to biopsies) t= -90 min and postprandially at t=60 min for determination of fasting and postprandial energy expenditure and respiratory quotient.

Exercise test (50% VO2max)

At T=300 min, the participant is exercised at 60 W for 4 minutes after which work load is increased until oxygen consumption is 50% of estimated VO2max. Pulse rate is recorded with a chest mounted pulse rate monitor and oxygen consumption with a mask mounted indirect calorimeter. Blood is sampled to characterise glucose, lipid and amino acid metabolism (Box 4 and 5). After 30 min, VO2max is estimated by increasing workload by 50W until a pulse rate increase of 30. When pulse is steady for 2 min, oxygen consumption and pulse rate are recorded, and the test is stopped. VO2max is estimated by linear extrapolation to the theoretical maximum pulse rate (220-age).

Ad libitum meal test

SGLT2 inhibition is associated with a lower weight reduction than predicted from the urinary energy loss. SGLT2 inhibition does not change resting energy expenditure or blunt the thermogenic

effect of feeding, suggesting that energy intake is increased [38]. Therefore, the metabolic study day is ended with an ad *libitum meal*, consisting of thoroughly mixed pasta bolognese (fixed nutrient composition and energy content). Patients are placed in a quiet corner and instructed to eat until full. Two glasses of water (total 300 mL) are allowed with the meal. The meal is weighed before and after serving and ad libitum meal intake defined as the difference. Throughout the day patients are asked to score their hunger, satiety and sensation of fullness on a visual analogue scale [51].

Cardiac evaluation

Two CMR days are performed during each visit (V1-V4). In addition, diurnal blood pressure and Holter monitoring are performed. CMR is conducted at the Department of Cardiology, Rigshospitalet, Copenhagen, whereas Holter monitoring and diurnal blood pressure monitoring are performed at the Department of Cardiology, Hvidovre Hospital.

Participants meet fasting and morning medication, including investigational medicinal product (V2 and V4), is administered. Anthropometric data are recorded, and two intravenous catheters are inserted into an antecubital and the contralateral dorsal hand vein for infusion of adenosine, gadolinium contrast and glycopyrrolate and for blood sampling respectively (Box 5). Prior to CMR a transthoracic echocardiography is performed (Box 3)

CMR is performed on a 1.5 Tesla scanner (Siemens Aera; Siemens; Erlangen; Germany) with the patient lying supine on the back, using an 18-channel cardiac coil with continuous ECG gating.

Cine 2-, 3- and 4-chamber images, complete transverse and short axis cine stacks covering the whole heart are acquired. All images are obtained during end-expiratory breath-holds. Myocardial perfusion images during rest and stress are obtained at the basal, mid-ventricular and apical cardiac short-axis level. Rest perfusion images of the myocardium is acquired using an intravenous bolus of gadolinium contrast (Gadovist®, Bayer AG, Germany) 0.075 mmol/kg bodyweight. The time of gadolinium contrast entry into the right and the left ventricle is accurately determined, and this transit time of gadovist multiplied by cardiac output is used to calculate the pulmonary and central (pulmonary + cardiac) blood volume.

Myocardial stress perfusion images are obtained with an i.v dose of 0.075 mmol/kg of gadovist during and 10 min after an intravenous adenosine (140 ug/min) administrated for maximum 4 minutes). This is followed by evaluation of cardiac function during chronotropic stress, where short axis cine stack will be reacquired 10 minutes after the administration of intravenous glycopyrrolate (4 μ g/kg, max. 400 μ g, given as a bolus). This approach has been shown to unmasque subclinical diastolic dysfunction as has been demonstrated in normal healthy elderly [50].

Cardiac MRI, Acipimox

CMR scans will follow the same procedure as described above, but participants are instructed to ingest 250 mg acipimox p.o. twice, 4 hours before and right before the scan, to determine

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myocardial function. This repeated administration of acipimox is required for adequate suppression of hormone sensitive lipase activity and depletion of plasma FFAs (28). This has been shown to gradually impair cardiac function [52], and is done to disclose any coupling between FFA availability and cardiac function.

CMR image analysis

Is performed using Circle42 (Circle Cardiovascular Imaging Inc., Calgary Canada, v5.5.1). LV volumes, LV mass, LV ejection fraction (LVEF) and LV peak filling rate are determined by tracing of the endo- and epicardial contours in end-diastolic and end-systolic phases. The papillary muscles are excluded from the myocardium. On native and post-contrast T1-mappings, endocardial and epicardial borders are traced, and the mean extra cellular volume (ECV) is calculated from areas outside late gadolinium enhancement (LGE) lesions. For determination of the ECV within an LGE lesion, myocardium without LGE in the segment is excluded. Myocardial perfusion scans are inspected for perfusion defects. Regions with infarctions, sub-endocardial perfusion defects or darkrim artefacts will be excluded. Blinded to clinical data, the analyses will be reviewed and finalised by two CMR specialist.

Diurnal blood pressure and Holter monitoring

Between study days during each visit, diurnal blood pressure is recorded (ScottCare, ABP 320, Cleveland, OH)) for 24 hours with 15 minutes intervals between 6.00 to 22.00- and 60-min intervals during night-time. Cardiac rhythm is evaluated with Holter monitoring (SCOTTCARE, CHROMA, model RZ153C, Cleveland, OH) for 48 hours.

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Patient and public involvement No patient involved.

ANALYSES

Blood and tissue samples

Subcutaneous fat- and muscle biopsies: Local analgesia is applied before sampling with a Bergströms cannula. Samples are immediately frozen in liquid nitrogen and stored at -80°C. Blood and urine samples: Samples are spun, aliquoted and stored at -20°C (GLP-1, PYY, Glucagon) or – 80°C for later analysis. Bedside plasma glucose measurements are performed using the glucose oxidase technique (YSI model 2300 STAT Plus; YSI,Yellow Springs, OH). Home blood glucose measurements are carried out on Contour XT (Ascensia Diabetes Care Holdings AG, Basel, Switzerland). Safety blood- and urine samples are analysed on the same day at the Department of Clinical Biochemistry, Hvidovre Hospital.

- Statistical methods
- Sample size calculation

Measures of myocardial function are highly reproducible when assessed using CMR, and interstudy and cohort coefficients of variation are in the range of 3-5% [53–55]

Using the same CMR protocol as the present, Ahtarovski et al found a mean difference of 92 ml/s in Left Ventricular Peak Filling Rate between healthy young (585±62 ml/s) and healthy elderly

subjects (493±55 ml/s) [55]. We assume that T2D patients have LVPFR corresponding to healthy elderly subjects, and we assume that empagliflozin treatment improves LVPFR by 30 ml/s (Δ LVPFR=30 ml/s) from baseline and that insulin treatment does not improve LVPFR (Δ LVPFR=0 ml/s).

Conservatively setting the standard deviation of between treatment differences of Δ LVPFR at 30 ml/s, a number of 20 patients would be adequate to determine a 30 ml/s difference between the two treatments with a power of 93% and a two-sided significance level of 0.01, when evaluating data with the paired student's t-test. In case of a 20% dropout rate, power would still be acceptable (83%, p=0.01).

Statistical analysis plan

The primary and secondary endpoints are analysed assuming no period effect or treatment-period interaction. This assumption is reasonable, given results from similar studies, where no such interactions or effects have been reported [56]. Normally distributed data are presented using standard descriptive statistics, and reported as mean (SD) for normally distributed and median (Q1;Q3) for non-normally distributed data. Likewise, comparisons of normally distributed data is done using the paired Student's t-test for all completers, whereas Wilcoxon's paired signed rank test will be used if data is non-normally distributed.

Ethics and dissemination

The study is conducted according to ICH GCP guidelines E6 (R2) and registered with the Danish Medicines Agency (ref. id: 2017061587), The Capital Region Ethical Committee (H-17018846) and the Danish Data Protection Agency (2012-58-0004; AHH-2017-093, I-Suite nr.: 06012). Our results, regardless of outcome, will be published in relevant scientific journals. In addition, we will seek to disseminate results through presentations at scientific meetings. Publication will take place as soon as scientifically feasible. No later than 12 months after unregistering of the study, will results be made available at www.clinicalregister.eu.

Timeframe

Screenings are performed from January 2018. Last patient, last visit is expected second half of 2021 after which the study will be unregistered with the Danish Medicines Agency and the Capital Region Municipal Ethical Committee within 90 days. Data analyses are expected to be completed by Winter 2022.

DISCUSSION

The profound and swift benefits of SGLT2i on cardiovascular risk in T2D have inspired the discussion of metabolism and its importance for cardiac function in patients with T2D [57–61]. Especially since, SGLT2 inhibitors have metabolic effects that by and large are opposite to those of insulin treatment. Thus, insulin treatment is associated with increased tissue glucose uptake and utilisation, but suppression of lipid mobilisation and oxidation as well as lowering of plasma concentrations of ketone bodies [62]. SGLT2 inhibitors increase lipid mobilisation and oxidation, increase plasma ketone body concentrations and reduce tissue glucose uptake [38,63]. Both treatments lower plasma glucose, but insulin treatment increases whereas SGLT2i treatment decreases plasma insulin concentrations. Whether such changes in metabolism affect cardiac function, is still unsettled, but forcing cardiac glucose uptake and utilisation through insulin

treatment has been suggested by some to benefit and by others to impair cardiac function [47,64– 67], while yet others have suggested increased lipid and ketone body oxidation to be important for proper cardiac function in T2D [46,58,67]. Studies on SGLT2i treatments and the effects on cardiac function are beginning to emerge. In a recent study, 42 patients with T2D were randomised to 12 weeks of empagliflozin 10 mg or placebo once daily. SGLT2 inhibition was shown to rapidly improve diastolic cardiac function as evaluated with echocardiography [68]. In a placebo controlled cross-over design, after 4 weeks of empagliflozin treatment in patients with T2D, myocardial glucose uptake was reduced and fatty acid oxidation unaltered, but this did not significantly change myocardial oxygen consumption or cardiac efficiency, nor any measure of cardiac function [69]. In a Swedish study, 6 weeks dapagliflozin treatment showed unchanged cardiac fatty acid uptake, a trend toward reduced left atrial maximal volume, and reduced LV oxygen consumption and external work compared to placebo [70], and in the only study found, where an active comparator was used, 10 mg empagliflozin once daily for 12 weeks did not change cardiac lipid accumulation (as measured by MR spectrometry), cardiac function or cardiac metabolism compared to sitagliptin 50 mg daily [71].

In conclusion, existing studies in humans have shown divergent results regarding changes in cardiac diastolic function with little changes in cardiac metabolism. However, most studies have compared cardiac effects of SGLT2i to placebo, thus not accounting for the circumstances that characterised the EMPA-REG trial, where anti glycaemic treatment was intensified in the placebo group concurrently [26]. Thus, the CVD risk benefits of the study may have arisen from unfavourable metabolic consequences of the treatment in the placebo arm. In the one study with an active comparator empagliflozin was compared to sitagliptin, which not only affects the incretin system but also has less specific metabolic effect [72]. Therefore, to date our study, is the one to most directly pursue the coupling between metabolism and cardiac function, by choosing insulin as the comparator, and by including the effects of acute lowering of free fatty acid concentrations in plasma on cardiac function.

Disclosure summary

All authors have completed the ICMJE uniform disclosure form and declare: no support from any organisations for the submitted work; SM and NBJ have received research grants from Boehringer Ingelheim and JJH serves on advisory boards for Novo Nordisk, no other relationships or activities that could appear to have influenced the submitted work.

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Contributions

Author contributions

Roopameera Thirumathyam (RT) will be conducting the study, collect all the data, perform data analyses and co-submit the study.

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Erik A. Richter has contributed to the study design, designed the keton body- and biopsy analysis

Iona D	and is going to perform these analyses.
Jens P. (analyses	Goetze designed the pro-ANP and pro-BNP analysis scheme and is going to perform these
2	Fenger designed multiple metabolic analysis schemes (Triglycerides, insulin, free fatty
-	ric acid, urea and nitrogen) and is going to perform these analyses.
	an Hall designed the three-tracer measuring analysis schemes we are using in this study, and
	to perform these analyses.
0 0	ixen provided the study with analysis tools (holter-monitors and blood pressure monitors).
	perform the analyses of these data.
lens Jui	Il Holst participated in the protocol design, designed multiple metabolic analyses (GLP-1,
glucago	n and PYY) and will perform the analyses of these data.
Sten Ma	adsbad co-planned the study and contributed with several analysing tools.
	ejlstrup co-designed the cardiac MRI guideline for the study and contributed with analysing RI scanner).
	Madsen co-planned the study, co-designed the cardiac MRI guideline for the study and will the cardiac MRI data.
2	un Jørgensen co-planned the study, will perform all data analyses with RT and co-submit
	ne above mentioned authors contributed with writing and revising this manuscript.
	Eligibility criteria
Inclusi	on criteria
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Inclusi Age ≥ BMI ≥ HbA1¢ Fasting	on criteria 18 years 28 kg/m ² $c \le 9\%$ ($\le 10\%$ in diet or metformin treated only) g C-peptide ≥ 500 pmol/L
Inclusi Age ≥ BMI ≥ HbA1c Fasting Uncha	on criteria 18 years 28 kg/m ² $c \le 9\%$ ($\le 10\%$ in diet or metformin treated only) g C-peptide ≥ 500 pmol/L nged glycaemic treatment for 3 months prior to inclusion
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Inclusi Age ≥ BMI ≥ HbA1c Fasting Uncha	on criteria 18 years 28 kg/m ² $c \le 9\%$ ($\le 10\%$ in diet or metformin treated only) g C-peptide ≥ 500 pmol/L nged glycaemic treatment for 3 months prior to inclusion ardiovascular risk as one of the following: Previous myocardial infarction, stroke or peripheral arterial disease more than 2 months
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Inclusi Age ≥ BMI ≥ HbA10 Fasting Uncha High c • • • Excluss Insulin	on criteria 18 years 28 kg/m ² $c \le 9\%$ ($\le 10\%$ in diet or metformin treated only) g C-peptide ≥ 500 pmol/L nged glycaemic treatment for 3 months prior to inclusion ardiovascular risk as one of the following: Previous myocardial infarction, stroke or peripheral arterial disease more than 2 months prior to informed consent Evidence of multi-vessel coronary arterial disease (CAD) but without prior myocardial infarction, if more than 50% stenosis is present, if revascularised (CABG or PCI) more than 2 months prior or if 1 vessel is vascularised and the other with 50% stenosis. Single vessel CAD without prior myocardial infarction if more than 50% stenosis is present, not revascularised and positive stress test for ischemia.

	orug or alcohol abuse within 3 months from informed consent
Pe	oor compliance
A	naemia (Hb < 6.4 mmol/L) or other blood dyscrasias causing haemolysis or unstable
er	rythrocytes.
In	idication of liver disease (ALT or Alkaline phosphatase elevation above 3x upper normal
	npaired renal function (eGFR<45 ml/min/1.73 m ²)
	nti-obesity medication within 3 months from informed consent
	ystemic steroid treatment within 6 weeks from informed consent.
-	ny uncontrolled endocrine disorder except T2D
	ariatric surgery or other gastrointestinal conditions that may compromise gastrointestinal
	bsorption
	eptic ulcer – verified endoscopically
	ny form of surgery within 3 months of informed consent
	cute myocardial infarction, stroke or peripheral arterial disease within 2 months of inform
	onsent.
P	ersistent or permanent atrial fibrillation
Ŧ	
	nability to undergo experimental procedures including exclusion criteria for CMR scannin
	nplantable cardioverter defibrillator/pacemaker
	erromagnetic clips
С	laustrophobia.
	ontraindication to glycopyrrolate infusion:
	nown closed-angle glaucoma
kı	nown severe prostate hyperplasia
T	achycardia (HR > 100 at rest)
K	nown bladder atony
С	ardia insufficiency or non-congenital pylorus stenosis -verified endoscopically
K	nown gastroparesis
С	ontraindications to adenosine: nd or 3rd degree atrioventricular block
21	nd or 3rd degree atrioventricular block
	evere hypotension (BP \leq 90/50 mmHg)
	ong QT syndrome
	Instable angina pectoris
	becompensated heart failure
	inus node dysfunction
	hronic obstructive pulmonary disease or asthma bronchiale (FEV1 \leq 50% of expected)
U.	mome obstructive puthonary disease of astima biohemate ($1 \ge v_1 \ge 30\%$ of expected)
٨	llargy towards any of the drugs or diagnostics used in the protocol (inculin, empediated)
	llergy towards any of the drugs or diagnostics used in the protocol (insulin, empagliflozi
	cipimox, glycopyrrolate, adenosine, gadolinium contrast enhancer).
	ny condition which in the opinion of the investigator may jeopardize subject safety or
	ompliance with the protocol.
Ŵ	Vithdrawal criteria

Subjects may withdraw from the study without any notice or reason
Pregnancy discovered during the experiment
Unacceptable adverse reactions or reactions associated with the planned experiments, including
severe glycaemic dysregulation during washout periods.

Box 2: Endpoints	
Primary end point	Change in left ventricular peak filling rate
	(Δ LVPFR)
Secondary endpoints	Change in left atrial passive emptying fraction
	(Δ LAPEF)
	Change in left ventricular ejection fraction
	(Δ LVEF)
Explorative endpoints include	Cardiovascular:
	Change in VO2max and exercise tolerance test
	variables
	Change in central blood volume and
	haematocrite
	Change in heart rate variability
	Change in left ventricular volume
	Metabolic:
	Basal and postprandial AUC Free Fatty Acids
	and glycerol turnover
	Endogenous glucose production and tissue
	glucose disposal (metabolic clearance of
	glucose)
	Fasting and postprandial energy expenditure
	and respiratory quotient
	Glucagon-Insulin ratio
	Insulin sensitivity (AUC glucose metabolic
	clearance / AUC insulin concentration)
	Beta-cell function (prehepatic insulin secretion
	rate, correlated to ambient glucose)

Box 3: Screening procedures	
Blood samples	Haematology (haemoglobin, thrombocytes,
	haematocrit, leukocytes), liver and renal
	function tests (creatinine, eGFR (Cockroft-
	Gault formula), alkaline phosphatases, alanine
	aminotransferases, lactate dehydrogenase,

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	bilirubin, amylase, sodium, potassium), fasting
	P-glucose, C-peptide, HbA1c, TSH, Urinary
	Albumin/creatinine mass ratio, and in fertile
	women, U-hCG.
Echocardiography	Parasternal long axis view, parasternal short
	axis view at aortic, mitral and apex levels,
	apical 4-chamber view, LVEF, E/E', E',
	LVEDV/BSA.
Estimation of VO2max	Maximum oxygen uptake is estimated using
	Åstrøm's two-point test performed on a cycle
	ergometer during indirect calorimetry. From
	measurements of VO2 at two sub-maximal
	pulse rates VO2max is estimated by linear
	extrapolation to the theoretical maximal pulse
	rate (220-age) [73].

rate (220-age) [73].		
Box 4: Visit overview	Cardiac MR	Cardia MD Asiaina an
Metabolic study day		Cardiac MR, Acipimox
- DXA-scan and fasting safety	- Fasting blood samples,	Same protocol as Cardiac MR
and efficacy blood samples - Determination of 3-hour	before and after CMR.	day, but during
basal metabolism.	- Echocardiography - CMR Rest	pharmacological suppression
Infusion of glucose and	- Without enhancement	of hormone sensitive lipase activity and depletion of
glycerol tracers	With enhancement and	plasma free fatty acids.
Basal muscle and fat	adenosine infusion	- 48h Holter monitoring.
biopsies	- CMR Stress	- 48h Hoher monitoring.
Basal energy expenditure	Unenhanced repeated during	
and determination of	pharmacological chronotropic	
respiratory quotient	stress with glycopyrrolate	
- 5-hour OGTT	infusion.	
with oral glucose tracer and	- 24h ambulant blood pressure	
continued intravenous		
glucose and glycerol tracer.		
Fat- and muscle biopsies at		
maximum insulin stimulation		
- Exercise test and		
determination of VO2max		
- Ad libitum meal.		

Box 5: Blood samples on metabolic and CMR st	udy days include
Metabolic study day	Blood samples: glucose, insulin, C-peptide,
	glucagon, FFAs, triglycerides, total amino
	acids and ketone bodies (betahydroxybuturate),
	tracers/tracees, gut hormones.
	HbA1c, urate, blood urea nitrogen, cortisol, is
	sampled at baseline.
Cardiac MRI days	Markers of cardiac function, including pro-
	ANP and pro-BNP, glucose, insulin, C-peptide,
	glucagon, FFAs, triglycerides, ketone bodies,
	haematocrit are drawn before and after CMR.
Figure legends	

Figure legends

Figure 1. Study outline. Included patients undergo a 7-week program of washout of pre-existing antiglycemic treatment (except metformin) and run-in of empagliflozin. Hereafter they are randomised to treatment for 5 ± 1 weeks, followed by 3 ± 1 weeks wash-out and cross-over to 5 ± 1 weeks treatment with the remaining study drug. Tests performed at each visit are summarised in Box 4 and 5.

Figure 2. Metabolic profile of the two study drugs. Schematic representation of the metabolic changes expected with the two study drug treatments in a patient randomized to insulin first. Insulin treatment is characterized by low glucose, low FFAs and high insulin concentrations; empagliflozin treatment by low glucose, high FFAs and low insulin.

References:

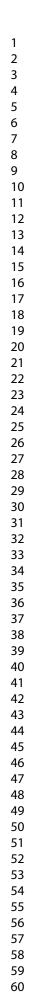
0	1	Defronzo RA. From the Triumvirate to the Ominous Octet : A New Paradigm for the
7 8	1	Treatment of Type 2 Diabetes Mellitus. ;:773–95. doi:10.2337/db09-9028
8 9	2	De Vegt F, Dekker JM, Ruhé HG, <i>et al.</i> Hyperglycaemia is associated with all-cause and
10	_	cardiovascular mortality in the Hoorn population: The Hoorn study. <i>Diabetologia</i>
11		1999; 42 :926–31. doi:10.1007/s001250051249
12	3	Pyorala M, Miettinen H, Laakso M, et al. Plasma insulin and all-cause, cardiovascular, and
13		noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study.
14		Diabetes Care 2000;23:1097–102. doi:10.2337/diacare.23.8.1097
15	4	Lakka HM, Lakka TA, Tuomilehto J, et al. Hyperinsulinemia and the risk of cardiovascular
16		death and acute coronary and cerebrovascular events in men: The Kuopio Ischaemic Heart
17		Disease Risk Factor Study. Arch Intern Med 2000;160:1160–8.
18	5	doi:10.1001/archinte.160.8.1160 Selvin E, Marinopoulos S, Berkenblit G, <i>et al.</i> Meta-analysis: Glycosylated hemoglobin and
19	5	cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141. doi:10.7326/0003-
20		4819-141-6-200409210-00007
21 22	6	Redfield MM, Jacobsen SJ, Burnett JC, <i>et al.</i> Burden of systolic and diastolic ventricular
22	U	dysfunction in the community: appreciating the scope of the heart failure epidemic. JAMA
24		2003; 289 :194–202. doi:10.1001/JAMA.289.2.194
25	7	Boyer JK, Thanigaraj S, Schechtman KB, et al. Prevalence of ventricular diastolic
26		dysfunction in asymptomatic, normotensive patients with diabetes mellitus. Am J Cardiol
27		2004; 93 :870–5. doi:10.1016/J.AMJCARD.2003.12.026
28	8	Jørgensen PG, Biering-Sørensen T, Mogelvang R, et al. Predictive value of
29		echocardiography in Type 2 diabetes. <i>Eur Heart J Cardiovasc Imaging</i> 2019; 20 :687–93.
30	0	doi:10.1093/ehjci/jey164
31	9	Tarquini R, Lazzeri C, Pala L, <i>et al.</i> The diabetic cardiomyopathy. <i>Acta Diabetol</i>
32	10	2011; 48 :173–81. doi:10.1007/s00592-010-0180-x
33	10	Komi S, Inoue Y, Hata H, <i>et al.</i> Cardiovascular magnetic resonance evaluation of left ventricular peak filling rate using steady-state free precession and phase contrast sequences.
34		Springerplus 2016;5. doi:10.1186/s40064-016-2878-x
35 36	11	Diamant M, Lamb HJ, Groeneveld Y, <i>et al.</i> Diastolic dysfunction is associated with altered
37		myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2
38		diabetes mellitus. J Am Coll Cardiol 2003;42:328-35. doi:10.1016/S0735-1097(03)00625-9
39	12	Rosano G, Coats A. Modulation of Cardiac Metabolism in Heart Failure. Int Cardiovasc
40		<i>Forum J</i> 2019; 17 :99–103. doi:10.17987/icfj.v17i0.597
41	13	Ungar I, Gilbert M, Siegel A, et al. Studies on myocardial metabolism. IV. Myocardial
42		metabolism in diabetes. Am J Med 1955;18:385–96. doi:10.1016/0002-9343(55)90218-7
43	14	Rijzewijk LJ, van der Meer RW, Lamb HJ, et al. Altered myocardial substrate metabolism
44		and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies
45		with cardiac positron emission tomography and magnetic resonance imaging. J Am Coll
46	15	<i>Cardiol</i> 2009; 54 :1524–32. doi:10.1016/j.jacc.2009.04.074 Stanley WC, Lopaschuk GD, Hall JL, <i>et al.</i> Regulation of myocardial carbohydrate
47	15	metabolism under normal and ischaemic conditions. Potential for pharmacological
48 49		interventions. Cardiovasc Res 1997; 33 :243–57. doi:10.1016/S0008-6363(96)00245-3
49 50	16	Mehta SR, Yusuf S, Díaz R, <i>et al.</i> Effect of glucose-insulin-potassium infusion on mortality
51		in patients with acute ST-segment elevation myocardial infarction: The CREATE-ECLA
52		randomized controlled trial. J Am Med Assoc 2005;293:437-46. doi:10.1001/jama.293.4.437
53	17	Malmberg K, Rydén L, Wedel H, et al. FASTTRACK intense metabolic control by means of
54		insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2):
55		Effects on mortality and morbidity. <i>Eur Heart J</i> 2005; 26 :650–61.
56	10	doi:10.1093/eurheartj/ehi199
57	18	Malmberg K, Rydén L, Efendic S, et al. Randomized trial of insulin-glucose infusion
58		followed by subcutaneous insulin treatment in diabetic patients with acute myocardial
59		infarction (DIGAMI study): Effects on mortality at 1 year. J Am Coll Cardiol 1995;26:57-
60		
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

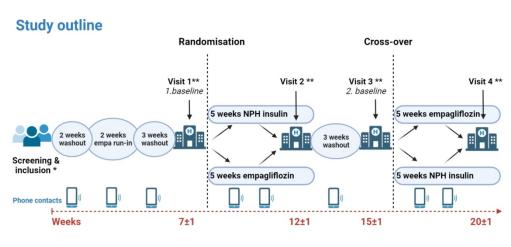
2		
3		
4		65. doi:10.1016/0735-1097(95)00126-K
5	19	NICE-SUGAR Study Investigators, Finfer S, Chittock DR, <i>et al.</i> Intensive versus
6	17	conventional glucose control in critically ill patients. <i>N Engl J Med</i> 2009; 360 :1283–97.
7		doi:10.1056/NEJMoa0810625
8	20	
9	20	Hemmingsen B, Lund SS, Gluud C, et al. Intensive glycaemic control for patients with type
10		2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised
11		clinical trials. BMJ
12		2011; 343 :d6898.http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3223424&tool=
		pmcentrez&rendertype=abstract (accessed 9 Sep 2015).
13	21	Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in
14		veterans with type 2 diabetes. N Engl J Med 2009; 360 :129–39.
15		doi:10.1056/NEJMoa0808431
16	22	ADVANCE Collaborative Group, Patel A, MacMahon S, et al. Intensive blood glucose
17		control and vascular outcomes in patients with type 2 diabetes. N Engl J Med
18		2008; 358 :2560–72. doi:10.1056/NEJMoa0802987
19	23	Basal Insulin and Cardiovascular and Other Outcomes in Dysglycemia. <i>N Engl J Med</i>
20	20	2012; 367 :319–28. doi:10.1056/nejmoa1203858
21	24	Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, et
22	27	<i>al.</i> Effects of intensive glucose lowering in type 2 diabetes. <i>N Engl J Med</i> 2008; 358 :2545–
23		59. doi:10.1056/NEJMoa0802743
24	25	
25	23	Sciences HH, Miller ME, Byington RP, <i>et al.</i> Effects of Intensive Glucose Lowering in Type
26	20	2 Diabetes. N Engl J Med 2008; 358 :2545–59. doi:10.1056/nejmoa0802743
27	26	Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, Cardiovascular Outcomes, and
28		Mortality in Type 2 Diabetes. N Engl J Med 2015; 373 :2117–28.
29		doi:10.1056/NEJMoa1504720
	27	McMurray JJV, Solomon SD, Inzucchi SE, et al. Dapagliflozin in Patients with Heart Failure
30		and Reduced Ejection Fraction. N Engl J Med 2019; 381 :1995–2008.
31		doi:10.1056/nejmoa1911303
32	28	Zou CY, Liu XK, Sang YQ, et al. Effects of SGLT2 inhibitors on cardiovascular outcomes
33		and mortality in type 2 diabetes: A meta-analysis. Med (United States) 2019;98.
34		doi:10.1097/MD.00000000018245
35	29	Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose
36		cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest 2014; 124 :499–508.
37		doi:10.1172/JCI72227
38	30	Merovci A, Solis-Herrera C, Daniele G, <i>et al.</i> Dapagliflozin improves muscle insulin
39	50	sensitivity but enhances endogenous glucose production. J Clin Invest 2014; 124 :509–14.
40		doi:10.1172/JCI70704
41	31	Ferrannini E, Baldi S, Frascerra S, <i>et al.</i> Renal Handling of Ketones in Response to Sodium –
42	51	Glucose Cotransporter 2 Inhibition in Patients With Type 2 Diabetes. 2017; 40 :771–6.
43		
44	22	doi:10.2337/dc16-2724
45	32	Chilton R, Tikkanen I, Cannon CP, et al. Effects of empagliflozin on blood pressure and
46		markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. <i>Diabetes</i>
47	~ ~	Obes Metab 2015;17:1180–93. doi:10.1111/dom.12572
	33	Trum M, Riechel J, Lebek S, et al. Empagli fl ozin inhibits Na + / H + exchanger activity in
48		human atrial cardiomyocytes. 2020;:4429–37. doi:10.1002/ehf2.13024
49	34	Baartscheer A, Schumacher CA, Wüst RCI, et al. Empagliflozin decreases myocardial
50		cytoplasmic Na + through inhibition of the cardiac Na + $/$ H + exchanger in rats and rabbits.
51		2017;:568–73. doi:10.1007/s00125-016-4134-x
52	35	Garvey WT, Gaal L Van, Leiter LA, et al. Effects of canagli fl ozin versus glimepiride on
53		adipokines and in fl ammatory biomarkers in type 2 diabetes \Rightarrow . 2018;85:32–7.
54		doi:10.1016/j.metabol.2018.02.002
55	36	Heerspink HJL, Perco P, Mulder S, <i>et al.</i> Canagliflozin reduces inflammation and fibrosis
56	20	biomarkers : a potential mechanism of action for beneficial effects of SGLT2 inhibitors in
57		diabetic kidney disease. 2019;:1154–66.
58	37	Lauritsen KM, Nielsen BRR, Tolbod LP, <i>et al.</i> SGLT2 Inhibition Does Not Affect
59	וכ	Launton Kivi, Incisch DKK, TOIOULLI, ei al. SOLTZ IIIIIOIIIOII DUCS NULAIICU
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4		Myocardial Fatty Acid Oxidation or Uptake, But Reduces Myocardial Glucose Uptake and
5		Blood Flow in Individuals With Type 2 Diabetes- a Randomized Double-Blind, Placebo-
6		Controlled Crossover Trial. Diabetes 2020;:db200921. doi:10.2337/db20-0921
7	38	Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose
8		cotransporter 2 inhibition in type 2 diabetic patients. <i>J Clin Invest</i> 2014; 124 :499–508.
9		doi:10.1172/JCI72227
10	39	Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: Glucose excretion and lipid mobilization
11		- not storage - saves lives. J Diabetes Complications 2016;30:753.
12 13		doi:10.1016/j.jdiacomp.2016.02.015
13 14	40	Ekanayake P, Hupfeld C, Mudaliar S. Sodium-Glucose Cotransporter Type 2 (SGLT-2)
14		Inhibitors and Ketogenesis: the Good and the Bad. Curr Diab Rep 2020;20.
16	4.1	doi:10.1007/s11892-020-01359-z
17	41	Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: A
18	10	thrifty substrate hypothesis. <i>Diabetes Care</i> 2016; 39 :1108–14. doi:10.2337/dc16-0330
19	42	Nielsen R, Møller N, Gormsen LC, et al. Cardiovascular Effects of Treatment With the
20		Ketone Body 3-Hydroxybutyrate in Chronic Heart Failure Patients. <i>Circulation</i>
21	12	2019;139:2129–41. doi:10.1161/CIRCULATIONAHA.118.036459
22	43	Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, <i>et al.</i> Empagliflozin Ameliorates
23		Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing
24	44	Myocardial Energetics. J Am Coll Cardiol 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056
25	44	Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces
26		myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i> 2016; 26 :387–92. doi:10.1016/j.numecd.2016.03.012
27	45	Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiac
28	43	work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006; 114 :2130–7.
29		doi:10.1161/CIRCULATIONAHA.106.645184
30	46	Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation
31	40	impairs postischemic recovery of the insulin-resistant rat heart. FASEB J 2013;27:3966–78.
32		doi:10.1096/fj.13-234914
33	47	Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and
34	17	contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012; 26 :3118–26. doi:10.1096/fj.12-
35		208991
36	48	Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a
37		randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG
38		OUTCOME TM). Cardiovasc Diabetol 2014; 13 :102. doi:10.1186/1475-2840-13-102
39	49	Li N, Zhou H. SGLT2 Inhibitors: A Novel Player in the Treatment and Prevention of
40		Diabetic Cardiomyopathy. Drug Des Devel Ther 2020; Volume 14:4775–88.
41		doi:10.2147/DDDT.\$269514
42	50	Ahtarovski K a, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during
43		dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac
44		magnetic resonance. Am J Physiol Heart Circ Physiol 2012;303:H1469-73.
45		doi:10.1152/ajpheart.00365.2012
46	51	Flint a, Raben a, Blundell JE, et al. Reproducibility, power and validity of visual analogue
47		scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab
48		Disord 2000;24:38-48.http://www.ncbi.nlm.nih.gov/pubmed/10702749
49 50	52	Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces
50		myocardial lipid content and systolic function in type 2 diabetes. Nutr Metab Cardiovasc Dis
51		2016; 26 :387–92. doi:10.1016/j.numecd.2016.03.012
52	53	Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of
53 54		cardiovascular magnetic resonance with two-dimensional echocardiography in normal
54 55		subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol
55 56		2002; 90 :29–34. doi:10.1016/S0002-9149(02)02381-0
50 57	54	Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance perfusion
58		imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012; 13 :954–60.
58 59		doi:10.1093/ehjci/jes103
60		
00		

- 55 Ahtarovski KA, Iversen KK, Lønborg JT, *et al.* Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. *Am J Physiol Hear Circ Physiol* 2012;**303**:1469–73. doi:10.1152/ajpheart.00365.2012
 - 56 Kumarathurai P, Anholm C, Larsen BS, *et al.* Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. *Diabetes Care* 2017;**40**:117–24. doi:10.2337/dc16-1580
 - 57 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis. *Diabetes Care* 2016;**39**:1108–14. doi:10.2337/dc16-0330
 - 58 Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: Glucose excretion and lipid mobilization - not storage - saves lives. *J Diabetes Complications* 2016;**30**:753. doi:10.1016/j.jdiacomp.2016.02.015
 - 59 The Lancet Diabetes Endocrinology. Getting to the heart of the matter in type 2 diabetes. *lancet Diabetes Endocrinol* 2015;**3**:827. doi:10.1016/S2213-8587(15)00384-8
 - 60 DeFronzo RA. The EMPA-REG study: What has it told us? A diabetologist's perspective. *J* Diabetes Complications 2016;**30**:1–2. doi:10.1016/j.jdiacomp.2015.10.013
- 61 Nirengi S, Peres Valgas da Silva C, Stanford KI. Disruption of energy utilization in diabetic cardiomyopathy; a mini review. Curr. Opin. Pharmacol. 2020;**54**:82–90. doi:10.1016/j.coph.2020.08.015
- 62 DeFronzo RÅ. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009;**58**:773–95. doi:10.2337/db09-9028
- 63 Merovci A, Solis-Herrera C, Daniele G, *et al.* Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest* 2014;**124**:509–14. doi:10.1172/JCI70704
- 64 Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res* 1997;**34**:25–33.http://www.ncbi.nlm.nih.gov/pubmed/9217869 (accessed 3 Feb 2016).
- Nolan CJ, Ruderman NB, Kahn SE, *et al.* Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. *Diabetes* 2015;64:673–86. doi:10.2337/db14-0694
- 66 Taegtmeyer H, Beauloye C, Harmancey R, *et al.* Comment on Nolan et al. Insulin Resistance as a Physiological Defense Against Metabolic Stress: Implications for the Management of Subsets of Type 2 Diabetes. Diabetes 2015;64:673-686. *Diabetes* 2015;**64**:e37. doi:10.2337/db15-0655
 - 67 Taegtmeyer H, Beauloye C, Harmancey R, *et al.* Insulin resistance protects the heart from fuel overload in dysregulated metabolic states. *Am J Physiol Heart Circ Physiol* 2013;**305**:H1693-7. doi:10.1152/ajpheart.00854.2012
 - 68 Rau M, Thiele K, Hartmann NUK, *et al.* Empagliflozin does not change cardiac index nor systemic vascular resistance but rapidly improves left ventricular filling pressure in patients with type 2 diabetes: a randomized controlled study. *Cardiovasc Diabetol* 2021;**20**. doi:10.1186/s12933-020-01175-5
 - 69 Lauritsen KM, Nielsen BRR, Tolbod LP, *et al.* SGLT2 Inhibition Does Not Affect Myocardial Fatty Acid Oxidation or Uptake, But Reduces Myocardial Glucose Uptake and Blood Flow in Individuals With Type 2 Diabetes– a Randomized Double-Blind, Placebo-Controlled Crossover Trial. *Diabetes* 2020;**70**:db200921. doi:10.2337/db20-0921
 - 70 Oldgren J, Laurila S, åkerblom A, *et al.* Effects of 6 weeks treatment with dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, on myocardial function and metabolism in patients with type 2 diabetes: a randomized placebo-controlled exploratory study. *Diabetes, Obes Metab* 2021;:dom.14363. doi:10.1111/dom.14363
- 71 Hiruma S, Shigiyama F, Hisatake S, *et al.* A prospective randomized study comparing effects of empagliflozin to sitagliptin on cardiac fat accumulation, cardiac function, and cardiac metabolism in patients with early-stage type 2 diabetes: the ASSET study. *Cardiovasc Diabetol* 2021;**20**. doi:10.1186/s12933-021-01228-3

2 3 4 5 6 7 8 9 10	72 73	Deacon CF. A review of dipeptidyl peptidase-4 inhibitors. Hot topics from randomized controlled trials. Diabetes, Obes. Metab. 2018; 20 :34–46. doi:10.1111/dom.13135 Nordsborg N, Timmerman M. <i>Testmanual - patientinterview og konditionstest</i> . 2.0. København: : Sundhedsstyrelsen 2006.
11 12 13 14 15 16 17 18 19		
20 21 22 23 24 25 26 27 28		
29 30 31 32 33 34 35 36 37		
38 39 40 41 42 43 44 45 46		
47 48 49 50 51 52 53 54		
55 56 57 58 59 60		





* See text box 3 for screening procedure ** See text box 4 & 5 for visit overview

Figure 1. Study outline. Included patients undergo a 7-week program of washout of pre-existing antiglycemic treatment (except metformin) and run-in of empagliflozin. Hereafter they are randomised to treatment for 5±1 weeks, followed by 3±1 weeks wash-out and cross-over to 5±1 weeks treatment with the remaining study drug. Tests performed at each visit are summarised in Box 4 and 5.

369x187mm (96 x 96 DPI)

FFA / Glucose /	nsulin		
Wash-out	First treatment: Insulin	Wash-out	Second treatment: Empagliflozin

Figure 2. Metabolic profile of the two study drugs. Schematic representation of the metabolic changes expected with the two study drug treatments in a patient randomized to insulin first. Insulin treatment is characterized by low glucose, low FFAs and high insulin concentrations; empagliflozin treatment by low glucose, high FFAs and low insulin.

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Investigating the roles of hyperglycemia, hyperinsulinemia, and elevated free fatty acids in cardiac function in patients with type 2 diabetes via treatment with insulin compared with empagliflozin: protocol for the HyperCarD2 randomised, crossover trial

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TITLE

"Investigating the roles of hyperglycemia, hyperinsulinemia, and elevated free fatty acids in cardiac function in patients with type 2 diabetes via treatment with insulin compared with empagliflozin: protocol for the HyperCarD2 randomised, crossover trial"

Short title: The HyperCarD2 study

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ABSTRACT

Introduction: Type 2 diabetes (T2D) is characterised by elevated plasma glucose, free fatty acid (FFA) and insulin concentrations, and this metabolic profile is linked to diabetic cardiomyopathy, a diastolic dysfunction at first, and increased cardiovascular disease (CVD) risk. Shifting cardiac metabolism towards glucose utilisation has been suggested to improve cardiovascular function and CVD risk, but insulin treatment increases overall glucose oxidation and lowers lipid oxidation, without reducing CVD risk, whereas SGLT-2 inhibitors (SGLT-2i) increase FFA, ketone body concentrations and lipid oxidation while decreasing insulin concentrations and CVD risk. The aim of the present study is to elucidate the importance of different metabolic profiles obtained during treatment with a SGLT2i versus insulin for myocardial function in patients with T2D.

Methods and analyses: Randomised, cross-over study, where 20 patients with T2D and BMI >28 kg/m2 receive 25 mg empagliflozin qd or NPH insulin bid first for 5 weeks followed by a 3-week washout before crossing over to the remaining treatment. Insulin treatment is titrated to achieve similar glycaemic control as with empagliflozin. In those randomised to insulin first, glycaemia during an initial empagliflozin run-in period prior to randomisation serves as target glucose. Metabolic and cardiac evaluation is performed before and at the end of each treatment period. The primary endpoint is change (treatment – washout) in left ventricular peak filling rate, as assessed by cardiac MR (CMR) with and without acute lowering of plasma FFAs with acipimox. Secondary and explorative endpoints are changes in left atrial passive emptying fraction, left ventricular ejection fraction, central blood volume and metabolic parameters.

Ethics and dissemination: This study is approved by the Danish Medicines Agency (ref. nr.: 2017061587), the Danish Data Protection Agency (ref. nr.: AHH-2017-093) and the Capital Region Ethics Committee (ref. nr.: H-17018846). The trial will be conducted in accordance with ICH-GCP guidelines and the Helsinki Declaration and all participants will provide oral and written informed consent (see supplementary appendix 1). Our results, regardless of outcome, will be published in relevant scientific journals and we also will seek to disseminate results through presentations at scientific meetings.

Trial registration number: EudraCT: 2017-002101.

STRENGTHS AND LIMITATIONS

- Comparison with NPH Insulin, which has opposite metabolic effects to empagliflozin, provides a strong basis for detecting metabolic effects on cardiac function.
- Repeated cardiac MR, during depletion of plasma FFAs with acipimox during treatments and washouts allows for dissection of the individual roles of hyperglycaemia, hyperinsulinemia and elevated free fatty acids on cardiac function in T2D.
- A cross-over design is vulnerable to dropout, but provides greater statistical power

 • Effects of metabolic changes on cardiac function are limited to the 5-week intervention period, which excludes effects arising from longer-term treatment.

INTRODUCTION

Type 2 diabetes (T2D) is characterised by hyperglycaemia, hyperinsulinemia, increased free fatty acids and impaired tissue glucose uptake and oxidation [1]. T2D is associated with an increased cardiovascular morbidity, and the more dysregulated the metabolic state, the greater the cardiovascular risk [2–5]. T2D develops when insulin secretion can no longer compensate for the ambient insulin resistance, and therefore previous treatments has focused on increasing insulin signalling by either exogenous insulin administration, stimulation of endogenous insulin secretion or enhancing insulin sensitivity [1].

Diabetic cardiomyopathy (DCM), is an early "silent" complication to T2D, independent of hypertension and/or coronary heart disease. It is characterised by left ventricular (LV) hypertrophy and diastolic dysfunction [6,7] and has been linked to the increased cardiovascular risk in T2D [8]. DCM may be accurately described by measuring left ventricular peak filling rate (LVPFR) and left ventricular ejection fraction (LVEF) using cardiac magnetic resonance imaging (CMR) [9,10]. Both diastole and systole are energy requiring processes and sensitive to changes in energy availability [11,12]. Interestingly, cardiac metabolism in patients with T2D is altered and depends more on lipid oxidation and less on glucose oxidation compared to non-diabetic controls [13,14]. It has been argued that glucose oxidation is a better source of energy for the heart than lipid oxidation, especially during stress such as myocardial ischemia, because this yields more ATP pr. unit oxygen [15]. However, manipulating cardiac metabolism towards glucose oxidation, by administering glucose-insulin (-potassium) infusions in patients with hyperglycaemia and myocardial infarction has been attempted, but did not improve survival in neither diabetic nor non-diabetic patients [16-18]. In intensive care unit patients, strict glycaemic control using insulin has been associated with increased mortality [19], and in patients with T2D and increased CVD risk, intensive glycaemic control has not reduced CVD risk compared to conventional glycaemic control [20–23] and in the ACCORD study, which involved aggressive insulin treatment resulted in excess mortality [24]. Thus, insulin treatment does not prevent cardiovascular events in patients with T2D nor improve prognosis when such occur [25].

SGLT2-inhibition (SGLT2i), on the other hand, is a newer treatment principle in T2D, which has proven effective in attenuating the risk of myocardial infarctions, worsening of heart failure, cardiovascular mortality and all-cause mortality in patients with T2D [26–28]

SGLT2i increases renal glucose excretion thereby lowering plasma glucose and insulin levels and increasing glucagon release, lipolysis and ketogenesis [29,30]. Additionally, tissue glucose uptake and oxidation is reduced and hepatic glucose production increased [31]. The exact cardioprotective mechanisms of SGLT2i are not yet understood, but has been proposed to be linked to improved

haemodynamics [32], inhibition of myocardial Na⁺/H⁺ exchange [33,34] or reductions in inflammatory activity [35,36].

An early and interesting hypothesis proposed that changes in cardiac metabolism may be responsible for the cardioprotective effect of SGLT2i. The lowered glucose and insulin concentrations, persistent hyperketonaemia and elevated free fatty acids, caused by SGLT2i treatment, leads to reduced glucose uptake, increased ketone body uptake and oxidation and unchanged uptake of free fatty acids in the heart while overall lipid oxidation is increased [37,38]. This altered energy metabolism may rapidly improve myocardial function, especially during myocardial stress [39–42]. The SGLT2i induced myocardial fuel switch from glucose to fatty acids and ketone bodies, has been suggested to ameliorate adverse cardiac remodelling and heart failure in nondiabetic porcine models [43], and it is noteworthy that eliminating the availability of free fatty acids to insulin resistant hearts can lead to cardiac dysfunction in rodents and in humans, suggesting an important role for lipid metabolism in cardiac function [44–47]. Cardiovascular endpoint trials with SGLT2 inhibitors have shown effects within weeks after initiation of treatment, coinciding with the metabolic effects of the treatment [26,38]

Altogether, SGLT2 inhibitors "amplify" some components of the dysmetabolic profile of T2D and works opposite the metabolic effects of insulin. This raises the question of how cardiac function in patients with T2D depends on lipid and glucose oxidation in the resting state and during stress, and how increasing or lowering blood glucose, free fatty acids, ketone bodies and insulin concentrations influence cardiac function.

Objective

The primary objective of the present study is to evaluate myocardial function in patients with T2D and high risk of CV events using advanced cardiac magnetic resonance imaging (CMR) scans during rest, chronotropic stress and under depletion of plasma free fatty acids before and after 5 weeks of empagliflozin treatment (high free fatty acid and ketone body concentrations, high lipid oxidation and low insulin concentrations) and before and after 5 weeks of human insulin treatment titrated to yield glycaemic control similar to the empagliflozin treatment period (low free fatty acid and ketone body concentrations, high insulin concentrations and glucose oxidation).

Hypothesis

We hypothesise that hyperinsulinemia and hyperglycaemia are conditions that negatively affect cardiac function in T2D, while the availability of free fatty acids and ketone bodies and switching metabolism towards lipid oxidation improves cardiac diastolic and systolic function. Thus, we expect that lowering plasma glucose insulin-independently, and increasing fatty acid concentrations, lipid oxidation and ketone body availability with empagliflozin treatment, improves myocardial function in patients with T2D, and that depleting plasma of free fatty acids during empagliflozin treatment will impair cardiac function.

METHODS AND ANALYSES

Design

This is a 20-week prospective, investigator-initiated, comparator controlled, open label, 2-arm cross-over, human study where subjects are randomised in blocks of 3-5 to NPH insulin or Empagliflozin treatment (25 mg once daily) for 5±1 weeks, followed by 3±1 weeks wash-out and cross-over of treatment for 5±1 weeks (figure 1). For 7 weeks preceding randomisation, but after inclusion, patients undergo a program of 2 weeks of washout of pre-existing antiglycaemic treatment (except metformin), 2 weeks of empagliflozin run-in (used for glycaemic target and titration of treatment in participants randomised to insulin first, see below) followed by 3 weeks of wash-out. During run-in and treatment periods, participants measure blood glucose twice daily (fasting and before evening meal), and during washouts patients measure fasting blood glucose. After the screening visit (V0) there are four study visits (V1-4) – before and at the end of each treatment period. Each visit consists of three study days – a metabolic study day (MET) and two CMR study days. Randomisation is performed at V1 after the metabolic study day.

Participants

Twenty subjects older than 18 years diagnosed with T2D, a BMI \geq 28 kg/m², HbA1c \leq 9%, fasting C-peptide >500 pmol/L and unchanged antiglycaemic treatment for 12 weeks prior to screening, and who are at a risk of cardiovascular disease (CVD), are eligible for the study. High CVD risk is modified from the EMPA-REG protocol [48]. Inclusion, exclusion and withdrawal criteria are listed in Box 1.

Recruitment

Participants are recruited from the Department of Endocrinology and Cardiology at Hvidovre Hospital and are identified by reviewing laboratory results and patient files. Potential participants will be contacted by means of a recruitment letter, in which they are informed of the opportunity to participate in a scientific research project. We also will advertise for participant in local newspapers and on the internet as well as social media (e.g. www.forsøgsperson.dk; www.sundhed.dk and www.facebook.com).

Outcomes

The primary outcome is change in myocardial diastolic function. This was chosen because firstly, diastole is a highly energy requiring process [11,12], and secondly, because diastolic dysfunction (with or without LV hypertrophy) is the notable early manifestation of diabetic cardiomyopathy [49]. Thus, if changes in overall energy metabolism are to affect cardiac function in patients with T2D, it may well occur in diastole at the earliest. Diastolic cardiac function can be accurately assessed using CMR by measuring left ventricular peak filling rate (LVPFR) and left atrial passive emptying fraction (LAPEF) [50]. Our primary outcome measure is change (LVPFR_{treatment} – LVPFR_{wash-out}) in LVPFR (Δ LVPFR). All endpoints are listed in Box 2.

Randomisation and intervention

Participants are randomised consecutively by lottery in blocks of 3-5 to treatment with either subcutaneous NPH insulin (Insulatard®) twice daily or oral empagliflozin (Jardiance®) 25 mg once daily first. All patient will receive both treatments during the trial. Randomisation is performed at V1. NPH insulin is initiated at a dose of 0.2 IU/kg body weight/day and is titrated daily over phone (phone contacts, figure 1) by 0.05 IU/kg body weight/day until average blood glucose over three consecutive days is within ±1 mmol/L of the individual glycaemic target. In participants randomised to insulin first, the glycaemic target is average fasting and evening glucose concentrations during the second week of empagliflozin run-in. In patients randomised to insulin second, the glycaemic target is average fasting and pre-prandial evening BG values of week 3 and 4 during the first (empagliflozin) treatment period.

As previously discussed, insulin and empagliflozin represents two metabolically opposing methods for lowering plasma glucose concentrations. By titrating insulin treatment to match the glycaemic control found with empagliflozin in the same participants, the result is two distinct metabolic phenotypes: one with hyperinsulinemia and suppressed levels of FFAs (NPH insulin treatment), and one with reduced insulin levels and increased levels of FFAs (empagliflozin treatment) - but both with the same levels of glycaemic control (Figure 2). NPH Insulin has been chosen over more modern human insulin analogues, as it is not albumin bound and can be measured in an ordinary insulin assay.

Safety considerations

During washout periods blood glucose concentrations will increase – that is a separate point of the study, but severely dysregulated diabetes is an exclusion criterium to ensure participant safety. The risk of severe hyperglycaemia is reduced in several ways in the study:

- Existing metformin treatment is continued throughout the whole study as background antiglycaemic treatment.
- In case of fasting BG concentrations of more than 13 mmol/L, patients are instructed to contact study personnel.
- Phone contacts by study investigator are planned in the second week of washout periods to follow up on the patient and enquire to hyperglycaemic events or other adverse events.
- As soon as the final day (CMR with acipimox) of a washout visit (visit 1 or 3) is completed, antiglycaemic treatment according to study drug sequence is commenced to minimise time spent in hyperglycaemia.

In case of fasting BG > 13 mmol/L, the patient will be contacted daily for two additional days. If average fasting BG over the 3 days > 13 mmol/L that triggers an extra safety visit, where fasting plasma glucose (PG) is measured. If PG > 13 mmol/L on the day of the extra visit, then the patient is withdrawn from the study and antihyperglycaemic treatment is initiated.

Screening visit (V0)

Once oral and written informed consent is obtained by the study investigator, the screening procedure follows. Medical history is recorded, screening blood samples drawn, and an ECG, recording of blood pressure, pulse rate and registration of anthropometric data are performed, and patients are screened according to in- and exclusion criteria. A standard transthoracic echocardiography is performed, and VO2max is estimated (Box 3).

Study visits

All study visits consist of three study days – a metabolic study day and two CMR study days (Box 4).

The metabolic study day

The metabolic study is conducted at the Department of Endocrinology, Hvidovre Hospital, to document the metabolic effects of each study drug.

Participants meet in the morning after an overnight fast. Anthropometric data, blood pressure, pulse rate and an ECG are recorded, and two catheters, one in each arm are inserted for infusion of tracers and for repeated drawing of arterialised blood samples respectively. Baseline and safety blood samples are taken (Box 5), the participant empties bladder and the investigational drug (V2, V4) and the participants usual medications are administered at 0800h. Body composition is determined by Dual energy x-ray absorptiometry scan (DXA).

Basal metabolism

Primed infusions of stable glucose ($[6,6-D_2]$ -glucose) and glycerol ($[1,1,2,3,3-D_5]$ -glycerol) tracers are initiated (T=-180 min). Blood is sampled at -30, -15 and -2 min to characterise glucose, lipid and amino acid metabolism. The patient empties bladder, urine is weighed, and samples are taken for determination of tracer concentrations and urinary nitrogen excretion, and the 5h-OGTT is initiated.

5h-OGTT

The patient ingests anhydrous glucose (72 g) with added [U-13C6]-glucose tracer (3 g) dissolved in 250 mL of water over 5 minutes (T=0 min). Intravenous tracer infusions continue unchanged. Blood is sampled regularly for 5 hours for characterisation of postprandial glucose, lipid and amino acid metabolism (Box 4 and 5). The patient empties bladder regularly during and at the end of the OGTT. Urine is sampled for nitrogen excretion and tracers/tracees.

Fat and muscle biopsies: Biopsies are obtained during the basal (T=-60 min) and the maximally insulin stimulated (T=60 min) state. Muscle biopsies are considered proxies for cardiomyocyte metabolic status. 30 min ventilated hood indirect calorimetry (Vyaire Vyntus® CPX) is performed during the basal period (prior to biopsies) t= -90 min and postprandially at t=60 min for determination of fasting and postprandial energy expenditure and respiratory quotient.

Exercise test (50% VO2max)

At T=300 min, the participant is exercised at 60 W for 4 minutes after which work load is increased until oxygen consumption is 50% of estimated VO2max. Pulse rate is recorded with a chest mounted pulse rate monitor and oxygen consumption with a mask mounted indirect calorimeter. Blood is sampled to characterise glucose, lipid and amino acid metabolism (Box 4 and 5). After 30 min, VO2max is estimated by increasing workload by 50W until a pulse rate increase of 30. When pulse is steady for 2 min, oxygen consumption and pulse rate are recorded, and the test is stopped. VO2max is estimated by linear extrapolation to the theoretical maximum pulse rate (220-age).

Ad libitum meal test

SGLT2 inhibition is associated with a lower weight reduction than predicted from the urinary energy loss. SGLT2 inhibition does not change resting energy expenditure or blunt the thermogenic effect of feeding, suggesting that energy intake is increased [38]. Therefore, the metabolic study day is ended with an ad *libitum meal*, consisting of thoroughly mixed pasta bolognese (fixed nutrient composition and energy content). Patients are placed in a quiet corner and instructed to eat until full. Two glasses of water (total 300 mL) are allowed with the meal. The meal is weighed before and after serving and ad libitum meal intake defined as the difference. Throughout the day patients are asked to score their hunger, satiety and sensation of fullness on a visual analogue scale [51].

Cardiac evaluation

Two CMR days are performed during each visit (V1-V4). In addition, diurnal blood pressure and Holter monitoring are performed. CMR is conducted at the Department of Cardiology, Rigshospitalet, Copenhagen, whereas Holter monitoring and diurnal blood pressure monitoring are performed at the Department of Cardiology, Hvidovre Hospital.

Participants meet fasting and morning medication, including investigational medicinal product (V2 and V4), is administered. Anthropometric data are recorded, and two intravenous catheters are inserted into an antecubital and the contralateral dorsal hand vein for infusion of adenosine, gadolinium contrast and glycopyrrolate and for blood sampling respectively (Box 5). Prior to CMR a transthoracic echocardiography is performed (Box 3)

CMR is performed on a 1.5 Tesla scanner (Siemens Aera; Siemens; Erlangen; Germany) with the patient lying supine on the back, using an 18-channel cardiac coil with continuous ECG gating.

Cine 2-, 3- and 4-chamber images, complete transverse and short axis cine stacks covering the whole heart are acquired. All images are obtained during end-expiratory breath-holds. Myocardial perfusion images during rest and stress are obtained at the basal, mid-ventricular and apical cardiac short-axis level. Rest perfusion images of the myocardium is acquired using an intravenous bolus of gadolinium contrast (Gadovist®, Bayer AG, Germany) 0.075 mmol/kg bodyweight. The time of gadolinium contrast entry into the right and the left ventricle is accurately determined, and this transit time of gadovist multiplied by cardiac output is used to calculate the pulmonary and central (pulmonary + cardiac) blood volume.

Myocardial stress perfusion images are obtained with an i.v dose of 0.075 mmol/kg of gadovist during and 10 min after an intravenous adenosine (140 ug/min) administrated for maximum 4 minutes). This is followed by evaluation of cardiac function during chronotropic stress, where short axis cine stack will be reacquired 10 minutes after the administration of intravenous glycopyrrolate

 $(4 \mu g/kg, max. 400 \mu g, given as a bolus)$. This approach has been shown to unmasque subclinical diastolic dysfunction as has been demonstrated in normal healthy elderly [50].

Cardiac MRI, Acipimox

CMR scans will follow the same procedure as described above, but participants are instructed to ingest 250 mg acipimox p.o. twice, 4 hours before and right before the scan, to determine myocardial function. This repeated administration of acipimox is required for adequate suppression of hormone sensitive lipase activity and depletion of plasma FFAs (28). This has been shown to gradually impair cardiac function [52], and is done to disclose any coupling between FFA availability and cardiac function.

CMR image analysis

Is performed using Circle42 (Circle Cardiovascular Imaging Inc., Calgary Canada, v5.5.1). LV volumes, LV mass, LV ejection fraction (LVEF) and LV peak filling rate are determined by tracing of the endo- and epicardial contours in end-diastolic and end-systolic phases. The papillary muscles are excluded from the myocardium. On native and post-contrast T1-mappings, endocardial and epicardial borders are traced, and the mean extra cellular volume (ECV) is calculated from areas outside late gadolinium enhancement (LGE) lesions. For determination of the ECV within an LGE lesion, myocardium without LGE in the segment is excluded. Myocardial perfusion scans are inspected for perfusion defects. Regions with infarctions, sub-endocardial perfusion defects or dark-rim artefacts will be excluded. Blinded to clinical data, the analyses will be reviewed and finalised by two CMR specialist.

Diurnal blood pressure and Holter monitoring

Between study days during each visit, diurnal blood pressure is recorded (ScottCare, ABP 320, Cleveland, OH)) for 24 hours with 15 minutes intervals between 6.00 to 22.00- and 60-min intervals during night-time. Cardiac rhythm is evaluated with Holter monitoring (SCOTTCARE, CHROMA, model RZ153C, Cleveland, OH) for 48 hours.

Patient and public involvement No patient involved.

ANALYSES

Blood and tissue samples

Subcutaneous fat- and muscle biopsies: Local analgesia is applied before sampling with a Bergströms cannula. Samples are immediately frozen in liquid nitrogen and stored at -80°C. Blood and urine samples: Samples are spun, aliquoted and stored at -20°C (GLP-1, PYY, Glucagon) or – 80°C for later analysis. Bedside plasma glucose measurements are performed using the glucose oxidase technique (YSI model 2300 STAT Plus; YSI,Yellow Springs, OH). Home blood glucose measurements are carried out on Contour XT (Ascensia Diabetes Care Holdings AG, Basel,

Switzerland). Safety blood- and urine samples are analysed on the same day at the Department of Clinical Biochemistry, Hvidovre Hospital.

- Statistical methods
- Sample size calculation

Measures of myocardial function are highly reproducible when assessed using CMR, and interstudy and cohort coefficients of variation are in the range of 3-5% [53–55]

Using the same CMR protocol as the present, Ahtarovski et al found a mean difference of 92 ml/s in Left Ventricular Peak Filling Rate between healthy young (585 ± 62 ml/s) and healthy elderly subjects (493 ± 55 ml/s) [55]. We assume that T2D patients have LVPFR corresponding to healthy elderly subjects, and we assume that empagliflozin treatment improves LVPFR by 30 ml/s (Δ LVPFR=30 ml/s) from baseline and that insulin treatment does not improve LVPFR (Δ LVPFR=0 ml/s).

Conservatively setting the standard deviation of between treatment differences of Δ LVPFR at 30 ml/s, a number of 20 patients would be adequate to determine a 30 ml/s difference between the two treatments with a power of 93% and a two-sided significance level of 0.01, when evaluating data with the paired student's t-test. In case of a 20% dropout rate, power would still be acceptable (83%, p=0.01).

Statistical analysis plan

The primary and secondary endpoints are analysed assuming no period effect or treatment-period interaction. This assumption is reasonable, given results from similar studies, where no such interactions or effects have been reported [56]. Normally distributed data are presented using standard descriptive statistics, and reported as mean (SD) for normally distributed and median (Q1;Q3) for non-normally distributed data. Likewise, comparisons of normally distributed data is done using the paired Student's t-test for all completers, whereas Wilcoxon's paired signed rank test will be used if data is non-normally distributed.

Timeframe

Screenings are performed from January 2018. Last patient, last visit is expected second half of 2021 after which the study will be unregistered with the Danish Medicines Agency and the Capital Region Municipal Ethical Committee within 90 days. Data analyses are expected to be completed by Winter 2022. No later than 12 months after unregistering of the study, will results be made available at www.clinicalregister.eu. Trial registration number: EudraCT: 2017-002101.

ETHICS AND DISSEMINATION

The study is conducted according to ICH GCP guidelines E6 (R2) and the Helsinki Declaration and all participants will provide oral and written informed consent. The study is approved by the Danish Medicines Agency (ref. id: 2017061587), The Capital Region Ethical Committee (H-17018846) and the Danish Data Protection Agency (AHH-2017-093). Our results, regardless of outcome, will be published in relevant scientific journals. In addition, we will seek to disseminate results through presentations at scientific meetings. Publication will take place as soon as scientifically feasible.

DISCUSSION

The profound and swift benefits of SGLT2i on cardiovascular risk in T2D have inspired the discussion of metabolism and its importance for cardiac function in patients with T2D [57–61]. Especially since, SGLT2 inhibitors have metabolic effects that by and large are opposite to those of insulin treatment. Thus, insulin treatment is associated with increased tissue glucose uptake and utilisation, but suppression of lipid mobilisation and oxidation as well as lowering of plasma concentrations of ketone bodies [62]. SGLT2 inhibitors increase lipid mobilisation and oxidation, increase plasma ketone body concentrations and reduce tissue glucose uptake [38,63]. Both treatments lower plasma glucose, but insulin treatment increases whereas SGLT2i treatment decreases plasma insulin concentrations. Whether such changes in metabolism affect cardiac function, is still unsettled, but forcing cardiac glucose uptake and utilisation through insulin treatment has been suggested by some to benefit and by others to impair cardiac function [47,64– 67], while yet others have suggested increased lipid and ketone body oxidation to be important for proper cardiac function in T2D [46,58,67]. Studies on SGLT2i treatments and the effects on cardiac function are beginning to emerge. In a recent study, 42 patients with T2D were randomised to 12 weeks of empagliflozin 10 mg or placebo once daily. SGLT2 inhibition was shown to rapidly improve diastolic cardiac function as evaluated with echocardiography [68]. In a placebo controlled cross-over design, after 4 weeks of empagliflozin treatment in patients with T2D, myocardial glucose uptake was reduced and fatty acid oxidation unaltered, but this did not significantly change myocardial oxygen consumption or cardiac efficiency, nor any measure of cardiac function [69]. In a Swedish study, 6 weeks dapagliflozin treatment showed unchanged cardiac fatty acid uptake, a trend toward reduced left atrial maximal volume, and reduced LV oxygen consumption and external work compared to placebo [70], and in the only study found, where an active comparator was used, 10 mg empagliflozin once daily for 12 weeks did not change cardiac lipid accumulation (as measured by MR spectrometry), cardiac function or cardiac metabolism compared to sitagliptin 50 mg daily [71].

In conclusion, existing studies in humans have shown divergent results regarding changes in cardiac diastolic function with little changes in cardiac metabolism. However, most studies have compared cardiac effects of SGLT2i to placebo, thus not accounting for the circumstances that characterised the EMPA-REG trial, where anti glycaemic treatment was intensified in the placebo group concurrently [26]. Thus, the CVD risk benefits of the study may have arisen from unfavourable metabolic consequences of the treatment in the placebo arm. In the one study with an active comparator empagliflozin was compared to sitagliptin, which not only affects the incretin system but also has less specific metabolic effect [72]. Therefore, to date our study, is the one to most directly pursue the coupling between metabolism and cardiac function, by choosing insulin as the comparator, and by including the effects of acute lowering of free fatty acid concentrations in plasma on cardiac function.

Disclosure summary

All authors have completed the ICMJE uniform disclosure form and declare: no support from any organisations for the submitted work; SM and NBJ have received research grants from Boehringer Ingelheim and JJH serves on advisory boards for Novo Nordisk, no other relationships or activities that could appear to have influenced the submitted work.

Funding

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Contributions

Author contributions

RT will be conducting the study, collect all the data, perform data analyses and co-submit the study. EAR has contributed to the study design, designed the keton body- and biopsy analysis scheme and is going to perform these analyses. JPG designed the pro-ANP and pro-BNP analysis scheme and is going to perform these analyses. MF designed multiple metabolic analysis schemes (Triglycerides, insulin, free fatty acids, uric acid, urea and nitrogen) and is going to perform these analyses.

GVH designed the three-tracer measuring analysis schemes we are using in this study and is going to perform these analyses. UD provided the study with analysis tools (holter-monitors and blood pressure monitors). He will perform the analyses of these data.

JJH participated in the protocol design, designed multiple metabolic analyses (GLP-1, glucagon and PYY) and will perform the analyses of these data.

SM is the principal investigator, co-planned the study and contributed with several analysing tools. NV co-designed the cardiac MRI guideline for the study and contributed with analysing tool (MRI scanner).PLM co-planned the study, co-designed the cardiac MRI guideline for the study and will analyse the cardiac MRI data.

NBJ co-planned the study, will perform all data analyses with RT and co-submit the study. All of the authors contributed with writing, revising and approving this manuscript.

• Single vessel CAD without prior myocardial infarction if more than 50% stenosis is present, not revascularised and positive stress test for ischemia.

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Exclusion criteria
Insulin treatment within 3 months from informed consent
Type 1 diabetes
Psychiatric disorder or mental retardation
Drug or alcohol abuse within 3 months from informed consent
Poor compliance
Anaemia (Hb < 6.4 mmol/L) or other blood dyscrasias causing haemolysis or unstable
erythrocytes.
Indication of liver disease (ALT or Alkaline phosphatase elevation above 3x upper normal limit)
Impaired renal function (eGFR<45 ml/min/1.73 m ²)
Anti-obesity medication within 3 months from informed consent
Systemic steroid treatment within 6 weeks from informed consent.
Any uncontrolled endocrine disorder except T2D
Bariatric surgery or other gastrointestinal conditions that may compromise gastrointestinal absorption
Peptic ulcer – verified endoscopically
Any form of surgery within 3 months of informed consent
Acute myocardial infarction, stroke or peripheral arterial disease within 2 months of informed
consent.
Persistent or permanent atrial fibrillation
Inability to undergo experimental procedures including exclusion criteria for CMR scanning:
Implantable cardioverter defibrillator/pacemaker
Ferromagnetic clips
Claustrophobia.
Contraindication to glycopyrrolate infusion:
Known closed-angle glaucoma
known severe prostate hyperplasia
Tachycardia (HR > 100 at rest)
Known bladder atony
Cardia insufficiency or non-congenital pylorus stenosis –verified endoscopically
Known gastroparesis
Known gastroparesis
Contraindications to adenosine:
2nd or 3rd degree atrioventricular block
Severe hypotension (BP \leq 90/50 mmHg)
Long QT syndrome
Unstable angina pectoris
Decompensated heart failure
Sinus node dysfunction
Chronic obstructive pulmonary disease or asthma bronchiale (FEV1 \leq 50% of expected)
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Allergy towards any of the drugs or diagnostics used in the protocol (insulin, empagliflozin, acipimox, glycopyrrolate, adenosine, gadolinium contrast enhancer). Any condition which in the opinion of the investigator may jeopardize subject safety or compliance with the protocol. Withdrawal criteria Subjects may withdraw from the study without any notice or reason Pregnancy discovered during the experiment Unacceptable adverse reactions or reactions associated with the planned experiments, including

severe glycaemic dysregulation during washout periods.		1					
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Box 2: Endpoints	
Primary end point	Change in left ventricular peak filling rate
	(ALVPFR)
Secondary endpoints	Change in left atrial passive emptying fraction
	(Δ LAPEF)
	Change in left ventricular ejection fraction
	(Δ LVEF)
Explorative endpoints include	Cardiovascular:
	Change in VO2max and exercise tolerance test
	variables
	Change in central blood volume and
	haematocrite
	Change in heart rate variability
	Change in left ventricular volume
	Metabolic:
	Basal and postprandial AUC Free Fatty Acids
	and glycerol turnover
	Endogenous glucose production and tissue
	glucose disposal (metabolic clearance of
	glucose)
	Fasting and postprandial energy expenditure
	and respiratory quotient
	Glucagon-Insulin ratio
	Insulin sensitivity (AUC glucose metabolic
	clearance / AUC insulin concentration)
	Beta-cell function (prehepatic insulin secretion
	rate, correlated to ambient glucose)

Box 3: Screening procedures	
Blood samples	Haematology (haemoglobin, thrombocytes,
	haematocrit, leukocytes), liver and renal
	function tests (creatinine, eGFR (Cockroft-
	Gault formula), alkaline phosphatases, alanine
	aminotransferases, lactate dehydrogenase,
	bilirubin, amylase, sodium, potassium), fasting
	P-glucose, C-peptide, HbA1c, TSH, Urinary
	Albumin/creatinine mass ratio, and in fertile
	women, U-hCG.
Echocardiography	Parasternal long axis view, parasternal short
	axis view at aortic, mitral and apex levels,
	apical 4-chamber view, LVEF, E/E', E',
	LVEDV/BSA.
Estimation of VO2max	Maximum oxygen uptake is estimated using
	Åstrøm's two-point test performed on a cycle
	ergometer during indirect calorimetry. From
	measurements of VO2 at two sub-maximal
	pulse rates VO2max is estimated by linear
	extrapolation to the theoretical maximal pulse
	rate (220-age) [73].

Box 4: Visit overview		
Metabolic study day	Cardiac MR	Cardiac MR, Acipimox
- DXA-scan and fasting safety	- Fasting blood samples,	Same protocol as Cardiac MR
and efficacy blood samples	before and after CMR.	day, but during
- Determination of 3-hour	- Echocardiography	pharmacological suppression
basal metabolism.	- CMR Rest	of hormone sensitive lipase
Infusion of glucose and	Without enhancement	activity and depletion of
glycerol tracers	With enhancement and	plasma free fatty acids.
Basal muscle and fat	adenosine infusion	- 48h Holter monitoring.
biopsies	- CMR Stress	
Basal energy expenditure	Unenhanced repeated during	
and determination of	pharmacological chronotropic	
respiratory quotient	stress with glycopyrrolate	
- 5-hour OGTT	infusion.	
with oral glucose tracer and	- 24h ambulant blood pressure	
continued intravenous		
glucose and glycerol tracer.		
Fat- and muscle biopsies at		
maximum insulin stimulation		
- Exercise test and		
determination of VO2max		
- Ad libitum meal.		

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Box 5: Blood samples on metabolic and CMR s	
Metabolic study day	Blood samples: glucose, insulin, C-peptide,
	glucagon, FFAs, triglycerides, total amino
	acids and ketone bodies (betahydroxybuturate),
	tracers/tracees, gut hormones.
	HbA1c, urate, blood urea nitrogen, cortisol, is
	sampled at baseline.
Cardiac MRI days	Markers of cardiac function, including pro-
	ANP and pro-BNP, glucose, insulin, C-peptide,
	glucagon, FFAs, triglycerides, ketone bodies,
	haematocrit are drawn before and after CMR.
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Figure legends	

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Figure 1. Study outline. Included patients undergo a 7-week program of washout of pre-existing antiglycemic treatment (except metformin) and run-in of empagliflozin. Hereafter they are randomised to treatment for 5 ± 1 weeks, followed by 3 ± 1 weeks wash-out and cross-over to 5 ± 1 weeks treatment with the remaining study drug. Tests performed at each visit are summarised in Box 4 and 5.

Figure 2. Metabolic profile of the two study drugs. Schematic representation of the metabolic changes expected with the two study drug treatments in a patient randomized to insulin first. Insulin treatment is characterized by low glucose, low FFAs and high insulin concentrations; empagliflozin treatment by low glucose, high FFAs and low insulin.

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References:

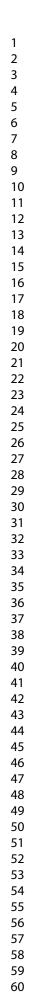
7	-	Treatment of Type 2 Diabetes Mellitus. ;:773–95. doi:10.2337/db09-9028
8	2	De Vegt F, Dekker JM, Ruhé HG, <i>et al.</i> Hyperglycaemia is associated with all-cause and
9	2	
10		cardiovascular mortality in the Hoorn population: The Hoorn study. <i>Diabetologia</i>
11	•	1999; 42 :926–31. doi:10.1007/s001250051249
12	3	Pyorala M, Miettinen H, Laakso M, et al. Plasma insulin and all-cause, cardiovascular, and
13		noncardiovascular mortality: the 22-year follow-up results of the Helsinki Policemen Study.
14		<i>Diabetes Care</i> 2000; 23 :1097–102. doi:10.2337/diacare.23.8.1097
15	4	Lakka HM, Lakka TA, Tuomilehto J, et al. Hyperinsulinemia and the risk of cardiovascular
16		death and acute coronary and cerebrovascular events in men: The Kuopio Ischaemic Heart
17		Disease Risk Factor Study. Arch Intern Med 2000;160:1160-8.
18		doi:10.1001/archinte.160.8.1160
19	5	Selvin E, Marinopoulos S, Berkenblit G, et al. Meta-analysis: Glycosylated hemoglobin and
20	U	cardiovascular disease in diabetes mellitus. Ann Intern Med 2004;141. doi:10.7326/0003-
		4819-141-6-200409210-00007
21	6	Redfield MM, Jacobsen SJ, Burnett JC, <i>et al.</i> Burden of systolic and diastolic ventricular
22	0	
23		dysfunction in the community: appreciating the scope of the heart failure epidemic. JAMA
24	7	2003; 289 :194–202. doi:10.1001/JAMA.289.2.194
25	7	Boyer JK, Thanigaraj S, Schechtman KB, et al. Prevalence of ventricular diastolic
26		dysfunction in asymptomatic, normotensive patients with diabetes mellitus. Am J Cardiol
27		2004; 93 :870–5. doi:10.1016/J.AMJCARD.2003.12.026
28	8	Jørgensen PG, Biering-Sørensen T, Mogelvang R, et al. Predictive value of
29		echocardiography in Type 2 diabetes. <i>Eur Heart J Cardiovasc Imaging</i> 2019; 20 :687–93.
30		doi:10.1093/ehjci/jey164
31	9	Tarquini R, Lazzeri C, Pala L, et al. The diabetic cardiomyopathy. Acta Diabetol
32		2011; 48 :173–81. doi:10.1007/s00592-010-0180-x
33	10	Komi S, Inoue Y, Hata H, et al. Cardiovascular magnetic resonance evaluation of left
34		ventricular peak filling rate using steady-state free precession and phase contrast sequences.
35		Springerplus 2016;5. doi:10.1186/s40064-016-2878-x
36	11	Diamant M, Lamb HJ, Groeneveld Y, et al. Diastolic dysfunction is associated with altered
37		myocardial metabolism in asymptomatic normotensive patients with well-controlled type 2
		diabetes mellitus. <i>J Am Coll Cardiol</i> 2003; 42 :328–35. doi:10.1016/S0735-1097(03)00625-9
38	12	Rosano G, Coats A. Modulation of Cardiac Metabolism in Heart Failure. <i>Int Cardiovasc</i>
39	14	<i>Forum J</i> 2019; 17 :99–103. doi:10.17987/icfj.v17i0.597
40	13	Ungar I, Gilbert M, Siegel A, <i>et al.</i> Studies on myocardial metabolism. IV. Myocardial
41	15	metabolism in diabetes. Am J Med 1955; $18:385-96$. doi:10.1016/0002-9343(55)90218-7
42	14	
43	14	Rijzewijk LJ, van der Meer RW, Lamb HJ, <i>et al.</i> Altered myocardial substrate metabolism
44		and decreased diastolic function in nonischemic human diabetic cardiomyopathy: studies
45		with cardiac positron emission tomography and magnetic resonance imaging. J Am Coll
46	1.5	<i>Cardiol</i> 2009; 54 :1524–32. doi:10.1016/j.jacc.2009.04.074
47	15	Stanley WC, Lopaschuk GD, Hall JL, et al. Regulation of myocardial carbohydrate
48		metabolism under normal and ischaemic conditions. Potential for pharmacological
49		interventions. Cardiovasc Res 1997;33:243-57. doi:10.1016/S0008-6363(96)00245-3
50	16	Mehta SR, Yusuf S, Díaz R, et al. Effect of glucose-insulin-potassium infusion on mortality
51		in patients with acute ST-segment elevation myocardial infarction: The CREATE-ECLA
52		randomized controlled trial. J Am Med Assoc 2005;293:437-46. doi:10.1001/jama.293.4.437
53	17	Malmberg K, Rydén L, Wedel H, et al. FASTTRACK intense metabolic control by means of
54		insulin in patients with diabetes mellitus and acute myocardial infarction (DIGAMI 2):
55		Effects on mortality and morbidity. <i>Eur Heart J</i> 2005; 26 :650–61.
		doi:10.1093/eurheartj/ehi199
56 57	18	Malmberg K, Rydén L, Efendic S, <i>et al.</i> Randomized trial of insulin-glucose infusion
57	10	followed by subcutaneous insulin treatment in diabetic patients with acute myocardial
58		infarction (DIGAMI study): Effects on mortality at 1 year. J Am Coll Cardiol 1995;26:57–
59		infarction (DIOAIvir study). Effects on mortanty at 1 year. J Am Con Curulor 1995,20.57-
60		
		For peer review only - http://bmjopen.bmj.com/site/about/guidelines.xhtml

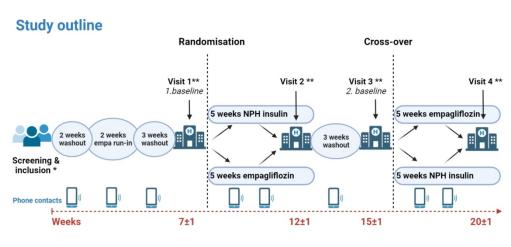
2 3		
4		(5, 1, 10, 101) (107) (1007) (05) (0010) W
5	10	65. doi:10.1016/0735-1097(95)00126-K
6	19	NICE-SUGAR Study Investigators, Finfer S, Chittock DR, <i>et al.</i> Intensive versus
7		conventional glucose control in critically ill patients. <i>N Engl J Med</i> 2009; 360 :1283–97.
8	20	doi:10.1056/NEJMoa0810625
9	20	Hemmingsen B, Lund SS, Gluud C, <i>et al.</i> Intensive glycaemic control for patients with type
10		2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised
11		clinical trials. <i>BMJ</i>
12		2011; 343 :d6898.http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3223424&tool=
13	21	pmcentrez&rendertype=abstract (accessed 9 Sep 2015).
14	21	Duckworth W, Abraira C, Moritz T, <i>et al.</i> Glucose control and vascular complications in
15		veterans with type 2 diabetes. <i>N Engl J Med</i> 2009; 360 :129–39.
16	22	doi:10.1056/NEJMoa0808431
17	22	ADVANCE Collaborative Group, Patel A, MacMahon S, <i>et al.</i> Intensive blood glucose
18		control and vascular outcomes in patients with type 2 diabetes. <i>N Engl J Med</i>
19	23	2008; 358 :2560–72. doi:10.1056/NEJMoa0802987 Basal Insulin and Cardiovascular and Other Outcomes in Dysglycemia. <i>N Engl J Med</i>
20	23	2012; 367 :319–28. doi:10.1056/nejmoa1203858
21	24	Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, <i>et</i>
22	24	<i>al.</i> Effects of intensive glucose lowering in type 2 diabetes. <i>N Engl J Med</i> 2008; 358 :2545–
23		59. doi:10.1056/NEJMoa0802743
24	25	Sciences HH, Miller ME, Byington RP, et al. Effects of Intensive Glucose Lowering in Type
25	23	2 Diabetes. <i>N Engl J Med</i> 2008; 358 :2545–59. doi:10.1056/nejmoa0802743
26	26	Zinman B, Wanner C, Lachin JM, <i>et al.</i> Empagliflozin, Cardiovascular Outcomes, and
27	20	Mortality in Type 2 Diabetes. <i>N Engl J Med</i> 2015; 373 :2117–28.
28		doi:10.1056/NEJMoa1504720
29	27	McMurray JJV, Solomon SD, Inzucchi SE, et al. Dapagliflozin in Patients with Heart Failure
30	_ /	and Reduced Ejection Fraction. N Engl J Med 2019; 381 :1995–2008.
31		doi:10.1056/nejmoa1911303
32	28	Zou CY, Liu XK, Sang YQ, et al. Effects of SGLT2 inhibitors on cardiovascular outcomes
33	-	and mortality in type 2 diabetes: A meta-analysis. Med (United States) 2019;98.
34		doi:10.1097/MD.00000000018245
35	29	Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose
36		cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest 2014;124:499–508.
37		doi:10.1172/JCI72227
38	30	Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin
39		sensitivity but enhances endogenous glucose production. J Clin Invest 2014;124:509–14.
40		doi:10.1172/JCI70704
41	31	Ferrannini E, Baldi S, Frascerra S, et al. Renal Handling of Ketones in Response to Sodium -
42		Glucose Cotransporter 2 Inhibition in Patients With Type 2 Diabetes. 2017;40:771–6.
43		doi:10.2337/dc16-2724
44	32	Chilton R, Tikkanen I, Cannon CP, et al. Effects of empagliflozin on blood pressure and
45		markers of arterial stiffness and vascular resistance in patients with type 2 diabetes. <i>Diabetes</i>
46		Obes Metab 2015;17:1180–93. doi:10.1111/dom.12572
47 49	33	Trum M, Riechel J, Lebek S, et al. Empagli fl ozin inhibits Na + / H + exchanger activity in
48 49		human atrial cardiomyocytes. 2020;:4429-37. doi:10.1002/ehf2.13024
50	34	Baartscheer A, Schumacher CA, Wüst RCI, et al. Empagliflozin decreases myocardial
51		cytoplasmic Na + through inhibition of the cardiac Na + $/$ H + exchanger in rats and rabbits.
52	25	2017;:568–73. doi:10.1007/s00125-016-4134-x
53	35	Garvey WT, Gaal L Van, Leiter LA, et al. Effects of canagli fl ozin versus glimepiride on
54		adipokines and in fl anmatory biomarkers in type 2 diabetes 2018 ;85:32–7.
55	20	doi:10.1016/j.metabol.2018.02.002
56	36	Heerspink HJL, Perco P, Mulder S, <i>et al.</i> Canagliflozin reduces inflammation and fibrosis
57		biomarkers : a potential mechanism of action for beneficial effects of SGLT2 inhibitors in diabatic bidray diagona 2010;1154 66
58	37	diabetic kidney disease. 2019;:1154–66.
59	51	Lauritsen KM, Nielsen BRR, Tolbod LP, et al. SGLT2 Inhibition Does Not Affect
60		

1		
2		
3		
4		Myocardial Fatty Acid Oxidation or Uptake, But Reduces Myocardial Glucose Uptake and
5		Blood Flow in Individuals With Type 2 Diabetes- a Randomized Double-Blind, Placebo-
6		Controlled Crossover Trial. Diabetes 2020;:db200921. doi:10.2337/db20-0921
7	38	Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose
8 9		cotransporter 2 inhibition in type 2 diabetic patients. <i>J Clin Invest</i> 2014; 124 :499–508.
9 10	20	doi:10.1172/JCI72227
11	39	Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: Glucose excretion and lipid mobilization
12		- not storage - saves lives. J Diabetes Complications 2016; 30 :753.
13	40	doi:10.1016/j.jdiacomp.2016.02.015
14	40	Ekanayake P, Hupfeld C, Mudaliar S. Sodium-Glucose Cotransporter Type 2 (SGLT-2) Inhibitors and Ketogenesis: the Good and the Bad. <i>Curr Diab Rep</i> 2020; 20 .
15		doi:10.1007/s11892-020-01359-z
16	41	Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: A
17	71	thrifty substrate hypothesis. <i>Diabetes Care</i> 2016; 39 :1108–14. doi:10.2337/dc16-0330
18	42	Nielsen R, Møller N, Gormsen LC, <i>et al.</i> Cardiovascular Effects of Treatment With the
19		Ketone Body 3-Hydroxybutyrate in Chronic Heart Failure Patients. <i>Circulation</i>
20		2019; 139 :2129–41. doi:10.1161/CIRCULATIONAHA.118.036459
21	43	Santos-Gallego CG, Requena-Ibanez JA, San Antonio R, et al. Empagliflozin Ameliorates
22		Adverse Left Ventricular Remodeling in Nondiabetic Heart Failure by Enhancing
23		Myocardial Energetics. J Am Coll Cardiol 2019;73:1931–44. doi:10.1016/j.jacc.2019.01.056
24 25	44	Wolf P, Winhofer Y, Krssak M, et al. Suppression of plasma free fatty acids reduces
25 26		myocardial lipid content and systolic function in type 2 diabetes. Nutr Metab Cardiovasc Dis
20	4.5	2016; 26 :387–92. doi:10.1016/j.numecd.2016.03.012
28	45	Tuunanen H, Engblom E, Naum A, <i>et al.</i> Free fatty acid depletion acutely decreases cardiac
29		work and efficiency in cardiomyopathic heart failure. <i>Circulation</i> 2006; 114 :2130–7. doi:10.1161/CIRCULATIONAHA.106.645184
30	46	Harmancey R, Vasquez HG, Guthrie PH, <i>et al.</i> Decreased long-chain fatty acid oxidation
31	40	impairs postischemic recovery of the insulin-resistant rat heart. FASEB J 2013;27:3966–78.
32		doi:10.1096/fj.13-234914
33	47	Harmancey R, Lam TN, Lubrano GM, <i>et al.</i> Insulin resistance improves metabolic and
34	.,	contractile efficiency in stressed rat heart. <i>FASEB J</i> 2012; 26 :3118–26. doi:10.1096/fj.12-
35		208991
36	48	Zinman B, Inzucchi SE, Lachin JM, et al. Rationale, design, and baseline characteristics of a
37		randomized, placebo-controlled cardiovascular outcome trial of empagliflozin (EMPA-REG
38		OUTCOME TM). Cardiovasc Diabetol 2014; 13 :102. doi:10.1186/1475-2840-13-102
39 40	49	Li N, Zhou H. SGLT2 Inhibitors: A Novel Player in the Treatment and Prevention of
40 41		Diabetic Cardiomyopathy. Drug Des Devel Ther 2020; Volume 14:4775–88.
41	50	doi:10.2147/DDDT.S269514
43	50	Ahtarovski K a, Iversen KK, Lønborg JT, et al. Left atrial and ventricular function during
44		dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac
45		magnetic resonance. <i>Am J Physiol Heart Circ Physiol</i> 2012; 303 :H1469-73. doi:10.1152/ajpheart.00365.2012
46	51	Flint a, Raben a, Blundell JE, <i>et al.</i> Reproducibility, power and validity of visual analogue
47	51	scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab
48		Disord 2000;24:38–48.http://www.ncbi.nlm.nih.gov/pubmed/10702749
49	52	Wolf P, Winhofer Y, Krssak M, <i>et al.</i> Suppression of plasma free fatty acids reduces
50	<i>v</i> <u>-</u>	myocardial lipid content and systolic function in type 2 diabetes. <i>Nutr Metab Cardiovasc Dis</i>
51		2016; 26 :387–92. doi:10.1016/j.numecd.2016.03.012
52	53	Grothues F, Smith GC, Moon JCC, et al. Comparison of interstudy reproducibility of
53		cardiovascular magnetic resonance with two-dimensional echocardiography in normal
54		subjects and in patients with heart failure or left ventricular hypertrophy. Am J Cardiol
55 56		2002; 90 :29–34. doi:10.1016/S0002-9149(02)02381-0
56 57	54	Morton G, Jogiya R, Plein S, et al. Quantitative cardiovascular magnetic resonance perfusion
58		imaging: Inter-study reproducibility. <i>Eur Heart J Cardiovasc Imaging</i> 2012; 13 :954–60.
59		doi:10.1093/ehjci/jes103
60		

- 55 Ahtarovski KA, Iversen KK, Lønborg JT, *et al.* Left atrial and ventricular function during dobutamine and glycopyrrolate stress in healthy young and elderly as evaluated by cardiac magnetic resonance. *Am J Physiol Hear Circ Physiol* 2012;**303**:1469–73. doi:10.1152/ajpheart.00365.2012
 - 56 Kumarathurai P, Anholm C, Larsen BS, *et al.* Effects of liraglutide on heart rate and heart rate variability: A randomized, double-blind, placebo-controlled crossover study. *Diabetes Care* 2017;**40**:117–24. doi:10.2337/dc16-1580
 - 57 Ferrannini E, Mark M, Mayoux E. CV Protection in the EMPA-REG OUTCOME Trial: A "Thrifty Substrate" Hypothesis. *Diabetes Care* 2016;**39**:1108–14. doi:10.2337/dc16-0330
 - 58 Jørgensen NB, Pedersen J, Vaag AA. EMPA-REG: Glucose excretion and lipid mobilization - not storage - saves lives. *J Diabetes Complications* 2016;**30**:753. doi:10.1016/j.jdiacomp.2016.02.015
 - 59 The Lancet Diabetes Endocrinology. Getting to the heart of the matter in type 2 diabetes. *lancet Diabetes Endocrinol* 2015;**3**:827. doi:10.1016/S2213-8587(15)00384-8
 - 60 DeFronzo RA. The EMPA-REG study: What has it told us? A diabetologist's perspective. *J* Diabetes Complications 2016;**30**:1–2. doi:10.1016/j.jdiacomp.2015.10.013
- 61 Nirengi S, Peres Valgas da Silva C, Stanford KI. Disruption of energy utilization in diabetic cardiomyopathy; a mini review. Curr. Opin. Pharmacol. 2020;**54**:82–90. doi:10.1016/j.coph.2020.08.015
- 62 DeFronzo RÅ. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* 2009;**58**:773–95. doi:10.2337/db09-9028
- 63 Merovci A, Solis-Herrera C, Daniele G, *et al.* Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J Clin Invest* 2014;**124**:509–14. doi:10.1172/JCI70704
- 64 Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. *Cardiovasc Res* 1997;**34**:25–33.http://www.ncbi.nlm.nih.gov/pubmed/9217869 (accessed 3 Feb 2016).
- Nolan CJ, Ruderman NB, Kahn SE, *et al.* Insulin resistance as a physiological defense against metabolic stress: implications for the management of subsets of type 2 diabetes. *Diabetes* 2015;64:673–86. doi:10.2337/db14-0694
- 66 Taegtmeyer H, Beauloye C, Harmancey R, *et al.* Comment on Nolan et al. Insulin Resistance as a Physiological Defense Against Metabolic Stress: Implications for the Management of Subsets of Type 2 Diabetes. Diabetes 2015;64:673-686. *Diabetes* 2015;**64**:e37. doi:10.2337/db15-0655
 - 67 Taegtmeyer H, Beauloye C, Harmancey R, *et al.* Insulin resistance protects the heart from fuel overload in dysregulated metabolic states. *Am J Physiol Heart Circ Physiol* 2013;**305**:H1693-7. doi:10.1152/ajpheart.00854.2012
 - 68 Rau M, Thiele K, Hartmann NUK, *et al.* Empagliflozin does not change cardiac index nor systemic vascular resistance but rapidly improves left ventricular filling pressure in patients with type 2 diabetes: a randomized controlled study. *Cardiovasc Diabetol* 2021;**20**. doi:10.1186/s12933-020-01175-5
 - 69 Lauritsen KM, Nielsen BRR, Tolbod LP, *et al.* SGLT2 Inhibition Does Not Affect Myocardial Fatty Acid Oxidation or Uptake, But Reduces Myocardial Glucose Uptake and Blood Flow in Individuals With Type 2 Diabetes– a Randomized Double-Blind, Placebo-Controlled Crossover Trial. *Diabetes* 2020;**70**:db200921. doi:10.2337/db20-0921
 - 70 Oldgren J, Laurila S, åkerblom A, *et al.* Effects of 6 weeks treatment with dapagliflozin, a sodium-glucose co-transporter 2 inhibitor, on myocardial function and metabolism in patients with type 2 diabetes: a randomized placebo-controlled exploratory study. *Diabetes, Obes Metab* 2021;:dom.14363. doi:10.1111/dom.14363
- 71 Hiruma S, Shigiyama F, Hisatake S, *et al.* A prospective randomized study comparing effects of empagliflozin to sitagliptin on cardiac fat accumulation, cardiac function, and cardiac metabolism in patients with early-stage type 2 diabetes: the ASSET study. *Cardiovasc Diabetol* 2021;**20**. doi:10.1186/s12933-021-01228-3

2 3 4 5 6 7 8 9 10 11	72 73	Deacon CF. A review of dipeptidyl peptidase-4 inhibitors. Hot topics from randomized controlled trials. Diabetes, Obes. Metab. 2018; 20 :34–46. doi:10.1111/dom.13135 Nordsborg N, Timmerman M. <i>Testmanual - patientinterview og konditionstest</i> . 2.0. København: : Sundhedsstyrelsen 2006.
12 13 14 15 16 17 18 19 20 21		
22 23 24 25 26 27 28 29 30 31 32		
33 34 35 36 37 38 39 40 41 42		
43 44 45 46 47 48 49 50 51 52		
53 54 55 56 57 58 59 60		





* See text box 3 for screening procedure ** See text box 4 & 5 for visit overview

Figure 1. Study outline. Included patients undergo a 7-week program of washout of pre-existing antiglycemic treatment (except metformin) and run-in of empagliflozin. Hereafter they are randomised to treatment for 5±1 weeks, followed by 3±1 weeks wash-out and cross-over to 5±1 weeks treatment with the remaining study drug. Tests performed at each visit are summarised in Box 4 and 5.

369x187mm (96 x 96 DPI)

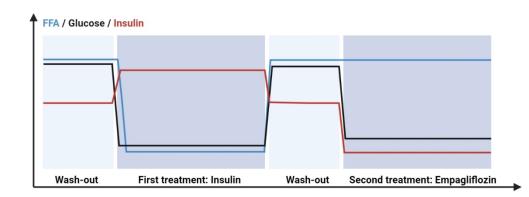


Figure 2. Metabolic profile of the two study drugs. Schematic representation of the metabolic changes expected with the two study drug treatments in a patient randomized to insulin first. Insulin treatment is characterized by low glucose, low FFAs and high insulin concentrations; empagliflozin treatment by low glucose, high FFAs and low insulin.

396x151mm (96 x 96 DPI)

(S4)

Informed consent to participate in a health science research study.

Title of the research project:" The role of hyperglycemia, hyperinsulinemia and elevated free fatty acids for cardiac function in patients with type 2 diabetes – the HyperCarD2 study".

Declaration from the patient:

I have received written and oral information and I know enough about the purpose, method, benefits and

disadvantages of saying yes to participating.

I know that <u>participating is voluntary</u> and that I can always withdraw my consent without losing my current or future rights to treatment.

I give my consent to participate in the research study and to have my biological material collected and stored in a research biobank. I have received a copy of this consent form as well as a copy of the written information about the study for my own use.

Data	
Date:	Signature:
would like to de	nealth information about you appears in the research study, you will be informed. If you line receiving any new information about your health that appears in the research nark here: (insert an x)
Do you want to by you?	e informed about the result of the research study and any possible consequences for
Yes (inser	t an x) No (insert an x)
Declaration fro	m the person providing the information:
I declare that the	e patient has received oral and written information about the research study.
In my opinion, se in the study.	ifficient information has been provided to enable a decision to be taken on participation
The name of the	person who provided the information: Roopameera Thirumathyam
Date:	Signature:

Studytidentification: (E.g comiteé ID, EudraCT no., version no./date or similar.)

EudraCT 2017-002101-35