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

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Abstract

Sodium-glucose cotransporter 2 (SGLT2) inhibition reduces cardiovascular morbidity and mortality in individuals with type 2 diabetes. Beneficial effects have been attributed to increased ketogenesis, reduced cardiac fatty acid oxidation and diminished cardiac oxygen consumption. We therefore studied whether SGLT2 inhibition altered cardiac oxidative substrate consumption, efficiency, and perfusion.

13 individuals with type 2 diabetes were studied after four weeks treatment with empagliflozin and placebo in a randomized, double-blind, placebo-controlled crossover study. Myocardial palmitate and glucose uptake were measured with ^{11}C-palmitate and ^{18}F-FDG PET/CT. Oxygen consumption and myocardial external efficiency (MEE) were measured with ^{11}C-acetate PET/CT. Resting and adenosine stress myocardial blood flow (MBF) and myocardial flow reserve (MFR) were measured using ^{15}O-H_2O PET/CT. Empagliflozin did not affect myocardial FFA uptake but reduced myocardial glucose uptake by 57% ($p<0.001$). Empagliflozin did not change myocardial oxygen consumption or MEE. Empagliflozin reduced resting MBF by 13% ($p<0.01$), but did not significantly affect stress MBF or MFR.

In conclusion, SGLT2 inhibition did not affect myocardial FFA uptake, but channeled myocardial substrate utilization from glucose towards other sources and reduced resting MBF. However, the observed metabolic and hemodynamic changes were modest and most likely contribute only partially to the cardioprotective effect of SGLT2 inhibition.

Clinical Trial Registration: EudraCT nr.: 2017-001779-22.

Treatment with SGLT2 inhibitors has consistently shown marked reductions in the risk of cardiovascular disease in individuals with type 2 diabetes(1-3). However, SGLT2 inhibitor treatment is also associated with substantial reductions in cardiovascular events in individuals with heart failure without diabetes, where the effect on plasma glucose is minimal(4). The cardioprotective effects of SGLT2 inhibitors can therefore not be mediated merely through lowering of blood glucose levels. However, exactly which alternative mechanisms may be responsible for such beneficial effects of SGLT2 inhibition remain unclear(5).

It has been suggested that SGLT2 inhibitors exert their beneficial effect through an increase in ketogenesis(6). A shift towards myocardial ketone body oxidation potentially improves myocardial energetics(7), since ketone bodies require less oxygen to generate ATP than the quantitatively most important myocardial substrate, free fatty acids (FFA)(7). In addition, increased levels of circulating ketone bodies may also have other potential beneficial effects, since elevated ketone body levels have been demonstrated to increase myocardial perfusion(8), ejection fraction(9) and cardiac output (CO)(9). Another potential cardioprotective mechanism is through the natriuresis and osmotic diuresis induced by SGLT2 inhibitors(10). The diuretic effect reduces extracellular fluid volume and blood pressure leading to a reduction in cardiac preload and afterload(5), which may improve left ventricular function and reduce cardiac workload and oxygen demand(5).

To further explore the cardioprotective effects of SGLT2 inhibitors, we performed a 4-week randomized, double-blind, placebo-controlled crossover trial of empagliflozin to determine the effects on myocardial substrate metabolism, oxygen consumption and perfusion in individuals with type 2 diabetes. We used a range of positron emission tomography (PET) tracers to measure myocardial FFA and glucose utilization, myocardial oxygen consumption, myocardial external efficiency, myocardial blood flow (MBF) and myocardial flow reserve (MFR) *in-vivo*.

We hypothesized that SGLT2 inhibition would induce a metabolic shift from FFA and glucose towards oxidation of other substrates leading to a reduced myocardial FFA oxidation and a reduced myocardial FFA and glucose uptake. We also hypothesized that SGLT2 inhibition would reduce cardiac workload and myocardial blood flow secondary to a reduction in cardiac afterload.

Methods

The study was approved by the Danish Medicines Agency and the regional ethics committee. The study was registered at eudract.ema.europa.eu (EUDRA-CT nr: 2017-001779-22). Informed, written consent was obtained from all participants.

Trial Design and Participants

The study was a randomized double-blind, placebo-controlled crossover study of once-daily empagliflozin 25 mg. Each treatment period was four weeks with a one-week washout in-between. Inclusion criteria were a) type 2 diabetes, b) diabetes duration > 1 year, c) HbA1c 6.5-9.0% (48-75 mmol/mol), d) age 50-70, e) metformin as only antidiabetic pharmacological treatment, and f) unchanged glucose-lowering treatment for three months (one month for other medications). Exclusion criteria were a) active or prior cancer, b) impaired renal function (eGFR < 60 ml/min), c) recent myocardial infarction (< 1 year), d) anemia (Hb < 6.5 mmol/L), e) recurrent genital infections, f) prior ketoacidosis, and g) alcohol abuse. Participants were recruited through advertisements in local press. All participants underwent physical examination, routine blood samples and electrocardiography to evaluate eligibility. Participants were randomly assigned in a 1:1 ratio to receive empagliflozin or placebo in the first study period. Randomization and encapsulation of medicine were handled by the hospital pharmacy. Excess trial medication was returned, and compliance was ensured by counting the remaining number of capsules. Participants were examined once weekly with blood samples. Three days prior to the end of each study period, participants were studied after an overnight fast. During this visit, whole-body DXA (Horizon, Hologic and Discovery, Hologic) scan was performed to assess body composition and indirect calorimetry (Jaeger Oxycon Pro, Intra medic and Deltatrac II, Datex) to measure resting energy expenditure (EE) and respiratory quotient (RQ). In addition, flash glucose monitoring (Flash Libre, Abbott, Chicago, IL) was applied for the 72 hours preceding the PET/CT study day. Measurements from the first day

were discarded due to the greater imprecision of measurements during the first 24 hours. To prevent impact of results on patient behavior, the display of the reader unit was blinded with black tape. Finally, equipment for measuring 24-hour blood pressure (Arteriograph 24, Tensiomed, Budapest, Hungary) and activity level (Fitbit charge II, Fitbit, San Francisco, CA) were applied. Participants were instructed to maintain an activity level equal to 6,000-10,000 steps/day to ensure comparable activity levels in the two study periods.

Positron Emission Tomography protocols and data acquisition

Participants were studied in the postabsorptive state after an overnight fast and took the last dose of EMPA/placebo in the morning of the PET/CT study day. PET/CT examinations were done on either a Siemens Biograph TruePoint TrueV 64 or a Siemens Biograph Vision (Siemens Healthcare, Erlangen, Germany). Images were reconstructed with a voxel size of 4×4×4 mm and all participants underwent PET/CT examinations on the same PET scanner on both study days. Due to tracer production difficulties, not all PET examinations were carried out on all participants. The number of successful PET examinations are listed below and in the figure legends for each radiotracer.

Participants were placed with the heart in the field of view and a low-dose CT scan (16 mAs, 100 kV) was obtained for attenuation and anatomic localization purposes. Timing of the PET/CT's were planned in order to allow for radiotracer decay. PET/CT flowchart is displayed in figure 1B and detailed description of PET protocols and data acquisition are available in the online supplemental material. *PET Image analysis*

Dynamic PET image analyses were performed using the in-house developed software package aQuant Research by an assessor blinded to study day sequence. PET image analysis is described in the online supplemental material.

Blood samples

Glucose was analyzed immediately after sampling using YSI 2300 STAT Plus glucose analyzer (YSI, Yellow Springs, Ohio). 3-hydroxybutyrate (3-OHB) were stored at -20 ^{o}C and other samples were stored at -80^{o}C until batch analysis. 3-OHB concentrations in serum were quantified using liquid chromatography tandem mass spectrometry(11), serum FFA concentrations with an enzymatic colorimetric method assay NEFA-HR (Wako Chemicals GmbH, Germany), lactate concentrations with immobilized enzyme biosensor technology (YSI 2300 model Stat Plus, YSI Life Sciences, Yellow Springs, OH) and insulin with AutoDELFIA immunoassay (PerkinElmer, Waltham, MA).

Statistics

Data are presented as mean±SD or median (CI95%) as appropriate. Data were inspected with QQ-plots for normal distribution and data were log transformed when appropriate. Paired samples t-test was used to detect effects of empagliflozin compared to placebo on PET/CT measurements. Mixed model analysis was used for repeated measurements during the study period (3-OHB) and during the study days (3-OHB, glucose, FFA, lactate) with treatment, time and the interaction between treatment and time as fixed factors. P-values less than 0.05 were considered statistically significant. The primary endpoint was myocardial FFA oxidation rate since we hypothesized a shift from FFA to ketone body oxidation. To detect a clinically significant reduction of 20% in FFA oxidation, a sample size of 10 subjects was required. To account for potential missing values, we planned to include 12 subjects. The remaining outcomes, except left ventricular mass and ejection fraction, were predefined in our protocol as secondary outcomes.

Data and Resource Availability

The data is available from the corresponding author upon reasonable request. No applicable resources were generated or analyzed.

Results

23 volunteers were screened for eligibility and 13 participants were included in the study. The study was planned to include 12 participants, but an extra participant was recruited after one participant withdrew consent after the first study day due to claustrophobia during PET scans. Baseline characteristics are presented in table 1.

Metabolic variables, blood pressure, body composition and energy expenditure

EMPA reduced 48-hour mean glucose measured by FGM (8.0±0.9 vs. 9.4±2.2 mmol/L, p<0.01), 24-hour systolic (124±8 vs. 129±12 mmHg, p<0.05) and diastolic blood pressure (70±6 vs. 74±7 mmHg, p<0.05) compared to placebo. Body weight was unaltered (94.6±9.6 vs. 95.2±9.7 kg, p=0.15), but EMPA led to a decrease in lean body mass (59.4±5.6 vs. 60.4±5.4 kg, p=0.03). Total body fat mass (31.4±12.2 vs. 31.2±11.3 kg, p=0.53) and fat percentage (32.9±10.1 vs. 32.4±9.3%, p=0.26) were similar after EMPA and placebo. EE was similar (7435±544 vs. 7443±481 KJ/d, p=0.95), but RQ decreased during EMPA treatment (0.81±0.03 vs. 0.83±0.03, p=0.02). Plasma creatinine (75±17 vs. 70±17 μmol/L, p=0.01) and hematocrit (0.42±0.02 vs. 0.41±0.03, p<0.01) increased during EMPA.

EMPA treatment increased FFA (0.86±0.30 vs. 0.72±0.27 mmol/L, p=0.02) and 3-OHB concentration (92 (CI95% 50-169) vs. 49 (CI95% 31-79) μmol/L, p<0.01) compared to placebo. The increase in 3-OHB was present from week one and remained stable throughout the treatment period (treatment: p=0.01, time: p=0.05, interaction; p=0.47) (figure 1A). 3-OHB increased more during the study day with EMPA and was 83% (22-173%) higher at the beginning of the study day and 226% (117-390%) higher at the end of the study day (interaction: p=0.02) compared to placebo (figure 1B). FFA concentration increased during the study day and was consistently higher during EMPA than during placebo (treatment: p=0.01; time effect: p=0.01; interaction: p=0.42) (figure 1C). Lactate levels were unaffected by EMPA, but decreased during the study day (treatment: p=0.25; time: p<0.01; interaction: 0.86) (figure

1D). Plasma glucose concentration decreased over the study day and was consistently lower during EMPA (treatment: p<0.001; time effect: p<0.0001; interaction: p=0.20) (figure 1E). Insulin levels were lower after EMPA (65±47 vs. 84±51 pmol/L, p=0.01) and were consistently lower throughout the study day (treatment: p=0.02, time effect: p<0.0001; interaction: p=0.31) (figure 1F).

Myocardial ^{11}C-Palmitate Metabolism

EMPA treatment reduced relative myocardial FFA uptake rate (8.0±1.4 vs. 9.8±1.7 mL/100g/min, p<0.001) and relative myocardial FFA oxidation rate (6.9±1.2 vs. 8.7±1.7 mL/100g/min, p<0.001), with no change in relative myocardial FFA re-esterification rate (1.2±0.4 vs. 1.2±0.5 mL/100g/min, p=0.98) (figure 2A). When absolute metabolism rates were calculated by multiplying the relative metabolism rates by plasma FFA concentrations, similar myocardial FFA uptake rate (7.7±3.7 vs. 8.2±3.6 μmol/100g/min, p=0.75), myocardial FFA oxidation rate (primary endpoint) (6.6±3.2 vs. 7.3±3.3 μmol/100g/min, p=0.54) and myocardial FFA re-esterification rate (1.1±0.7 vs. 1.0±0.6 μmol/100g/min, p=0.31) were observed (figure 2B).

Myocardial ^{18}F-FDG Uptake

EMPA treatment reduced relative myocardial glucose uptake rate (0.11±0.10 vs. 0.20±0.10 mL/100g/min, p<0.01) (figure 2C). When absolute myocardial glucose uptake was calculated by multiplying relative uptake rates with plasma glucose concentration, EMPA treatment reduced absolute myocardial glucose uptake by more than 50% (0.6±0.6 vs. 1.4±0.6 μmol/100g/min (p<0.001)) (figure 2D).

Myocardial blood flow

EMPA decreased resting MBF (0.74±0.10 vs. 0.85±0.10 mL/g/min (p<0.01)), but did not significantly affect stress MBF (3.08±0.79 vs. 3.10±0.80 mL/g/min (p=0.92)), MFR (4.14±1.06 vs. 3.60±0.86, p=0.09), or CO (5.6±0.9 vs. 5.5±0.8 L/min, p=0.55) (figure 3A-D). Since MBF is dependent on cardiac work, we also analyzed the data after adjustment for rate pressure product (RPP) (pulse x systolic blood pressure/10.000). Here, we also observed a reduction in resting MBF (1.01 vs. 1.06 mL/g/min, p=0.04).

Myocardial oxygen consumption, MEE, left ventricular mass and ejection fraction

EMPA did not significantly change myocardial oxygen consumption (8.8±1.0 vs. 9.7±1.4 mL/100g/min (p=0.12)) or MEE (29.5±7.7 vs. 27.0±4.1% (p=0.22)) (figure 3E-F).

EMPA did not affect left ventricular mass (127±29 vs. 134±24 g, p=0.67) or ejection fraction (69±7 vs. 70±7 %, p=0.90).

Discussion

The present study was performed to determine the effects of SGLT2 inhibition on cardiac substrate metabolism, oxygen consumption and perfusion in individuals with type 2 diabetes. We specifically aimed to investigate if changes in cardiac intermediary metabolism could be involved in the cardioprotective effects of SGLT2 inhibitors. First, EMPA did not affect myocardial FFA metabolism, but reduced the low myocardial glucose uptake by 57%, presumably due to an increase in oxidation of other substrates. Second, the EMPA driven shift in substrate utilization did not result in measurable changes in myocardial oxygen consumption or MEE. Third, EMPA reduced resting MBF by 13%, which was significant even after adjustment for cardiac workload. Collectively, these results indicate that SGLT2 inhibition is associated with an altered composition of oxidative energy substrates, but these changes are quantitatively of a minor degree and is unlikely to explain the cardioprotective effect of SGLT2 inhibition. Of interest, SGLT2 inhibition reduced resting myocardial perfusion, but the mechanisms behind this remain to be elucidated.

Empagliflozin reduces myocardial glucose uptake, but not FFA uptake or oxidation

SGLT2 inhibition increases ketogenesis and concentrations of circulating ketone bodies moderately(6). Recently, it has been hypothesized that such increases in circulating ketone bodies could serve as an oxygen-sparing energy-efficient substrate for the heart, since oxidation of ketone bodies require less oxygen to generate the same amount of ATP compared to FFA (the *thrifty substrate hypothesis*)(7). We have previously shown that acute experimental hyperketonemia in healthy subjects results in glucose being replaced by ketone bodies as the preferred myocardial oxidative substrate(8). However, that study was performed during a hyperinsulinemic-euglycemic clamp, with lipolysis and circulating free fatty acids suppressed to a minimum coupled with maximal myocardial glucose uptake. This particular metabolic milieu prevented us from determining whether ketone bodies could also replace FFA as a

substrate for the heart. To address this question, our current study was designed to investigate the effect of SGLT2 inhibition on cardiac FFA metabolism in the physiological, postabsorptive range. Therefore, participants were investigated after an overnight fast to avoid insulin-mediated suppression of ketogenesis and lipolysis. Consequently, we observed 10-fold higher FFA levels than in our previous study. As observed in most other SGLT2 inhibitor studies(6; 12), EMPA treatment resulted in significantly increased circulating FFAs compared with placebo but somewhat surprisingly, absolute rates of MFAU, MFAO and MFAE were unaltered. Since circulating FFA concentration is the primary driver of FFA uptake and oxidation(13; 14), an increase in MFAU and MFAO might have been expected. However, despite similar *absolute* FFA uptake and oxidation rates, we observed a decrease in *relative* fatty acid uptake and oxidation capacity (k-values). This downregulation may be mediated through the regulation of fatty acid translocase (FAP)/CD36, the primary transport protein for myocardial FFA uptake(15). FAP/CD36 is regulated by FFA and insulin concentration and the higher FFA and lower insulin concentration observed during SGLT2 inhibition, may have increased FAP/CD36 degradation and thereby reduced myocardial FFA uptake capacity(16).

Whereas EMPA treatment had no effect on fatty acid metabolism, EMPA reduced myocardial glucose uptake more than 50%. This finding is in accordance with a study in pigs, where SGLT2 inhibition increased ketone body and FFA uptake at the expense of glucose after iatrogenic myocardial infarction(17). Therefore, even though the increased delivery of ketone bodies appears to be readily utilized in the myocardium, this does not occur at the expense of less oxygen-efficient FFA, but as a substitute for glucose. However, the reduction in glucose uptake could also reflect an altered oxidation of other cardiac substrates. Recently, it was shown that myocardial branched chain amino acid uptake was increased by EMPA(17), but since amino acid oxidation only accounts for a very small percentage of cardiac substrate oxidation(18), it is unlikely to explain the findings of this study. Another important cardiac

substrate is lactate, but we did not observe any difference in lactate concentrations indicating similar lactate oxidation on the two study days. Finally, increased oxidation of glucose from myocardial glycogenolysis or circulating or intramyocellular triglyceride could have replaced plasma glucose as an oxidative substrate. All in all, we find that the most likely explanation for the reduction in glucose uptake is a shift towards ketone body oxidation due to an increased availability of ketone bodies.

Even though we observed a significant reduction in myocardial glucose uptake during EMPA, the absolute uptake rates were 10-fold lower (1.4 vs. 0.8 μmol/100g/min) than uptake rates observed in studies of healthy subjects, where fasting myocardial glucose uptake is in the range from 10-20 μmol/100g/min when measured with PET(19) or isotopic tracer infusion(20). It has previously been observed that fasting myocardial glucose uptake is reduced in type 2 diabetes(21), but the magnitude observed in this study was surprising to us. In fact, we found that myocardial glucose uptake only contributes ~2% of the energy required to the total cardiac work (cardiac work ≈20 J per mL $O_{2(22)}$) during placebo and ~1% during EMPA. This is lower than reported for healthy individuals, where the myocardial glucose uptake has been estimated to account for ~8% of cardiac work when measured as arteriovenous differences over the heart(23). Therefore, glucose appears to contribute minimally as a cardiac substrate in the postabsorptive state in individuals with type 2 diabetes. Based on these findings, we find it unlikely that the observed reduction in myocardial glucose uptake during EMPA is an important mediator of the cardioprotective effect.

Cardiac oxygen consumption or myocardial external efficiency

MEE is the ratio between cardiac workload and oxygen consumption and a reduction in MEE typically reflects a condition in which oxygen consumption increases more than cardiac work(24). MEE is reduced in heart failure(9) and is considered as part of the pathogenesis for heart failure(25). As discussed above, SGLT2 inhibition could potentially improve MEE

through a shift towards less oxygen demanding substrates. However, only limited data from animal studies are available regarding the effect of SGLT2 inhibition on MEE and cardiac oxygen consumption. Two recent studies have shown that EMPA treatment improves MEE in pigs after myocardial infarction(17) and during acute myocardial ischemia(26). In the latter study, MEE increased independently of oxygen consumption and myocardial fuel switching. This implies that SGLT2 inhibition affects MEE via mechanisms not necessarily mediated through the putative shift in cardiac substrate utilization. SGLT2 inhibition could also affect MEE through the reduction in blood pressure and thus cardiac work, which is supported by data showing that MEE is reduced in patients with hypertension and ventricular hypertrophy(27). In this study, the four-week intervention with EMPA did not significantly affect cardiac oxygen consumption or MEE. However, although not statistically significant, we did observe a decrease in oxygen consumption ($p=0.12$) and an increase in MEE ($p=0.23$) and it is tempting to speculate that a subtle decrease in oxygen consumption of 15% could have been picked up by a study primarily powered to assess ^{11}C-acetate PET(28). Also, we performed ^{11}C-acetate PET as one of the first scans during the study day, where the difference in ketone body concentration was smaller than at the end of the study day. To further complicate our ability to detect an effect on MEE, none of our participants had heart failure as judged by their normal LVEF and MEE, rendering it unlikely to see an improvement in MEE. It is therefore possible that the results had been different if subjects with established heart failure and a reduced MEE had been included. It is also possible that the relatively short duration of the intervention partly explains the absence of effect of EMPA on MEE. There are indications that longer term EMPA treatment results in left ventricular remodeling secondary to reduction in preload and afterload(17; 29). However, we observed no change in left ventricular mass indicating that the intervention period could be too short to detect such morphological changes. In conclusion, additional studies are required to

determine whether SGLT2 inhibition improves MEE in individuals with and without heart failure.

EMPA reduces resting myocardial blood flow

Myocardial perfusion is predictive of cardiovascular morbidity and mortality(30). Resting MBF increases with age(31) and primarily depend on cardiac workload and oxygen demand(32). The MFR (ratio between resting and stress MBF) reflects the combined function of epicardial arteries and the myocardial microcirculation and a reduced MFR is predictive of heart failure(33), cardiovascular events(34) and cardiovascular mortality(30). In the present study, EMPA reduced resting MBF but had no significant effect on stress MBF or MFR although the latter showed a tendency towards improvement ($p=0.09$). Improved MFR driven by increased stress-induced hyperemia has previously been demonstrated after 10 weeks of SGLT2 inhibition in prediabetic mice(35), but no studies in humans have thus far been published. Our observation of a reduction in resting MBF during SGLT2 inhibition could very well be caused by the reduced blood pressure. However, resting MBF was still reduced in the EMPA period after adjustment for cardiac workload (RPP), indicating that the decrease in resting MBF is not merely a consequence of reduced cardiac afterload.

In our previous studies, experimental acute hyperketonemia increased resting MBF(8) and CO(9). The increase in CO was dose-dependent, with a significant increase at 3-OHB concentrations as low as 0.7 mmol/L(9). By contrast, 4 weeks of EMPA treatment resulted in a more modest increase in 3-OHB to ≈ 0.1-0.2 mmol/L, which had no effect on CO and resting MBF was actually decreased. These observations appear to suggest that the moderate ketosis induced by SGLT2 inhibition does not have a quantitatively important impact on cardiac hemodynamics.

Strengths and limitations

A significant strength of this study is the comprehensive *in-vivo* characterization of the effects of EMPA on myocardial blood flow, oxygen consumption and substrate metabolism in humans using a range of robust PET/CT tracers and validated kinetic models. However, the study also has limitations. First, the sample size is small, which may have limited our possibility to detect more subtle effects of EMPA on our secondary end points. Second, only one participant had a history of cardiovascular disease. We did not restrict our inclusion criteria to individuals with heart failure or cardiovascular disease to investigate the cardiac effects of SGLT2 inhibitor treatment as second line therapy for the broad population of individuals with type 2 diabetes. Therefore, results could have been different if more participants with cardiovascular disease, especially heart failure, had been included. Subgroup analyses of the DECLARE-TIMI 58(36) indicated that the beneficial effect of SGLT2 inhibition was primarily observed in subjects with established cardiovascular disease. However, a meta-analysis has shown that the cardiovascular benefit of SGLT2 inhibitors appear to be independent of a history of heart failure (37). Third, we performed an adenosine stress test in the beginning of the study day, which potentially could have affected the estimates from the subsequent PET scans. However, since our two study days were identical and the participants served as their own controls, this is unlikely to explain the observed effects of EMPA.

Conclusions

SGLT2 inhibition does not affect myocardial FFA oxidation or uptake, but induces a shift in myocardial substrate utilization from glucose towards other sources, possibly ketone bodies. However, this shift in myocardial substrate utilization is quantitatively of minor importance and does not appear to improve either MEE or myocardial oxygen consumption. Therefore, it is unlikely to explain the striking cardioprotective benefit of SGLT2 inhibition. Of interest, SGLT2 inhibition reduces resting MBF, even when adjusting for cardiac workload.

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Disclosures

None.

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Table 1. Patient characteristics	
	Mean ± SD
Age (years)	62 ± 6
BMI (kg/m2)	31.5 ±5.0
Diabetes duration (years)	4.6 ± 3.0
HbA1c (%, mmol/mol)	7.3 ± 2.7, 56.7 ± 5.5
	N (%)
Sex	
Male	10 (77)
Female	3 (23)
Race	
White	12 (92)
Black or african american	1 (8)
IHD	1 (8)
Antidiabetic drugs	
Metformin	13 (100)
Antihypertensive drugs	
RAAS inhibitor	8 (62)
Thiazid	2 (15)
Beta blocker	2 (15)
Calcium Channel blockers	2 (15)
Lipid-lowering drugs	8 (62)

Figure 1: 3-OHB concentration during the study period and substrate concentrations during the study day

A: Empagliflozin increased 3-OHB concentration from week one during the four-week intervention compared to placebo. Data is plotted as median with 95% CI.

B: 3-OHB concentration was higher from the start of the study day and increased more during the study day after empagliflozin compared to placebo. Data is plotted as medians with 95% CI.

C: Empagliflozin increased FFA concentration during the study day compared to placebo. Data is mean with 95% CI.

D: Lactate decreased during the study day but was similar during empagliflozin and placebo. Data is median with 95% CI.

E: Plasma glucose was lower on the study day during empagliflozin compared to placebo. Data is mean with SEM.

F: Insulin decreased during both study days and were lower during empagliflozin treatment. Data is median with 95% CI.

Data was analyzed using linear mixed model analysis. 3-OHB; 3-hydroxybutyrate, FFA; free fatty acids. White circles = placebo, black circles = Empagliflozin.

Figure 2: Myocardial FFA and glucose metabolism

A: Empagliflozin reduced FFA relative uptake and oxidation rate compared to placebo. B; Absolute FFA metabolism rates did not change during empagliflozin compared to placebo. N = 12.

C: Empagliflozin reduced both relative myocardial glucose uptake rate and D; absolute myocardial glucose uptake rate compared to placebo. N=11.

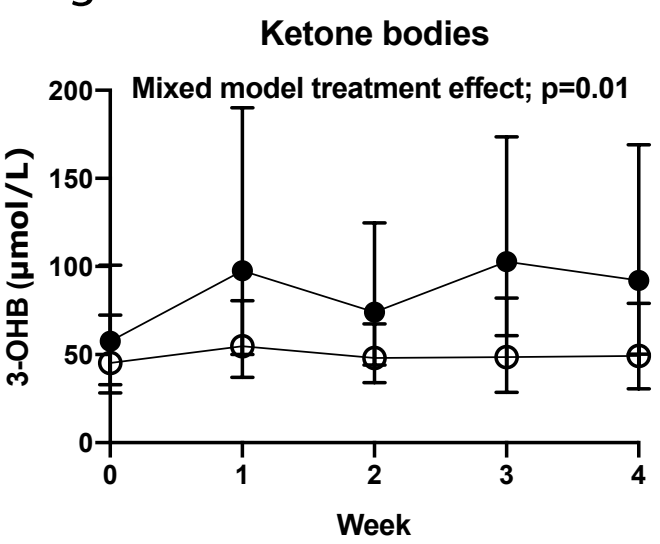
Data was analyzed with a paired samples t-test. FFA; free fatty acids. White circles = placebo, black circles = Empagliflozin.

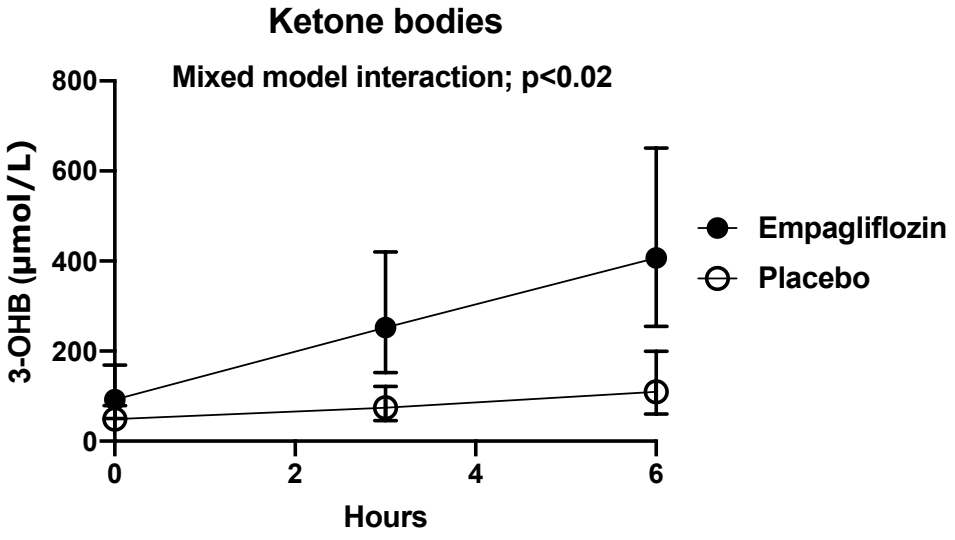
Figure 3: Myocardial blood flow, cardiac output, myocardial external efficiency and myocardial oxygen consumption

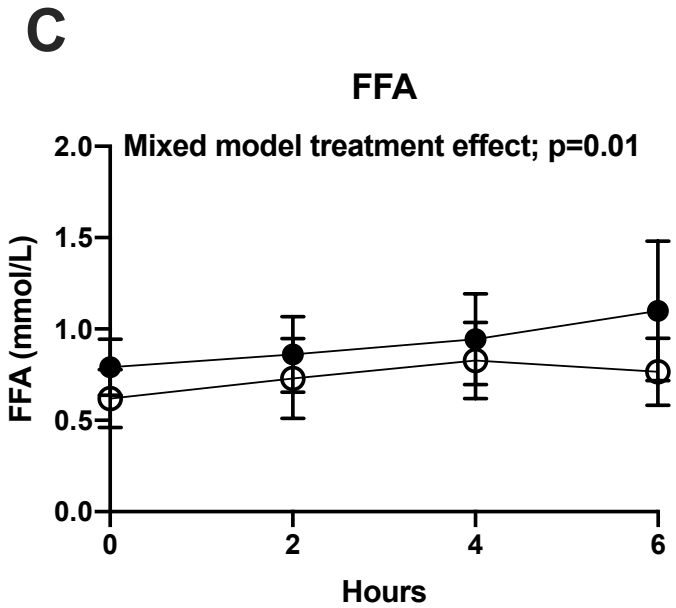
A: Empagliflozin reduced resting MBF compared to placebo. B, C, D; Empagliflozin did not significantly affect stress MBF, MFR and cardiac output. MBF: N=10, stress MBF: N=9.

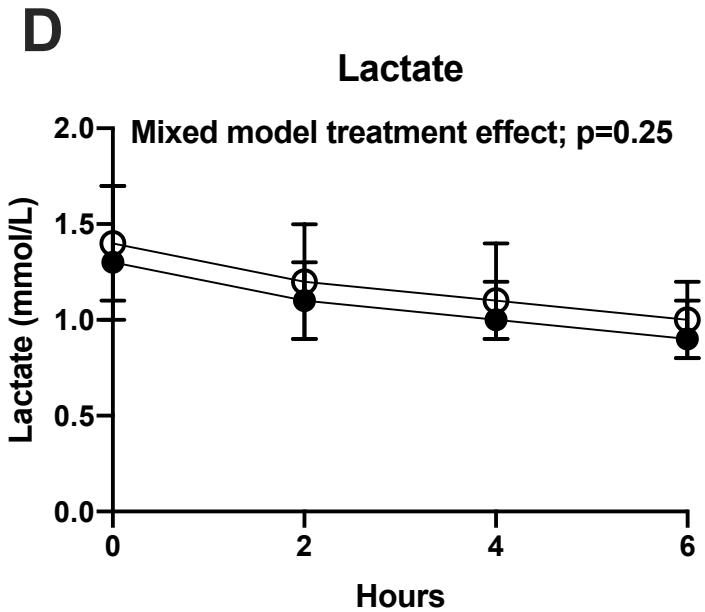
E, F; Myocardial external efficiency and myocardial oxygen consumption were unaffected by empagliflozin compared to placebo. N=10.

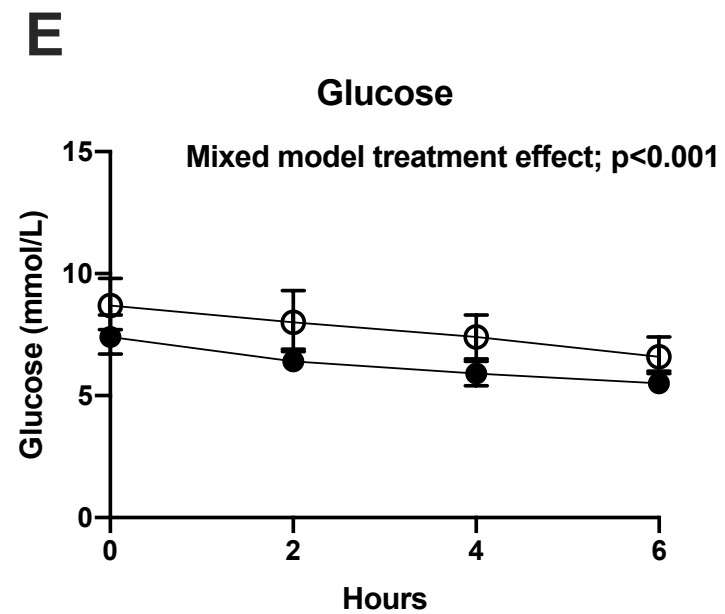
Data was analyzed with a paired samples t-test. MBF: myocardial blood flow, MFR; myocardial flow reserve. White circles = placebo, black circles = Empagliflozin.

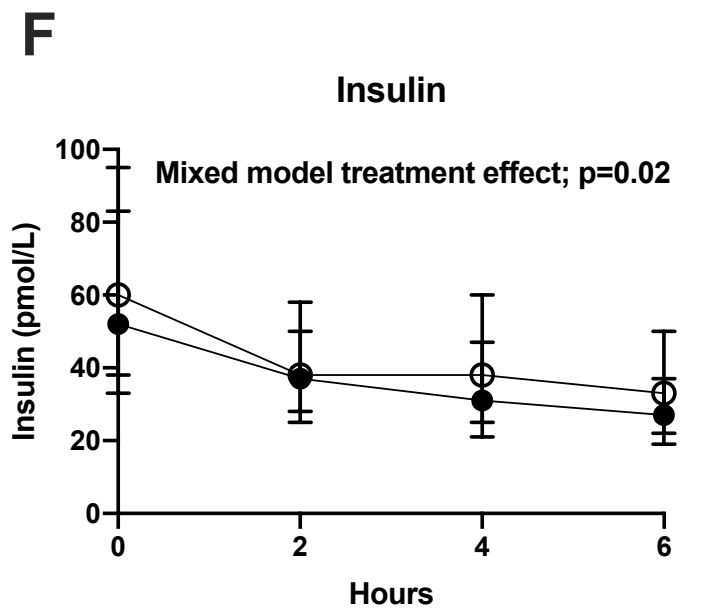

A
Ketone bodies
Mixed model treatment effect; p=0.01
3-OHB (µmol/L)
200
150
100
50
0
0
1
2
3
4
Week

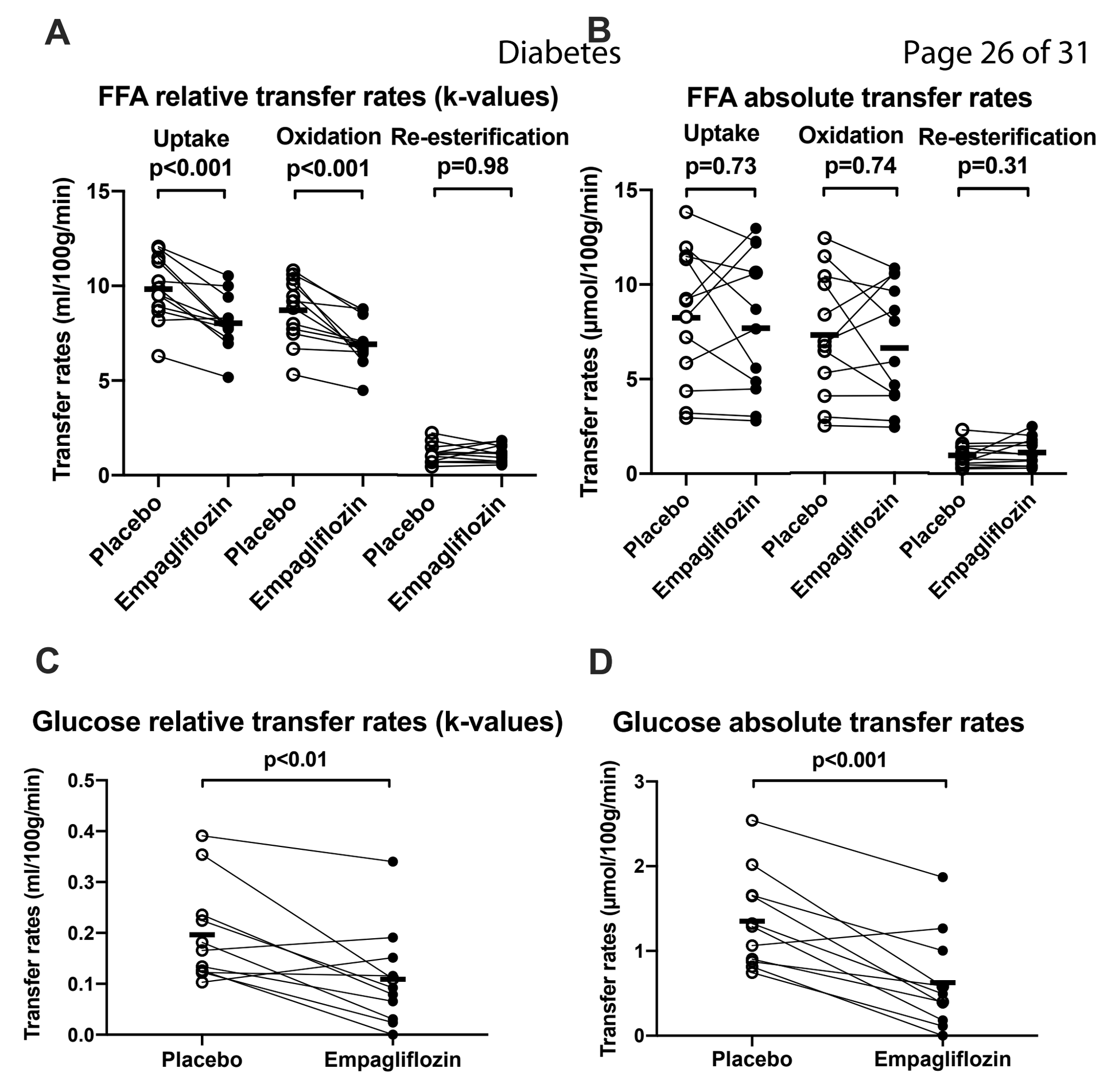

B
Ketone bodies
Mixed model interaction; p<0.02
3-OHB (µmol/L)
800
600
400
200
0
0
2
4
6
Hours
Empagliflozin
Placebo

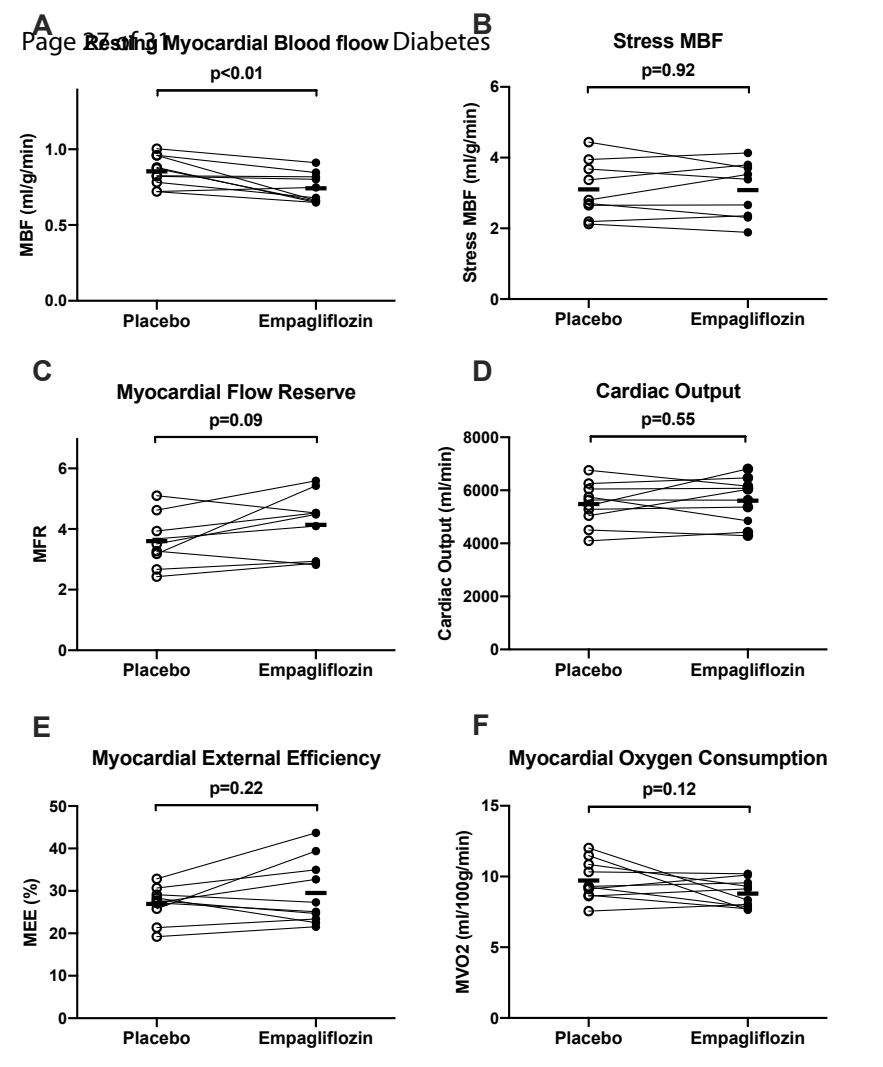

C
FFA
Mixed model treatment effect; p=0.01
FFA (mmol/L)
2.0
1.5
1.0
0.5
0.0
0
2
4
6
Hours


D
Lactate
Mixed model treatment effect; p=0.25
Lactate (mmol/L)
2.0
1.5
1.0
0.5
0.0
0
2
4
6
Hours


E
Glucose
Mixed model treatment effect; p<0.001
Glucose (mmol/L)
15
10
5
0
0
2
4
6
Hours


F
Insulin
Mixed model treatment effect; p=0.02
Insulin (pmol/L)
100
80
60
40
20
0
0
2
4
6
Hours


A
FFA relative transfer rates (k-values)
Uptake
p<0.001
Oxidation
p<0.001
Re-esterification
p=0.98
Transfer rates (ml/100g/min)
15
10
5
0
Placebo
Empagliflozin
Placebo
Empagliflozin
Placebo
Empagliflozin
B
FFA absolute transfer rates
Uptake
p=0.73
Oxidation
p=0.74
Re-esterification
p=0.31
Transfer rates (µmol/100g/min)
15
10
5
0
Placebo
Empagliflozin
Placebo
Empagliflozin
Placebo
Empagliflozin
C
Glucose relative transfer rates (k-values)
p<0.01
Transfer rates (ml/100g/min)
0.5
0.4
0.3
0.2
0.1
0.0
Placebo
Empagliflozin
D
Glucose absolute transfer rates
p<0.001
Transfer rates (µmol/100g/min)
3
2
1
0
Placebo
Empagliflozin


A
Resting Myocardial Blood flow
p<0.01
MBF (ml/g/min)
1.0
0.5
0.0
Placebo
Empagliflozin
B
Stress MBF
p=0.92
Stress MBF (ml/g/min)
6
4
2
0
Placebo
Empagliflozin
C
Myocardial Flow Reserve
p=0.09
MFR
6
4
2
0
Placebo
Empagliflozin
D
Cardiac Output
p=0.55
Cardiac Output (ml/min)
8000
6000
4000
2000
0
Placebo
Empagliflozin
E
Myocardial External Efficiency
p=0.22
MEE (%)
50
40
30
20
10
0
Placebo
Empagliflozin
F
Myocardial Oxygen Consumption
p=0.12
MVO2 (ml/100g/min)
15
10
5
0
Placebo
Empagliflozin

Online supplemental material

Positron Emission Tomography protocols and data acquisition

Two ^{15}O-H_2O perfusion scans were initiated at time = 0 min as 6-min scans with the following time-frame sequence: 1×10, 8×5, 4×20, 2×15, 3×20, 2×30, and 2×60 s. To reconstruct images a 3-dimensional (D) iterative algorithm was used (3 iterations (Vision 4), 21 subsets (Vision 5), 5-mm Gaussian postfilter), applying all appropriate corrections for normalization, dead time, scatter, random coincidences, and attenuation. The first ^{15}O-H_2O scan was an examination for resting MBF (n=10) whereas the second scan was performed as an adenosine stress test to determine maximal MBF (n=9) and MFR (n=9).

The 27-minute ^{11}C-acetate scan (n=10) was begun at time = 30 min with frame structure: 1x10, 12x5, 5x10, 2x30, 3x60, 3x120, and 3x300 s. Data were reconstructed with a 3D iterative algorithm (3 iterations (Vision 4), 21 subsets (Vision 5), 5-mm Gaussian postfilter). Blood pressure and heartrate was measured during the examination at time = 1, 5, 10, and 20 minutes.

The ^{11}C-palmitate scan (n=12) was initiated at time = 130 min in a 50-minute list mode scan (frame structure 6×5, 6×10, 3×20, 5×30, 5×60, 8×150, 4×300 s). Data were reconstructed with a 3D iterative algorithm (3 iterations (Vision 4), 21 subsets (Vision 5), 5-mm Gaussian postfilter). Blood and dynamic PET data were decay corrected to scan start.

The 50-minute ^{18}F-FDG scan (n=11)) was initiated at time = 230 min. 200 MBq ^{18}F-FDG was injected and a 50-minute list mode scan (frame structure 1×10, 8×5, 4×10, 3×20, 5×30, 5×60, 4×150, 4×300, and 1×600 s) was performed using 3D iterative reconstruction (3 iterations (Vision 4), 21 subsets (Vision 5), 4-mm Gaussian postfilter).

PET image analysis

Myocardial fatty acid metabolism was analyzed using a 3-tissue compartment model in which 3 rate constants was fitted. The input function was corrected for ^{11}C-metabolites using validated population-based estimates(1) The efflux rate of $^{11}CO_2$ was fixed to the oxidation rate and a slow esterification compartment was included. Macroparameters myocardial fatty acid oxidation (MFAO), myocardial fatty acid esterification (MFAE), and total myocardial fatty acid uptake (MFAU) were defined according to the suggestions by Bergmann(2):

$$MFAE = C_{NEFA} = \frac{k_{p1}k_{12}}{k_{1p} + k_{12} + k_{13}}$$

$$MFAO = C_{NEFA} = \frac{k_{p1}k_{13}}{k_{1p} + k_{12} + k_{13}}$$

$$MFAO = MFAE + MFAO$$

Myocardial glucose uptake (MGU) was estimated by Patlak analysis as previously described(3), with automatic segmentation of the left ventricle performed using K1 parametric images (due to low myocardial FDG retention). The relative uptake rate, Ki, was multiplied by the plasma glucose concentration to obtain absolute MGU (μmol/100 g /min). The lumped constant was not assumed to change between the two visits and was hence fixed at 1.

Myocardial oxygen consumption and efficiency were measured by ^{11}C-acetate PET. The examination was performed to obtain the global clearance rate (k_2) and to calculate MVO_2 as previously described(4)

$$MVO_2 = \frac{135xk2 - 0.96}{100}$$

Myocardial external efficiency, MEE, was calculated as:

$$MEE = \frac{LVexternal\ work}{Total\ LV\ MVO_2} = \frac{(FCO\ x\ MAP\ x\ 1.33x\ 10^{-4})}{MVO_2\ x\ LVmass\ x\ 20}$$

Left ventricular mass ($LV_{mass)}$ and forward cardiac output (FCO) was obtained from the PET dataset, whereas MAP was measured manually during the PET scan.

Rest and stress MBF were measured by ^{15}O-H_2O and analyzed using a previously described method allowing for highly automated calculation of parametric MBF imaging(5). In brief, parametric MBF images were generated using cluster analysis and implementation of a basis function method of the single-tissue model with additional RV spillover correction. All parametric images were automatically segmented according to the 17-segment model advocated by the American Heart Association (AHA)(6).

Left ventricular ejection fraction (LVEF) was calculated using the 11C-acetate scans as previously reported (7). In brief, the left ventricle was segmented automatically using parametric images of K_1 (myocardial 11C-acetate uptake rate) and V_A (Arterial blood fraction). End Systolic Volumes (ESV) were calculated based on V_A>0.7 and End Diastolic Volume (EDV) on V_A>0.175. LVEF calculated this way has been shown to correlate very well with LVEF measured by the Gold Standard of CMR (7).

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