The SGLT2 inhibitor dapagliflozin reduces liver fat, but does not affect tissue insulin sensitivity: a randomized, double-blind, placebo controlled study with 8-week treatment in type 2 diabetes patients

Running title: Dapagliflozin effect on tissue glucose uptake

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Objective: The aim of this study was to investigate tissue specific effects of dapagliflozin on insulin sensitivity and liver and body fat in patients with type 2 diabetes.

Research Design and Methods: 32 patients with type 2 diabetes were recruited for this randomized, double-blind, parallel group, placebo controlled study. Enrolled patients were to have HbA1c 6.5-10.5 % (48-91 mmol/mol), and ≥3 months of stable treatment with metformin, DPP-IV-inhibitor or their combination. Patients were randomized 1:1 to receive either 10 mg dapagliflozin or placebo daily for 8 weeks. Before and after the intervention, tissue insulin sensitivity was measured using [^{18}F]-fluorodeoxyglucose and positron emission tomography (PET) during hyperinsulinemic euglycemic clamp. Liver proton density fat fraction (PDFF) and adipose tissue volumes were assessed using MRI, and blood biomarkers were analyzed.

Results: After 8 weeks, glycemic control was improved by dapagliflozin (placebo-corrected change in HbA1c -0.39 % [p<0.01]), but whole-body glucose uptake was not increased (p=0.90). Tissue-specific insulin-stimulated glucose uptake did not change in skeletal muscle, liver, myocardium, or white and brown adipose tissue and endogenous glucose production remained unaffected. However, there were significant placebo-corrected decreases in liver PDFF (-3.74 %, p<0.01), liver volume (-0.10 L, p<0.05), visceral adipose tissue volume (-0.35 L, p<0.01), interleukin-6 (-1.87 pg/ml, p<0.05) and NT-proBNP (-96 ng/L, p=0.03).

Conclusions: In this study, 8 weeks of treatment with dapagliflozin reduced liver PDFF and the volume of visceral adipose tissue in obese patients with type 2 diabetes. Although glycemic control was improved, no effect on tissue-level insulin sensitivity was observed.

KEY WORDS: dapagliflozin, insulin resistance, liver proton density fat fraction, glucose uptake, positron emission tomography

Dapagliflozin is a highly selective inhibitor of renal sodium-glucose transporter 2 (SGLT2) approved for the treatment of patients with type 2 diabetes (T2D). The effect of this class of drugs is based on suppressing renal glucose reabsorption and increasing excretion of glucose to urine, resulting in improved glycemic control independent of insulin actions, as well as reduced body weight (1, 2).

Although SGLT2 is expressed almost exclusively in the kidney (3) and its inhibition is not expected to have a direct effect on tissue glucose metabolism elsewhere, previous studies have shown increased whole-body glucose consumption during hyperinsulinemia both after acute dosing, and after two weeks up to 3 months of treatment with dapagliflozin (4, 5, 6). In these reports, the amelioration of insulin resistance was assumed to reflect an increase in insulin-stimulated glucose uptake in skeletal muscle (4, 5) possibly explained by increased non-oxidative glucose disposal (6). Furthermore, the urinary glucose excretion is associated with increase in endogenous glucose production, which show differential mechanisms of action of SGLT2 inhibitors on glucose control in different tissues (7). Previous studies are lacking the ability to measure tissue-specific changes in metabolic rates, including the possible role of white and brown adipose tissue for SGLT2 inhibitor mediated increase in insulin-stimulated glucose uptake. Furthermore, with the recent interest in the effects of SGLT2 inhibitors on myocardial metabolism, it is notable that studies focusing on changes in myocardial substrate metabolism are limited.

The reduction in body weight associated with dapagliflozin treatment in obese T2D patients seems to mainly result from a reduction in both visceral and subcutaneous adipose tissue volumes (8), in addition to decreased fluid volume due to mild osmotic diuresis.

More recently, results from two open-label studies and one randomized, placebo-controlled study have suggested a decline in liver fat following treatment with SGLT2 inhibitors in obese T2D patients with non-alcoholic fatty liver disease (9, 10, 11).

The primary aim of this study was to investigate the effect of 8 weeks of dapagliflozin treatment on insulin-stimulated glucose uptake (GU) in insulin sensitive tissues, as measured by positron emission tomography (PET) and [^{18}F]-fluorodeoxyglucose ([^{18}F]-FDG) in patients with T2D to determine which tissues contribute to the reported increase in whole-body insulin sensitivity. Based on earlier results (4, 5, 6), we hypothesized that the intervention should have a measurable effect on skeletal muscle insulin mediated glucose uptake. The second aim was to assess changes in liver proton density fat fraction (PDFF) and volume, and in visceral and abdominal subcutaneous adipose tissue volumes, using magnetic resonance imaging (MRI).

RESEARCH DESIGN AND METHODS

Study subjects

Patients with previously diagnosed T2D and HbA1c 6.5-10.5 % (48-91 mmol/mol) were recruited for the study. Other main inclusion criteria were age 35 to 70 years, body mass index less than 40 kg/m^2, and at least 3 months of stable medication with either metformin, DPP-IV-inhibitor or their combination. Patients with any other concomitant diabetes medication, decreased renal function (creatinine clearance <60 mL/min using the Cockcroft-Gault equation [12]), significantly elevated liver enzymes (alanine aminotransferase [ALT] or aspartate aminotransferase [AST] >3 times above the upper limit of normal, total bilirubin >2.0 mg/dL), blood pressure over 160/100 mmHg at screening, unstable coronary syndrome, symptomatic heart failure, or alcohol abuse were excluded. The sample size was determined to detect a 25

% change in skeletal muscle glucose uptake, with about 90 % power at significance level α=5%.

Study design

The study design is illustrated in Figure 1. The study comprised five visits: a screening visit (Visit 1) 4 to 1 weeks before the second visit, a randomization visit (Visit 2), followed by a treatment follow-up visit after 4 weeks (Visit 3), and an end of treatment visit after 8 weeks of treatment (Visit 4) and a final visit (Visit 5) as a telephone follow-up 2 weeks after the end of treatment. PET/CT- and MRI-scans were performed at the Turku PET Centre on visits 2 and 4, whereas the other visits were organized by the recruiting site either at the Turku PET Centre or at a satellite site in Jyväskylä. A total of 55 volunteers were recruited from outpatient clinics, patient databases and by ads in local newspapers. After their eligibility was assessed on the first visit, 32 subjects were included in the study. On visit 2, the subjects were randomly assigned 1:1 to two parallel groups stratified by sex, to receive either 10 mg dapagliflozin (Forxiga®, AstraZeneca) or placebo (produced by AstraZeneca) daily, starting from the following day as add-on to their previous medication. Randomization was performed in balanced blocks in each stratum. Compliance was evaluated based on the amount of returned study medicine.

The study medication was administered in double-blinded fashion and all PET image, MRI, laboratory analyses and statistical analyses were performed by investigators blinded to the treatment. The study protocol was approved by Finnish Medicines Agency Fimea and the Independent Ethics Committee in Southwest Finland Hospital District. The study was conducted according to the principles of the Declaration of Helsinki. All subjects gave written informed consent prior to any study procedures.

Measuring insulin-stimulated whole-body and tissue glucose uptake using PET/CT

The PET/CT studies were conducted after an overnight fast of 10-12 hours and withholding of any medications on the day of the visit. Two catheters were inserted in opposite forearms of the subject; one to obtain venous blood, arterialized by using hot water bottles distally in the arm, samples and the other for injection of the PET radiotracer, and for insulin and glucose infusions. After collection of fasting laboratory samples, a hyperinsulinemic euglycemic clamp was performed as previously described (13, 14). The rate of insulin infusion was 40 mU m^{-2} body surface area min^{-1} (Actrapid, Novo Nordisk, Copenhagen, Denmark) and the rate of 20 % glucose infusion was adjusted based on plasma glucose levels, as determined every 5-10 minutes to maintain euglycemia (plasma glucose level of 5.0 ± 0.5 mmol/L). Whole body glucose uptake (M-value) was calculated by subtracting estimated urinary glucose excretion and space correction (change in glucose level in the glucose pool) from the glucose infusion rate (GIR). M-value is presented as the average of three to four 20-minute periods during steady euglycemia. Urinary [^{18}F]-FDG was measured at the end-of-study visit in all subjects, but glucose in urine only in eight subjects on dapagliflozin. Because the excretion rates of glucose and [^{18}F]-FDG correlated linearly (r=0.74, p=0.04, Supplemental Figure S1) in the subjects with both measurements, urine radioactivity was used to estimate urinary glucose excretion for the remaining subjects (see Supplementary info). In the dapagliflozin group, the mean excretion rate was 0.8 (SD 0.4) μmol/kg/min (range 0.2-1.3 μmol/kg/min), whereas urinary glucose concentrations were diminutive in the placebo group and were therefore not used for correction of M-values.

75 minutes (SD 15 min) after the start of the insulin infusion, subjects were injected with 155 MBq (SD 8 MBq) of [^{18}F]-FDG, produced as described earlier (15) and the PET scanning (Discovery 690, General Electric (GE) Medical systems, Milwaukee, WI, USA) was started

right after with the clamp ongoing. All tissues were scanned in one session sequentially, starting from the thoracic area (40 min) and followed by the upper abdomen (15 min), thighs (15 min) and neck (10 min). Radioactivity from arterialized plasma samples collected at 5-15 min intervals during the scanning and from an urine sample obtained at the end of scan was measured in an automatic gamma counter (Wizard 1480 3, Wallac, Turku, Finland).

PET data analysis

PET data was corrected for dead time, decay and photon attenuation before analysis. Tracer uptake into tissues was measured by determining volumes of interest (VOIs) with Carimas software version 2.9 (Turku PET Centre, downloadable at www.turkupetcentre.fi/carimas). Tissue time activity curves were obtained by a segmenting tool for the left ventricle, and free-hand drawing for other tissues, including both quadriceps femoris muscles, and a portion of the right liver lobe avoiding large vessels. For adipose tissue (AT), several VOIs were drawn in waistline subcutaneous AT, intraperitoneal visceral AT and bilateral supraclavicular depots of brown AT, and respective averages were reported.

Tissue time activity curves and an input function combined from PET image data and plasma samples, were used to estimate the fractional uptake (Ki) of tracer in each tissue by graphical analysis (16). Tissue glucose uptake (μmol kg^{-1} min^{-1}) was calculated by multiplying Ki by steady-state plasma glucose levels divided by tissue density and a previously established lumped constant (1.2 for skeletal muscle, 1.0 for liver and myocardium, 1.14 for adipose tissue [17-20]). Endogenous glucose production (EGP) was assessed by subtracting glucose infusion rate from rate of glucose disposal derived from [^{18}F]-FDG consumption and estimated urinary glucose loss (21).

MRI assessment of liver fat, liver volume and abdominal adipose tissue volumes

MRI was performed at 3T using the MRI part of a clinical PET-MR system (Philips Ingenuity TF, Philips Healthcare, OH, USA). Subjects were positioned in supine position with the arms extended above the head. Imaging was performed using the integrated body coil. Liver and abdominal adipose tissue (AT) volumes were assessed using multi-echo water-fat MRI and liver volume using a T1-weighted fat suppressed single echo sequence. Liver fat was measured by use of a proton density fat fraction (PDFF) measurement. All imaging was performed during breath holds in exhaled position. Details on scan parameters and image reconstruction are given in supplementary materials. To optimize precision, liver fat was assessed by manual delineation of a large volume of interest including as much liver tissue as possible, while avoiding the tissue borders to limit partial volume effects. The median liver fat content was reported. Liver volume was determined using semi-automated segmentation and the software Smartpaint (version 1.0) (22). Volumes of abdominal subcutaneous and visceral AT were determined from whole-body scans by using an automated algorithm (23). Coefficients of variations (CVs) from repeated imaging and analysis have been previously determined to be 5.4% and 2.1% for liver PDFF and liver volume, respectively (n=10, unpublished data) and 2.3% and 1.9% for subcutaneous and visceral AT, respectively (23).

Laboratory measurements

A detailed description of biochemical and immunological analyses is provided in supplementary materials.

Statistical analyses

All statistical analyses were performed using SAS® version 9.4 (SAS Institute Inc., Cary, NC, USA). Changes in parameters measured at baseline, week 4 and week 8 including body weight, systolic and diastolic blood pressure, blood HbA1c, serum FFAs, and plasma levels of fasting glucose, HbA1c, insulin, glucagon were analyzed using mixed model for repeated

measurements (MMRM), with the fixed categorical effects of treatment, week, treatment-by-week interaction, the randomization strata of sex and the sex-by-week interaction, as well as fixed covariates of baseline measurement and baseline measurement-by-week interaction. For other variables measured at baseline and week 8, a two-way analysis of covariance (ANCOVA) was used to detect a two-sided change at the 5 % level of significance. Fixed effects of treatment, sex and baseline value were included in the model. M-values are reported either as original values or as corrected values divided by the average of plasma insulin levels during steady state in clamp. Difference in baseline NT-proBNP in subjects with or without previous hypertension or cardiovascular disease was analyzed using Wilcoxon Rank-sum test. Baseline values are reported as mean ± SD, and end-of-treatment results as placebo-corrected adjusted least square means changes in the dapagliflozin arm from baseline with 95 % confidence intervals. Correlations were tested using Spearman rank correlation. The one discontinued patient was not included in the analyses.

RESULTS

Subject characteristics

Baseline characteristics of both treatment arms can be seen in Table 1. There were no significant differences between groups concerning sex distribution (with the majority being male in both groups, 87 % in dapagliflozin vs. 75 % in placebo group), age, BMI, glycemic control or time since diagnosis. All subjects were on metformin and 9 (60 %) in the dapagliflozin and 7 (44 %) in the placebo group were also on sitagliptin. Groups were similar in the prevalence of hypertension (53 % in dapagliflozin vs. 52 % in placebo group). One subject in the dapagliflozin group was discontinued on the day after randomization due to elevated liver enzymes on Visit 2. Compliance was high within both groups (≥95 %).

Dapagliflozin was well tolerated, with no difference in the occurrence of infections between groups (2 subjects in the dapagliflozin group, 3 subjects in the placebo group).

Improved glycemic control and weight loss with dapagliflozin

There was a significant reduction in HbA1c, fasting plasma glucose and BMI already after 4 weeks, and also after 8 weeks of treatment with dapagliflozin (Table 1). Fasting insulin, free fatty acids, glucagon and GLP-1 levels did not change significantly, although in the dapagliflozin-group plasma insulin levels decreased numerically, and glucagon and GLP-1 levels increased numerically after 4 weeks of treatment but returned close to baseline after 8 weeks of treatment. (Supplemental Figure S2). Moreover, glucagon/insulin ratio was not significantly altered (p=0.42).

Unchanged insulin sensitivity

Baseline whole-body insulin-stimulated glucose uptake (M-value) was low in both groups: 6.2 µmol/kg/min in the dapagliflozin group and 7.8 µmol/kg/min in the placebo group (Table 1), and the placebo-corrected changes in the M-values were not significant (-0.12 µmol/kg/min, 95 % CI -2.1, 1.9, p=0.90) (Figure 2C), also when correcting for steady state insulin levels (0.01, 95 % CI -0.01, 0.04, p=0.40). Change in rate of endogenous glucose production was not different from placebo (-0.02 µmol/kg/min, 95% CI -3.2, 3.2, p=1.0) (Table 1, Figure 2C).
There was no effect of dapagliflozin on skeletal muscle glucose uptake (-0.003 µmol/kg/min, 95 % CI -3.1, 3.1, p=1.0) (Figure 2C, Supplemental table S2), but the changes in M-value and skeletal muscle glucose uptake were correlated (r=0.64, p<0.01). Levels of plasma glucose or insulin levels during the steady state did not change in either group (Supplemental figure S3).

Decrease in liver fat and volume

Baseline PDFF was similar between groups, 22 ± 11 % in the dapagliflozin group and 21 ± 9.3 % in the placebo group. At the end of treatment, there was a significant reduction of both liver PDFF (-3.7 %, 95 % CI -6.18, -1.30, p<0.01) and volume (-0.10 L, 95 % CI -0.19, -0.003, p=0.04) (Figure 2A). Post-hoc analyses showed that introducing changes in BMI or visceral AT volume in the model had a significant effect on reduction of liver fat (p=0.02 and p=0.01, respectively). In the dapagliflozin group, hepatic glucose uptake did not change significantly (-1.3 µmol/kg/min, 95 % CI -5.6, 3.0, p=0.53) (Figure 2C, Supplemental table S2), and the levels of ALT and AST remained at baseline level (Table 1). In addition, fibroblast growth factor 21 (FGF21) tended to lower (-111 pg/mL, 95 % CI -232, 9.4, p=0.07). Including the change in liver PDFF in the model, showed a statistically significant effect on the decrease in FGF21 (p=0.01).

Reduction of adipose tissue volume

Dapagliflozin treatment resulted in significant changes in adipose tissue measured by MRI: volume of visceral adipose tissue was reduced by -0.35 L (95 % CI -0.59, -0.12, p<0.01) and the volume of abdominal subcutaneous adipose tissue was reduced by -0.28 L (95 % CI -0.52, -0.05, p=0.02) (Figure 2B). There was no significant change in lean body mass (-1.2 L, 95 % CI -2.8, 0.41, p=0.14). Insulin-stimulated glucose uptake was not altered in visceral, subcutaneous or brown adipose tissue (-0.02 µmol/kg/min, 95 % CI -0.13, 0.09, p=0.71; 1.14 µmol/kg/min, 95 % CI -0.6, 2.9, p=0.19; -0.38 µmol/kg/min, 95 % CI -2.1, 1.3, p=0.65) (Figure 2C, Supplemental table S2).

Effects on inflammatory biomarkers

Dapagliflozin-intervention decreased the level of interleukin-6 (IL-6) by 1.9 pg/mL (95 % CI -3.6, -0.14, p=0.04). There was no change in levels of tumor necrosis factor α (TNF- α) (0.103 pg/mL, 95 % CI -0.136, 0.343, p=0.40), or monocyte chemotactic protein 1 (MCP-1) (-0.60,

95 % CI -76, 75, p=1.0). In post hoc analysis, changes in IL-6 and subcutaneous AT volume correlated significantly in the dapagliflozin group (r=-0.62, p=0.02), but including change in subcutaneous AT volume in the model did not significantly influence the treatment effect on IL-6 (p=0.07).

Lowering of NT-proBNP by dapagliflozin

In this study, dapagliflozin did not have a significant effect on systolic or diastolic blood pressure (Table 1), or myocardial left ventricular glucose uptake (-19.0 µmol/kg/min, 95 % CI -70, 32, p=0.46). However, the level of NT-proBNP decreased significantly by -0.96 ng/L in the dapagliflozin group (Table 1). Although subjects with pre-existing hypertension or other cardiovascular diagnosis (N=8 in dapagliflozin and N=9 in placebo group, including 1 subject with atrial fibrillation in both groups and 3 subjects with coronary artery disease in the dapagliflozin group) had higher baseline NT-proBNP (p=0.04), this did not significantly predict the treatment response.

CONCLUSIONS

This randomized, parallel-group, double-blind, placebo-controlled study showed that in obese T2D patients, 8 weeks of treatment with dapagliflozin did not change skeletal muscle insulin sensitivity, as measured directly with PET. Also, in contrast to previous studies (5, 6, 7), we did not find an effect on whole-body insulin sensitivity. However, comparing results with previous studies is not completely straightforward due to different methodologies, including how the clamp was performed. As compared to previous studies, the two (4, 6, 7) to three times (5) lower insulin infusion rate in this study likely did not inhibit endogenous glucose production completely. The duration of the clamp was also shorter compared to earlier reports (4-7). Moreover, in this study the drug was not administered on the day of visits, and the participants

were characterized by more severe insulin resistance associated obesity and liver steatosis, and not only hyperglycemia. These differences plausibly explain why the rate of endogenous glucose production remained slightly higher during hyperinsulinemia in our study compared to what has been reported by Merovci et al (4), and possibly explain why we did not see an effect by dapagliflozin treatment. It might also be that the change in EGP would have been measurable at fasting rather than euglycemia, as reported by Daniele et al (6). In addition, the patients had low M-values indicating that the insulin infusion rate could have been too low to detect small changes in insulin sensitivity.

Previous studies have assumed that changes in whole body glucose disposal (M-value) reflect an improvement in skeletal muscle insulin sensitivity by dapagliflozin, considering that muscle is the predominant glucose user during insulin stimulation. The method used in this study, PET imaging during clamp, enables direct quantitation of insulin sensitivity in multiple tissues simultaneously (14). In line with unchanged M-value, we found no change in skeletal muscle insulin sensitivity by dapagliflozin, and no difference compared to placebo. Also, in other tissues, including liver, myocardium, subcutaneous, visceral and brown adipose tissue no changes in glucose uptake could be detected. Thus, the tissue uptake of glucose measured with PET during the clamp was not able to reveal which tissues could have been responsible of the increase in M-value shown in other studies (4, 5, 6).

One important finding of the study was the significant reduction in whole-liver fat content after 8 weeks of treatment with dapagliflozin in obese T2D patients; results similar to a recent report (11). This decrease is consistent with the associated reduction in body weight and visceral adipose tissue as shown in other studies (24, 25). Except from reduced body weight, an alternative hypothesis explaining loss of liver fat is the metabolic substrate shift from glucose to fatty acids (5, 6, 26) and possibly increased fatty acid oxidation in the liver presumed to be

associated with reduced night-time hepatic glycogen depots and increased gluconeogenesis (27).

The numerical lowering of FGF-21 by dapagliflozin treatment, also reported by Eriksson et al (11), can be attributed to the changes in liver fat in this study, supported by the previously recognized association between higher concentrations of FGF21 and NAFLD (28). Reduced FGF21 can also be attributed to improved mitochondrial function as exemplified by the very high FGF21 levels in patients with inherited mitochondrial dysfunction (29) or alleviated endoplasmic reticulum stress. Interestingly, both higher circulating NT-proBNP and FGF-21 levels are associated with myocardial diastolic dysfunction (30).

We did not observe changes in myocardial glucose utilization after 8 weeks of treatment, which is supported by the evidence that glucose is not the primary substrate for myocardium at rest. Neither did we see significant changes in fasting plasma beta-hydroxybutyrate nor serum free fatty acid levels (Table 1). As insulin-stimulated glucose metabolism did not change, our findings do not contradict the hypothesis that it is the substrate shift in favor of fatty acids and ketones which results in improved cardiac energy usage, efficacy and contractility resulting in the rapidly decreased risk of heart failure and cardiovascular mortality in the EMPA-REG OUTCOME study (31, 32). We observed a significant decrease in NT-proBNP, despite none of the subjects in this study had a history of heart failure. Even a moderately increased level of NT-proBNP has been shown to predict cardiovascular mortality independent of traditional risk factors in T2D patients (33). Therefore, the decline in NT-proBNP may indicate a reduced risk to develop heart failure in T2D patients. We also saw an increase in hematocrit by 3.7 ± 3.2 % in the dapagliflozin group at the end of treatment, similar to previous reports (34).

Several previous studies have reported an increase in the levels of glucagon and glucagon/insulin ratio during SGLT2i treatment, and inhibition of SGLT2 has also been shown

to directly stimulate secretion of glucagon from pancreatic alpha cells (35). It could be speculated that we were not able to see these changes in this study because of the time elapsing between study drug administration and sampling. Another possibility is the use of DPP-4 inhibitors in about half of the study population. However, a post hoc analysis showed that patients on sitagliptin had a similar change in glucagon/insulin ratio as compared to those on no sitagliptin treatment.

Changes in different inflammatory biomarkers were inconsistent in this study. Even though up to 35 % of the circulating IL-6 is excreted by adipose tissue (36), the decrease was not affected by loss of subcutaneous AT mass in our small study sample and, surprisingly, the association between these reductions was negative, so some other factor might contribute to the lowering of IL-6 during dapagliflozin treatment. Interestingly an association between higher circulating concentrations of IL-6 and increased risk of myocardial infarction has been observed (37). Therefore, reduced IL-6 levels may contribute to the cardioprotective effects of SGLT2 inhibitors (34, 38).

The strengths of this study are its well-established methods in measuring insulin-stimulated glucose uptake comprehensively from several different tissues, adipose tissue volumes and liver proton density fat fraction, as well as the double-blinded, randomized design. The PET method used for quantifying tissue glucose uptake takes into account potential changes in biodistribution and urinary loss of [^{18}F]-FDG. Limitations of the study include a small number of patients, which could help to explain why no significant effects were observed on well-known effects of SGLT2 inhibition such as blood pressure and plasma levels of beta-hydroxybutyrate. Another limitation is that glucose loss in urine was not quantified in all subjects which lead to the need to use [^{18}F]-FDG to estimate urinary glucose. This might have caused reduced precision concerning M-values and EGP.

To conclude, eight weeks of treatment with dapagliflozin did not significantly change tissue insulin-stimulated glucose uptake directly measured with PET. However, the treatment reduced liver fat content, as well as subcutaneous and visceral adipose tissue, when measured using MRI data from the whole liver and adipose tissue depots from the abdominal region. Dapagliflozin also seems to have a positive effect on plasma NT-proBNP and IL-6 levels, which could help to understand the positive effects on cardiovascular deaths and hospitalization due to heart failure.

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Author Contributions and Guarantor Statement

A.L.-R. performed study visits and drafted the manuscript. A.L.-R., M.-J.H, J.M., and J.C.H. participated in PET data analysis. L.J. and J.K. analyzed MRI data. J.S. was the PI of the Jyväskylä satellite site and performed study visits there. A.K.K. is accounted for [^{18}F]-FDG production. V.S. was involved as an on-site expert on MRI. P.I. offered consultation on methodological issues and critical manuscript revision. A.L.-R., J.O. and P.N. contributed to statistical analysis. All authors commented on initial version of the manuscript. P.N. is the principal investigator and guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflicts of Interest

L.J., J.K. are employees of Antaros Medical and J.O. is employed by AstraZeneca Gothenburg.

Other authors report no potential conflicts of interest.

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Prior Presentation Information

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FOOTNOTES

Clinical trial reg. no. NCT02426541, clinicaltrials.gov

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TABLES

Table 1

Variable	Placebo		Dapagliflozin 10 mg		p-value
	Baseline	At 8 weeks	Baseline	At 8 weeks	
Group size	N= 16	N=16	N= 15	N=15	
Sex (m/f)	12/4		13/2		
Age (yr)	60 ± 7.4		62 ± 8.4		
Diabetes duration (yr)	7.3 ± 3.7		7.8 ± 3.8		
MET / MET+SITA	9/7		6/9		
Hypertension yes/no	9/7		8/7		
BMI (kg/m^2)	31.7 ± 5.0	31.8 ± 4.8	32.1 ± 3.9	31.3 ± 3.7	<0.0001
FPG (mmol/L)	8.7 ± 1.7	9.0 ± 1.5	9.5 ± 1.9	7.8 ± 0.9	<0.01
HbA1c (%)	6.8 ± 0.5	6.8 ± 0.4	7.0 ± 0.6	6.6 ± 0.6	<0.01
HbA1c (mmol/mol)	51 ± 6	51 ± 5	53 ± 7	49 ± 7	
Systolic BP (mmHg)	147 ± 14	139 ± 15	151 ± 13	144 ± 15	0.79
Diastolic BP (mmHg)	86 ± 9.6	81 ± 7.6	84 ± 6.8	82 ± 9.0	0.48
M-value (µmol/kg/min)	7.8 ± 5.2	8.3 ± 5.1	6.2 ± 3.3	6.9 ± 3.5	0.90
EGP (µmol/kg/min)	9.4 ± 3.9	7.8 ± 4.0	7.6 ± 4.4	7.0 ± 4.2	1.0
Fasting insulin (mU/L)	19 ± 12	17 ± 8	20 ± 11	17 ± 8	0.52
FFA (mmol/L)	0.69 ± 0.18	0.66 ± 0.21	0.64 ± 0.15	0.67 ± 0.14	0.62
OHBut (mmol/L)	0.12 ± 0.11	0.12 ± 0.09	0.09 ± 0.05	0.17 ± 0.18	0.33
ALT (U/L)	38 ± 14	39 ± 15	50 ± 21	45 ± 16	0.47
AST (U/L)	32 ± 12	31 ± 10	30 ± 10	30 ± 10	0.92
NT-proBNP (ng/L)	75 ± 146	120 ± 193	99 ± 140	44 ± 48	0.03
IL-6 (pg/mL)	3.5 ± 2.4	4.0 ± 4.4	6.6 ± 8.2	5.8 ± 8.9	0.04
FGF-21 (pg/mL)	293 ± 194	362 ± 272	388 ± 315	334 ± 198	0.07

Table 1. Baseline and after treatment values are reported as mean ± SD. P-values placebo-corrected adjusted mean changes from baseline for the dapagliflozin group. MET/MET+SITA, subjects on metformin monotherapy or metformin + sitagliptin, FPG fasting plasma glucose, BP blood pressure, EGP endogenous glucose production, FFA fasting free fatty acids, OHBut hydroxybutyrate, ALT alanine aminotransferase, AST aspartate aminotransferase, NT-proBNP N-terminal pro-brain natriuretic peptide, IL-6 interleukin-6, FGF-21 fibroblast growth factor 21.

FIGURE LEGENDS

Figure 1. General study outline.

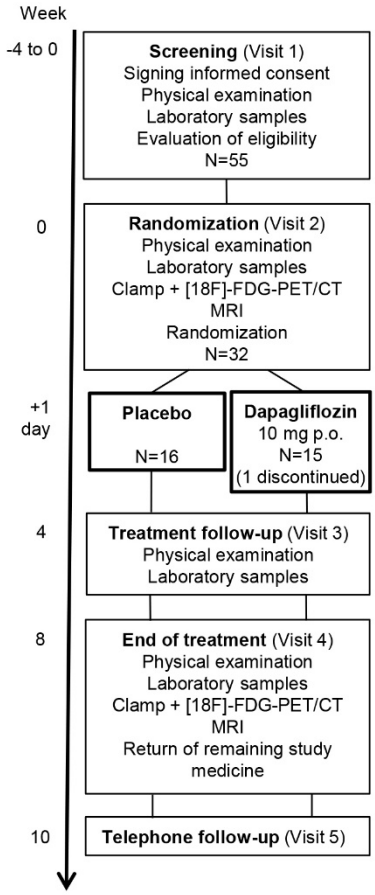
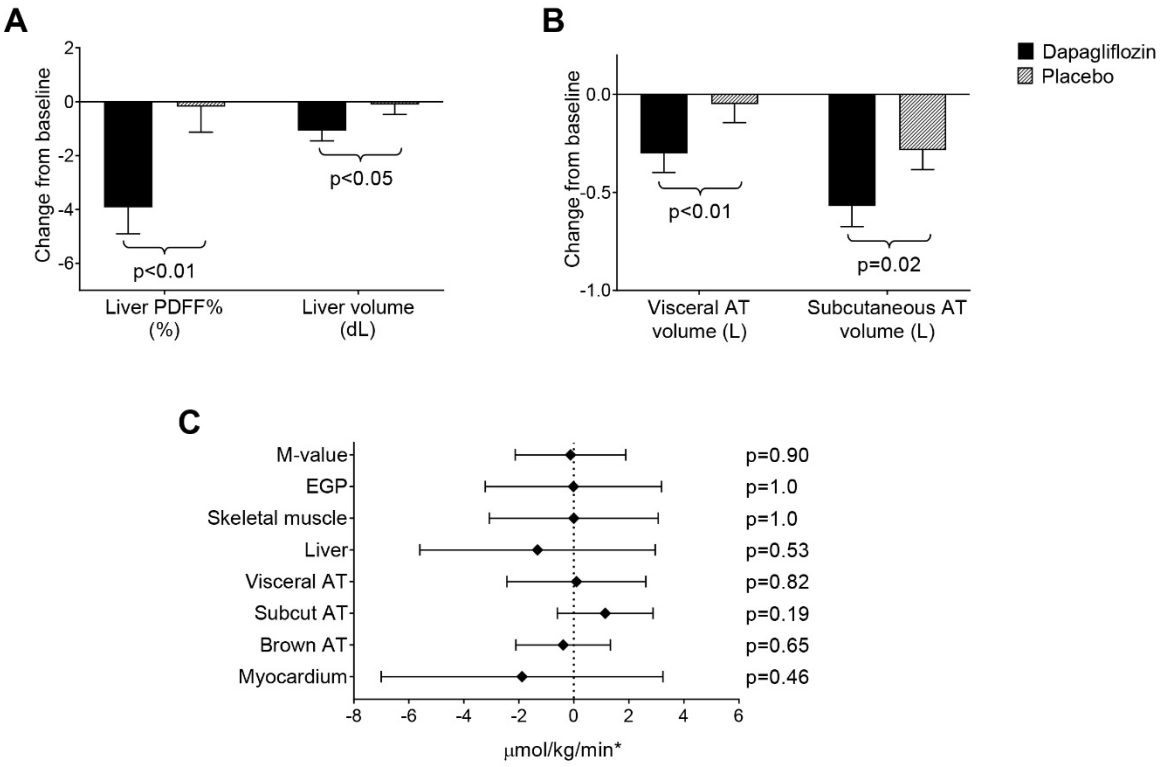


Figure 2. A. Adjusted mean change from baseline for liver PDFF% and volume in dapagliflozin and placebo treatment groups. **B.** Adjusted mean change from baseline in visceral adipose tissue (AT) and abdominal subcutaneous AT volumes in dapagliflozin and placebo treatment groups. **C.** Placebo-corrected mean changes and 95 % confidence intervals of M-value, endogenous glucose production and of glucose uptake in different tissues. EGP endogenous glucose production, AT adipose tissue, Subcut subcutaneous. *Unit for myocardial glucose uptake is µmol/100 g/min.



The SGLT2 inhibitor dapagliflozin reduces liver fat, but does not affect tissue insulin sensitivity: a randomized, double-blind, placebo controlled study with 8-week treatment in type 2 diabetes patients

SUPPLEMENTARY MATERIALS

Estimation urinary glucose loss

FDG clearance (ml/min) was calculated as $Renal\ clearance_{FDG} = \frac{FDG_{urine}}{AUC_{0 \rightarrow sampling\ time}}$, where FDGurine was urine [^{18}F]-FDG decay corrected activity in the sample (kBq) and $AUC_{0->sampling}$ time decay corrected area under the curve for FDG in plasma from the injection until the urine sample ($\frac{\text{min} \times \text{kBq}}{\text{ml}}$). Eight subjects in the dapagliflozin group had measurement of amount of glucose lost to urine during the study. Glucose flux to urine (µmol/min) was calculated as $Flux_{urine\ glucose} = \frac{Glucose_{urine}}{Time_{urine\ collection}}$, where glucose in urine was expressed as (µmol) and time for urine collection (min) was determined as interval from voiding the bladder before the study and urine sampling at the end of the study. There was a significant correlation between FDG clearance and glucose flux to urine during the study, r=0.74, p=0.038 (Supplementary figure S1). This allowed us to create a linear regression model to estimate glucose flux to urine from [^{18}F]-FDG clearance in subjects whose actual urine glucose loss had not been measured: $Flux_{urine\ glucose} = -13.47 + 1.92 \times Renal\ clearance_{FDG}$, unit (µmol/min). Measured glucose in the urine in five subjects of the placebo group was negligible, thus glucose flux to urine is assumed as 0 for the placebo group.

Figure S1

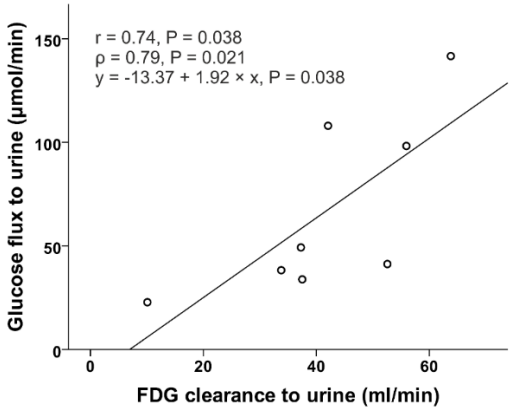


Figure S1. Association between measured [^{18}F]-FDG clearance to urine and glucose flux to urine among eight subjects with dapagliflozin treatment.

MRI parameters

Detailed MRI parameters are given in table 1. The water-fat reconstruction of the liver fat scans was performed using a Matlab implementation of the algorithm described in (1). The method was applied without application of the final ICM-step and minor parameter adjustments were made to improve the performance on the current data. The water-fat reconstructions of the whole-body scans was performed using station wise reconstructions using the method described previously (2).

Supplemental table S1: Detailed MRI parameters.

Scan name	FOV (mm)	Resolution (mm)	Num echoes	TR / TE1 / dTE (ms)	Flip angle (degrees)
Liver fat	384x288x150	3x3x10	6 unipolar	9.1 / 0.88 / 1.41	4
Liver volume	450x356x275	2.34x2.34x5	1	2.80 / 0.89 / -	10
Whole-body	502x340x152	1.96x1.96x8	3 unipolar	5.37 / 0.99 / 1.61	6

Dimension are given in the directions SAG x COR x AX.

Laboratory analyses

Plasma glucose during clamp was analyzed on site in duplicates using glucose oxidase method (Analox GM9 Analox Instruments, London, UK). Plasma insulin was measured with automated automated electrochemiluminescence immunoassay, ECLIA, fasting plasma glucose amd urinary glucose with enzymatic hexokinase method (all Cobas 8000, Roche Diagnostics, Mannheim, Germany), and HbA1c with immunoturbidimetry (Cobas 6000, Roche Diagnostics, Mannheim, Germany). Plasma ALT, AST, ALP, and total bilirubin were defined with photometric methods (Cobas 8000, Roche Diagnostics, Mannheim, Germany).
Rest of the samples were separated and stored at -70 ^{0}C until all the enrolled subjects had completed the study. Serum free fatty acid level was quantified in fasting and at 60 min intervals during the clamp, and the analysis was performed by enzymatic colorimetric method assay (NEFA-HR2, ACS-ACOD, Wako Chemicals, Neuss, Germany; Cobas 8000 c502 Analyzer, Roche Diagnostics GmbH, Mannheim, Germany). Plasma NT-proBNP and TnT were assessed by ECLIA (Cobas 8000, Roche Diagnostics, Mannheim, Germany). Chilled EDTA tubes added with final concentrations of 500 KIE/ml of trypsin inhibitor aprotinin (Bayer AG, Leverkusen, Germany) and 0.1 mmol/l of DPP-IV-inhibitor Diprotin A (Sigma Aldrich, St Louis, MO, USA) were used for glucagon and active GLP-1 samples. Glucagon was analyzed with radioimmunoassay, RIA (GL-32K, EMD Millipore, Billerica, MA, USA)

and active GLP-1 with enzyme-linked immunosorbent assay, ELISA (EGLP-35K, EMD Millipore, Billerica, MA, USA) which has been shown to be one of the most sensitive and specific commercial kits, although some cross reaction is possible (3). Serum IL-6, MCP-1 and TNF-alpha were measured using immunoassay (Milliplex® MAP Human Cytokine/ Chemokine Magnetic Bead Panel, cat.no. HCYTOMAG-60K, EMD Millipore, Billerica, MA, USA) and FGF-21 using ELISA (Quantikine® Human FGF-21 Immunoassay, R&D Systems, Inc., MN, USA).

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Table S2

Variable	Placebo		Dapagliflozin 10 mg		p-value
	Baseline	At 8 weeks	Baseline	At 8 weeks	
Skeletal muscle	13.6 ± 6.2	13.2 ± 5.0	10.7 ± 3.5	11.6 ± 4.7	1.0
Liver	14.3 ± 4.9	15.4 ± 5.5	11.9 ± 3.6	14.0 ± 5.4	0.53
Myocardium	143 ± 95	142 ± 94	139 ± 154	116 ± 102	0.46
Visceral AT	13.4 ± 3.5	13.2 ± 4.3	10.1 ± 3.9	10.6 ± 4.6	0.82
Subcutaneous AT	7.9 ± 2.6	7.1 ± 2.8	6.7 ± 2.0	7.3 ± 2.9	0.19
Brown AT	8.9 ± 3.3	8.6 ± 3.0	6.7 ± 1.7	6.8 ± 2.2	0.65

Table S2. Tissue glucose uptake (µmol/kg/min) in analyzed tissues without corrections reported as mean ± SD. P-values are placebo-corrected adjusted mean changes from baseline for the dapagliflozin group. AT adipose tissue.

Figure S2

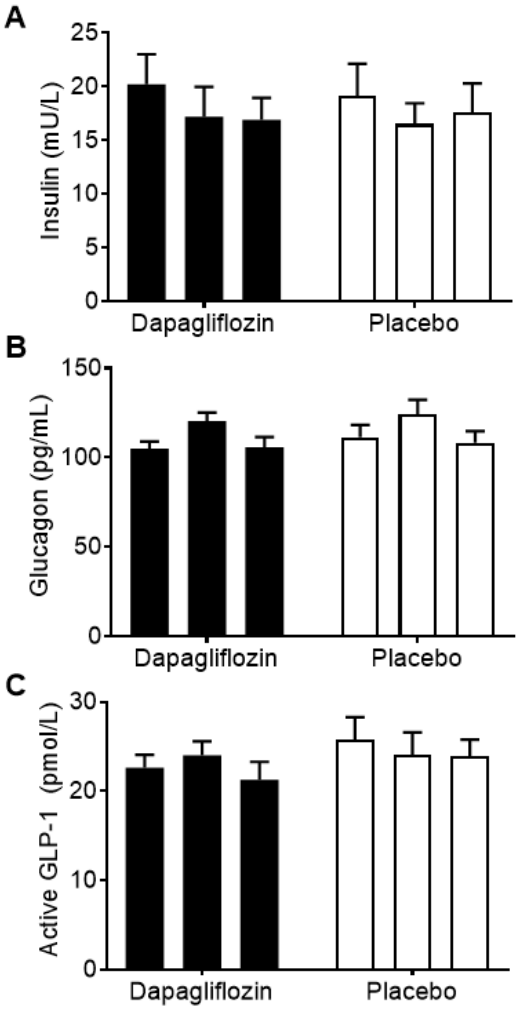

Figure S2. Levels of fasting plasma insulin (**A**), fasting plasma glucagon (**B**) and fasting active plasma glucagon-like peptide-1 (GLP-1) (**C**) in dapagliflozin and placebo treatment groups at baseline (left), after 4 weeks of treatment (middle) and after 8 weeks of treatment (right). All the changes and differences between groups remained not significant.

Figure S3

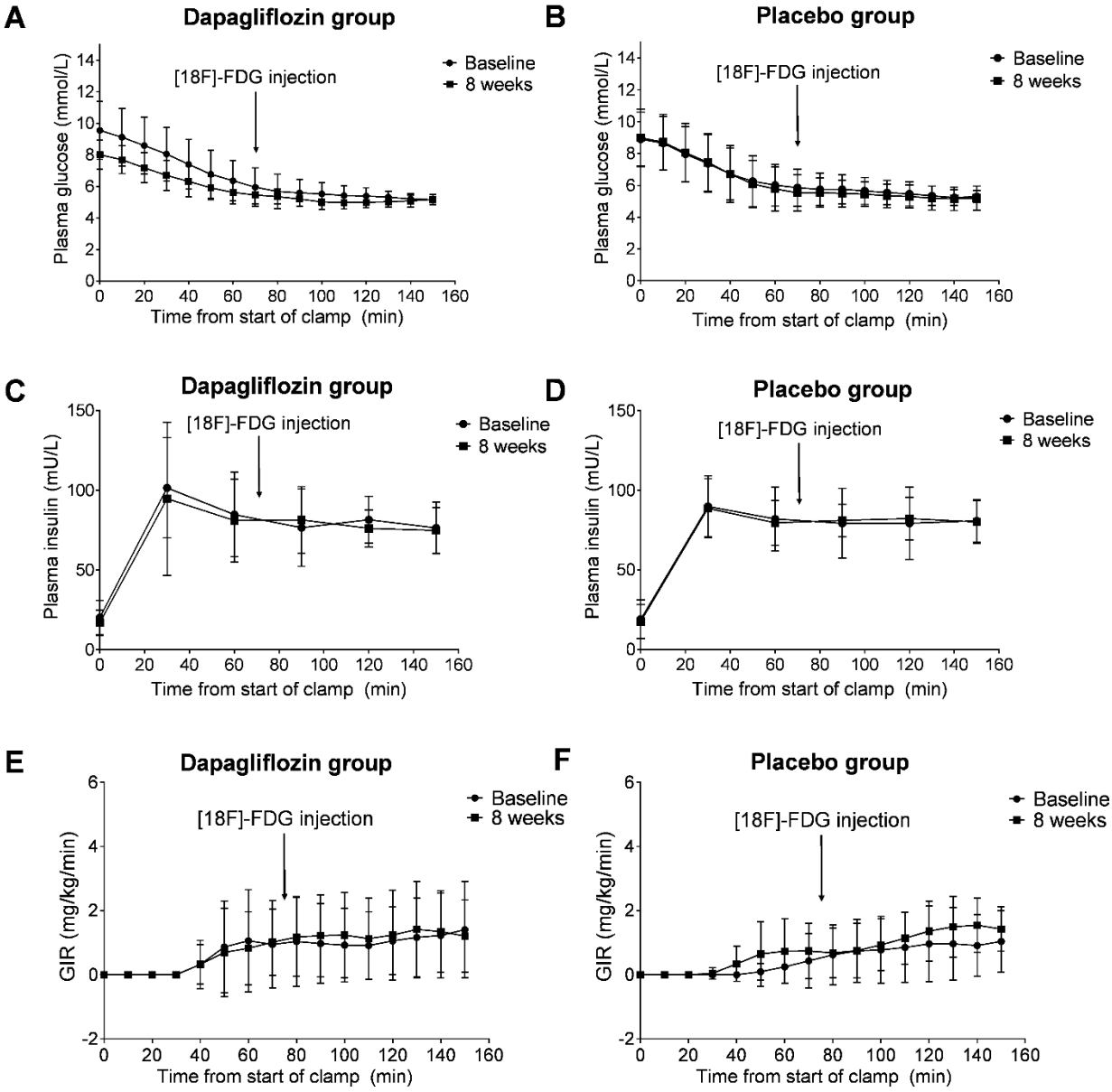

Figure S3. Plasma glucose (A and B) and insulin (C and D) levels, and glucose infusion rates (GIR) (E and F) during hyperinsulinemic euglycemic clamp and PET/CT scanning at baseline and after 8 weeks of treatment in dapagliflozin group (A, C and E) and in placebo group (B, D and F).