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

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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

This randomized, double-blind, parallel group, placebo-controlled study recruited 32 patients with type 2 diabetes. Enrolled patients were to have HbA_{1c} 6.5–10.5% (48–91 mmol/mol) and ≥3 months of stable treatment with metformin, dipeptidyl peptidase 4 inhibitor, or their combination. Patients were randomized 1:1 to receive 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 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 HbA_{1c} −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 N-terminal prohormone of brain natriuretic peptide (−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.

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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 2 weeks up to 3 months of treatment with dapagliflozin (4–6). In these reports, the amelioration of insulin resistance was assumed to reflect an increase in insulin-stimulated glucose uptake (GU) in skeletal muscle (4,5), possibly explained by increased nonoxidative glucose disposal (6). Furthermore, urinary glucose excretion is associated with an increase in endogenous glucose production (EGP), which shows differential mechanisms of action of SGLT2 inhibitors on glucose control in different tissues (7). Previous studies lacked the ability to measure tissue-specific changes in metabolic rates, including the possible role of white and brown adipose tissue (AT) for SGLT2 inhibitor–mediated increase in insulin-stimulated GU. 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 patients with T2D seems to mainly result from a reduction in visceral and subcutaneous AT 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 after treatment with SGLT2 inhibitors in obese patients with T2D and nonalcoholic fatty liver disease (9–11).

The primary aim of this study was to investigate the effect of 8 weeks of dapagliflozin treatment on insulin-stimulated 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. On the basis of earlier results (4–6), we hypothesized that the intervention should have a measurable effect on skeletal muscle insulin-mediated GU. The second aim was to use MRI to assess changes in the liver proton density fat fraction (PDFF) and volume and in visceral and abdominal subcutaneous AT volumes.

RESEARCH DESIGN AND METHODS

Study Subjects

Patients with previously diagnosed T2D and HbA_{1c} 6.5–10.5% (48–91 mmol/mol) were recruited for the study. Other main inclusion criteria were age 35–70 years, BMI $<$40 kg/m^2, and at least 3 months of stable medication with metformin, dipeptidyl peptidase 4 inhibitor, or their combination. We excluded 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 AST more than three times above the upper limit of normal, and total bilirubin $>$2.0 mg/dL), blood pressure $>$160/100 mmHg at screening, unstable coronary syndrome, symptomatic heart failure, or alcohol abuse. The sample size was determined to detect a 25% change in skeletal muscle GU uptake, with ~90% power at a significance level α of 5%.

Study Design

The study design is illustrated in Fig. 1. The study comprised five visits: a screening visit (visit 1) 4 weeks to 1 week before the second visit, a randomization visit (visit 2), followed by a treatment follow-up visit after 4 weeks (visit 3), 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, and the other visits were organized by the recruiting site at the Turku PET Centre or at a satellite site in Jyväskylä, Finland.

A total of 55 volunteers were recruited from outpatient clinics, patient databases, and by advertisements 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 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.

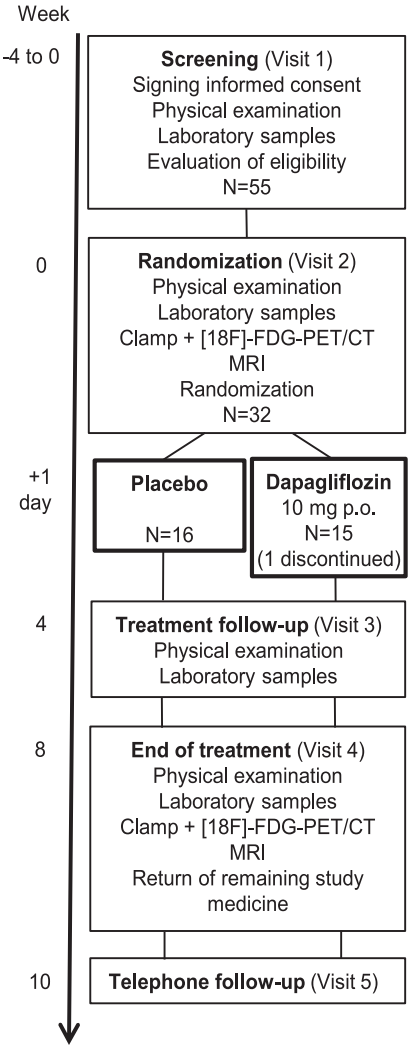

Figure 1—General study outline.

The study medication was administered in double-blinded fashion, and all PET imaging, 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 Southwest Finland Hospital District Independent Ethics Committee. The study was conducted according to the

principles of the Declaration of Helsinki. All subjects gave written informed consent before any study procedures.

Measuring Insulin-Stimulated Whole-Body and Tissue GU Using PET/CT

The PET/CT studies were conducted after an overnight fast of 10–12 h 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 samples, arterialized by using hot water bottles distally in the arm, and the other for injection of the PET radiotracer and for insulin and glucose infusions. After fasting laboratory samples were collected, 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 (Actrapid; Novo Nordisk, Copenhagen, Denmark), and the rate of 20% glucose infusion was adjusted based on plasma glucose levels, as determined every 5–10 min to maintain euglycemia (plasma glucose level of 5.0 $\pm$ 0.5 mmol/L). Whole-body GU (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. M-value is presented as the average of three to four 20-min 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) (Supplementary Fig. 1) in the subjects with both measurements, urine radioactivity was used to estimate urinary glucose excretion for the remaining subjects (Supplementary Data). In the dapagliflozin group, the mean excretion rate was 0.8 $\pm$ 0.4 μmol/kg/min (range 0.2–1.3), whereas urinary glucose concentrations were diminutive in the placebo group and were therefore not used for correction of M-values.

At 75 $\pm$ 15 min after the start of the insulin infusion, subjects were injected with 155 $\pm$ 8 MBq of [^{18}F]-FDG, produced as described earlier (15), and the PET scanning (Discovery 690; General Electric Medical Systems, Milwaukee, WI) 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 intervals of 5–15 min during the scanning and from an urine sample obtained at the end of scan was measured in an automatic gamma counter (Wizard 1480 3-inch; Wallac, Turku, Finland).

PET Data Analysis

PET data were corrected for dead time, decay, and photon attenuation before analysis. Tracer uptake into tissues was measured by determining volumes of interest with Carimas 2.9 software (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 AT, several volumes of interest 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 GU (μmol/kg/min) was calculated by multiplying Ki by steady-state plasma glucose levels divided by tissue density and a previously established lumped constant of 1.2 for skeletal muscle, 1.0 for liver and myocardium, and 1.14 for AT (17–20). EGP was assessed by subtracting the glucose infusion rate from the rate of glucose disposal derived from [^{18}F]-FDG consumption and estimated urinary glucose loss (21).

MRI Assessment of Liver Fat, Liver Volume, Abdominal AT Volumes

MRI was performed at 3T using the MRI part of a clinical PET-MR system (Philips Ingenuity TF; Philips Healthcare, Cleveland, OH). Subjects were positioned supine with the arms extended above the head. Imaging was performed using the integrated body coil. Abdominal AT volume was assessed using multiecho water-fat MRI and liver volume was assessed using a T1-weighted fat-suppressed single-echo sequence. Liver fat was measured by use of a PDFF measurement. All imaging was performed during breath holds in exhaled position. Details on scan parameters and image reconstruction are given in the 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 semiautomated segmentation and the SmartPaint 1.0 software (22). Volumes of abdominal subcutaneous and visceral AT were determined from whole-body scans by using an automated algorithm (23). Coefficients of variations from repeated imaging and analysis have been previously determined to be 5.4% for liver PDFF, 2.1% for liver volume (n = 10; J.K., unpublished data), 2.3% for subcutaneous AT, and 1.9% for visceral AT (23).

Laboratory Measurements

A detailed description of biochemical and immunological analyses is provided in the Supplementary Data.

Statistical Analyses

All statistical analyses were performed using SAS 9.4 software (SAS Institute, Cary, NC). Changes in parameters measured at baseline, week 4, and week 8, including body weight, systolic and diastolic blood pressure, blood HbA_{1c}, serum free fatty acids, and plasma levels of fasting glucose, HbA_{1c}, insulin, and glucagon were analyzed using mixed model for repeated measurements 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 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 as original values or as corrected values divided by the average of plasma insulin levels during steady state in clamp. Difference in baseline N-terminal prohormone of brain natriuretic peptide (NT-proBNP) in subjects with or without previous hypertension or cardiovascular

disease was analyzed using the 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% CI. 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 are reported in Table 1. There were no significant differences between groups concerning sex distribution (with 87% men in the dapagliflozin vs. 75% in placebo group), age, BMI, glycemic control, or time since diagnosis. All subjects were on metformin, and nine (60%) in the dapagliflozin and seven (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 the groups (two subjects in the dapagliflozin group and three subjects in the placebo group).

Improved Glycemic Control and Weight Loss with Dapagliflozin

There was a significant reduction in HbA_{1c}, 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 glucagon-like peptide 1 levels did not change significantly, although plasma insulin levels in the dapagliflozin-group decreased numerically, and glucagon and glucagon-like peptide 1 levels increased numerically after 4 weeks of treatment but returned close to baseline after 8 weeks of treatment (Supplementary Fig. 2). Moreover, the glucagon-to-insulin ratio was not significantly altered (P = 0.42).

Unchanged Insulin Sensitivity

Baseline whole-body insulin-stimulated M-value was low in both groups, at 6.2 μmol/kg/min in the dapagliflozin group and 7.8 μmol/kg/min in the placebo group (Table 1). 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) (Fig. 2*C*), also when correcting for steady-state insulin levels (0.01, 95% CI −0.01, 0.04, P = 0.40). Change in rate of EGP was not different from placebo (−0.02 μmol/kg/min, 95% CI −3.2, 3.2, P = 1.0) (Table 1 and Fig. 2*C*).

Dapagliflozin had no effect on skeletal muscle GU (−0.003 μmol/kg/min, 95% CI −3.1, 3.1, P = 1.0) (Fig. 2*C* and Supplementary Table 2), but the changes in the M-value and skeletal muscle GU

Table 1—Characteristics of the treatment arms at baseline and after 8 weeks

	Placebo		Dapagliflozin 10 mg		
Variable	Baseline (n = 16)	At 8 weeks (n = 16)	Baseline (n = 15)	At 8 weeks (n = 15)	P value
Sex, *n*					
Male	12		13		
Female	4		2		
Age (years)	60 ± 7.4		62 ± 8.4		
Diabetes duration (years)	7.3 ± 3.7		7.8 ± 3.8		
Metformin monotherapy, *n*	9		6		
Metformin + sitagliptin, *n*	7		9		
Hypertension, *n*					
Yes	9		8		
No	7		7		
BMI (kg/m^2)	31.7 ± 5.0	31.8 ± 4.8	32.1 ± 3.9	31.3 ± 3.7	<0.0001
Fasting plasma glucose (mmol/L)	8.7 ± 1.7	9.0 ± 1.5	9.5 ± 1.9	7.8 ± 0.9	<0.01
HbA_{1c} (%)	6.8 ± 0.5	6.8 ± 0.4	7.0 ± 0.6	6.6 ± 0.6	<0.01
HbA_{1c} (mmol/mol)	51 ± 6	51 ± 5	53 ± 7	49 ± 7	
Blood pressure (mmHg)					
Systolic	147 ± 14	139 ± 15	151 ± 13	144 ± 15	0.79
Diastolic	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
Fasting free fatty acids (mmol/L)	0.69 ± 0.18	0.66 ± 0.21	0.64 ± 0.15	0.67 ± 0.14	0.62
β-Hydroxybutyrate (mmol/L)	0.12 ± 0.11	0.12 ± 0.09	0.09 ± 0.05	0.17 ± 0.18	0.33
ALT (units/L)	38 ± 14	39 ± 15	50 ± 21	45 ± 16	0.47
AST (units/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
FGF21 (pg/mL)	293 ± 194	362 ± 272	388 ± 315	334 ± 198	0.07

Values at baseline and after treatment are reported as mean ± SD. P values are placebo-corrected adjusted mean changes from baseline for the dapagliflozin group.

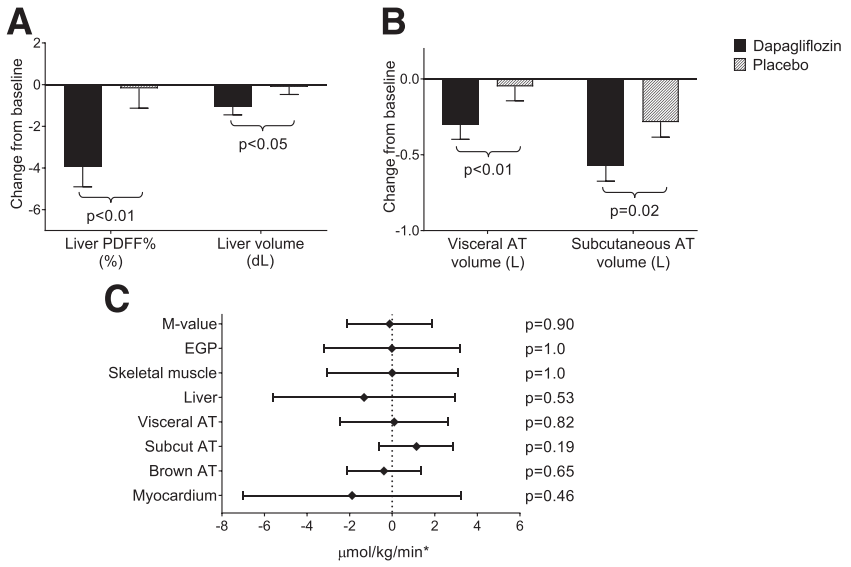

Figure 2—*A*: Adjusted mean change from baseline for liver PDFF% and volume in dapagliflozin and placebo treatment groups. *B*: Adjusted mean change from baseline in visceral AT and abdominal subcutaneous AT volumes in dapagliflozin and placebo treatment groups. *C*: Placebo-corrected mean changes and 95% CI of M-value, EGP, and of GU in different tissues. Subcut, subcutaneous. *Unit for myocardial GU is μmol/100 g/min.

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 (Supplementary Fig. 3).

Decrease in Liver Fat and Volume

Baseline PDFF was similar between groups, at 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 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) (Fig. 2*A*). Post hoc analyses showed that introducing changes in BMI or visceral AT volume in the model had a significant effect on the reduction of liver fat (P = 0.02 and P = 0.01, respectively). In the dapagliflozin group, hepatic GU did not change significantly (−1.3 μmol/kg/min, 95% CI −5.6, 3.0, P = 0.53) (Fig. 2*C* and Supplementary Table 2), 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 AT Volume

Dapagliflozin treatment resulted in significant changes in AT measured by MRI: the volume of visceral AT was reduced by −0.35 L (95% CI −0.59, −0.12, $P < 0.01$), and the volume of abdominal subcutaneous AT was reduced by −0.28 L (95% CI −0.52, −0.05, P = 0.02) (Fig. 2*B*). There was no significant change in lean body mass (−1.2 L, 95% CI −2.8, 0.41, P = 0.14). Insulin-stimulated GU was not altered in visceral (−0.02 μmol/kg/min, 95% CI −0.13, 0.09, P = 0.71), subcutaneous (1.14 μmol/kg/min, 95% CI −0.6, 2.9, P = 0.19), or brown AT (−0.38 μmol/kg/min, 95% CI −2.1, 1.3, P = 0.65) (Fig. 2*C* and Supplementary Table 2).

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 pg/mL, 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 on myocardial left ventricular GU (−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 preexisting hypertension or other cardiovascular diagnosis (n = 8 in dapagliflozin and n = 9 in placebo group, including one subject with atrial fibrillation in both groups and three 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 patients with T2D, 8 weeks of treatment with dapagliflozin did not change skeletal muscle insulin sensitivity, as measured directly with PET. Also, in contrast to previous studies (5–7), 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. Compared with previous studies, the two (4,6,7) to three times (5) lower insulin infusion rate in this study likely did not inhibit EGP completely. The duration of the clamp was also shorter compared with 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 EGP remained slightly higher during hyperinsulinemia in our study compared with what was 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 the 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 the unchanged M-value, we found no change in skeletal muscle insulin sensitivity by dapagliflozin and no difference compared with placebo. Also, no changes in GU could be detected in other tissues, including liver, myocardium, and subcutaneous, visceral, and brown AT. Thus, the tissue uptake of glucose measured with PET during the clamp was not able to reveal which tissues could have been responsible for the increase in the M-value shown in other studies (4–6).

One important finding of the study was the significant reduction in whole-liver fat content after 8 weeks of treatment with dapagliflozin in obese patients with T2D, results similar to a recent report (11). This decrease is consistent with the associated reduction in body weight and visceral AT 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 nighttime hepatic glycogen depots and increased gluconeogenesis (27).

The numerical lowering of FGF21 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 nonalcoholic fatty liver disease (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, higher circulating NT-proBNP and FGF21 levels are both 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, nor did we see significant changes in fasting plasma β-hydroxybutyrate nor serum free fatty acid levels (Table 1). Because 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 that 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 (BI 10773 [Empagliflozin] Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients) study (31,32). We observed a significant decrease in NT-proBNP, even though 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 patients with T2D (33). Therefore, the decline in NT-proBNP may indicate a reduced risk to develop heart failure in patients with T2D. We also saw an increase in the hematocrit by 3.7 ± 3.2% in the dapagliflozin group at the end of treatment, similar to previous reports (34).

Several previous studies reported an increase in the levels of glucagon and the glucagon-to-insulin ratio during SGLT2 inhibitor treatment, and inhibition of SGLT2 has also been shown to directly stimulate secretion of glucagon from pancreatic α-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 dipeptidyl peptidase 4 inhibitors in about half of the study population. However, a post hoc analysis showed that patients on sitagliptin had a similar change in the glucagon-to-insulin ratio compared with 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 AT (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 GU comprehensively from several different tissues, AT volumes, and liver PDFF, as well as the double-blinded, randomized design. The PET method used for quantifying tissue GU 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 β-hydroxybutyrate. Another limitation is that glucose loss in urine was not quantified in all subjects, which led 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, 8 weeks of treatment with dapagliflozin did not significantly change tissue insulin-stimulated GU uptake directly measured with PET. However, the treatment reduced liver fat content, as well as subcutaneous and visceral AT, when measured using MRI data from the whole liver and AT 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. A.L.-R. performed study visits and drafted the manuscript. A.L.-R., M.-J.H., J.K., N.M., T.L., J.S., A.K.K., V.S., P.I., L.J., J.O., J.C.H., and P.N. commented on the initial version of the manuscript. A.L.-R., M.-J.H., and J.C.H. participated in PET data analysis. A.L.-R., J.O., and

P.N. contributed to statistical analysis. J.K. and L.J. analyzed MRI data. J.S. was the principal investigator of the Jyväskylä satellite site and performed study visits there. A.K.K. 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. 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.

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References

1. Meng W, Ellsworth BA, Nirschl AA, et al. Discovery of dapagliflozin: a potent, selective renal sodium-dependent glucose cotransporter 2 (SGLT2) inhibitor for the treatment of type 2 diabetes. J Med Chem 2008;51:1145–1149
2. Ferrannini E, Ramos SJ, Salsali A, Tang W, List JF. Dapagliflozin monotherapy in type 2 diabetic patients with inadequate glycemic control by diet and exercise: a randomized, double-blind, placebo-controlled, phase 3 trial. Diabetes Care 2010;33:2217–2224
3. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. Physiol Rev 2011;91:733–794
4. Merovci A, Solis-Herrera C, Daniele G, et al. Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. J Clin Invest 2014;124:509–514
5. Mudaliar S, Henry RR, Boden G, et al. Changes in insulin sensitivity and insulin secretion with the sodium glucose cotransporter 2 inhibitor dapagliflozin. Diabetes Technol Ther 2014;16:137–144
6. Daniele G, Xiong J, Solis-Herrera C, et al. Dapagliflozin enhances fat oxidation and ketone production in patients with type 2 diabetes. Diabetes Care 2016;39:2036–2041
7. Merovci A, Abdul-Ghani M, Mari A, et al. Effect of dapagliflozin with and without acipimox on insulin sensitivity and insulin secretion in T2DM males. J Clin Endocrinol Metab 2016;101:1249–1256
8. Bolinder J, Ljunggren Ö, Kullberg J, et al. Effects of dapagliflozin on body weight, total fat mass, and regional adipose tissue distribution in patients with type 2 diabetes mellitus with inadequate glycemic control on metformin. J Clin Endocrinol Metab 2012;97:1020–1031
9. Ito D, Shimizu S, Inoue K, et al. Comparison of ipragliflozin and pioglitazone effects on nonalcoholic fatty liver disease in patients with type 2 diabetes: a randomized, 24-week, open-label, active-controlled trial. Diabetes Care 2017;40:1364–1372
10. Shibuya T, Fushimi N, Kawai M, et al. Luseogliflozin improves liver fat deposition compared to metformin in type 2 diabetes patients with non-alcoholic fatty liver disease: a prospective randomized controlled pilot study. Diabetes Obes Metab 2018;20:438–442
11. Eriksson JW, Lundkvist P, Jansson PA, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people with type 2 diabetes: a double-blind randomised placebo-controlled study. Diabetologia 2018;61:1923–1934
12. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41
13. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979;237:E214–E223
14. Nuutila P, Koivisto VA, Knuuti J, et al. Glucose-free fatty acid cycle operates in human heart and skeletal muscle in vivo. J Clin Invest 1992;89:1767–1774
15. Hamacher K, Coenen HH, Stöcklin G. Efficient stereospecific synthesis of no-carrier-added 2-[^{18}F]-fluoro-2-deoxy-D-glucose using aminopolyether supported nucleophilic substitution. J Nucl Med 1986;27:235–238
16. Patlak CS, Blasberg RG. Graphical evaluation of blood-to-brain transfer constants from multiple-time uptake data. Generalizations. J Cereb Blood Flow Metab 1985;5:584–590
17. Peltoniemi P, Lönnroth P, Laine H, et al. Lumped constant for [^{18}F]fluorodeoxyglucose in skeletal muscles of obese and nonobese humans. Am J Physiol Endocrinol Metab 2000;279:E1122–E1130
18. Iozzo P, Järvisalo MJ, Kiss J, et al. Quantification of liver glucose metabolism by positron emission tomography: validation study in pigs. Gastroenterology 2007;132:531–542
19. Bøtker HE, Böttcher M, Schmitz O, et al. Glucose uptake and lumped constant variability in normal human hearts determined with [^{18}F]-fluorodeoxyglucose. J Nucl Cardiol 1997;4:125–132
20. Virtanen KA, Peltoniemi P, Marjamäki P, et al. Human adipose tissue glucose uptake determined using [^{18}F]-fluoro-deoxy-glucose ([^{18}F]FDG) and PET in combination with microdialysis. Diabetologia 2001;44:2171–2179
21. Iozzo P, Gastaldelli A, Järvisalo MJ, et al. ^{18}F-FDG assessment of glucose disposal and production rates during fasting and insulin stimulation: a validation study. J Nucl Med 2006;47:1016–1022
22. Malmberg F, Nordenskjöld R, Strand R, Kullberg J. SmartPaint – a tool for interactive segmentation of medical volume images. Comput Methods Biomech Biomed Eng Imaging Vis 2017;5:36–44
23. Kullberg J, Johansson L, Ahlström H, et al. Automated assessment of whole-body adipose tissue depots from continuously moving bed MRI: a feasibility study. J Magn Reson Imaging 2009;30:185–193
24. Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia 2012;55:885–904
25. van der Poorten D, Milner KL, Hui J, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. Hepatology 2008;48:449–457
26. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest 2014;124:499–508
27. Esterline RL, Vaag A, Oscarsson J, Vora J. Mechanisms in endocrinology: SGLT2 inhibitors: clinical benefits by restoration of normal diurnal metabolism? Eur J Endocrinol 2018;178:R113–R125
28. Giannini C, Feldstein AE, Santoro N, et al. Circulating levels of FGF-21 in obese youth: associations with liver fat content and markers of liver damage. J Clin Endocrinol Metab 2013;98:2993–3000
29. Suomalainen A, Elo JM, Pietiläinen KH, et al. FGF-21 as a biomarker for muscle-manifesting mitochondrial respiratory chain deficiencies: a diagnostic study. Lancet Neurol 2011;10:806–818
30. Chou RH, Huang PH, Hsu CY, et al. Circulating fibroblast growth factor 21 is associated with diastolic dysfunction in heart failure patients with preserved ejection fraction. Sci Rep 2016;6:33953
31. Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME Trial: a "thrifty substrate" hypothesis. Diabetes Care 2016;39:1108–1114
32. Mudaliar S, Alloju S, Henry RR. Can a shift in fuel energetics explain the beneficial cardiorenal outcomes in the EMPA-REG OUTCOME study? A unifying hypothesis. Diabetes Care 2016;39:1115–1122
33. Tarnow L, Gall MA, Hansen BV, Hovind P, Parving HH. Plasma N-terminal pro-B-type natriuretic peptide and mortality in type 2 diabetes. Diabetologia 2006;49:2256–2262
34. Zinman B, Wanner C, Lachin JM, et al.; EMPA-REG OUTCOME Investigators. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med 2015;373:2117–2128
35. Bonner C, Kerr-Conte J, Gmyr V, et al. Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic alpha cells triggers glucagon secretion. Nat Med 2015;21:512–517
36. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-alpha, in vivo. J Clin Endocrinol Metab 1997;82:4196–4200
37. Ridker PM, Rifai N, Stampfer MJ, Hennekens CH. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. Circulation 2000;101:1767–1772
38. Wiviott SD, Raz I, Bonaca MP, et al.; DECLARE–TIMI 58 Investigators. Dapagliflozin and cardiovascular outcomes in type 2 diabetes. N Engl J Med 2018;380:347–357