Empagliflozin Effectively Lowers Liver Fat Content in Well-Controlled Type 2 Diabetes: A Randomized, Double-Blind, Phase 4, Placebo-Controlled Trial

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OBJECTIVE

To evaluate whether the sodium–glucose cotransporter 2 inhibitor empagliflozin (EMPA) reduces liver fat content (LFC) in recent-onset and metabolically well-controlled type 2 diabetes (T2D).

RESEARCH DESIGN AND METHODS

Patients with T2D ($n = 84$) (HbA_{1c} 6.6 ± 0.5% [49 ± 10 mmol/mol], known disease duration 39 ± 27 months) were randomly assigned to 24 weeks of treatment with 25 mg daily EMPA or placebo. The primary end point was the difference of the change in LFC as measured with magnetic resonance methods from 0 (baseline) to 24 weeks between groups. Tissue-specific insulin sensitivity (secondary outcome) was assessed by two-step clamps using an isotope dilution technique. Exploratory analysis comprised circulating surrogate markers of insulin sensitivity and liver function. Statistical comparison was done by ANCOVA adjusted for respective baseline values, age, sex, and BMI.

RESULTS

EMPA treatment resulted in a placebo-corrected absolute change of −1.8% (95% CI −3.4, −0.2; $P = 0.02$) and relative change in LFC of −22% (−36, −7; $P = 0.009$) from baseline to end of treatment, corresponding to a 2.3-fold greater reduction. Weight loss occurred only with EMPA (placebo-corrected change −2.5 kg [−3.7, −1.4]; $P < 0.001$), while no placebo-corrected change in tissue-specific insulin sensitivity was observed. EMPA treatment also led to placebo-corrected changes in uric acid (−74 mol/L [−108, −42]; $P < 0.001$) and high-molecular-weight adiponectin (36% [16, 60]; $P < 0.001$) levels from 0 to 24 weeks.

CONCLUSIONS

EMPA effectively reduces hepatic fat in patients with T2D with excellent glycemic control and short known disease duration. Interestingly, EMPA also decreases circulating uric acid and raises adiponectin levels despite unchanged insulin sensitivity. EMPA could therefore contribute to the early treatment of nonalcoholic fatty liver disease in T2D.

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Patients with type 2 diabetes (T2D) are prone to develop nonalcoholic fatty liver disease (NAFLD) (1), and NAFLD itself is associated with a doubled risk of incident T2D (2). NAFLD associates not only with cardiovascular disease but also with diabetes-related chronic kidney disease and retinopathy (1). Moreover, patients with T2D are at a higher risk of progressing from steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, and cirrhosis (1).

Pronounced weight loss is effective for the treatment of NAFLD but difficult to achieve in many cases. Although well-known (glucagon-like peptide 1 receptor agonists, thiazolidinediones) and novel (e.g., pegbelfermin, elafibranor) compounds have demonstrated beneficial effects in patients with T2D and NAFLD, there is no accepted pharmacological treatment for these patients (3).

Sodium–glucose cotransporter 2 inhibitors (SGLT2is) not only improve glycemia by increasing urinary glucose excretion but also reduce body weight and blood pressure (4) and improve cardiovascular and renal outcomes (5). Some open-label and placebo-controlled studies have reported that SGLT2is may also alleviate NAFLD (6,7), while canagliflozin and dapagliflozin trended toward decreased liver fat content (LFC) compared with placebo (8,9). Body weight loss and glycated hemoglobin (HbA_{1c}) reduction may be mainly responsible for LFC reduction with canagliflozin (8,10), but empagliflozin (EMPA) could improve NAFLD independently of body weight and glycemia (7,11). Of note, SGLT2is ameliorated inflammation, oxidative stress, and dysregulated hormone secretion in preclinical studies (4). The current randomized, placebo-controlled clinical trial examined the effectiveness of EMPA on LFC reduction in patients with recent-onset, well-controlled T2D and explored its effects on tissue-specific insulin sensitivity.

RESEARCH DESIGN AND METHODS

Study Design

This randomized, parallel-group, double-blind, phase 4 trial was performed at five centers in Germany (Düsseldorf, Potsdam-Rehbrücke, Dresden, Tübingen, and Heidelberg) with a 1:1 allocation to treatment arms. The lead ethics committee of Heinrich-Heine University Düsseldorf approved all trial procedures.

Patients

The study population consisted of well-controlled patients with T2D with short known disease duration to exclude that the observed effects of EMPA were mainly driven by improvement of glycemic control. The rationale for this selection was the research question of whether SGLT2is would also be effective in early T2D, where effects on glycemia and changes in additional antihyperglycemic treatment during the intervention would be minimized. Participants were recruited by newspaper and Internet advertisements. Before inclusion, all patients gave written informed consent. Principal inclusion criteria were age 18–75 years, BMI <45 kg/m^2, known diabetes duration ≤ 7 years, HbA_{1c} of 6–8%, and no previous antihyperglycemic treatment or a 1-month washout period. Principal exclusion criteria included uncontrolled hyperglycemia at screening (fasting blood glucose [FBG] ≥ 240 mg/dL), liver disease other than NAFLD, previous thiazolidinedione treatment, and use of immunomodulatory, antiobesity, anti-NASH drugs. Full inclusion and exclusion criteria are listed in the Supplementary Data.

Randomization and Masking

All participants were randomized by a stratified computed randomization procedure to account for age, sex, and BMI to EMPA or placebo and were masked to the treatment assignment. The electronic master randomization list was only accessible to the assigned randomization list managers, and study sites received sealed opaque envelopes for unblinding in cases of emergency. Enrollment was performed at the respective site. Randomization and assignment to the double-blind study drug was done by central pharmacy personnel, who had access to the computer-generated randomization scheme. No open access to the code was available at study centers to monitors, statisticians, or sponsors' teams. Blinding of investigators and patients was achieved by providing EMPA and placebo tablets with identical appearance and packaging. Unblinding was performed after the final database lock.

Procedures

All procedures are summarized in the Supplementary Data. Eligibility of patients was assessed at screening and at the end of the 1-month washout period (for patients with previous antihyperglycemic treatment only). Participants received one individual dietary counseling before the baseline visit according to recommendations of the American Diabetes Association (12).

All baseline measures were performed before the first intake of study medication. From screening on, FBG levels were self-monitored daily with a glucose meter.

Enrolled patients were allocated to one treatment arm (EMPA 25 mg once daily or matching placebo orally; both from Boehringer Ingelheim, Ingelheim/Rhein, Germany) and returned to the study center at baseline; at weeks 1, 4, 8, 12, 16, 20, and 24 for efficacy and safety (including adverse events) assessments; and at 2 weeks after discontinuation of study medication.

Assessments of visceral adipose tissue (VAT) and subcutaneous adipose tissue (SCAT) volume and LFC, respectively, were performed at baseline and 12 and 24 weeks.

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LFC was assessed at each center using volume-selective proton MRS (^{1}H-MRS) using a stimulated echo acquisition mode (coefficient of variation [CV] 0.3–1.7%) as reported previously (13) or chemical shift–selective in-phase/opposed-phase imaging technique in one center (14). All measurements were performed in liver segment 7, and LFC was calculated as fat / (water + fat) * 100% by central reading.

SCAT (CV 1.5% [J.M., personal communication]) and VAT (CV 1.1% [15]) were measured using T1-weighted axial fast spin-echo (16) and quantified using an automated algorithm on the basis of fuzzy clustering and orthonormal snakes (15). Central reading was done at the Institute for Diabetes Research and Metabolic Diseases of the Helmholtz Center Munich at University of Tübingen by a spectroscopist blinded to patients' treatment allocation.

Two-step euglycemic insulin clamps with [6,6-$^2H_2$]glucose (17) were done to assess whole-body, mainly skeletal muscle, insulin sensitivity (M value, R_d) and adipose tissue (% suppression of free fatty acids [FFAs]) insulin sensitivity as well as parameters related to endogenous glucose production (EGP) (absolute EGP rates, % EGP suppression) during low and high insulinemia at baseline and week 24. Briefly, participants fasted overnight for at least 10 h and refrained from any exercise and alcohol for at least 24 h before the test. [6,6-$^2H_2$]glucose was given as primed-continuous intravenous infusion throughout the clamp. After 120 min, a primed (40 mU/m^2/min for 8 min) insulin intravenous infusion (Insuman Rapid; Sanofi, Paris, France) was given for the next 120 min at 20 mU/m^2/min (low insulin) and for the final 120 min at 40 mU/m^2/min (high insulin). A variable 20% glucose infusion enriched with [6,6-$^2H_2$]glucose was used to maintain blood glucose at ~90 mg/dL. The M value was calculated from glucose infusion rates during the last 20–30 min of both low- and high-insulin periods. Patients in whom steady-state conditions were not achieved were excluded from analysis. Because the study drug was discontinued at least 3 days before the clamps to account for the half-life of EMPA 25 mg (~10.7 h [8]), urinary glucose excretion was not measured.

Fasting hepatic insulin resistance (HIR) was calculated as fasting plasma insulin * basal EGP (8). Fasting adipose tissue insulin resistance was calculated as fasting FFA * fasting plasma insulin.

Daily energy intake was analyzed from 3-day food diaries, which were filled in by patients before each visit at the site using the Prodi system (Prodi 6.3.0.1 [Nbase 3.60]; Nutri-Science GmbH, Freiburg, Germany). Physical activity was assessed by Baecke index (18).

Glucose, insulin (hemolytic blood samples were excluded from analysis), C-peptide, and FFA concentrations were measured as previously described (19). Serum levels of cytokeratin 18 (CK18)-M30 and -M65, adiponectin, fibroblast growth factor 21 (FGF-21), tumor necrosis factor-α (TNF-α, interleukin-1 receptor antagonist (IL-1Ra), IL-6, and resistin were measured at baseline and after 12 and 24 weeks. IL-6 and TNF-α were measured with Quantikine High Sensitivity ELISA Kits (R&D Systems, Abington, U.K.), and IL-1Ra, FGF-21, and resistin were measured with Quantikine ELISA Kits (R&D Systems). High-molecular-weight (HMW) adiponectin was measured with the HMW and Total Adiponectin ELISA Kit (ALPCO, Salem, NH), and CK18-M30 (apoptosis-associated capase-cleaved keratin 18, K18Asp396, or M30 neoepitope) and CK18-M65 (soluble keratin K18) were measured using the M30 Apoptosense ELISA and M65 ELISA Kits (VLVbio, Nacka, Sweden).

Outcomes

The primary efficacy end point was defined as the difference in change of LFC (in %) between EMPA and placebo from baseline to 24 weeks of treatment. Secondary end points comprised the differences in changes of measures of whole-body/skeletal muscle (M value, R_d) and hepatic insulin sensitivity (HIR, insulin-stimulated EGP suppression, fasting EGP) measures between EMPA and placebo from baseline to 24 weeks. All assessments except LFC were exploratory. Safety was monitored by assessment of vital signs, physical examination, electrocardiogram, adverse events, and laboratory results (blood chemistry, hematological and coagulation parameters) at each visit.

Power Calculation

An ~3% reduction from baseline in body weight was observed for EMPA 25 mg in a phase 3 study with patients with T2D (20). In patients with T2D with a short disease duration and excellent glycemic control, an ~5% reduction in body weight corresponded to an ~7% reduction in LFC (19). Thus, the current study required a sample size of 30 patients/arm to detect a 4% absolute decrease in LFC from baseline with a pairwise comparison within a 95% CI, assuming an SD of 5.4% and a power of at least 80%. An estimated dropout rate of 15% resulted in 36 participants/arm.

Statistical Analyses

All analyses for efficacy parameters were performed in the intention-to-treat population, including all patients, of which at least the baseline and 12-week and/or 24-week LFC data were available. For patient characteristics, data are shown as mean with SD for normally distributed data and median with first and third quartiles for log-normally distributed parameters. Values of parameters at week 0 in both treatment arms are presented as means for normally distributed data and geometric means for log-normally distributed data with 95% CIs. Placebo-corrected changes from baseline to 24 weeks for normally distributed parameters are presented as absolute changes and for log-normally distributed data, as relative changes with corresponding 95% CIs adjusted for age, sex, BMI, and respective baseline parameter (least square means). Comparison of changes between treatments was done by an ANCOVA adjusted for age, sex, BMI, and the baseline value of the respective parameter. Calculations were performed with SAS 9.4 TS1M2 (SAS Institute, Cary, NC). No data monitoring committee was foreseen for this small-scale phase 4 trial.

RESULTS

Between 4 March 2016 and 1 February 2018, 84 patients were randomized to EMPA ($n = 42$) or placebo ($n = 42$) and received at least one dose of the study medication. Of all randomized patients, 65 (77%) completed the trial (Fig. 1).

Patient Characteristics

Baseline anthropometric and metabolic measures were all comparable between EMPA and placebo (Table 1). Physical activity and daily calorie intake neither differed at baseline nor changed from baseline to 24 weeks between the groups (data not shown).

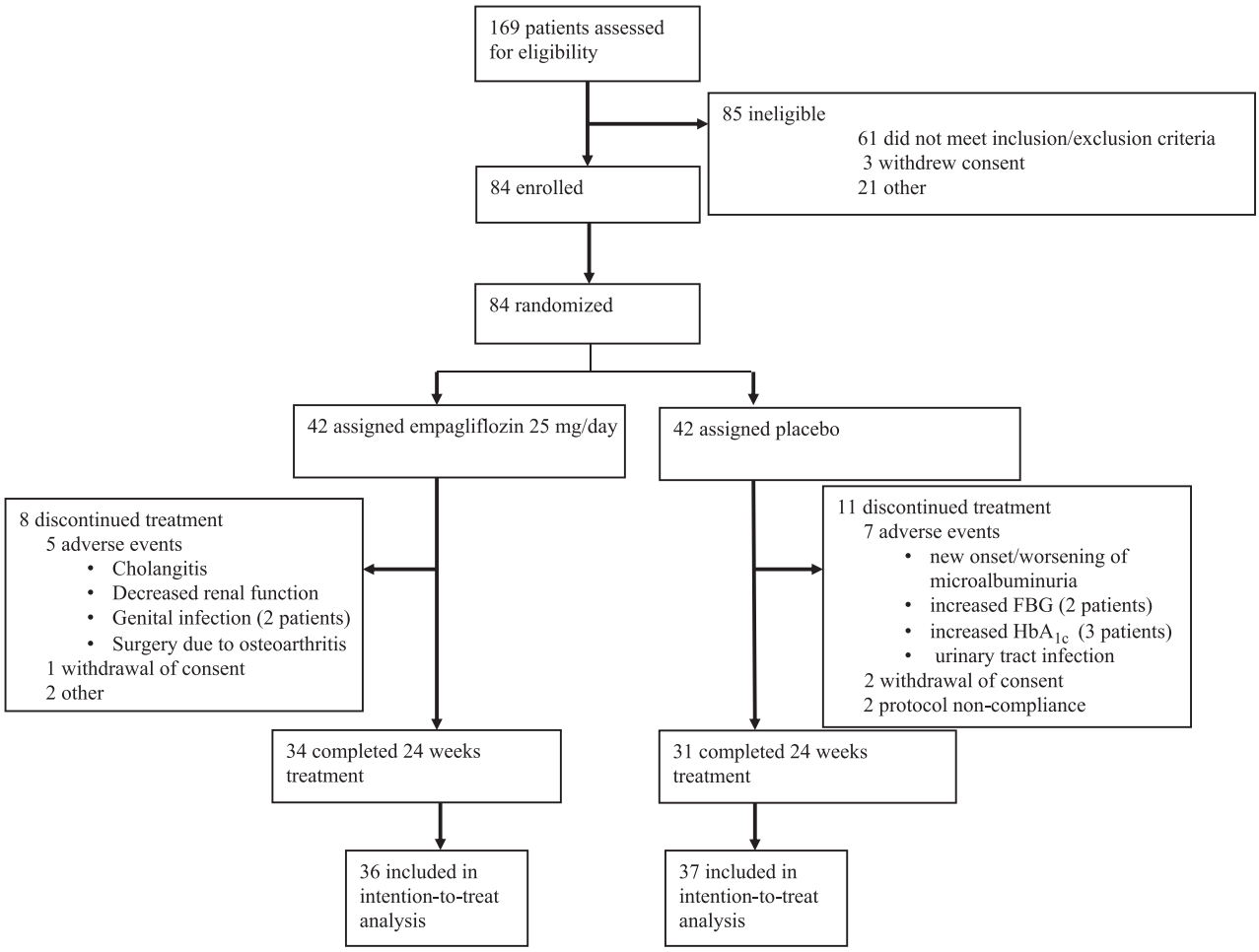

Figure 1—Trial profile.

Effect of EMPA on LFC

In the intention-to-treat population, 29 (81%) of 36 patients in the EMPA arm and 29 (78%) of 37 in the placebo arm had NAFLD at week 0. LFC was comparable between groups (EMPA 9.6% [95% CI 7.3, 12.7]; placebo 11.3% [8.6, 14.7]) and decreased in both groups already at week 12 (relative reduction: EMPA −21%, placebo −15%). At 24 weeks, a placebo-corrected absolute (−1.8% [−3.4, −0.2]; $P = 0.02$) and relative decrease in LFC (−22% [−36, −7]; $P = 0.009$) was observed, corresponding to a 2.3-fold higher relative reduction in EMPA (Fig. 2*A* and Supplementary Table 2). Further adjustment for change in body weight attenuated the difference in LFC reduction of EMPA and placebo (placebo-corrected decrease −6% [−23, 14]; $P = 0.50$).

Applying maximum likelihood methods to account for missing values for LFC at week 24 did not affect the results (data not shown). NAFLD resolution (LFC <5.56% [21]) occurred in 5 (20%) of 25 patients in the EMPA group and 2 (8%) of 24 patients in the placebo group at 24 weeks.

To examine the impact of the presence of NAFLD on EMPA-mediated reduction of LFC, an interaction term of treatment and NAFLD status (yes/no) was added to the model. Interaction of NAFLD status and treatment was not significant ($P = 0.94$).

The impact of sex on the EMPA-related reduction in LFC was examined by including an interaction term of treatment and sex in our model. There was a placebo-corrected decrease in LFC in males (−31% [95% CI −44, −14]; $P = 0.002$) but not in females (−1% [−28, 37]; $P = 0.96$). The test of interaction between sex and treatment did not achieve significance ($P = 0.075$).

Effect of EMPA on Skeletal Muscle and Hepatic and Adipose Tissue Insulin Sensitivity

During low-insulin clamp conditions, placebo-corrected whole-body/skeletal muscle R_d increased by 30% (95% CI 9, 55; $P = 0.005$) (Supplementary Table 1). However, there were no significant placebo-corrected changes in M value both at low (50% [0, 126]; $P = 0.05$) and high (12% [−12, 42]; $P = 0.36$) insulin with EMPA (Fig. 2*B* and Supplementary Table 1). Changes in HIR and insulin-mediated suppression of EGP at low- and high-insulin conditions were also comparable between groups (Fig. 2*C* and Supplementary Table 1). Likewise, changes in adipose tissue insulin resistance and insulin-stimulated FFA suppression at low- and high-insulin conditions did not differ between groups (Fig. 2*D* and Supplementary Table 1).

Effect of EMPA on Body Composition, Glycemia, and Lipidemia

EMPA resulted in a placebo-corrected weight loss of −2.5 kg (95% CI −3.7, −1.4; $P < 0.001$) at 24 weeks (Fig. 3*A* and Supplementary Table 2). The body weight reduction occurred in 31 (86%)

Table 1—Patient characteristics at week 0

	EMPA (n = 42)	Placebo (n = 42)
Sex		
Male	29 (69)	29 (69)
Female	13 (31)	13 (31)
Age (years)	62.7 ± 7.0	61.5 ± 10.0
Ethnicity		
Caucasian	42 (100)	41 (98)
Hispanic/Latino	0 (0)	1 (2)
BMI (kg/m^2)	32.1 ± 4.6	32.4 ± 4.2
Known diabetes duration (months)	36 ± 27	40 ± 27
Hepatic steatosis*	33 (79)	33 (79)
Concomitant medication		
Antihyperglycemic drugs#	28 (67)	26 (62)
Antihypertensive drugs	21 (50)	29 (69)
Lipid-lowering drugs	19 (45)	15 (36)
Glycemia		
HbA_{1c}		
%	6.8 ± 0.5	6.7 ± 0.7
mmol/mol	51 ± 6	50 ± 8
FBG (mmol/L)	7.5 ± 1.4	7.2 ± 1.3
Serum lipid concentrations		
Triglycerides (mg/dL)	159 (122; 202)	181 (103; 251)
HDL cholesterol (mg/dL)	50 ± 15	48 ± 10
LDL cholesterol (mg/dL)	133 ± 40	120 ± 30
Liver transaminases		
ALT (μmol/s/L)	0.54 (0.42; 0.80)	0.62 (0.42; 0.88)
AST (μmol/s/L)	0.42 (0.36; 0.49)	0.43 (0.37; 0.55)

Data are mean ± SD for normally distributed parameters, median (25%; 75%) for log-normally distributed parameters, or n (%). ALT, alanine aminotransferase; AST, aspartate aminotransferase. *LFC ≥5.56% measured by magnetic resonance–based methods. #Antihyperglycemic medication was stopped from at least 4 weeks before randomization until the end of the intervention period.

of 36 patients in the EMPA group and 18 (49%) of 37 patients in the placebo group. Weight loss of ≥5% occurred in 27% of patients on EMPA and in 16% on placebo. There were no placebo-corrected changes in VAT (−290 cm^3 [−694, 114]; P = 0.16) and SCAT (−2% [−10, 6]; P = 0.55) with EMPA. Of note, patients who underwent both VAT and SCAT measurements (n = 21 of 29) also exhibited a placebo-corrected decrease in body weight with EMPA (−2.6 kg [−4.0, −1.1]; $P < 0.001$).

EMPA led to a placebo-corrected change in FBG (−0.7 mmol/L [95% CI −1.3, −0.2]; $P = 0.01$) (Fig. 3*B*) but not in HbA_{1c} (Supplementary Table 2). Placebo-corrected changes in fasting insulin, C-peptide, and FFA levels did not reach significance (all $P > 0.2$) (Supplementary Table 2). Also, serum HDL and LDL cholesterol, serum total cholesterol, and plasma triglycerides were unaffected by EMPA treatment (data not shown).

Effect of EMPA on Adiponectin and Inflammation- and Liver-Related Parameters

Serum uric acid markedly decreased (placebo-corrected change −74 mol/L [95% CI −108, −42]; $P < 0.001$), and HMW adiponectin concentrations increased (placebo-corrected change 36% [16, 60]; $P < 0.001$) from 0 to 24 weeks (Fig. 3*C* and *D*). Placebo-corrected changes in IL-1Ra, TNF-α, IL-6, and FGF-21 did not differ between groups (all $P > 0.2$) (Supplementary Table 3).

Serum alanine aminotransferase and γ-glutamyl transferase were reduced with similar effect sizes in EMPA and placebo after 24 weeks (Supplementary Table 3). CK18-M30 and -M65 numerically decreased in the EMPA group, but no placebo-corrected changes were detectable (Supplementary Table 3).

CONCLUSIONS

This trial provides evidence that empagliflozin effectively reduces LFC compared with placebo but has no major effects on tissue-specific insulin sensitivity. Exploratory analyses revealed a marked decrease in serum uric acid and a rise in serum HMW adiponectin levels. Of interest, these effects occurred in the presence of moderate weight loss and despite only minor changes in glycemia in a cohort of metabolically well-controlled patients with T2D with a short disease duration.

Effects of SGLT2is on LFC and Body Weight

Recent randomized controlled trials demonstrated that SGLT2is can induce a reduction of LFC compared with baseline (6–9), but only one trial also reported a statistically significant effect on LFC compared with placebo (6). The magnitude of the reduction in LFC may depend on trial medication and design; duration of the intervention; cohort characteristics, such as NAFLD status, T2D duration, glycemic control, and sex distribution; and, finally, statistical power (22,23). The current study reports that EMPA leads to a nominally greater placebo-corrected decrease in LFC than dapagliflozin (6) but a slightly smaller decrease in change from baseline than canagliflozin (8). However, the absence of studies on dose dependency and head-to-head comparisons does not allow any conclusions about drug-specific effects at present. As indicated in other NAFLD trials (22,23), the guideline-based dietary counseling for all groups could have been responsible for the higher rates of LFC improvement observed in the placebo groups of this study and one previous (8) but not in other SGLT2i trials (6,9).

On the other hand, study duration may play a role as illustrated by the observation that alanine aminotransferase, as a crude surrogate marker of NAFLD, decreased only during the first 28 weeks of EMPA treatment (11). At the least, the nominally greater baseline-corrected decrease in LFC in the 24-week placebo-controlled trials (i.e., in one previous [8] and the current trial) than in the 8- and 12-week trials could support this contention (6,9).

As to cohort characteristics, the better metabolic control and shorter known diabetes duration compared with previous trials (6–9) and the possible inclusion of patients without NAFLD could have led to an underestimation of the efficacy of EMPA on LFC in our cohort (11). Indeed, incidence of NAFLD positively associates

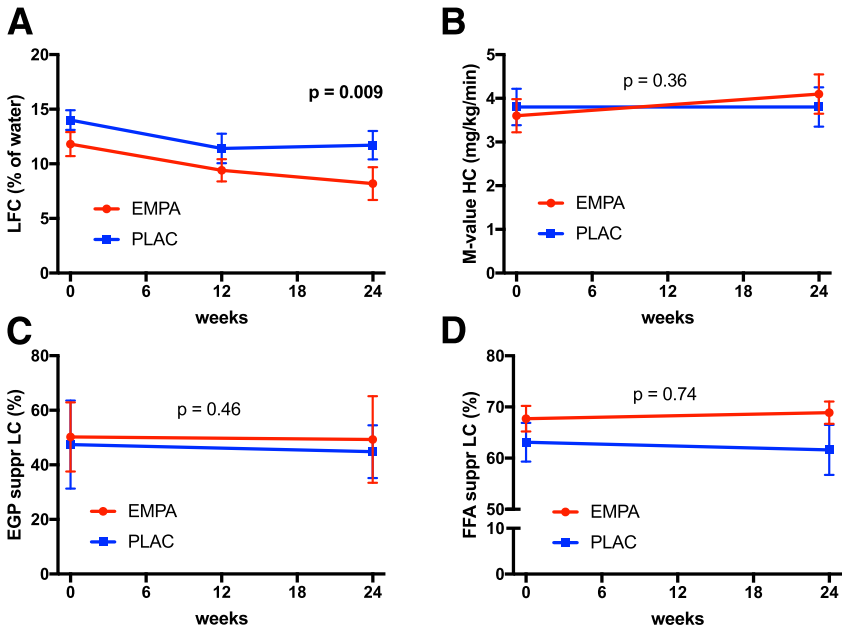

Figure 2—Effects of EMPA on LFC (*A*), whole-body insulin sensitivity (M value in high-insulin condition [40 IU/m^2 body surface area/min] [HC]) (*B*), hepatic insulin sensitivity as insulin-stimulated suppression of EGP under low-insulin conditions (20 IU/m^2 body surface area) (EGP suppr LC) (*C*), and adipose tissue insulin sensitivity as insulin-stimulated suppression of FFA under low-insulin conditions (FFA suppr LC) (*D*). Numbers of patients in EMPA and placebo (PLAC), respectively, of which week 0 and 24 data were obtained are as follows: 31 and 31 (*A*), 28 and 26 (*B*), 24 and 25 (*C*), and 27 and 27 (*D*). Unadjusted values of parameters are mean ± SEM. *P* values indicate significance level for PLAC-corrected EMPA effect and are based on ANCOVA with adjustment for age, sex, BMI, and the respective baseline parameter.

with higher HbA_{1c} and most likely also longer diabetes duration (24), and NAFLD frequency may affect the magnitude of LFC reduction in T2D (8,11).

Finally, this study found a placebo-corrected reduction of LFC in males but not in females, although the interaction of sex and treatment was not significant and the number of females small. Given that the percentage of males ranged from 60 to 81% in the previous randomized SGLT2i studies (6–9), sex-dependent differences in metabolic effects on LFC cannot be excluded. In this context, a recent study suggested sex differences in the effects of EMPA on arterial stiffness (25), whereas a post hoc analysis of the BI 10773 (Empagliflozin) Cardiovascular Outcome Event Trial in Type 2 Diabetes Mellitus Patients (EMPA-REG OUTCOME) did not detect any changes in outcomes between females and males (26).

This study shows that the changes in LFC occur in parallel to the decline in body weight during SGLT2i treatment. While significant reduction in LFC was considered to require weight loss of ≥5% (8), studies have indicated that even minor weight loss up to 5% can initiate a decrease in LFC by 33% (3,27). Because a body weight reduction of ≥5% was observed in only 27% of the EMPA group, the 34% decline in LFC underlines the role of minor weight loss for the effect of SGLT2is on LFC.

EMPA and Insulin Sensitivity

During low-insulin conditions, EMPA resulted in a borderline, but nonsignificant (M value) or significant (R_d) increase in whole-body glucose disposal. Under these conditions, R_d and M value represent the amount of glucose taken up not only by skeletal muscle but also by other organs like adipose tissue and the splanchnic bed (28). However, measures of adipose tissue (insulin-stimulated FFA suppression) or hepatic (insulin-stimulated EGP suppression) insulin sensitivity were not different between EMPA and placebo. Thus, the higher R_d could have resulted from EMPA-induced glucosuria, but study medication was stopped at least 3 days before the clamps (to account for the half-life of EMPA [~10.7 h for EMPA 25 mg (29)]), rendering urinary glucose loss unlikely. Moderate increases in R_d with SGLT2is have also been attributed to improvements in hyperglycemia and glucose toxicity (8,30). Thus, despite the (very) good glucometabolic control and rather short known diabetes duration, the minor decrease in fasting glycemia with EMPA could have contributed to the small increase in R_d during low-grade insulinemia. In contrast, the current study found no placebo-corrected effects of EMPA on R_d and M value during high-insulin clamps, which is in line with the trials on dapagliflozin (6) and canagliflozin (8). Under these conditions, R_d almost exclusively reflects insulin-stimulated skeletal muscle glucose uptake (28).

Interestingly, the decrease in LFC was not paralleled by improved hepatic insulin sensitivity, which is comparable to one dapagliflozin study (6) but in contrast to the canagliflozin trial (8). The latter study also reported lower HbA_{1c} and discussed reduction of glucotoxicity by canagliflozin as the cause (8). The absence of changes in HbA_{1c} in the current study supports this contention.

Previous studies demonstrated that the antihyperglycemic efficacy of SGLT2i is partly counteracted by a rise in EGP (31,32). This could result from a chronic SGLT2i-induced rise in plasma glucagon and decreased insulin concentrations. However, a recent clinical trial showed that canagliflozin still increases EGP when liraglutide prevents the changes in plasma insulin and glucagon levels (33). Similarly, hyperglucagonemia per se does not mediate the SGLT2i-induced increase in EGP (34). Of note, glycemia per se may regulate EGP in that a decrease in plasma glucose concentration can stimulate EGP independent of changes in plasma insulin and glucagon (35).

Finally, fasting FFA and insulin levels as well as adipose tissue insulin resistance were unchanged during this study. The previous placebo-controlled SGLT2i trials on LFC yielded contradictory results, showing elevated (8) or unchanged FFA levels (6,9) in the presence of decreased (8) or unchanged (6,9) fasting insulinemia. The previously reported SGLT2i-associated FFA elevation has been explained by glucosuria-induced relative hypoinsulinemia, which would reduce inhibition of lipolysis and tissue glucose uptake with a compensatory increase in lipid oxidation and hyperketonemia (4). In our cohort of patients with well-controlled, recent-onset T2D, EMPA did not decrease circulating insulin levels so that the ambient insulinemia

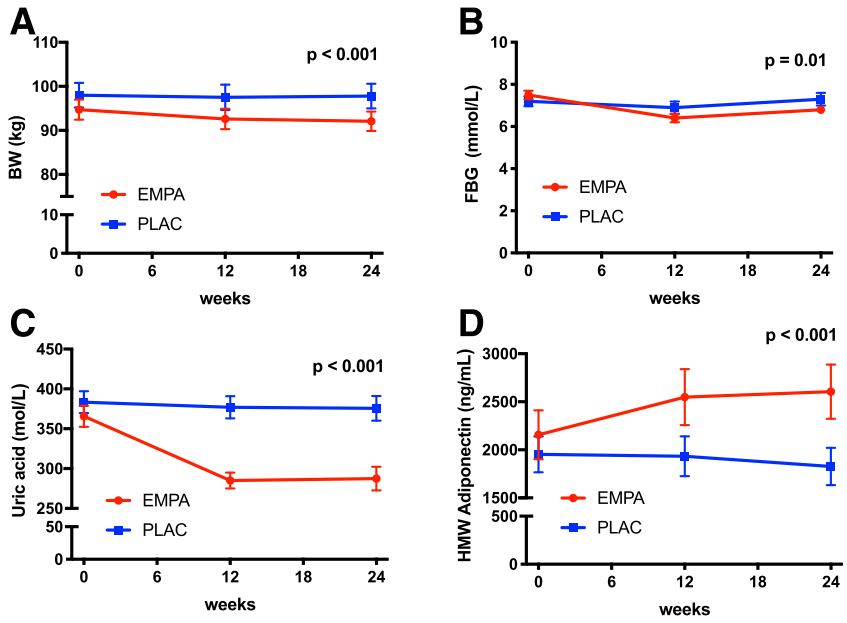

Figure 3—Effects of EMPA on body weight (BW) (*A*), FBG (*B*), serum uric acid levels (*C*), and serum HMW adiponectin levels (*D*). Comparison of changes in the respective parameters between treatment arms. Numbers of patients in EMPA and placebo (PLAC) groups, respectively, of which week 0 and 24 data were obtained are as follows: 32 and 32 (*A*), 31 and 31 (*B*), 31 and 31 (*C*), and 31 and 30 (*D*). Unadjusted values of parameters are mean ± SEM. *P* values indicate significance level for PLAC-corrected EMPA effect and are based on ANCOVA with adjustment for age, sex, BMI, and the respective baseline parameter.

might have sufficed to inhibit lipolysis as shown in humans without diabetes (4).

Exploratory Analyses of Circulating Parameters

A recent uncontrolled pilot study provided some evidence that EMPA treatment for 24 weeks could improve histological components of NASH and its resolution despite a mean reduction in BMI of only −0.7 kg/m^2 (36). The current trial did not observe placebo-corrected changes in circulating surrogate markers of liver injury, such as transaminases or CK18-M30 fragment. This is partly in line with some studies (6,7) but not in another that reported improvements in transaminases as well as CK18 fragments with dapagliflozin (9). The lack of an effect of EMPA could be due to the absence of NASH and fibrosis or masked by the greater decrease in LFC in the placebo group, which is a major trigger for reduction of these surrogate markers (27).

EMPA treatment markedly reduced serum uric acid and raised serum adiponectin concentrations. High uric acid levels trigger adipose tissue inflammation, insulin resistance, and hypoadiponectinemia (37). Of note, increased uric acid and decreased adiponectin levels associate with body weight; metabolic syndrome features, including T2D; and NAFLD (37–39).

Limitations

The patient cohort comprised exclusively metabolically well-controlled patients with T2D with short known disease duration with and without NAFLD. Thus, results cannot be necessarily extrapolated to the general population of patients with T2D, particularly to those with uncontrolled glycemia, longer disease duration, and more severe liver disease. On the other hand, this limitation represents a specific strength by showing that EMPA is effective in reducing LFC in the absence of major changes in glycemia. This trial provides no information about the efficacy and safety of EMPA in glucose-tolerant individuals with NAFLD, a collective at increased risk of T2D (2). Moreover, this study used detailed metabolic phenotyping with two-step euglycemic clamps but not mixed-meal tests, which would have allowed the assessment of postprandial β-cell function and metabolism, and did not include liver biopsies because of the expected early stages of NAFLD in these patients and the short duration of intervention. Finally, this study did not use multiple imputation to account for missing values but performed maximum likelihood methods for the primary end point (40).

In conclusion, this proof-of-concept trial shows that the SGLT2i EMPA decreases LFC in near-normoglycemic patients with recent-onset T2D with and without NAFLD. EMPA induced minor weight loss and no effect on tissue-specific insulin sensitivity. The marked decrease in serum uric acid and the rise in HMW adiponectin levels with EMPA treatment calls for further studies on the clinical relevance of these observations. Because future NAFLD treatment in T2D will require strategies that simultaneously address the different mechanisms underlying metabolic liver disease, EMPA could serve as a partner for such combination treatments because of its favorable effects on liver fat and body weight.

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References

1. Tilg H, Moschen AR, Roden M. NAFLD and diabetes mellitus. Nat Rev Gastroenterol Hepatol 2017;14:32–42
2. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care 2018;41:372–382
3. Toplak H, Stauber R, Sourij H. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease: guidelines, clinical reality and health economic aspects. Diabetologia 2016;59:1148–1149
4. Ferrannini E. Sodium-glucose co-transporters and their inhibition: clinical physiology. Cell Metab 2017;26:27–38
5. Zelniker TA, Wiviott SD, Raz I, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet 2019; 393:31–39
6. Latva-Rasku A, Honka MJ, Kullberg J, et al. 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. Diabetes Care 2019;42:931–937
7. Kuchay MS, Krishan S, Mishra SK, et al. Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: a randomized controlled trial (E-LIFT trial). Diabetes Care 2018;41:1801–1808
8. Cusi K, Bril F, Barb D, et al. Effect of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes. Diabetes Obes Metab 2019;21:812–821
9. 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
10. Leiter LA, Forst T, Polidori D, Balis DA, Xie J, Sha S. Effect of canagliflozin on liver function tests in patients with type 2 diabetes. Diabetes Metab 2016;42:25–32
11. Sattar N, Fitchett D, Hantel S, George JT, Zinman B. Empagliflozin is associated with improvements in liver enzymes potentially consistent with reductions in liver fat: results from randomised trials including the EMPA-REG OUTCOME® trial. Diabetologia 2018;61:2155–2163
12. American Diabetes Association. Standards of medical care in diabetes—2014. Diabetes Care 2014;37(Suppl. 1):S14–S80
13. Machann J, Thamer C, Schnoedt B, et al. Hepatic lipid accumulation in healthy subjects: a comparative study using spectral fat-selective MRI and volume-localized ^{1}H-MR spectroscopy. Magn Reson Med 2006;55:913–917
14. Reeder SB, McKenzie CA, Pineda AR, et al. Water-fat separation with IDEAL gradient-echo imaging. J Magn Reson Imaging 2007;25:644–652
15. Würslin C, Machann J, Rempp H, Claussen C, Yang B, Schick F. Topography mapping of whole body adipose tissue using A fully automated and standardized procedure. J Magn Reson Imaging 2010;31:430–439
16. Machann J, Thamer C, Stefan N, et al. Follow-up whole-body assessment of adipose tissue compartments during a lifestyle intervention in a large cohort at increased risk for type 2 diabetes. Radiology 2010;257:353–363
17. Phielix E, Brehm A, Bernroider E, et al. Effects of pioglitazone versus glimepiride exposure on hepatocellular fat content in type 2 diabetes. Diabetes Obes Metab 2013;15:915–922
18. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr 1982;36:936–942
19. Nowotny B, Zahiragic L, Bierwagen A, et al. Low-energy diets differing in fibre, red meat and coffee intake equally improve insulin sensitivity in type 2 diabetes: a randomised feasibility trial. Diabetologia 2015;58:255–264
20. Roden M, Weng J, Eilbracht J, et al.; EMPA-REG MONO trial investigators. Empagliflozin monotherapy with sitagliptin as an active comparator in patients with type 2 diabetes: a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Diabetes Endocrinol 2013;1:208–219
21. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 2005;288:E462–E468
22. Loomba R, Wesley R, Pucino F, Liang TJ, Kleiner DE, Lavine JE. Placebo in nonalcoholic steatohepatitis: insight into natural history and implications for future clinical trials. Clin Gastroenterol Hepatol 2008;6:1243–1248
23. Han MAT, Altayar O, Hamdeh S, et al. Rates of and factors associated with placebo response in trials of pharmacotherapies for nonalcoholic steatohepatitis: systematic review and meta-analysis. Clin Gastroenterol Hepatol 2019;17: 616–629.e26
24. Hazlehurst JM, Woods C, Marjot T, Cobbold JF, Tomlinson JW. Non-alcoholic fatty liver disease and diabetes. Metabolism 2016;65: 1096–1108
25. Bosch A, Ott C, Jung S, et al. How does empagliflozin improve arterial stiffness in patients with type 2 diabetes mellitus? Sub analysis of a clinical trial. Cardiovasc Diabetol 2019;18:44
26. Zinman B, Inzucchi SE, Wanner C, et al.; EMPA-REG OUTCOME® investigators. Empagliflozin in women with type 2 diabetes and cardiovascular disease - an analysis of EMPA-REG OUTCOME®. Diabetologia 2018;61:1522–1527
27. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. J Hepatol 2017;67:829–846
28. Roden M. *Clinical Diabetes Research: Methods and Techniques*. Chichester, U.K., John Wiley & Sons, 2007
29. Heise T, Seman L, Macha S, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of multiple rising doses of empagliflozin in patients with type 2 diabetes mellitus. Diabetes Ther 2013;4:331–345
30. O'Brien TP, Jenkins EC, Estes SK, et al. Correcting postprandial hyperglycemia in Zucker diabetic fatty rats with an SGLT2 inhibitor restores glucose effectiveness in the liver and reduces insulin resistance in skeletal muscle. Diabetes 2017;66:1172–1184
31. 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
32. 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
33. Martinez R, Al-Jobori H, Ali AM, et al. Endogenous glucose production and hormonal changes in response to canagliflozin and liraglutide combination therapy. Diabetes 2018;67: 1182–1189
34. Perry RJ, Rabin-Court A, Song JD, et al. Dehydration and insulinopenia are necessary and sufficient for euglycemic ketoacidosis in SGLT2 inhibitor-treated rats. Nat Commun 2019;10:548
35. Cherrington AD. Banting Lecture 1997. Control of glucose uptake and release by the liver in vivo. Diabetes 1999;48:1198–1214
36. Lai LL, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK. Empagliflozin for the treatment of nonalcoholic steatohepatitis in patients with type 2 diabetes mellitus. Dig Dis Sci. 25 January 2019 [Epub ahead of print]. DOI: 10.1007/s10620-019-5477-1
37. Johnson RJ, Nakagawa T, Sanchez-Lozada LG, et al. Sugar, uric acid, and the etiology of diabetes and obesity. Diabetes 2013;62:3307–3315
38. Gastaldelli A, Harrison SA, Belfort-Aguilar R, et al. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. Hepatology 2009; 50:1087–1093
39. Bellou V, Belbasis L, Tzoulaki I, Evangelou E. Risk factors for type 2 diabetes mellitus: an exposure-wide umbrella review of meta-analyses. PLoS One 2018;13:e0194127
40. Schafer JL, Graham JW. Missing data: our view of the state of the art. Psychol Methods 2002;7:147–177