Effect of Empagliflozin on Liver Fat in Patients With Type 2 Diabetes and Nonalcoholic Fatty Liver Disease: A Randomized Controlled Trial (E-LIFT Trial)

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OBJECTIVE

Sodium–glucose cotransporter 2 (SGLT-2) inhibitors have been shown to reduce liver fat in rodent models. Data regarding the effect of SGLT-2 inhibitors on human liver fat are scarce. This study examined the effect of empagliflozin (an SGLT-2 inhibitor) on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease (NAFLD) by using MRI-derived proton density fat fraction (MRI-PDFF).

RESEARCH DESIGN AND METHODS

Fifty patients with type 2 diabetes and NAFLD were randomly assigned to either the empagliflozin group (standard treatment for type 2 diabetes plus empagliflozin 10 mg daily) or the control group (standard treatment without empagliflozin) for 20 weeks. Change in liver fat was measured by MRI-PDFF. Secondary outcome measures were change in alanine transaminase (ALT), aspartate transaminase (AST), and γ-glutamyl transferase (GGT) levels.

RESULTS

When included in the standard treatment for type 2 diabetes, empagliflozin was significantly better at reducing liver fat (mean MRI-PDFF difference between the empagliflozin and control groups −4.0%; $P < 0.0001$). Compared with baseline, significant reduction was found in the end-of-treatment MRI-PDFF for the empagliflozin group (16.2% to 11.3%; $P < 0.0001$) and a nonsignificant change was found in the control group (16.4% to 15.5%; $P = 0.057$). The two groups showed a significant difference for change in serum ALT level ($P = 0.005$) and nonsignificant differences for AST ($P = 0.212$) and GGT ($P = 0.057$) levels.

CONCLUSIONS

When included in the standard treatment for type 2 diabetes, empagliflozin reduces liver fat and improves ALT levels in patients with type 2 diabetes and NAFLD.

Nonalcoholic fatty liver disease (NAFLD) often coexists with type 2 diabetes (1). The presence of type 2 diabetes in patients with NAFLD is a risk factor for progression to nonalcoholic steatohepatitis (NASH), a severe form of NAFLD that can further progress to liver fibrosis, cirrhosis, and hepatocellular cancer (2). NAFLD also leads to various extrahepatic complications. For instance, NAFLD is an independent risk factor for cardiovascular disease (3), type 2 diabetes (4), and chronic kidney disease (5).

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The pathogenesis of NAFLD is complex, involving insulin resistance, oxidative stress, lipid peroxidation, and mitochondrial dysfunction (6). Insulin resistance is the key pathogenic factor for the development of both type 2 diabetes and NAFLD (7,8). Several antidiabetic therapies have been investigated in the treatment of NAFLD with varying results, including lifestyle modification (9,10), metformin (11), pioglitazone (12,13), and liraglutide (14).

Empagliflozin is a potent oral antidiabetic agent that inhibits sodium–glucose cotransporter 2 (SGLT-2) (15). By inhibiting this transporter, SGLT-2 inhibitors promote urinary glucose excretion, which, in turn, decreases blood levels of glucose and improves insulin resistance in patients with type 2 diabetes (16,17). Improvement in hyperglycemia downregulates carbohydrate-responsive element-binding protein (ChREBP), a transcription factor responsible for activating the machinery for fatty acid synthesis (18). Improvement in insulin resistance (hyperinsulinemia) results in downregulation of SREBP-1c and the blockage of de novo hepatic lipogenesis (19). Thus, SGLT-2 inhibitors should improve NAFLD and/or NASH and provide a mechanistic rationale to conduct human trials with SGLT-2 inhibitors in patients with NAFLD.

In preclinical studies on rodent models, SGLT-2 inhibitors ameliorated NAFLD and NASH (16,20–22). In clinical trials with humans, ipragliflozin (an SGLT-2 inhibitor) reduced liver fat in patients with type 2 diabetes and NAFLD. However, liver fat was estimated indirectly by calculating liver fat index, a surrogate marker for fatty liver (23). The data on SGLT-2 inhibitors and human liver fat are scarce; therefore, this proof-of-concept study examined the effect of empagliflozin on liver fat in patients with type 2 diabetes and NAFLD. Liver fat was measured by MRI proton density fat fraction (MRI-PDFF). In addition, we evaluated the effect of empagliflozin on serum alanine transaminase (ALT), aspartate transaminase (AST), and γ-glutamyl transferase (GGT) levels.

RESEARCH DESIGN AND METHODS

Participants and Setting

The E-LIFT (Effect of Empagliflozin on Liver Fat Content in Patients With Type 2 Diabetes) trial was an investigator-initiated, prospective, open-label, randomized clinical study to examine the effect of empagliflozin 10 mg/day when included in the standard treatment of type 2 diabetes versus standard treatment without empagliflozin in patients with type 2 diabetes and NAFLD. Hepatic steatosis (intracellular fat accumulation in hepatocytes) was measured by MRI-PDFF, a robust and quantitative biomarker. The study was designed and conducted according to Consolidated Standards of Reporting Trials guidelines. Some changes were made to the methods after study commencement. Initially, we intended to recruit a larger sample size to power for other outcomes such as weight change and changes in AST, ALT, and GGT levels. Later, we recalculated the sample size to adequately power the study for the primary outcome only because recruitment was taking longer than expected. Another change is that we extended the duration of the treatment period from the initially intended 12 weeks to 20 weeks because, in the meantime, a few similar types of studies were published where duration of treatment was >12 weeks. Apart from these two changes, no other changes to the trial design were made. We did not perform an interim analysis. The study population comprised patients seen at the Medanta-The Medicity Hospital endocrine outpatient clinic who were visiting primarily for management of type 2 diabetes and other comorbidities. The study was conducted in Medanta-The Medicity Hospital, which is a tertiary care center in northern India. Patients deemed eligible were screened for the trial. The study was registered as a clinical trial and its protocol was approved by the institutional ethics review board (MICR-562/2015; Gurugram, Haryana, India). Informed written consent was obtained from all participants.

Eligibility Criteria

Patients were included if they were >20 years of age, had documented hepatic steatosis (MRI-PDFF >6%), were having uncontrolled type 2 diabetes (HbA_{1c} >7.0% to <10.0%), and provided written informed consent. Exclusion criteria were highly uncontrolled diabetes (HbA_{1c} >10.0%); alcohol intake >30 g/day (three drinks per day) within the previous 10 years or >10 g/day within the previous year; evidence of other forms of liver disease, including hepatitis B (positive serum hepatitis B surface antigen), hepatitis C (positive anti–hepatitis C virus), autoimmune hepatitis (positive autoimmune serology and consistent biopsy specimen), drug-induced liver disease on the basis of exposure and history, and biliary duct obstruction on the basis of imaging studies; history of gastrointestinal bypass or use of drugs known to cause hepatic steatosis (e.g., amiodarone, valproate, tamoxifen, methotrexate, steroids); recent initiation or change of antidiabetic drugs that influence liver fat, including thiazolidinediones and glucagon-like peptide 1 receptor agonists, or recent initiation of any SGLT-2 inhibitor, within 90 days of randomization; evidence of cirrhosis (on basis of ultrasonography and MRI [none of the patients had suspicion of cirrhosis on the basis of clinical features and biochemical profile]) or hepatocellular carcinoma (evidence on MRI); positive HIV test; active substance abuse; pregnant or trying to become pregnant; renal insufficiency (glomerular filtration rate <90 mL/min/1.73 m^2 as estimated by the MDRD equation); contraindications to empagliflozin use (history of recurrent urinary tract or genital infections, current or previous gangrene, or known allergy to the molecule); and contraindications to MRI (cardiac pacemakers, claustrophobia, foreign bodies, and implanted medical devices with ferromagnetic properties).

Randomization and Allocation Concealment

A research assistant randomly assigned the patients into either the empagliflozin group or the control group in a 1:1 ratio by using computer-generated numbers. The patients were then sent to their respective consultants (M.S.K., S.K.M., K.J.F., J.S.W., and A.M.) in the endocrine department for adjustment of treatment for type 2 diabetes (according to randomization into empagliflozin or control groups) and other comorbidities. Treatment allocation was open label. Investigators involved in imaging data analysis (i.e., radiology technician, radiologists) were blinded to patient information and the allocation sequence. Although aware of the treatment group, the endocrinologists were blinded to the imaging results until final data analysis. The drug used was

empagliflozin 10 mg (Jardiance 10 mg; Boehringer Ingelheim, Ingelheim am Rhein, Germany).

Study Visits

After careful assessment at the baseline visit, patients meeting all inclusion and exclusion criteria were randomly assigned to receive empagliflozin 10 mg orally daily plus standard treatment for type 2 diabetes. The control group received standard treatment for type 2 diabetes, and uptitration of treatment was done by antidiabetic medicines other than SGLT-2 inhibitors. Patients returned to the outpatient endocrine clinic for follow-up visits at weeks 8 and 20.

Outcomes

The primary outcome measure was change in liver fat content from baseline as quantified by MRI-PDFF in colocalized regions of interest (ROIs) within each of the nine liver segments. Secondary outcome measures were change in serum AST, ALT, and GGT levels.

Sample Size Calculation

We assumed that a 5% difference between the empagliflozin and control groups would be the minimally appreciable and clinically relevant difference. On the basis of the results of previous similar clinical studies involving colesevelam, ezetimibe, and sitagliptin (24–26), we expected the empagliflozin group to have a liver fat reduction of >5% compared with baseline, the control group to have <1% reduction in liver fat compared with baseline, and a dropout rate of <10%. With these assumptions, the calculated sample size per group needed to be ≥20 to achieve a power of ≥90% with $\beta = 0.05$. Therefore, we planned to randomize 50 patents, 25 in each group, to ensure adequate study power even with dropouts.

MRI-PDFF Protocol

MRI-PDFF for Detailed Fat Mapping of the Entire Liver

MRI-PDFF is a noninvasive and quantitative MRI-based biomarker that can accurately estimate liver fat content (27,28). It is a robust technique for assessing treatment response in NASH clinical trials (25–27). In this study, the mean (SD) time interval from obtaining the baseline MRI-PDFF to initiating the study drug was 6 (3) days. All MRI examinations were done by an experienced MRI technologist in the Medanta radiology department under the direction of the radiologist investigator (S.K.).

ROI Colocalization Before and After Treatment

To assess longitudinal changes in liver fat content, one colocalized ROI was placed in each of the nine liver segments (nine separate ROIs) on the baseline and follow-up MRI examinations.

Reason for Using MRI-PDFF for Liver Fat Quantification

We used MRI-PDFF for assessing liver fat change because it is a robust technique. Unlike computed tomography, it is accurate and does not subject patients to ionizing radiation, and unlike ultrasonography, it is not operator dependent. In addition, MRI-PDFF allows for objective, quantitative fat fraction measurements throughout the various segments of the liver with minimal sampling variability (29). In previous NAFLD clinical trials, MRI-PDFF was shown to be more sensitive than histology for assessing quantitative changes in liver fat (24,27).

MRI-PDFF Validation in Our Institution

We have generated normative data for liver fat in the Indian population by using MRI-PDFF liver fat quantification. We performed an MRI-PDFF estimation in 219 subjects with confirmed <5% liver fat on histopathology. The mean liver fat in this population was 2.6% (SD 1.9%, range 1.3–6.6%). The mean liver fat in segments I, II, III, $IV_{(A)}$, $IV_{(B)}$, V, VI, VII, and VIII were 2.56%, 2.71%, 2.70%, 2.63%, 2.52%, 2.48%, 2.41%, 2.60%, and 2.58%, respectively. The least significant change that could be measured reliably was 2.1%. No statistically significant differences in liver fat between the right- and the left-side lobes ($P = 0.07$) or among various segments ($P = 0.32$) were found (S.K., unpublished observations) (Supplementary Fig. 2).

Validation of MRI-PDFF for Liver Fat Quantification in NAFLD Clinical Trials

Previously, at least three trials have used MRI-PDFF for liver fat estimation in studies evaluating the effects of various drugs on NAFLD and NASH (24–26). They also validated MRI-PDFF with magnetic resonance spectroscopy (gold standard for hepatic fat quantification) and found a robust correlation between the two techniques. The three studies were from one center in the U.S. The current study is the first in our knowledge from India to use MRI-PDFF for liver fat quantification in an Indian population. This study provides independent validation of the methodology used in those trials and adds to the evidence that quantification of liver fat with MRI-PDFF is accurate and may be used longitudinally to measure liver fat changes over time.

Statistical Analysis

SAS 24.0 software (SAS Institute, Cary, NC) was used to perform all statistical analyses. For between-group comparisons, the χ^2 or Fisher exact test was used for categorical variables, and independent samples *t* test or Wilcoxon-Mann-Whitney *U* test was used for differences between continuous variables. Pearson correlation coefficient was used to evaluate correlations between variables. Additional analyses of primary and secondary outcomes within treatment groups were performed by using two-tailed independent sample *t* tests, paired *t* tests, or nonparametric tests, when indicated. Two-tailed $P < 0.05$ was considered significant. Statistical analyses were performed by a biostatistician (M.K.S.). All authors had access to study data and approved the final data analysis and submission.

RESULTS

Description of Study Population

From March 2016 to May 2017, 50 patients with type 2 diabetes and NAFLD were randomly assigned to receive either empagliflozin 10 mg/day orally in addition to standard treatment for type 2 diabetes (empagliflozin group) or standard treatment for type 2 diabetes without empagliflozin (control group). Seventy-eight patients were screened for the study (Fig. 1). In the empagliflozin arm, 22 patients completed the study, with 3 developing complications related to the study medication. In the control arm, 20 patients completed the study, with 3 lost to follow-up and 2 discontinuing because of work schedule conflicts. Forty percent of the study population comprised women, and all were of Indian origin. The two groups had similar baseline characteristics (Supplementary Table 1).

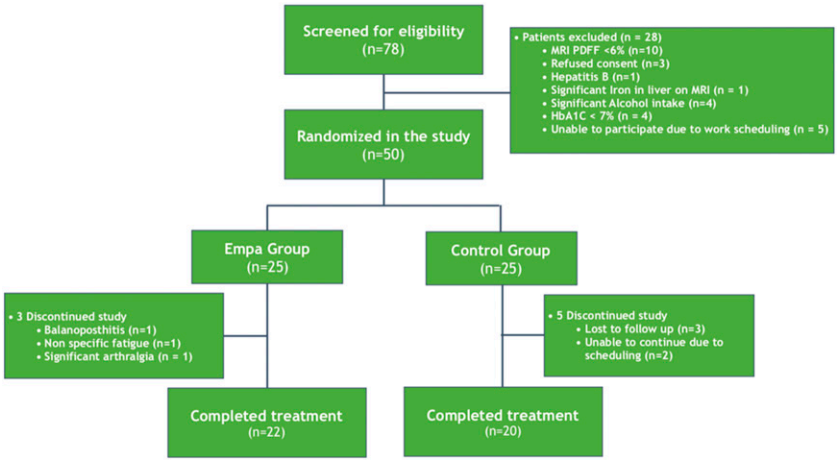

Figure 1—Derivation of the study cohort. Empa, empagliflozin.

The estimated compliance rate for the treatment was >95% as estimated by direct interview on follow-up visits. One patient had discontinued empagliflozin for 8 days because of bereavement in his family. Other medications for type 2 diabetes were metformin (100%), dipeptidyl peptidase 4 (DPP-4) inhibitor (73.6%), sulfonylurea (52%), and insulin (11.6%) (Supplementary Table 2).

Effect of Empagliflozin on Liver Fat

Liver fat, as measured by MRI-PDFF, was significantly reduced in the empagliflozin group compared with the control group (Table 1). The mean difference in liver fat change between the groups was −4.0% ($P < 0.0001$) (Table 2). Compared with baseline, a significant difference was found in end-of-treatment MRI-PDFF in the empagliflozin group (reduction from 16.2% to 11.3%; $P < 0.0001$), and an insignificant change was found in the control group (from 16.4% to 15.5%; $P = 0.054$) (Table 2 and Fig. 2). Four (18%) patients in the empagliflozin group achieved liver fat content <6.0% on MRI-PDFF (upper limit of normal in this population) compared with one (5%) in the control group.

Effect of Empagliflozin on Serum AST, ALT, and GGT Levels

The two groups showed significant differences for change in serum ALT (−10.9 IU/L; $P = 0.005$) and nonsignificant differences in AST (−7.7 IU/L; $P = 0.212$) and GGT (−11 IU/L; $P = 0.057$) levels (Table 2). Compared with baseline, a significant difference was found in end-of-treatment AST levels in the empagliflozin group (64.3 to 49.7 IU/L; $P = 0.001$), and an insignificant change was found in the control arm (65.3 to 61.6 IU/L; $P = 0.422$). Posttreatment changes in serum AST, ALT, and GGT levels relative to baseline are shown in Supplementary Fig. 1. Changes in biochemical and anthropometric variables between the empagliflozin and control groups are summarized in Table 2. Multivariate logistic regression analysis did not show any biochemical or anthropometric parameter as an independent predictor of liver fat reduction.

Maintenance of Glycemic Equipoise in the Two Groups

A significant decrease in glucose and HbA_{1c} was found in both the empagliflozin (glucose 173 to 124 mg/dL [$P < 0.0001$], HbA_{1c} 9.0% to 7.2% [$P < 0.0001$]) and the control (glucose 176 to 120 mg/dL [$P < 0.0001$], HbA_{1c} 9.1% to 7.1% [$P < 0.0001$]) groups (Table 2). We intended to maintain glycemic equipoise in the two groups by adjusting other antidiabetic medicines so that the changes in glycemic parameters had no effect on liver fat. Our prespecified targets for glycemic parameters for both the groups were according to the American Diabetes Association 2017 guidelines (fasting glucose 80–130 mg/dL, postprandial glucose <180 mg/dL, HbA_{1c} <7.0%). No significant differences were found between fasting plasma glucose (FPG) and HbA_{1c} between the empagliflozin and control groups at the end of treatment (FPG $P = 0.850$, HbA_{1c} $P = 0.880$).

Adverse Events

A few significant adverse events were documented as part of this study. One patient in the empagliflozin group developed balanoposthitis within 1 week of initiation of the drug, leading to

Table 1—Empagliflozin versus control group: longitudinal, full liver fat mapping by MRI-PDFF

	Control group (n = 20)			Empagliflozin group (n = 22)			Difference between groups
Liver segment	Baseline	Posttreatment	P value	Baseline	Posttreatment	P value	P value
I	16.3 (6.6)	15.1 (6.8)	0.254	16.3 (6.5)	11.5 (6.5)	<0.0001	0.013
II	16.1 (7.5)	15.4 (6.5)	0.573	15.6 (7.3)	11.2 (6.1)	<0.0001	0.032
III	16.6 (8.1)	15.7 (7.0)	0.311	16.3 (6.8)	11.1 (5.2)	<0.0001	0.007
$IV_{(A)}$	16.7 (8.5)	15.9 (6.8)	0.399	16.0 (7.9)	11.1 (5.4)	<0.0001	0.009
$IV_{(B)}$	16.0 (8.3)	14.7 (7.2)	0.190	16.2 (7.0)	11.5 (5.7)	0.001	0.041
V	16.7 (7.5)	16.2 (7.2)	0.693	16.6 (6.9)	11.4 (4.9)	<0.0001	0.004
VI	16.5 (7.1)	15.5 (7.0)	0.200	15.8 (6.8)	11.4 (4.6)	0.001	0.020
VII	15.8 (6.4)	15.5 (6.5)	0.711	16.1 (7.3)	10.9 (5.1)	<0.0001	0.002
VIII	16.8 (7.1)	15.3 (6.8)	0.055	16.9 (8.0)	11.3 (5.0)	<0.0001	0.010
MRI-PDFF, average (%)	16.4 (7.3)	15.5 (6.7)	0.054	16.2 (7.0)	11.3 (5.3)	<0.0001	<0.0001

Data are mean (SD) unless otherwise indicated. MRI-PDFFs measured in all nine liver segments were used to calculate segmental and overall fat fraction averages at baseline and posttreatment between the empagliflozin and control groups.

Table 2—Baseline characteristics and changes in parameters after 20 weeks of treatment

	Control group (n = 20)			Empagliflozin group (n = 22)			Difference between groups
	Baseline	Posttreatment	P value	Baseline	Posttreatment	P value	P value
Demographic							
Weight (kg)	81.1 (16.1)	79.5 (14.9)	0.022	80.8 (13.0)	77.5 (11.0)	0.001	0.154
BMI (kg/m^2)	29.4 (3.1)	28.8 (2.8)	0.019	30.0 (3.8)	28.7 (3.5)	0.001	0.124
Seated SBP (mm/Hg)	130 (19)	123 (13)	0.099	125 (13)	124 (9)	0.835	0.253
Seated DBP (mm/Hg)	81 (12)	81 (7)	0.855	79 (10)	81 (5)	0.444	0.620
Biochemical profile							
Fasting glucose (mg/dL)	176 (57)	120 (19)	<0.0001	173 (44)	124 (17)	<0.001	0.850
HbA_{1c} (%)	9.1 (1.4)	7.1 (0.9)	<0.0001	9.0 (1.0)	7.2 (0.6)	<0.001	0.880
HbA_{1c} (mmol/mol)	76.1 (16.3)	54.3 (9.8)	<0.001	75.1 (10.9)	55.2 (5.9)	<0.001	0.601
Serum creatinine (mg/dL)	0.89 (0.25)	0.91 (0.19)	0.319	0.81 (0.25)	0.90 (0.24)	0.018	0.420
Total bilirubin (mg/dL)	0.7 (0.3)	0.6 (0.2)	0.458	0.7 (0.3)	0.6 (0.3)	0.041	0.256
AST (units/L)	45.3 (24.3)	44.6 (23.8)	0.931	44.6 (23.5)	36.2 (9.0)	0.040	0.212
ALT (units/L)	65.3 (40.3)	61.6 (38.4)	0.422	64.3 (20.2)	49.7 (25.8)	0.001	0.005
GGT (units/L)	63.9 (45.3)	60.0 (39.0)	0.421	65.8 (36.1)	50.9 (24.6)	0.002	0.057
Albumin (units/L)	4.26 (0.25)	4.28 (0.35)	0.839	4.16 (0.35)	4.30 (0.35)	0.231	0.411
Triglycerides (mg/dL)	212 (115)	175 (43)	0.019	201 (124)	155 (52)	0.010	0.678
HDL (mg/dL)	45 (15)	47 (12)	0.097	42 (12)	45 (12)	0.087	0.752
LDL (mg/dL)	114 (30)	96 (17)	0.001	112 (35)	95 (22)	0.018	0.512

Data are mean (SD). DBP, diastolic blood pressure; SBP, systolic blood pressure.

discontinuation. One patient developed nonspecific fatigue after 5 days of drug initiation. Her serum electrolytes were in the normal range. The drug was discontinued and the symptoms improved. Another patient in the empagliflozin group developed arthralgia of the big joints. There were no inflammatory changes and no response to oral paracetamol. After discontinuation of empagliflozin, the arthralgia improved completely within a few days. Five patients in the control group dropped out of the study. The dropouts from the control arm were not associated with study adverse events (Fig. 1).

CONCLUSIONS

The current study demonstrated for the first time in our knowledge that empagliflozin 10 mg daily, when included in the standard treatment for type 2 diabetes, reduces liver fat in patients with type 2 diabetes and NAFLD. The mean difference in liver fat change between the empagliflozin and control groups was −4.0% ($P < 0.0001$). A Japanese study showed that ipragliflozin (an SGLT-2 inhibitor) reduced liver fat in patients with type 2 diabetes and NAFLD (23). However, the authors used fatty

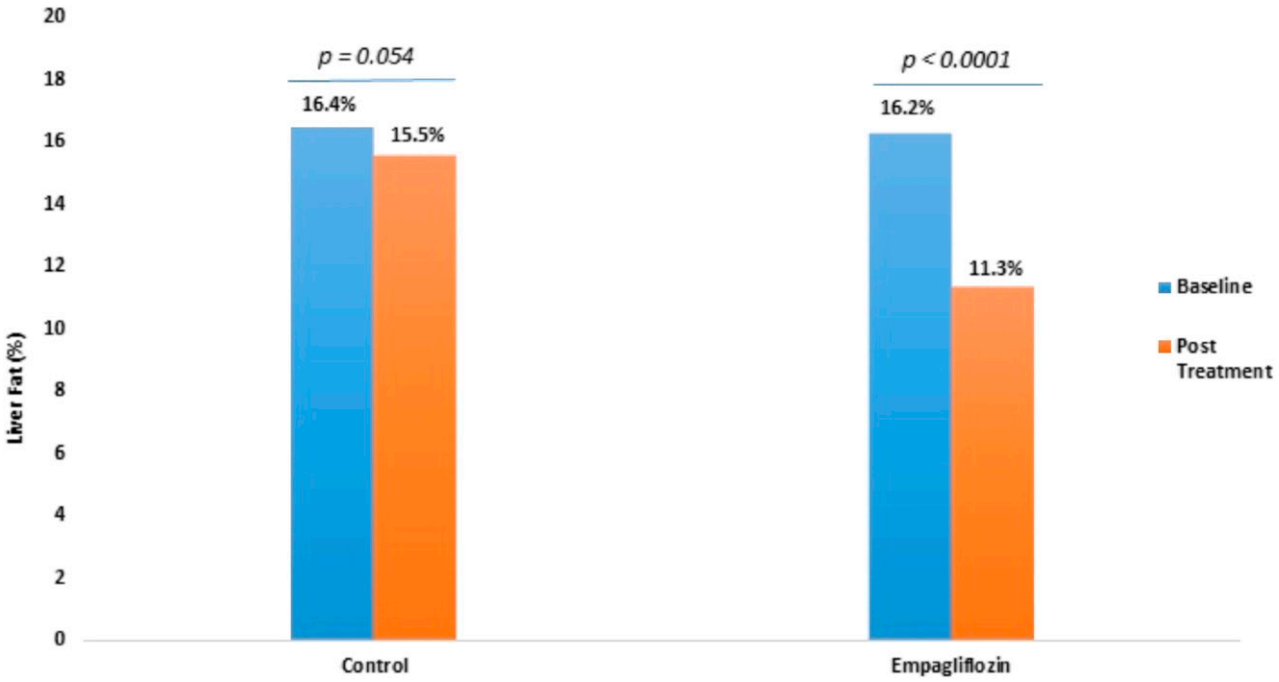

Figure 2—Baseline and posttreatment changes in liver fat in the empagliflozin and control groups as assessed by MRI-PDFF. Change in liver fat relative to baseline as assessed by MRI-PDFF. A significant difference was found in change in liver fat between the study groups ($P < 0.0001$).

liver index for the assessment of liver fat, which is a calculation-based parameter that uses BMI, waist circumference (in centimeters), GGT (in IU/L), and triglycerides (in milligrams per deciliter). They also found that reduction in liver fat was positively correlated with improvement in glycemic parameters, such as FPG and HbA_{1c}. A recent study showed that luseogliflozin (an SGLT-2 inhibitor) significantly reduced liver fat in patients with type 2 diabetes and NAFLD, but the liver-to-spleen attenuation ratio was used to estimate liver fat, which is not an accurate method of liver fat quantification (30).

The current study used MRI-PDFF for liver fat estimation, which is a robust and accurate technique. We maintained glycemic equipoise between the two groups, meaning that the reduction in FPG and HbA_{1c} was similar in both groups. Therefore, the effect of glycemic reduction on liver fat improvement was nullified between the groups. The reduction in liver fat in the empagliflozin group was greater than the improvement (if any) resulting from the glycemic reduction. Moreover, we did not find a correlation between liver fat reduction and glycemic improvement (HbA_{1c} r = 0.271; P = 0.222) (Supplementary Fig. 3).

We also did not find a correlation between body weight reduction and liver fat reduction (r = 0.218; P = 0.329) (Supplementary Fig. 4). The aforementioned Japanese study demonstrated that reduction in fatty liver index after ipragliflozin treatment did not correlate with body weight reduction (r = 0.3978; P = 0.0741) (24). Another study in mice demonstrated that ipragliflozin improved hepatic steatosis irrespective of body weight reduction in obese mice with insulin resistance (21). In the current study, 7 of 22 patients (32%) in the empagliflozin group had no significant body weight reduction (weight loss $<$2.0 kg), but all patients had significant liver fat reduction (MRI-PDFF $>$3.0%), demonstrating that liver fat reduction after empagliflozin treatment is irrespective of body weight reduction.

The current study shows a statistically significant reduction in serum ALT levels (P = 0.005) and some nonsignificant reductions in serum GGT levels (P = 0.057). This finding was also demonstrated by the Japanese study, which found a statistically significant decrease in serum ALT levels (P = 0.0063) and some nonsignificant reduction in serum GGT levels (P = 0.0537) after ipragliflozin treatment (23). Another study showed that the use of ipragliflozin in patients with type 2 diabetes and NAFLD improved serum AST and GGT levels irrespective of change in body weight (21). We also did not find a correlation between changes in serum ALT levels and body weight (r = 0.028; P = 0.902) (Supplementary Fig. 5). A clinical study reported that canagliflozin (an SGLT-2 inhibitor) improved serum ALT and GGT levels in patients with type 2 diabetes (31); therefore, the current study is in agreement with other studies that have demonstrated improvement in serum ALT and GGT levels after SGLT-2 inhibitor therapy. However, serum liver enzymes are only surrogate indices and do not predict liver histological responses. Raised liver enzymes do not correlate with liver histological grades of NAFLD, and reduction and/or normalization of liver enzymes after any intervention does not predict improvement in liver histology (32–34).

In a preclinical study in a mouse model, ipragliflozin treatment accelerated β-oxidation and export of VLDL by upregulation of expression of peroxisome proliferator–activated receptor-α (*PPARα*), carnitine palmitoyltransferase 1A (*CPT1 A*), and microsomal triglyceride transfer protein (*MTTP*) genes. These genes are negatively regulated by systemic inflammation (e.g., insulin resistance) (20). Liver fibrosis is a marker for the progression of liver disease. In the preclinical study, ipragliflozin treatment decreased areas of sirius red and α-smooth muscle actin staining and lowered mRNA levels of collagen 1α1 and α-smooth muscle actin. Therefore, the preclinical study suggested that ipragliflozin treatment affects the pathogenesis of NAFLD, at least in rodent models (20). A case report demonstrated that after ipragliflozin treatment for type 2 diabetes, there was a significant histological improvement (steatosis, inflammation, and ballooning but not fibrosis) in a female patient with NASH (35). Recently, another study showed histological improvement (defined as a decrease in an NAFLD activity score of $\geq$1 point without a worsening of fibrosis stage) in five patients with NASH and type 2 diabetes after canagliflozin therapy for 24 weeks (36). These reports are encouraging. The current study needs a longitudinal histopathological counterpart to prove the efficacy of empagliflozin in improving NAFLD.

Although absolute liver fat reduction by 4.1% (as measured by MRI-PDFF) has been demonstrated to improve histological steatosis by grade 1 and similarly to improve ballooning degeneration in patients with NAFLD (37), it is hepatic fibrosis that dictates the natural course of both hepatic and probably extrahepatic consequences of NAFLD (38,39). The current study demonstrates that empagliflozin treatment reduces liver fat, but whether this reduction has clinical relevance needs further study.

This study population involved other medications for diabetes and/or concurrent ailments (Supplementary Table 2). Among various other medications used by our study population, metformin has some favorable effect on liver fat reduction (11). All patients in both groups (100% in each group) were taking metformin. Therefore, the effect of empagliflozin on liver fat is over and above the improvements with metformin. Seventy percent (14 of 20) of patients in the control group and 77.3% (17 of 22) in the empagliflozin group were taking DPP-4 inhibitors, which have been shown to have a minimal effect on liver fat in patients with prediabetes or mild type 2 diabetes (26). Furthermore, an additional 7.3% of patients taking DPP-4 inhibitors in the empagliflozin group might not have influenced the results in a significant way. Twenty percent (4 of 20) of patients in the control group and 9% (2 of 22) in the empagliflozin group were also on levothyroxine therapy. They had stable thyrotropin levels in the normal reference range for $\geq$6 months before recruitment. Furthermore, levothyroxine therapy in patients with subclinical hypothyroidism has been shown to improve liver fat. More patients in the control group (11%) were on levothyroxine therapy. Even if levothyroxine therapy had influenced the results by reducing liver fat, we would have seen it in the control group, and if so, the results would be in favor of empagliflozin.

The strength of this study lies in its use of a randomized controlled trial design

to evaluate the efficacy of empagliflozin for reducing liver fat. It was conducted in a real-world scenario where patients were receiving standard treatment for type 2 diabetes and other comorbidities. A second strength of this study is the use of MRI-PDFF, a precise and accurate imaging biomarker, for liver fat quantification.

We also acknowledge some limitations. First, we did not use placebo in the control group because this study was conducted in a real-world scenario and we were already providing standard of care for type 2 diabetes and other comorbidities. Second, MRI-PDFF only provides information about changes in liver fat and not about inflammation, hepatocyte ballooning degeneration, and fibrosis. Finally, the study population was using other medications for type 2 diabetes and comorbidities. Although, patients taking medications that have known effects on liver fat were excluded, minor interactions of coprescribed medications on liver fat cannot be ruled out.

In conclusion, this randomized clinical trial showed that empagliflozin included in the standard treatment for type 2 diabetes significantly reduces liver fat and improves serum ALT levels. This study used MRI-PDFF for liver fat mapping, which is a validated and robust technique. The study suggests that SGLT-2 inhibitors are useful agents for improving NAFLD, which often coexists with type 2 diabetes. Histopathological studies are needed to see whether liver fat reduction after empagliflozin treatment leads to improvement in steatohepatitis and/or steatofibrosis. MRI-PDFF was successfully used to assess liver fat change over time, and future studies in patients with type 2 diabetes and NAFLD could use this noninvasive biomarker to assess liver fat content and its response to treatment in larger clinical trials.

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