The effects of ipragliflozin on the liver-to-spleen attenuation ratio as assessed by computed tomography and on alanine transaminase levels in Japanese patients with type 2 diabetes mellitus

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Abstract We assessed the effects of a 12-week ipragliflozin treatment on the liver-to-spleen attenuation ratio (*L/S* ratio) using computed tomography and on alanine transaminase (ALT) levels in Japanese patients with type 2 diabetes mellitus (T2DM). Sixty-two patients with T2DM [age, 56 ± 8 years; hemoglobin A1c (HbA1c) levels, $8.1 \pm 0.9\%$; body mass index (BMI), 27.5 ± 3.3 kg/m²] were randomly assigned in a 2:1 ratio to receive ipragliflozin (50 mg/day; ipragliflozin group; $n = 40$) or continued treatment (control group; $n = 22$) for 12 weeks. The primary endpoints were changes in ALT levels; the secondary endpoints included changes in the *L/S* ratio and in the visceral fat area (VFA) and subcutaneous fat area (SFA) before and after 12 weeks of the treatment as assessed by computed tomography. ALT levels (-12.45 vs. $+5.82$ IU/l, $P < 0.001$), *L/S* ratio ($+0.07$ vs. -0.08 , $P < 0.001$), SFA (-5.8 vs. $+13.3$ cm², $P < 0.05$), and VFA ($+1.4$ vs. $+20.4$ cm², $P < 0.05$) significantly changed from baseline in the ipragliflozin group compared with the values in the control group. Multiple regression analysis among all subjects revealed that the independent factor contributing to the $\% \Delta$ ALT and $\% \Delta L/C$ ratio was treatment group alone (ipragliflozin group = 1; control group = 0; β coefficient = -32.08 , $P < 0.001$ and β coefficient = 19.98 , $P < 0.05$, respectively). Thus,

ipragliflozin may lower ALT levels associated with increased *L/S* ratios, indicating its potential therapeutic efficacy in T2DM-associated hepatic steatosis.

Keywords Alanine transaminase · *L/S* ratio on computed tomography · Ipragliflozin · Type 2 diabetes mellitus

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the excessive lipid accumulation in the liver associated with obesity and insulin resistance and is the most common chronic liver disease worldwide [1]. NAFLD includes a spectrum of conditions ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), a severe form of NAFLD histologically characterized by steatosis with lobular inflammation and fibrosis. Approximately 10–20% of patients with NAFLD develop NASH, and it can progress to cirrhosis and hepatocellular carcinoma. In patients with type 2 diabetes mellitus (T2DM), the prevalence of NAFLD is as high as 75%, and they are prone to develop NASH [2]. However, no established pharmacological treatments are currently used for NAFLD associated with T2DM, and medical interventions have focused on diet control and exercise [3]. Therefore, there is a need for effective pharmacological therapies.

In a meta-analysis of patients with NASH, treatment with thiazolidinedione improved the liver histology relative to placebo [4, 5]. Recently, 36 months of pioglitazone treatment was reported to be safe and effective in patients with prediabetes or T2DM and NASH and was associated with improvements in fibrosis scores [6]. However, concerns about potential side effects, including significant weight gain [6], as well as the long-term safety profile of

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this drug class still limit its clinical use [7]. On the other hand, recently, Armstrong et al. reported that the glucagon-like protein-1 (GLP-1) receptor agonist liraglutide showed therapeutic efficacy in histological resolution of NASH in patients with T2DM in a double-blinded, randomized, placebo-controlled phase-2 trial using a very limited number of patients ($n = 26$) [8].

Sodium glucose co-transporter 2 (SGLT2) inhibitors are unique in terms of their mechanism of action. These drugs increase urinary glucose excretion, thereby lowering the blood glucose concentration and body weight [9]. Because of their role in improving obesity, these drugs could be useful for the treatment of NAFLD. A number of previous studies using rodent models demonstrated that several of these SGLT2 inhibitors ameliorated fatty liver with significant body weight loss [10, 11]. More recently, Komiya et al. reported that the SGLT2 inhibitor ipragliflozin improved hepatic steatosis in high-fat diet-induced and leptin-deficient (ob/ob) obese mice irrespective of body weight reduction [12]. Yet, few data exist on the clinical efficacy of ipragliflozin in humans with NAFLD and T2DM.

Here, we conducted a preliminary open-label, randomized, parallel-group prospective comparative study to evaluate the efficacy of 50 mg ipragliflozin once daily for the reduction of liver enzymes in comparison with a control group. In addition, this study assessed the effect of ipragliflozin on L/S ratios using computed tomography, a method that can determine the level of hepatic steatosis and the association with the change in visceral and subcutaneous fat distribution.

Subjects and materials and methods

Ethics statement

This study was conducted in accordance with Good Clinical Practice, the International Conference on Harmonization guidelines, and applicable laws and regulations. The study protocol was approved by the ethics committee of Fukui-ken Saiseikai Hospital. After receiving a full explanation of the study, all patients provided written informed consent before enrollment.

Study population

Eligible patients were aged 20–65 years, had been diagnosed with T2DM for at least 12 weeks prior to study enrollment, had baseline body mass indices (BMIs) of 24.0–40.0 kg/m², and had HbA1c levels of 7.0–10.0% (7.5–10.0% when the patient had taken sulfonylureas or glinides regularly). Patients were instructed to continue with their recommended diets and exercise habits during

the entire study period. We excluded patients using insulin, with type 1 diabetes, fasting triglyceride levels ≥ 4.5 mmol/l (400 mg/dl), an estimated glomerular filtration rate (eGFR) of < 60 ml/min/1.73 m², positivity for hepatitis B virus, hepatitis C virus or autoimmune antibodies and whose alcohol intake was > 20 g/day, and past history of cardiac events (e.g., angina pectoris, nonfatal myocardial infarction, and coronary revascularization as adjudicated hospitalized cardiovascular events). Additionally, subjects were excluded with dysuria caused by a neurogenic bladder or benign prostatic hypertrophy, recurrent urinary tract infections (UTIs) or a UTI at screening, difficulty reaching sufficient water intake due to an attenuated sense of thirst, chronic disease requiring continuous use of steroids or immunosuppressants, those who were pregnant or breast-feeding, and those who were considered unlikely to comply with the study requirements.

Finally, 62 eligible patients were randomly assigned in a 2:1 ratio using the EDC system to receive either 50 mg ipragliflozin once daily (ipragliflozin group, $n = 40$) or continued treatment (control group, $n = 22$) for 12 weeks. Of these subjects, abdominal computed tomography scans were acquired for 57 (ipragliflozin group, $n = 37$; control group, $n = 20$) before and 3 months after starting ipragliflozin to evaluate visceral fat accumulation [visceral fat area (VFA), cm²], subcutaneous fat accumulation [subcutaneous fat area (SFA), cm²], and the liver-to-spleen attenuation ratio (L/S ratio), which has previously been shown to be a valid tool for measuring the presence and severity of hepatic steatosis [13, 14].

The primary endpoint was a change in alanine transaminase (ALT) levels between the two treatment groups. The secondary endpoints included changes in the L/S ratio and VFA and SFA as assessed by computed tomography before and after 12 weeks of treatment. The tertiary endpoints were changes in HbA1c, glycated albumin levels, and body weight from baseline between the groups. To assess the safety profile, the incidence and details of adverse events and laboratory abnormalities were investigated.

This was a sub-study of the main study protocol that was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR; Japan), no. UMIN000014422 [15]. For purposes of this analysis, serum ALT was used as a surrogate marker to estimate the proportion of the participants of this study with liver injury. The new ALT cutoffs as recommended by Prati et al. (> 30 IU/l in males, > 19 IU/l females) were used to define abnormality [16]. We used the FIB4 index, calculated as $[\text{age (year)} \times \text{AST (IU/l)}] / [\text{PLT (10}^9/\text{l)} \times \sqrt{\text{ALT (IU/l)}}]$, as a parameter of fibrosis progression in patients with NAFLD. A FIB4 index ≥ 2.67 had an 80% positive predictive value and an FIB4 index ≤ 1.30 had a 90% negative predictive value for identifying advanced fibrosis

equivalent to bridging fibrosis or cirrhosis [17]. Compliance with treatment was assessed at 4, 8, and 12 weeks by interview. In principal, any change in the dosage regimen of concomitant anti-diabetes drugs was prohibited during the study. Laboratory tests (including biochemistry tests, hematology tests, urinalysis, and other parameters) were performed after an overnight fast at randomization and 12 weeks after randomization. All blood tests were performed using standard methods.

The principal investigator diagnosed T2DM in accordance with the standards of the Japan Diabetes Society. The BMI was calculated as weight (kg) divided by height squared (m^2). The eGFR was calculated using the formula reported by Matsuo et al. [18]. The presence of diabetic retinopathy was evaluated by fundus examination performed by an ophthalmologist. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg or current use of antihypertensive agents. Dyslipidemia was defined as total cholesterol ≥ 220 mg/dl, and/or high-density lipoprotein cholesterol < 40 mg/dl, and/or triglyceride ≥ 150 mg/dl after an overnight fast or current use of antihyperlipidemic agents.

Evaluation of liver fat deposition on computed tomography scan images

Abdominal computed tomography scans using a Aquilion64 Global Standard Version (Toshiba Medical System Co., Ltd., Tochigi, Japan) were acquired in the supine position in the morning after a 12-h overnight fast. A single 10-mm slice image obtained prior to the intravenous administration of contrast medium through the right lobe of the liver was used for the analysis. A 100-mm² region of interest (ROI) was drawn on two areas of this section, and the Hounsfield units (HU) of each ROI were recorded. Care was taken not to include any vessels in the ROIs. The sections were viewed on liver window settings. The mean liver attenuation was calculated from the two liver ROIs. The attenuation of the spleen was measured in the same manner. The CT attenuation characteristics of the liver were recorded as: (1) the mean liver attenuation and (2) the L/S attenuation ratio, where L is the mean hepatic attenuation (HU) and S is the mean splenic attenuation (HU). The presence of hepatic steatosis was defined as an L/S ratio < 1.0 [13] with increases in the ratio indicating reductions in steatosis [14].

Measurement of VFA and SFA on computed tomography scan images

Visceral and subcutaneous fat volumes were evaluated by measuring the VFA and SFA determined in a CT scan

image using commercially available software (ZIOS-TATION 2 CT Body Fat Measurement; ZIOSOFT, Co., Ltd., Tokyo, Japan). A single slice image of a cross-sectional CT scan (Aquilion64 Global Standard Version; Toshiba Medical System Co., Ltd., Tochigi, Japan) was acquired at the level of the umbilicus (from L4 to L5). The software automatically defined an ROI by tracking its contour on each scan, and the attenuation range of the CT values (in HUs) for fat tissue was calculated. A histogram for fat tissue was automatically computed by the software on the basis of the mean attenuation ± 1 standard deviation (SD). Tissue with attenuation values within the mean ± 1 SD was considered to be fat tissue within that ROI on the basis of a previously reported concept [19]. Trained radiologists made manual adjustments if needed, choosing a midway point between adipose and non-adipose tissue peaks if the peaks had considerable overlap and misclassification could occur [20]. The software automatically divided the total fat area into SFA and VFA, and radiologists also made manual adjustments if needed. To test the variability of SFA and VFA, the inter- and intra-observer coefficients for all 57 subjects were calculated. The inter-observer intra-class correlation coefficient for SFA and VFA was 0.976 and 0.955 [coefficient variation (CV), 3.9 and 9.2%], respectively. To assess the intra-observer variability, the same observer repeated the adjustment of the SFA and VFA measurements on two different occasions. The intra-observer intra-class correlation coefficient for the SFA and VFA was 0.989 and 0.980 (CV, 2.5 and 5.8%). The ratio of VFA to SFA was designated as the V/S ratio.

Statistical analysis

The data are expressed as the mean $\pm$ SD unless otherwise noted. Comparisons of discrete variable data were analyzed using the chi-square test or Fisher's direct test, as appropriate. Differences between two variables were analyzed for statistical significance using a two-tailed Student's paired or unpaired t test, as appropriate. Changes in variables were analyzed by a two-way analysis of variance with repeated measures, e.g., changes in ALT levels or in the L/S ratio. As the relationships between two variables were nonlinear, the correlations between sets of two independent continuous variables were investigated using Spearman's rank correlation coefficient method. Multiple linear regression analysis was conducted to determine the independent predictors of the percent change in ALT levels ($\%\Delta\text{ALT}$) and the percent change in the L/S ratio ($\%\Delta L/S$ ratio). All variables at baseline considered clinically relevant parameters for a patient's background were employed as

independent variables in multivariate analysis [i.e., sex, age, treatment group, BMI, eGFR, HbA1c, ALT levels, and homeostasis model assessment of insulin resistance (HOMA-IR)]. The HbA1c and ALT levels at baseline were calculated as averaged HbA1c and ALT levels measured within 12 weeks before starting the ipragliflozin treatment.

The $\% \Delta \text{ALT}$ and $\% \Delta \text{L/S}$ ratios were both calculated as follows: [the respective levels at 12 weeks after the treatment—the respective baseline levels]/the respective baseline levels $\times 100$). The HOMA-IR was calculated using the following formula: $\text{HOMA-IR} = \text{fasting blood glucose (mg/dl)} \times \text{insulin (U/ml)} / 405$. For all tests, $P < 0.05$ was considered statistically significant. All of the statistical analyses were performed using the JMP version 5.1 software (SAS Institute, Inc., Cary, NC, USA).

Results

Subject characteristics at baseline

The mean $\pm$ SD values for age, BMI, HbA1c, and glycated albumin levels at baseline for all study subjects ($n = 62$) were 55.6 ± 7.7 years, $27.6 \pm 3.3 \text{ kg/m}^2$, $8.1 \pm 1.0\%$, and $19.5 \pm 3.2\%$, respectively.

The relative percentage of users of concomitant anti-hyperglycemic agents (sulfonylureas, metformin, and DPP-4 inhibitors) and antihyperlipidemic agents (statins, fibrates, and ezetimibe) among all study subjects at baseline was 56, 82, and 74% and 79, 7, and 5%, respectively.

The subjects' baseline clinical characteristics (treatment group, $n = 40$ and control group, $n = 22$) are presented in Table 1. The two groups were well matched for gender,

Table 1 Baseline clinical characteristics of study subjects with type 2 diabetes mellitus between the ipragliflozin and control groups

Characteristic	Ipragliflozin group	Control group	<i>P</i> value
<i>n</i>	40	22	
Gender (male/female)	26/14	14/8	ns
Age (years)	54.8 ± 9.3	55.4 ± 7.5	ns
BMI (kg/m^2)	27.8 ± 3.9	27.3 ± 3.1	ns
Diabetes duration (years)	9.7 ± 4.6	9.5 ± 4.4	ns
HbA1c (%)	8.1 ± 1.0	8.2 ± 1.1	ns
Glycated albumin (%)	19.4 ± 3.4	19.8 ± 2.8	ns
Concomitant antihyperglycemic agents SU/Met/DPP-4i (%)	55/83/80	59/82/73	ns
Concomitant antihyperlipidemic agents (statin/fibrates/ezetimibe) (%)	80/8/5	77/5/5	ns
Parameters associated with liver function			
AST (U/l)	35 ± 19	30 ± 17	ns
ALT (U/l)	49 ± 36	41 ± 35	ns
ALP (U/l)	237 ± 60	262 ± 82	ns
LDH (U/l)	188 ± 35	176 ± 30	ns
FIB4 index	1.35 ± 0.5	1.20 ± 0.6	ns
Parameters associated with lipids			
Total cholesterol (mg/dl)	169 ± 38	171 ± 33	ns
LDL cholesterol (mg/dl)	95 ± 31	92 ± 26	ns
Triglycerides (mg/dl)	133 ± 72	154 ± 71	ns
HDL cholesterol (mg/dl)	48 ± 9	45 ± 10	ns
Non-HDL cholesterol (mg/dl)	122 ± 34	126 ± 31	ns
eGFR (ml/min/1.73 m)	79.5 ± 14.9	76.1 ± 13.6	ns
Complications			
Retinopathy (<i>n</i>)	5 (13%)	1 (5%)	ns
Hypertension (<i>n</i>)	21 (53%)	13 (59%)	ns
Dyslipidemia (<i>n</i>)	33 (83%)	18 (82%)	ns

FIB4 index was calculated as follows: $[\text{age (years)} \times \text{AST (IU/l)}] / [\text{PLT} (10^9/\text{l}) \times \sqrt{\text{ALT (IU/l)}}]$. All values are mean $\pm$ standard deviations or numbers of subjects with percentages in parentheses. *P* values between two groups of subjects were obtained using the unpaired *t* test, chi-square test, or Fisher's direct test, as appropriate

BMI body mass index, *HbA1c* hemoglobin A1c, *Met* metformin, *DPP-4i* DPP-4 inhibitors, *AST* aspartate transaminase, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *LDH* lactate dehydrogenase, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *eGFR* estimated glomerular filtration rate, *ns* not significant

age, BMI, eGFR, baseline liver enzyme levels [including levels of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH)], and FIB4-index. Only one patient from each group showed an FIB4 index ≥ 2.67 . Furthermore, the two groups were well matched for diabetes characteristics, including disease duration, baseline HbA1c, lipid parameters, oral antihyperglycemic therapy, and disease complications. Diabetic retinopathy, hypertension, and dyslipidemia between the treatment and control groups were observed in 5 (13%) vs. 1 (5%), 5 (13%) vs. 1 (5%), and 5 (13%) vs. 1 (5%) subjects, respectively, all of which were not significantly different between the two groups.

All patients completed the study protocol without any withdrawal due to treatment-related serious adverse events. The mean compliance was $\geq 95\%$ during the study period. No clinically significant elevations in ALT levels (>3 times the upper limit of the normal physiological levels on two consecutive occasions) or creatine kinase levels (>10 times the upper limit of the normal physiological levels) were observed during the study period.

Changes in ALT levels

Among all patients, 70.1% (44/62) had elevated baseline ALT levels, with mean values of 56.5 ± 40.4 IU/l in male patients and 28.9 ± 14.0 IU/l in female patients. The 12-week treatment with ipragliflozin (50 mg) significantly reduced ALT levels compared to those in the control group (-12.45 vs. $+5.82$ IU/l; $P < 0.001$, Table 1). In particular, among patients with elevated baseline ALT readings (>30 IU/l in male patients, >19 IU/l in female patients), the reduction of ALT levels in the ipragliflozin group was more pronounced compared to that in the control group (-16.28 vs. $+8.71$ IU/l; $P < 0.001$), while no significant differences in the reduction of ALT levels were observed among patients with normal baseline ALT readings in either of the two groups (-2.36 vs. $+0.75$ IU/l; $P = 0.07$, Fig. 1). In the ipragliflozin group, the $\% \Delta$ ALT was significantly correlated with baseline ALT levels ($r = -0.424$, $P < 0.01$).

Changes in the L/S ratio, SFA, and VFA

In this trial, the presence of hepatic steatosis defined as an L/S ratio < 1.0 was confirmed on CT imaging scans acquired at baseline in 94.7% (54/57) of the subjects. Compared with the control group, the ipragliflozin group exhibited a statistically significant change from baseline in the L/S ratio ($+0.07$ vs. -0.08 , $P < 0.001$), SFA (-5.8 vs. $+13.3$ cm², $P < 0.05$), and VFA ($+1.4$ vs. $+20.4$ cm², $P < 0.05$) (Table 2).

Spearman's rank correlation analysis showed that in the ipragliflozin group, $\% \Delta$ ALT and $\% \Delta$ VFA were both negatively correlated with the $\% \Delta$ L/S ratio ($r = -0.554$, $P < 0.001$

Fig. 1 Changes in the ALT levels in NAFLD patients with T2DM enrolled in the ipragliflozin treatment group (50 mg/day, 12-week treatment) vs. those enrolled in the control group. The ALT levels at baseline of the total study population (left), of patients with elevated ALT levels (center), and of patients with normal ALT levels (right)

and $r = -0.563$, $P < 0.001$, respectively), whilst among the control group, no significant relationship was found between $\% \Delta$ ALT or $\% \Delta$ VFA and the $\% \Delta$ L/S ratio ($r = -0.028$, $P = 0.901$ and $r = -0.215$, $P = 0.363$, respectively).

Additionally, Spearman's rank correlation analysis showed that among the two treatment groups, $\% \Delta$ SFA and the $\% \Delta$ V/S ratio were not significantly correlated with the $\% \Delta$ L/S ratio ($r = -0.180$, $P = 0.33$ and $r = -0.234$, $P = 0.10$, respectively in the ipragliflozin group; $r = -0.003$, $P = 0.93$ and $r = -0.124$, $P = 0.38$, respectively in the control group). The plots of the distribution between the $\% \Delta$ L/S ratio and $\% \Delta$ ALT or $\% \Delta$ VFA in the two treatment groups are shown in Fig. 2.

Multiple regression analysis of all the subjects' data revealed that the independent factor contributing to $\% \Delta$ ALT and the $\% \Delta$ L/C ratio was the treatment group (ipragliflozin group = 1, control group = 0) alone (β coefficient = -32.08 , $P < 0.001$ and β coefficient = 19.98 , $P < 0.05$, respectively, Table 3).

Changes in hemoglobin A1c, glycated albumin levels, and body weight

The ipragliflozin group exhibited a statistically significant decrease in hemoglobin A1c (HbA1c) and glycated albumin levels (-0.61 ± 0.52 vs. $0.52 \pm 0.74\%$, $P < 0.0001$ and

Fig. 2 Distribution plots of the %ΔL/S ratio and %ΔALT (upper panels) and %ΔVFA (lower panels) in the ipragliflozin (left panels) and control groups (right panels). ALT alanine transaminase, L/S ratio

-2.92 ± 2.48 vs. $+0.89 \pm 2.45\%$, $P < 0.0001$, respectively) 12 weeks after starting 50 mg ipragliflozin once daily compared with that in the control group. Similarly, the ipragliflozin group exhibited a statistically significant decrease in body weight compared with that in the control group (-1.51 ± 1.28 vs. $+0.45 \pm 0.77$ kg, $P < 0.0001$). Changes in various clinical parameters, except the secondary endpoints, between the two treatment groups are shown in Table S1 in Supplementary Appendix 1.

Safety profile

No gastrointestinal and hepatobiliary serious adverse events were observed in either treatment group during the 12-week observation period.

Discussion

In obesity and T2DM, excess energy accumulates as triglycerides not only in adipose tissues but also ectopically in non-adipose tissues, especially in the liver, where it

the liver-to-spleen attenuation ratio; %ΔL/S ratio the percentage change in the L/S ratio, %ΔALT the percentage change in ALT levels, %ΔVFA the percentage change in the visceral fat area

causes hepatic steatosis, which is strongly associated with whole-body and tissue-specific insulin resistance and inflammatory processes [21]. On the contrary, during SGLT2 inhibitor treatment, alterations in the hepatic metabolism in the liver are anticipated in response to urinary glucose excretion. Several recent studies demonstrated that the SGLT2 inhibitor ipragliflozin [12, 22–25] and other agents of this drug class [10, 26] improved hepatic steatosis and areas of fibrosis in rodent models of NAFLD [12, 22, 23, 26] and NASH [10, 24, 25] with [10, 22, 23, 25] and without [12, 24, 26] diabetes. Further, Leiter et al. recently reported that canagliflozin caused improvements in liver function tests compared to either placebo or sitagliptin treatment in a study comprising 3801 individuals with T2DM in a pooled analysis [27]. However, little is known about the efficacy of SGLT2 inhibitors in NAFLD itself in humans with T2DM.

In the present clinical study, the patients treated with 50 mg ipragliflozin once daily exhibited a statistically significant reduction in ALT levels, SFA, and VSA and a significant increase in the L/S ratio from baseline compared to those in the control group. In particular, among the

Table 2 Change in parameters for primary and secondary endpoints

	Ipragliflozin group	Control group	<i>P</i> value
<i>n</i>	37	20	
ALT(IU/l)			
Baseline	49 ± 36	41 ± 35	
12 weeks	37 ± 25	47 ± 42	<0.001
% change	−18 ± 25	13 ± 35	<0.001
<i>L/S</i> ratio			
Baseline	0.92 ± 0.26	1.04 ± 0.28	
12 weeks	0.98 ± 0.25	0.97 ± 0.26	<0.001
% change	8.7 ± 17.9	−2.9 ± 25.3	<0.05
SFA (cm ²)			
Baseline	186 ± 83	181 ± 89	
12 weeks	180 ± 82	194 ± 95	<0.05
% change	−2.0 ± 20.2	9.8 ± 23.9	0.06
VFA (cm ²)			
Baseline	158 ± 59	126 ± 54	
12 weeks	159 ± 61	143 ± 60	<0.05
% change	1.4 ± 18.9	20.4 ± 39.4	<0.05

All values are means ± standard deviation. The *P* values for the comparison before and after treatment within each group were obtained using paired *t* tests. The *P* values between two treatment groups were obtained using two-way repeated-measures ANOVA with post hoc Bonferroni tests

ALT alanine aminotransferase, *L/S ratio* liver-to-spleen attenuation ratio, SFA subcutaneous fat area, VFA visceral fat area

patients with elevated baseline ALT readings, the ipragliflozin group had a more pronounced decrease in ALT levels compared to the control group, and %ΔALT showed a significant negative correlation with baseline ALT levels in the ipragliflozin group. Additionally, in the ipragliflozin group, a significant negative relationship was observed between the basal *L/S* ratio and amount of change in the *L/S* ratio after treatment ($r = -0.442$, $P < 0.01$), suggesting that the efficacy of ipragliflozin for hepatic steatosis may become more evident with disease severity. Multiple regression analysis among all subjects revealed that the independent factor contributing to the %ΔALT and %Δ*L/C* ratio was treatment group alone. Thus, ipragliflozin might have prevented hepatic steatosis under the conditions of the present study, the underlying mechanism of which warrants elucidation.

Komiya et al. [12] recently reported that ipragliflozin potentially prevents obesity-related hepatic steatosis through both decreased de novo lipogenesis and increase in fatty acid oxidation in high-fat diet-induced and leptin-deficient (ob/ob) obese mice irrespective of body weight reduction. Lipid accumulation in the liver occurs because of an imbalance between hepatic triglyceride synthesis and fatty acid oxidation. Both insulin and glucose stimulate the gene expression of Srebp1c, a major transcription factor that positively regulates de novo lipogenic enzymes in the liver [28–30]. De novo lipogenesis is involved in inducing

Table 3 Independent predictors of the %ΔALT and %Δ*L/C* ratio among all study subjects using multiple linear regression analysis

Variables	β coefficient	SE	Value of <i>t</i>	Value of <i>P</i>
(A)				
Intercept	48.88	65.3	0.748	0.458
Treatment group	−32.08	8.04	−3.990	0.0002
Multiple <i>R</i> -squared (<i>r</i> ²)	0.311			
(B)				
Intercept	−16.88	32.07	−0.526	0.601
Treatment group	19.98	6.99	1.427	0.036
Multiple <i>R</i> -squared (<i>r</i> ²)	0.187			

(A) Dependent variable: %ΔALT, percent change in ALT levels between baseline and week 12 after the treatment

(B) Dependent variable: %Δ*L/C* ratio, percent change in the *L/C* ratio between baseline and week 12 after the treatment

Independent variables were sex (female = 0, male = 1), age, treatment group (the ipragliflozin group = 1, the control group = 0), BMI, eGFR, HbA1c at baseline, ALT levels at baseline, and HOMA-IR at baseline. The HbA1c and ALT levels at baseline were calculated as the averaged HbA1c and ALT levels measured within 12 weeks before starting the ipragliflozin treatment, respectively. Sex, age, BMI, eGFR, HbA1c at baseline, the ALT levels at baseline, and the HOMA-IR at baseline were not retained in the model because they were not significant predictors

All values are mean ± SD. Solid and white bars indicate the ipragliflozin group and control group, respectively. *P* values comparing the two groups were obtained using an unpaired Student's *t*-test. Abbreviations: ALT, alanine aminotransferase; T2DM, type 2 diabetes mellitus; NAFLD, non-alcoholic fatty liver disease

BMI body mass index, HbA1c glycated hemoglobin, HOMA-IR homeostasis model assessment of insulin resistance, ALT alanine transaminase, eGFR estimated glomerular filtration rate

hepatic steatosis as demonstrated in a previous study showing that SREBP-1 ablation protected ob/ob mice from hepatic steatosis irrespective of adiposity and systemic glucose metabolism [31]. In the present study, ipragliflozin attenuated hyperinsulinemia (11.6 ± 9.7 vs. 8.2 ± 5.1 $\mu\text{U/ml}$, $P < 0.03$, Table S1), possibly via improving chronic hyperglycemia (HbA1c 8.1 ± 1.0 vs. $7.4 \pm 0.8\%$, $P < 0.0001$) in an insulin-independent manner, which resulted in a significant reduction of HOMA-IR (4.7 ± 4.9 vs. 2.6 ± 1.8 , $P < 0.02$) in the ipragliflozin group compared with that in the control group (Table S1). This positive effect in the ipragliflozin group might have been related to reduced de novo hepatic lipogenesis.

Further, Suzuki et al. [11] recently reported that administration of tofogliflozin for 9 weeks in diet-induced obese rats decreased the respiratory quotient and plasma triglyceride level and increased the plasma total ketone body level, which indicated that tofogliflozin induced a metabolic shift from carbohydrate oxidation to fatty acid oxidation, potentially preventing fat accumulation through an increase in fatty acid oxidation in adipose tissue and the liver. In accordance with this finding, in the present study, a significant elevation of fasting serum 3-hydroxybutyrate levels was observed after the ipragliflozin treatment (68 ± 88 vs. 111 ± 120 $\mu\text{mol/l}$, $P < 0.001$) as shown in Table S1, suggesting that ipragliflozin enhanced fatty acid oxidation in the liver [11]; however, we do not have data to confirm this hypothesis.

Another possible mechanism involves glucagon [10]. Recently, it was reported that SGLT2 is expressed in glucagon-secreting pancreatic islet α cells and that SGLT2 inhibitors might thus promote glucagon secretion [32]. Therefore, an increased serum glucagon concentration may underlie the liver-related reduction of lipid accumulation. However, our data showed that there was no significant change in the percentage elevation in serum glucagon levels between the ipragliflozin and control groups (2.5 ± 14.6 vs. $2.1 \pm 14.2\%$, respectively, Table S1). Taken together, these data indicate that ipragliflozin treatment may attenuate hepatic steatosis in subjects with T2DM along with decreased de novo lipogenesis by improving insulin resistance and increasing fatty acid oxidation through a metabolic shift from carbohydrate to fatty acid oxidation.

We further examined the relationship between the percentage change in the $\Delta L/S$ ratio and SFA or VFA in the two treatment groups. Although Spearman's rank correlation analysis showed that the $\%\Delta\text{SFA}$ and $\%\Delta\text{VFA}$ ratio were not significantly correlated with the $\%\Delta L/S$ ratio in the two treatment groups, correlation analysis in the ipragliflozin group showed that $\%\Delta\text{VFA}$ was negatively correlated with the $\%\Delta L/S$ ratio ($r = -0.563$, $P < 0.001$); in the control group, the correlation analysis showed no

significant relationship between the two parameters ($r = -0.215$, $P = 0.363$).

Using dual-energy X-ray absorptiometry (DEXA), it was found that compared to placebo, SGLT2 inhibitors reduced total body weight, predominantly by reducing the total-body fat mass [33, 34], visceral adipose tissue, and subcutaneous adipose tissue volume in T2DM inadequately controlled with metformin [33]. On the other hand, there are few data regarding the regional fat distribution in patients with T2DM after treatment with SGLT2 inhibitors. To the best of our knowledge, this is the first randomized control study to demonstrate a significant correlation between the percentage change in the L/S ratio and the VFA in NAFLD patients with T2DM after treatment with an SGLT2 inhibitor. Several epidemiologic studies have implicated visceral fat as a major risk factor for insulin resistance, T2DM, cardiovascular disease, stroke, metabolic syndrome, and death [35]. Studies have demonstrated that VFA, but not SFA, is independently correlated with the severity of hepatic steatosis in 177 healthy living liver donors [36] and in 129 subjects with NAFLD diagnosed by ultrasonography [37]. Using DEXA, Punthakee et al. found that the thiazolidinedione rosiglitazone reduced the hepatic and visceral fat and increased subcutaneous fat [38]. On the other hand, it has been reported that lifestyle intervention for 6–12 months without using any drugs significantly decreased the VFA and waist circumference along with a significant decrease in ALT levels [39] or the L/S ratio [40]. In this context, SGLT2 inhibitors induce calorie loss through urinary glucose excretion, which is similar to oral carbohydrate restriction along with lifestyle intervention for T2DM. Therefore, the finding of the present study demonstrating a significant correlation between the $\%\Delta L/S$ ratio and $\%\Delta\text{VFA}$, but not the $\%\Delta\text{SFA}$, in NAFLD patients with T2DM after treatment with ipragliflozin seems to be consistent with the mechanism of action of this drug.

The present study has several limitations. First, the number of enrolled subjects was relatively small. However, baseline characteristics, such as sex, age, diabetes characteristics, and lipid profiles, did not differ between the two treatment groups, and none of the 62 randomized patients withdrew from the study. Second, the relatively short study duration of 12 weeks may be a limitation. Therefore, we found the effect of 50 mg ipragliflozin on lowering ALT levels associated with an increase in the L/S ratio should be verified in a more long-term, randomized prospective study with a larger sample size. Third, fasting glucose, insulin, and average HOMA-IR levels were relatively high among all subjects, with a notable additional increase in the control group accompanied by a slight increase in body weight (Figure S1). This strongly indicated non-compliance with the proscribed diet. Further, this study was conducted primarily from October to January. In Japan, many individuals, likely including the subjects of this study, are prone to

excessive carbohydrate intake and reduced exercise during this period because of holidays such as New Year's, as well as the winter weather conditions. Therefore, there is a need to re-evaluate the effect of this drug under a strict diet regimen and exercise therapy.

Fourth, a lack of liver biopsies precluded the ability to accurately validate the severity of the underlying liver injury and, more importantly, to validate the accuracy of the ALT and *L/S* ratio as a serial marker of the presence and severity of hepatic steatosis in our cohort. Finally, all of the participants were Japanese; therefore, our findings cannot be generalized to other races or ethnic groups.

In conclusion, our preliminary study highlights that a 12-week treatment with ipragliflozin 50 mg/day significantly improves the liver enzyme profile and increases the *L/S* ratio assessed by computed tomography vs. placebo in NAFLD patients with T2DM. These effects of ipragliflozin appear to be partly mediated by the attenuation of hyperinsulinemia via an insulin-independent decrease in hyperglycemia and an increase in fatty acid oxidation. Our data support the rationale to prospectively investigate the effect of a long-term treatment with SGLT-2 inhibitors in NAFLD associated with T2DM.

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Compliance with ethical standards

Conflict of interest Yukihiro Bando has served on advisory boards for Astellas Pharma, Inc. Yukihiro Bando has received speaker honoraria from Astellas Pharma, Inc., Eli Lilly Japan K.K., Sanofi K.K., Novo Nordisk Pharma, Ltd., Novartis Pharma K.K., MSD K.K., and Takeda Pharmaceutical Co., Ltd. None of the other authors declare a conflict of interest.

Human rights statement and informed consent All procedures conducted in this study were in accordance with the ethics committee of Fukui-ken Saiseikai Hospital and with the Helsinki Declaration of 1964 and later revision. Informed consent or substitute for it was obtained from all patients for being included in the study. Identifying information of patients of human subjects, including names, initials, addresses, admission dates, hospital numbers, or any other data that might identify patients were not included in our written descriptions.

References

- Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA*. 2015;313:2263–73.
- Targher G, Byrne CD. Clinical review: nonalcoholic fatty liver disease: a novel cardiometabolic risk factor for type 2 diabetes and its complications. *J Clin Endocrinol Metab*. 2013;98:483–95.
- Thoma C, Day CP, Trenell MI. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: a systematic review. *J Hepatol*. 2012;56:255–66.
- Mahady SE, Webster AC, Walker S, et al. The role of thiazolidinediones in non-alcoholic steatohepatitis—a systematic review and meta analysis. *J Hepatol*. 2011;55:1383–90.
- Boettcher E, Csako G, Pucino F, et al. Meta-analysis: pioglitazone improves liver histology and fibrosis in patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2012;35:66–75.
- Cusi K, Orsak B, Bril F, et al. Long-term pioglitazone treatment for patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus: a randomized trial. *Ann Intern Med*. 2016;165:305–15.
- Ratziu V. Pharmacological agents for NASH. *Nat Rev Gastroenterol Hepatol*. 2013;10:676–85.
- Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet*. 2016;387:679–90.
- Hardman TC, Rutherford P, Dubrey SW, et al. Sodium-glucose co-transporter 2 inhibitors: from apple tree to 'Sweet Pee'. *Curr Pharm Des*. 2010;16:3830–8.
- Qiang S, Nakatsu Y, Seno Y, et al. Treatment with the SGLT2 inhibitor luseogliflozin improves nonalcoholic steatohepatitis in a rodent model with diabetes mellitus. *Diabetol Metab Syndr*. 2015;7:104.
- Suzuki M, Takeda M, Kito A, et al. Tofogliflozin, a sodium/glucose cotransporter 2 inhibitor, attenuates body weight gain and fat accumulation in diabetic and obese animal models. *Nutr Diabetes*. 2014;. doi:[10.1038/nutd.2014.20](https://doi.org/10.1038/nutd.2014.20).
- Komiya C, Tsuchiya K, Shiba K, et al. Ipragliflozin improves hepatic steatosis in obese mice and liver dysfunction in type 2 diabetic patients irrespective of body weight reduction. *PLoS One*. 2016;. doi:[10.1371/journal.pone.0151511](https://doi.org/10.1371/journal.pone.0151511).
- McKimmie RL, Daniel KR, Carr JJ, et al. Hepatic steatosis and subclinical cardiovascular disease in a cohort enriched for type 2 diabetes: the Diabetes Heart Study. *Am J Gastroenterol*. 2008;103:3029–35.
- Oliva MR, Mortelet KJ, Segatto E, et al. Computed tomography features of nonalcoholic steatohepatitis with histopathologic correlation. *J Comput Assist Tomogr*. 2006;30:37–43.
- Bando Y, Toyama H, Aoki K, et al. Ipragliflozin lowers small, dense low-density lipoprotein cholesterol levels in Japanese patients with type 2 diabetes mellitus. *J Clin Transl Endocrinol*. 2016;6:1–7.
- Prati D, Taioli E, Zanella A, et al. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med*. 2002;137:1–10.
- Shah AG, Lydecker A, Murray K, et al. Use of the Fib4 index for non-invasive evaluation of fibrosis in nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol*. 2009;7:1104–12.
- Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis*. 2009;53:982–92.
- Yoshizumi T, Nakamura T, Yamane M, et al. Abdominal fat: standardized technique for measurement at CT. *Radiology*. 1999;211:283–6.
- Van der Kooy K, Seidell JC. Techniques for the measurement of visceral fat: a practical guide. *Int J Obes Relat Metab Disord*. 1993;17:187–96.

21. Byrne CD, Targher G. Ectopic fat, insulin resistance, and non-alcoholic fatty liver disease: implications for cardiovascular disease. *Arterioscler Thromb Vasc Biol.* 2014;34:1155–61.
22. Nishimura N, Kitade M, Noguchi R, et al. Ipragliflozin, a sodium-glucose cotransporter 2 inhibitor, ameliorates the development of liver fibrosis in diabetic Otsuka Long-Evans Tokushima fatty rats. *J Gastroenterol.* 2016;51:1141–9.
23. Tahara A, Kurosaki E, Yokono M, et al. Effects of SGLT2 selective inhibitor ipragliflozin on hyperglycemia, hyperlipidemia, hepatic steatosis, oxidative stress, inflammation, and obesity in type 2 diabetic mice. *Eur J Pharmacol.* 2013;715:246–55.
24. Hayashizaki-Someya Y, Kurosaki E, Takasu T, et al. Ipragliflozin, an SGLT2 inhibitor, exhibits a prophylactic effect on hepatic steatosis and fibrosis induced by choline-deficient L-aminoacid-defined diet in rats. *Eur J Pharmacol.* 2015;754:19–24.
25. Honda Y, Imajo K, Kato T, et al. The selective SGLT2 inhibitor ipragliflozin has a therapeutic effect on nonalcoholic steatohepatitis in mice. *PLoS One.* 2016;. doi:[10.1371/journal.pone.0146337](https://doi.org/10.1371/journal.pone.0146337).
26. Nakano S, Katsuno K, Isaji M, et al. Remogliflozin etabonate improves fatty liver disease in diet-induced obese male mice. *J Clin Exp Hepatol.* 2015;5:190–8.
27. Leiter LA, Forst T, Polidori D, et al. Effect of canagliflozin on liver function tests in patients with type 2 diabetes. *Diabetes Metab.* 2016;42:25–32.
28. Yecies JL, Zhang HH, Menon S, et al. Akt stimulates hepatic SREBP1c and lipogenesis through parallel mTORC1-dependent and -independent pathways. *Cell Metab.* 2011;14:21–32.
29. Hasty AH, Shimano H, Yahagi N, et al. Sterol regulatory element-binding protein-1 is regulated by glucose at the transcriptional level. *J Biol Chem.* 2000;275:31069–77.
30. Matsuzaka T, Shimano H, Yahagi N, et al. Insulin-independent induction of sterol regulatory element-binding protein-1c expression in the livers of streptozotocin treated mice. *Diabetes.* 2004;53:560–9.
31. Yahagi N, Shimano H, Hasty AH, et al. Absence of sterol regulatory element-binding protein-1 (SREBP-1) ameliorates fatty livers but not obesity or insulin resistance in Lep(ob)/Lep(ob) mice. *J Biol Chem.* 2002;277:19353–7.
32. 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;21:512–7.
33. 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;97:1020–31.
34. Blonde L, Stenlöf K, Fung A, et al. Effects of canagliflozin on body weight and body composition in patients with type 2 diabetes over 104 weeks. *Postgrad Med.* 2016;128:371–80.
35. Finelli C, Sommella L, Gioia S, et al. Should visceral fat be reduced to increase longevity? *Ageing Res Rev.* 2013;12:996–1004.
36. Park BJ, Kim YJ, Kim DH, et al. Visceral adipose tissue area is an independent risk factor for hepatic steatosis. *J Gastroenterol Hepatol.* 2008;23:900–7.
37. Eguchi Y, Eguchi T, Mizuta T, et al. Visceral fat accumulation and insulin resistance are important factors in nonalcoholic fatty liver disease. *J Gastroenterol.* 2006;41:462–9.
38. Punthakee Z, Almras N, Desprs JP, et al. Impact of rosiglitazone on body composition, hepatic fat, fatty acids, adipokines and glucose in persons with impaired fasting glucose or impaired glucose tolerance: a sub-study of the DREAM trial. *Diabet Med.* 2014;31:1086–92.
39. Sun WH, Song MQ, Jiang CQ, et al. Lifestyle intervention in non-alcoholic fatty liver disease in Chengyang District, Qingdao, China. *World J Hepatol.* 2012;4:224–30.
40. Oza N, Eguchi Y, Mizuta T, et al. A pilot trial of body weight reduction for nonalcoholic fatty liver disease with a home-based lifestyle modification intervention delivered in collaboration with interdisciplinary medical staff. *J Gastroenterol.* 2009;44:1203–8.