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CLINICAL TRIAL

Effects of canagliflozin on body composition and hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease

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Keywords
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ABSTRACT

Aims/Introduction: Non-alcoholic fatty liver disease is frequently associated with type 2 diabetes, and constitutes an important risk factor for the development of hepatic fibrosis and hepatocellular carcinoma. Because there remains no effective drug therapy for non-alcoholic fatty liver disease associated with type 2 diabetes, we evaluated the efficacy of sodium–glucose cotransporter 2 inhibitor.

Methods and Materials: In the present pilot, prospective, non-randomized, open-label, single-arm study, we evaluated the effect of 100 mg canagliflozin administered once daily for 12 months on serological markers, body composition measured by bioelectrical impedance analysis method and hepatic fat fraction measured by magnetic resonance imaging in type 2 diabetes patients with non-alcoholic fatty liver disease.

Results: Canagliflozin significantly reduced body and fat mass, and induced a slight decrease in lean body or muscle mass that did not reach significance at 6 and 12 months. Reductions in fat mass in each body segment (trunk, arms and legs) were evident, whereas those in lean body mass were not. The hepatic fat fraction was reduced from a baseline of 17.6 ± 7.5% to 12.0 ± 4.6% after 6 months and 12.1 ± 6.1% after 12 months ($P < 0.0005$ and $P < 0.005$), whereas serum liver enzymes and type IV collagen concentrations improved. From a mean baseline hemoglobin A1c of 8.7 ± 1.4%, canagliflozin significantly reduced hemoglobin A1c after 6 and 12 months to 7.3 ± 0.6% and 7.7 ± 0.7% ($P < 0.0005$ and $P < 0.01$).

Conclusions: Canagliflozin reduced body mass, fat mass and hepatic fat content without significantly reducing muscle mass.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a clinical term describing two types of liver conditions: non-alcoholic simple fatty liver and non-alcoholic steatohepatitis (NASH)[1]. Accumulating evidence showed that NASH can lead to progressive fibrosis, and ultimately to cirrhosis and carcinoma[2–4], whereas NAFLD is strongly associated with insulin resistance, overweight/obesity and other metabolic disorders. In particular, a strong association between NAFLD and type 2 diabetes mellitus has been shown, with >70% of patients with type 2 diabetes mellitus having NAFLD[5–7]. Although liver biopsy is the gold standard for the diagnosis of NAFLD, sampling variability could undermine the reliability of this invasive diagnostic procedure[8–10]. Computed tomography can show fat filtration in the liver; however, associated hepatic conditions, such as cirrhosis, inflammation and iron deposition, can affect the density of liver parenchyma. Magnetic resonance imaging (MRI) allows quantitative estimation of intrahepatic fat content without radiation exposure. Magnetic resonance spectroscopy is accepted as a reference imaging method for the measurement of intrahepatic fat content. However, it is not widely used in routine clinical practice because of technical and evaluation difficulties. The IDEAL IQ sequence (GE Healthcare, Waukesha, Wisconsin, USA) is a chemical shift-based water-fat separation method that measures proton density fat fraction using complex-based techniques that allow us to keep the scan time within a single

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breath hold[11]. Hepatic fat fraction (HFF), hepatic proton density fat fraction, is capable of detecting hepatic fat infiltration and permitting the grading of liver steatosis in NAFLD[12].

To assess extrahepatic body composition, dual energy X-ray absorptiometry can be used to determine the regional distribution of fat and lean body mass, but its ability to quantify abdominal fat is limited. A body composition analyzer, which uses the latest eight-electrode multifrequency technology, is an easy, convenient and accurate method, and is similarly accurate to dual energy X-ray absorptiometry for the estimation of total and segmental body composition in healthy adults, but it has also been reported to better quantify total abdominal fat than MRI[13–15], although it reportedly underestimates fat mass (FM) and overestimates lean body mass in obese individuals[16].

To date, no pharmacological agent has been approved for the treatment of NAFLD. Pioglitazone and metformin have some beneficial effects on NAFLD, but their efficacy and safety have not been confirmed[17]. Sodium–glucose cotransporter 2 inhibitors (SGLT2i) are a novel class of drug that reduces renal glucose reabsorption, lowers plasma glucose independent of insulin action[18,19] and has some positive metabolic benefits. Empagliflozin and canagliflozin have been shown to reduce the incidence of cardiovascular events and delay the progression of kidney disease in patients with type 2 diabetes mellitus[20–22]. Ipragliflozin reduces plasma lipid levels and ameliorates liver steatosis in diabetic animal models[23,24]. In type 2 diabetes mellitus patients, it decreases serum liver enzymes, visceral and subcutaneous fat volumes, and liver-to-spleen attenuation ratio on computed tomography after 24 weeks of therapy[25]. Canagliflozin also improves liver function, and reduces hemoglobin A1c (HbA1c) and body mass[26]. Additionally, a recent study showed that empagliflozin reduced hepatic FM in type 2 diabetes mellitus patients with NAFLD in just 20 weeks[27]. Thus, it is anticipated that SGLT2i might have longer-term benefits for body composition and NAFLD in humans when evaluated using standard reference methods.

In the present pilot, prospective, non-randomized, open-label, single-arm study, we evaluated the effects of the SGLT2i, canagliflozin, on body composition using the latest eight-electrode multifrequency technology and HFF using 3-T MRI in type 2 diabetes mellitus patients with NAFLD for 12 months.

METHODS

Patients

The participants were Japanese type 2 diabetes mellitus patients, who were diagnosed as NAFLD by abdominal echography, had been consistently followed up, and were enrolled at Kitasato University Hospital between October 2015 and June 2016. They had been receiving dietary and/or exercise therapy since the first diagnosis, treated with insulin and/or oral hypoglycemic agents before the start of SGLT2i administration, and were absent from urinary ketone bodies, severe renal dysfunction or other hepatic diseases (e.g., hepatitis B or C virus, alcoholic hepatitis and autoimmune hepatitis). The present study was registered with the UMIN Clinical Trial Registry (registration number UMIN000020615). The protocols were approved by the Kitasato University Medical School Ethics Committee (C15-936), and informed consent was obtained from all participants. All study methods were carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments, and the regulations of Kitasato University Medical School.

Study Design

All patients started to receive canagliflozin hydrate 100 mg once daily in addition to their existing therapeutic regimen. Two patients reduced their insulin dosage, four patients stopped taking glinide and one patients stopped taking alpha-glucosidase during the study. No additions or adjustments were made to other antidiabetic, antihypertensive or antilipidemic agents during the entire 12-month study period, except that reductions in the dosage of insulin, glinide and sulfonylurea were permitted to avoid the risk of hypoglycemia. Dietary instructions were provided and/or dietary interviews were carried out before SGLT2i administration, and no additional changes to dietary or exercise therapies were made during the 12-month study period. We confirmed that no participants had any mental or physical problems, including abnormal eating behavior, that could affect diabetes management. To achieve a difference in ΔHFF, a sample size of $n = 19$ was required to achieve 90% power with a type 1 error of 0.05. We planned to include 20 type 2 diabetes mellitus participants with NAFLD.

Biochemical Measurements

Blood samples were drawn at the start of canagliflozin administration (0 months), and after 6 and 12 months. Glycated albumin (GA) was measured by an enzymatic synthesis method using a glycated albumin-L assay kit (Lucica™; Asahi Kasei Pharma, Tokyo, Japan; coefficient of variation <0.3%). The indirect non-invasive fibrosis scores of the Fibrosis-4 (FIB4) index and NAFLD fibrosis score were calculated using the following formulae: FIB4 index = age (years) × aspartate aminotransferase (AST; IU/L) / (platelet count [10^9/L] × $\sqrt{}$alanine aminotransferase [ALT; IU/L])[28]. The FIB4 index was reported as a non-invasive fibrosis marker, and indices ≥2.67 were reported to have 80% positive predictive value, compared with liver biopsy results[29]. NAFLD fibrosis score = −1.675 + 0.037 × age (years) + 0.094 × body mass index (BMI; kg/m^2) + 1.13 × impaired fasting glucose / diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet count (×10^9/L) − 0.66 × albumin (g/dL)[30]. The NAFLD fibrosis score was reported to accurately separate NAFLD patients into those with and without advanced fibrosis. By applying the lower cut-off score of −1.455, advanced hepatic fibrosis could be excluded with high accuracy[30].

Measurement of Body Composition

Body composition, including body mass (BM), FM, muscle mass (MM), lean body mass (LBM) and total body water were

measured by a body composition analyzer using the latest eight-electrode multifrequency bioelectrical impedance analysis technology (body composition analyzer MC-180; Tanita, Tokyo, Japan). To do this, patients stood with the ball and heel of each foot in contact with electrodes on the floor scale, after having urinated. Once their body mass had been recorded, they were instructed to grasp the hand grips and hold them down by their sides, with the metabolic electrodes in contact with their palms and thumbs. Their arms were extended and kept away from their body, according to the manufacturer's instructions. The coefficient of variance of the impedance measure has been reported to be 0.4%[16].

Measurement of Hepatic Fat Fraction

For the measurement of intrahepatic fat content, we acquired MRI of the liver and measured HFF using a modified Dixon method with previously reported methodology (IDEAL-IQ; GE Healthcare, Waukesha, WI, USA)[11,12] at the start of canagliflozin administration, and 6 and 12 months thereafter.

IDEAL-IQ images representing HFF were acquired during a single breath hold using a Discovery MR750w Expert 3.0 Tesla or a Discovery MR750 3.0 Tesla (GE Healthcare). Imaging parameters on the MR750w scanner were as follows: repetition time/first echo time/Δecho time: 8.3/1.0/0.9 ms; number of echoes, six; flip angle, 4°; matrix, 160 × 160; slice thickness, 6 mm; bandwidth, ±111.11 kHz; field of vision, 36–50 cm; and acquisition time, 22 s. When using the MR750 scanner, after modification was applied: repetition time/first echo time/Δecho time, 6.3/1.0/0.8 ms; flip angle, 3°; and acquisition time, 19 s, based on the manufacturer's recommendation.

Image analysis was carried out by two authors (MI, AH) blinded to the clinical records to determine quantitative estimates of HFF under the presence of a third-party doctor who was not involved in this study. As the region of interest, the whole liver was manually demarcated on the slice where the liver area was largest, avoiding major bile ducts and vascular structures.

Statistical Analysis

Statistical analyses were carried out with GraphPad Prism 5.02 software (GraphPad Software Inc., San Diego, CA, USA) and JMP version 5.0.1a (SAS Institute, Cary, NC, USA). Data are presented as the mean ± standard deviation, unless otherwise indicated. The Wilcoxon signed-rank test was used to evaluate differences in ordinal data between two groups. $P < 0.05$ was considered to show statistical significance.

RESULTS

Demographic and Baseline Characteristics

The clinical characteristics of the 20 participants enrolled, who completed 12 months of canagliflozin therapy, are shown in Table 1. Their mean BMI was 31.5 ± 8.0 and HbA1c 8.7 ± 1.4%. They showed a high HFF of 17.6 ± 7.5%, indicative of NAFLD (HFF >5.2%)[12], and 13 patients (65%) were classified as having moderate or severe NAFLD (HFF >15.0%). Their serum liver enzymes were slightly higher than normal.

Table 1 | Characteristics of enrolled patients

Characteristic	
n	20
Sex (male/female)	11/9
Age (years)	51 ± 9 (33–69)
Height (cm)	163.1 ± 10.7 (145.0–178.0)
Body mass (kg)	83.6 ± 20.3 (52.25–135.50)
BMI (kg/m^2)	31.5 ± 8.0 (23.2–56.7)
18.5–24.9: normal class (*n*)	1
25.0–29.9: obese class 1 (*n*)	10
30.0–34.9: obese class 2 (*n*)	5
35.0–39.9: obese class 3 (*n*)	2
>40.0: obese class 4 (*n*)	2
Duration of diabetes (years)	7.4 ± 5.6 (1–23)
Diabetic neuropathy (–)/(+)	13/7
Diabetic nephropathy (NA/MA/ON/CRF)	8/9/3/0
Diabetic retinopathy (none/SDR/prePDR/PDR/UNK)	15/2/0/1/2
Concomitant oral antidiabetic agents (%)	
SU/BG/DPP4i/TZD	5/80/80/5
HbA1c (%)	8.7 ± 1.4 (6.7–12.2)
GA (%)	20.2 ± 5.4 (13.6–31.4)
HFF (%)	17.6 ± 7.5 (5.4–38.7)
NAFLD fibrosis score	–1.4 ± 1.3
<–1.455 (*n*)	9
–1.455–0.675 (*n*)	10
>0.675 (*n*)	1
FIB4 index	1.2 ± 0.5

Data are mean ± standard deviation (minimum–maximum). Non-alcoholic fatty liver disease (NAFLD) fibrosis score was calculated by: –1.675 + 0.037 × age (years) + 0.094 × body mass index (BMI; kg/m^2) + 1.13 × impaired fasting glycemia or diabetes (yes = 1, no = 0) + 0.99 × aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio − 0.013 × platelet (×10^9/L) − 0.66 × albumin (g/dL). The Fibrosis-4 (FIB4) index was calculated by: age (years) × AST (IU/L)/platelet count (×10^9/L) × $\sqrt{}$ALT (U/L). BG, biguanide; BMI, body mass index; CRF, chronic renal failure; DPP4i, DPP4 inhibitors, GA, glycated albumin; HbA1c, hemoglobin A1c; HFF, hepatic fat fraction; MA, microalbuminuria; NA, normoalbuminuria; ON, overt nephropathy; PDR, proliferative diabetic retinopathy; prePDR, pre-proliferative diabetic retinopathy; SDR, simple diabetic retinopathy; SU, sulfonylurea; TZD, thiazolidine; UNK, unknown.

Efficacy of Canagliflozin

BM, BMI and FM significantly decreased during the study period (Table 2). FM decreased from 32.0 ± 17.8 kg at baseline to 29.0 ± 16.3 kg at 6 months, and this change persisted until 12 months (29.4 ± 17.1 kg), whereas LBM and MM showed a slight decrease that did not reach significance. Body water content, calculated as a ratio of total body water/BM, increased significantly at 6 months. Compartment analysis showed the

Table 2 | Changes in body composition of type 2 diabetes mellitus patients with non-alcoholic fatty liver disease

	Mean ± SD	Minimum-maximum	*P*-value
Body mass (kg)			
Baseline	83.6 ± 20.3	52.3–135.5	
6 months	80.5 ± 19.8	49.1–130.0	0.0003
12 months	80.7 ± 20.9	50.0–132.9	0.0007
BMI			
Baseline	31.5 ± 8.0	23.2–56.7	
6 months	30.3 ± 7.7	21.8–54.5	0.0003
12 months	30.3 ± 8.0	22.2–54.4	0.0005
FM (kg)			
Baseline	32.0 ± 17.8	12.8–87.6	
6 months	29.0 ± 16.3	8.1–81.1	0.0008
12 months	29.4 ± 17.1	11.0–84.3	0.0007
MM (kg)			
Baseline	48.7 ± 11.3	32.2–68.1	
6 months	48.7 ± 11.0	33.4–68.6	0.5713
12 months	48.5 ± 11.6	32.2–68.2	0.1549
LBM (kg)			
Baseline	51.5 ± 11.7	34.8–71.8	
6 months	51.5 ± 11.4	35.3–72.3	0.4591
12 months	51.3 ± 12.1	34.1–71.9	0.1182
TBW (kg)			
Baseline	37.1 ± 7.6	26.6–55.4	
6 months	37.3 ± 7.6	26.4–54.4	0.6012
12 months	36.9 ± 8.0	24.9–52.5	0.1873
BWC (%)			
Baseline	45.6 ± 7.0	23.7–58.1	
6 months	47.3 ± 7.0	25.6–63.1	0.0035
12 months	46.7 ± 7.1	23.4–59.1	0.0329
Truncal MM (kg)			
Baseline	25.6 ± 4.4	18.7–30.9	
6 months	25.3 ± 4.7	18.6–32.4	0.9381
12 months	25.3 ± 4.9	18.3–32.1	0.9547
Truncal FM (kg)			
Baseline	16.4 ± 5.9	6.4–32.5	
6 months	14.7 ± 5.6	4.1–30.0	0.0018
12 months	14.9 ± 6.0	5.5–32.8	0.0025
MM in right arm (kg)			
Baseline	2.80 ± 0.72	1.85–4.05	
6 months	2.70 ± 0.74	1.55–4.05	0.2319
12 months	2.65 ± 0.73	1.45–3.95	0.0157
FM in right arm (kg)			
Baseline	1.24 ± 0.58	0.50–2.45	
6 months	1.11 ± 0.58	0.10–2.20	0.0068
12 months	1.09 ± 0.55	0.35–2.15	0.0024
MM in left arm (kg)			
Baseline	2.63 ± 0.64	1.75–3.80	
6 months	2.54 ± 0.67	1.50–3.70	0.3776
12 months	2.50 ± 0.67	1.40–3.60	0.0773
FM in left arm (kg)			
Baseline	1.28 ± 0.61	0.50–2.60	
6 months	1.15 ± 0.61	0.10–2.30	0.0143
12 months	1.13 ± 0.56	0.30–2.35	0.0038
MM in right leg (kg)			
Baseline	9.83 ± 2.94	6.20–16.65	
6 months	9.50 ± 2.98	5.80–16.40	0.1705
12 months	9.51 ± 2.98	5.60–15.90	0.2804
FM in right leg (kg)			
Baseline	5.19 ± 2.47	2.75–12.95	
6 months	4.71 ± 2.26	1.95–11.70	0.0041
12 months	4.73 ± 2.27	2.45–11.75	0.0066
MM in left leg (kg)			
Baseline	9.74 ± 2.90	6.10–17.05	
6 months	9.34 ± 2.88	5.35–16.35	0.1087
12 months	9.36 ± 2.92	5.50–16.20	0.1406
FM in left leg (kg)			
Baseline	5.14 ± 2.50	2.70–13.20	
6 months	4.63 ± 2.22	1.85–11.50	0.0049
12 months	4.66 ± 2.27	2.40–11.90	0.0066

P-values are compared with baseline and were generated using the Wilcoxon signed-rank test. BMI, body mass index; BWC, body water content (TBW/BM × 100); FM, fat mass; LBM, lean body mass; MM, muscle mass; SD, standard deviation; TBW, total body water.

reduction of FM in the truncal compartment, bilateral arms and legs, whereas MM showed a significant reduction only in the right arm and did not reach significance in other compartments (Table 2). However, in two patients, body mass at 12 months increased by >3% compared with 6 months.

3-T MRI analysis of the liver showed significant reduction of HFF from 17.6 ± 7.5% at baseline to 12.0 ± 4.6% at 6 months ($P = 0.0004$), and to 12.1 ± 6.1% at 12 months ($P = 0.0013$; Figure 1). Meanwhile, HFF at 12 months in four patients increased by >3% compared with 6 months. AST, ALT, gamma glutamyl transferase and serum type IV collagen 7S concentrations also improved significantly at 6 and 12 months. Alkaline phosphatase and serum ferritin decreased significantly by 6 months. Blood platelet count, serum albumin, cholinesterase and hyaluronic acid did not show any significant change. NAFLD fibrosis score and FIB4 index also did not change significantly (Table 3). We analyzed the correlation between the changes in clinical parameters with canagliflozin treatment to the reduction in HFF. At 6 months, the changes in HFF significantly correlated with changes in HbA1c ($r = 0.614$, $P = 0.0040$) and GA ($r = 0.517$, $P = 0.0334$), but did not correlate with those in body composition parameters (BM, BMI, FM, MM and LBM) or NAFLD parameters (AST, ALT, alkaline phosphatase, gamma glutamyltranspeptidase, type IV collagen 7S concentration and hyaluronic acid). At 12 months, changes in HFF correlated only with changes in HbA1c ($r = 0.582$, $P = 0.0071$) and GA ($r = 0.522$, $P = 0.0317$), but not with changes in body composition and NAFLD parameters.

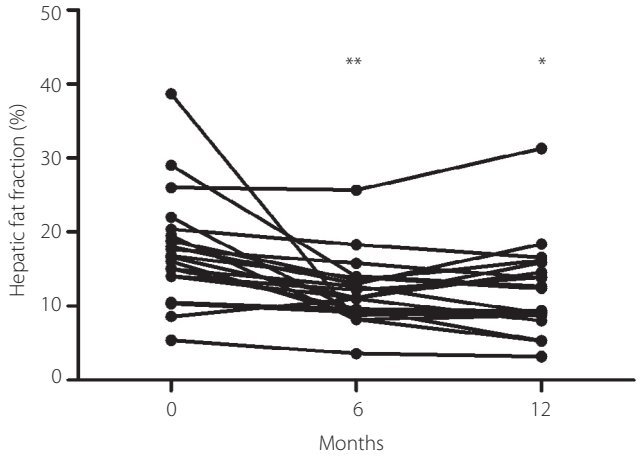

Figure 1 | Hepatic fat fraction before and after canagliflozin administration. Hepatic fat fraction was determined by 3-T magnetic resonance imaging immediately before (0), and 6 and 12 months after the administration of 100 mg canagliflozin daily. Each circle represents a single patient measurement. *$P < 0.005$, **$P < 0.0005$.

Canagliflozin significantly reduced HbA1c from 8.7 ± 1.4% at baseline to 7.3 ± 0.6% at 6 months ($P = 0.0003$) and to 7.7 ± 0.7% ($P = 0.0051$) at 12 months. GA decreased from 20.2 ± 5.4% at baseline to 15.6 ± 1.7% at 6 months ($P = 0.0024$) and to 17.1 ± 2.2% at 12 months ($P = 0.0522$). The only adverse events identified during the entire study period were thirst and increase in urinary volume in three patients, nausea in a single patient, and nocturia in four patients, which all improved within 3 months. No patient had to stop taking canagliflozin.

DISCUSSION

In the present study, administration of 100 mg canagliflozin hydrate daily for 12 months reduced total FM and HFF. Simultaneous reductions in serum aminotransferase concentrations and glycemic markers support the notion that canagliflozin might have beneficial effects, both on glycemic control and NAFLD. As for a hypocaloric diet, a study showed that a 6% reduction in BM by a 3-month hypocaloric diet was accompanied with a significant reduction in intrahepatic lipid content[31]. Previous studies have suggested that ipragliflozin ameliorates hepatic fibrosis, insulin resistance and lipotoxicity in animal models[23,24], and reduces BM and visceral fat volume more effectively than pioglitazone in type 2 diabetes mellitus patients[25]. In addition, recent studies suggested that while SGLT2i alone improves NASH, SGLT2i in combination with incretin-based treatment, such as dipeptidyl peptidase-4 inhibitor or glucagon-like peptide-1 analogs, can synergistically ameliorates NASH[31,32]. In the present study, most of type 2 diabetes mellitus patients were prescribed dipeptidyl peptidase-4 inhibitor (16 patients, 80% of all) and biguanide (16 patients, 80% of all) that might have a beneficial effect on NAFLD. The

Table 3 | Changes in clinical parameters of enrolled type 2 diabetes mellitus patients with non-alcoholic fatty liver disease

	Mean ± SD	Minimum–maximum	*P*-value
HbA1c (%)			
Baseline	8.7 ± 1.4	6.7–12.2	
6 months	7.3 ± 0.6	6.1–8.3	0.0003
12 months	7.7 ± 0.7	6.5–9.2	0.0051
GA (%)			
Baseline	20.2 ± 5.4	13.6–31.4	
6 months	15.6 ± 1.7	12.4–18.7	0.0024
12 months	17.1 ± 2.2	13.2–23.3	0.0522
AST (U/L)			
Baseline	52 ± 28	14–127	
6 months	37 ± 20	15–94	0.0024
12 months	43 ± 38	12–182	0.0168
ALT (U/L)			
Baseline	80 ± 54	16–269	
6 months	53 ± 31	13–152	0.0008
12 months	59 ± 41	14–146	0.0090
AP (U/L)			
Baseline	288 ± 103	136–531	
6 months	256 ± 79	139–399	0.0169
12 months	259 ± 84	130–447	0.0586
GGT (U/L)			
Baseline	132 ± 151	25–730	
6 months	95 ± 113	26–476	0.0034
12 months	92 ± 99	21–452	0.0008
Serum ferritin (ng/mL)			
Baseline	291 ± 247	84–863	
6 months	158 ± 156	22–583	0.0039
12 months	159 ± 133	23–475	0.0547
Cholinesterase (U/L)			
Baseline	423 ± 84	296–610	
6 months	403 ± 88	276–618	0.0803
12 months	415 ± 79	307–608	0.2439
Serum albumin (g/dL)			
Baseline	4.5 ± 0.4	3.3–5.1	
6 months	4.4 ± 0.3	3.6–4.7	0.4203
12 months	4.5 ± 0.3	4.0–5.0	0.8995
Blood platelet count ($\times 10^4/\mu L$)			
Baseline	276 ± 82	180–489	
6 months	267 ± 93	178–517	0.2266
12 months	264 ± 74	181–414	0.2761
Serum type IV collagen 7S (ng/mL)			
Baseline	6.4 ± 2.1	3.9–11.0	
6 months	5.6 ± 1.6	3.5–9.6	0.0006
12 months	5.0 ± 1.6	2.3–7.6	0.0015
Serum hyaluronic acid (ng/mL)			
Baseline	40.9 ± 33.1	9.0–133.0	
6 months	33.6 ± 27.4	9.0–117.0	0.2145
12 months	47.3 ± 43.1	9.0–170.0	0.3490
NAFLD fibrosis score			
Baseline	−1.43 ± 1.3	−4.21–2.28	
6 months	−1.35 ± 1.5	−4.45–2.47	0.2873
12 months	−1.37 ± 1.3	−4.47–1.75	0.5883

Table 3 (*Continued*)

	Mean ± SD	Minimum–maximum	*P*-value
FIB4 index			
Baseline	1.20 ± 0.5	0.29–2.39	
6 months	1.10 ± 0.5	0.33–2.10	0.0894
12 months	1.15 ± 0.7	0.36–3.75	0.2395

P-values are compared with baseline, and were generated using the Wilcoxon signed-rank test. AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; FIB4, Fibrosis-4; GA, glycated albumin; GGT, gamma glutamyltranspeptidase; HbA1c, hemoglobin A1c.

result in the present study might be influenced by the beneficial effect of these drugs. One patient showed a large decline in HFF from 38.7% at baseline to 8.2% at 6 months, and 5.3% at 12 months. We added an analysis of 19 patients after exclusion of this patient.The reduction of HFF was also significant in this subgroup; canagliflozin reduced HFF from 16.5 ± 5.7% at baseline to 12.2 ± 4.6% at 6 months ($P = 0.0006$), and to 12.5 ± 6.1% at 12 months ($P = 0.0022$). Furthermore, changes in HFF correlated with changes in HbA1c and GA, but not with changes in body composition or NAFLD clinical marker. The present results showed that HFF reduction by canagliflozin correlated with glycemic improvement markers, but not with body composition markers. A recent study[27] showed that empagliflozin significantly reduced HFF (from 16.2 ± 7.0% at baseline to 11.3 ± 5.3% at 20 weeks, $P < 0.0001$) in 22 type 2 diabetes patients with NAFLD, and standard treatment without empagliflozin did not change HFF (from 16.4 ± 7.3% at baseline to 15.5 ± 6.7% at 20 weeks, $P = 0.054$) in 20 type 2 diabetes patients with NAFLD. It showed a significant difference for change in serum ALT level (from 44.6 ± 23.5 units/L to 36.2 ± 9.0 units/L in the empagliflozin group, $P = 0.040$; from 45.3 ± 24.3 units/L to 44.6 ± 23.8 units/L in the control group, $P = 0.931$; $P = 0.005$), and non-significant changes for AST level ($P = 0.212$) and gamma glutamyltranspeptidase level ($P = 0.057$) between two groups. The present results showed that canagliflozin reduced HFF by >5% at 6 months, and maintained HFF until a longer period of 12 months in most of the cases (from 17.6 ± 7.5% at baseline to 12.0 ± 4.6% at 6 months, and to 12.1 ± 6.1% at 12 months).

Mean BM reductions of 3.7% at 6 months and 3.8% at 12 months in the present study might have contributed to the marked reductions in hepatic fat content, with little change in LBM or MM that did not reach significance. A previous report showed that some patients showed an increase in appetite and recovery of BM during the treatment with SGLT2i[33]. In the present study, although three of 20 patients showed an increase in BM at 12 months compared with 6 months, all maintained reduced HbA1c levels and HFF levels during this period. The effect of 100 mg canagliflozin to reduce FM and HFF, and to control glycemia was preserved even with a positive energy balance during this period.

Previous studies showed that bodyweight loss by SGLT2i is two-thirds fat and one-third lean mass. In animal models (high-fat diet-induced obese rats), reduction in bodyweight by ipragliflozin was accompanied by reduced visceral and subcutaneous fat masses, but not with lean mass or bone mineral content[34]. In type 2 diabetes mellitus patients, indirect calorimetric analysis showed that ipragliflozin mainly promoted fatty acids consumption instead of glucose for the energy source without changing the whole-body energy consumption[35,36]. The present results showing a significant decrease in FM and HFF suggest that SGLT2i promotes fatty acid utilization, and reduces subcutaneous and visceral fat as well as hepatic fat in patients with type 2 diabetes mellitus and NAFLD.

In the present study, we observed a slight decrease in LBM and MM, but they did not reach significance. The difference in methods used might have caused the undetectable change. We measured body and segmental composition by multifrequency bioelectrical impedance analysis to reduce participants' radiation exposure. Body composition analysis by this method is reported to underestimate total body FM and overestimate lean body mass in healthy young adults with >25% body fat, compared with the use of dual energy X-ray absorptiometry[16]. However, because the magnitude of the difference between the two methods was small (<4%), we speculated that bioelectrical impedance analysis can be used interchangeably with dual energy X-ray absorptiometry in the measurement of appendicular fat free mass.

The limitations of the present study are that the sample size was small; and that a single-arm, placebo-free, open-label design was used. In addition, HFF was measured by MRI as the primary outcome, whereas hepatic biopsy and histological evaluation were not carried out. A further investigation involving a larger sample size including histological evaluation should be undertaken to establish the overall effectiveness of SGLT2i for NAFLD in type 2 diabetes mellitus patients.

In conclusion, canagliflozin has beneficial effects on whole and segmental body composition, hepatic fat storage, liver enzymes, and glycemic control in patients with type 2 diabetes mellitus complicated by NAFLD for 12 months.

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DISCLOSURE

The authors declare no conflict of interest.

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