

Efficacy and safety of canagliflozin in type 2 diabetes mellitus patients with biopsy-proven nonalcoholic steatohepatitis classified as stage 1–3 fibrosis

Yuya Seko¹
Taichiro Nishikawa¹
Atsushi Umemura¹
Kanji Yamaguchi¹
Michihisa Moriguchi¹
Kohichiroh Yasui¹
Mayumi Kimura²
Hiroaki Iijima³
Toshio Hashimoto³
Yoshio Sumida⁴
Takeshi Okanoue⁵
Yoshito Itoh¹

¹Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, Japan; ²Ikuyaku. Integrated Value Development Division, Mitsubishi Tanabe Pharma Corporation, Osaka, Japan; ³Ikuyaku. Integrated Value Development Division, Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan; ⁴Division of Hepatology and Pancreatology, Department of Internal Medicine, Aichi Medical University, Aichi, Japan; ⁵Department of Gastroenterology and Hepatology, Saiseikai Suita Hospital, Osaka, Japan

Correspondence: Taichiro Nishikawa
Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan
Tel +81 75 251 5519
Fax +81 75 251 0710
Email taichi@koto.kpu-m.ac.jp

Aim: Nonalcoholic fatty liver disease (NAFLD), including nonalcoholic steatohepatitis (NASH), is known to be associated with type 2 diabetes mellitus (T2DM) in high rate. The improvement in hepatic function due to sodium-glucose co-transporter 2 (SGLT2) inhibitors has been reported in T2DM patients with and without NAFLD. However, only a few studies have attempted to evaluate the role of SGLT2 inhibitors in T2DM patients with biopsy-proven NASH, and no detailed prospective studies including the individual hepatic fibrosis stage have been reported. Therefore, we investigated the effect of canagliflozin on hepatic function in T2DM patients with biopsy-confirmed NASH.

Methods: T2DM patients with NASH (hepatic fibrosis stage 1–3 confirmed via liver biopsy, n=10) were enrolled and received canagliflozin (100 mg) once a day for 12 weeks. The primary end point was change in serum alanine aminotransferase (ALT) levels from baseline to week 12. Secondary end points were liver function/fibrosis markers, metabolic parameters, and safety.

Results: The change in ALT from baseline to week 12 was -23.9 U/L (95% CI -48.1 to 0.3 , $P=0.0526$). Significant improvements in several hepatic function/fibrosis markers, such as aspartate aminotransferase, fibrosis-4 index, and FM-fibro index, and metabolic parameters including hemoglobin A1c and body weight were found. No serious or liver-related adverse events were reported. Regarding individual patients, different trends in ALT-lowering effects between stage 1 and stage 2/3 subjects were observed; the degree of ALT-lowering effect tended to be greater in the stage 1 group than in the stage 2/3 group.

Conclusion: Our results suggest that canagliflozin is effective and well-tolerated in patients with T2DM and NASH. Canagliflozin may be useful for the treatment of T2DM patients with NASH, especially those in early stages of NASH.

Keywords: canagliflozin, Japanese, NASH, fibrosis stages, SGLT2 inhibitor, type 2 diabetes mellitus

Introduction

Nonalcoholic fatty liver disease (NAFLD), including nonalcoholic steatohepatitis (NASH), is associated with type 2 diabetes mellitus (T2DM).^{1–3} In Japan, the prevalence of T2DM is ~50% in patients with NAFLD and increases with progression of the fibrosis stage; DM is a significant risk factor for advanced fibrosis.⁴

Advanced NASH increases the risks of cirrhosis and hepatocellular carcinoma.³ A reduction in serum alanine aminotransferase (ALT) level ($\geq 30\%$ reduction from the baseline⁵ or $\geq 30\%$ reduction from the baseline and decrease to ≤ 40 U/L⁶) was

associated with amelioration of liver fibrosis progression in NASH patients. Therefore, control of serum ALT via suitable interventions is important to prevent NASH progression. Although pioglitazone has been shown to improve serum ALT levels and histological features in NASH patients with insulin resistance, concerns such as body weight gain and congestive heart failure exist.⁷ Therefore, new NASH treatment strategies are needed.

Sodium-glucose co-transporter 2 (SGLT2) inhibitors suppress glucose reabsorption in the renal tubules and exert antihyperglycemic effects. In addition to glucose lowering, SGLT2 inhibitors improve multiple risk factors such as body weight and blood pressure.^{8–10} Because some SGLT2 inhibitors including canagliflozin and empagliflozin have shown cardiovascular and renal protective effects in T2DM patients with a history or high risk of cardiovascular disease,^{11,12} they are a potential therapeutic option for preventing diabetic complications. Improvement in hepatic function due to SGLT2 inhibitors has also been reported in T2DM patients with and without NAFLD.^{13–18} We also previously reported that treatment of canagliflozin (100 mg) for 12 weeks significantly improved ALT levels compared with placebo in T2DM patients with ALT >31 U/L.¹⁹ However, studies on the role of SGLT2 inhibitors in T2DM patients with biopsy-proven NASH are limited, and no detailed prospective studies including individual hepatic fibrosis stage data have been reported.^{16,20,21} Therefore, we performed the pilot study to investigate the efficacy and safety of canagliflozin in T2DM patients with biopsy-confirmed NASH.

Methods

Study design and patients

A single-arm, exploratory study was performed to investigate the efficacy and safety of canagliflozin in T2DM patients with NASH. The inclusion criteria were as follows: T2DM patients with hemoglobin A1c (HbA1c) $\geq 6.0\%$ and $< 10.0\%$, ALT ≥ 31 U/L, age ≥ 20 and < 75 years, hepatic fibrosis stage 1–3^{22,23} confirmed via liver biopsies during 1 year prior to the start of the study, and daily alcohol consumption of < 30 g/day in males and < 20 g/day in females. The exclusion criteria were as follows: T1DM, history of class IV heart failure per the New York Heart Association functional classification, malignancy, platelet count $< 10^5/\mu\text{L}$, estimated glomerular filtration rate < 45 mL/min/1.73 m², and SGLT2 inhibitor treatment within 12 weeks prior to the start of the study. The severity of hepatic fibrosis stage was scored as: stage 1, zone 3 perisinusoidal fibrosis; stage 2, zone 3 perisinusoidal fibrosis with portal fibrosis; stage 3, zone 3 perisinusoidal

fibrosis and portal fibrosis with bridging fibrosis; and stage 4, cirrhosis.^{22,23}

Intervention and outcomes

Canagliflozin (100 mg) was orally administered to patients before breakfast for 12 weeks. Visits were scheduled every 4 weeks during the treatment. The primary end point was change in serum ALT levels from baseline. Secondary end points included changes from baseline to week 12 in aspartate aminotransferase (AST), γ -glutamyl transferase (γ -GTP), alkaline phosphatase (ALP), hyaluronic acid, type IV collagen 7S, waist circumference, NAFIC score, fibrosis-4 index (FIB-4 index), FM-fibro index calculated from hyaluronic acid and type IV collagen 7S,²⁴ liver stiffness measurement (LSM) and controlled attenuation parameter (CAP) measured via transient elastography (FibroScan; ECHOSENS, Paris, France), HbA1c, fasting plasma glucose, fasting serum insulin, serum C-peptide, platelet count, uric acid, total cholesterol, triglyceride, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, body composition measured using a multifrequency impedance body composition analyzer (InBody 720; InBody Japan, Tokyo, Japan), and responses to the Dutch Eating Behavior Questionnaire (DEBQ) and to the 8-Item Short Form Health Survey (SF-8). Exploratory end points included changes in other parameters including serum glucagon and ferritin. The safety evaluation included assessments of adverse events (AEs) described using the Medical Dictionary for Regulatory Activities/Japanese version 20.1 and laboratory values.

Sample size calculation

We assumed a mean difference of -10 U/L in the change in ALT levels from baseline to the end of the treatment period with a SD of 15 U/L based on results from a previous study¹⁹ and found that 20 patients were required for a power (paired *t*-test) of 81% at a two-sided significance level of 5%.

Statistical analyses

Data were represented as mean values with SD or 95% CI values. All changes between baseline and week 12 were analyzed using two-tailed paired *t*-tests. As subsidiary evaluations, we also analyzed changes after 4 and 8 weeks. A *P*-value < 0.05 was considered statistically significant. All statistical analyses were performed using the statistical analysis system version 9.4 (SAS Institute Inc., Cary, NC, USA). Data management and statistical analyses were conducted by the EP-CRSU Co. Ltd. (Tokyo, Japan).

Ethical considerations

The study was conducted in compliance with the Declaration of Helsinki and the “Ethical guidelines for medical and health research involving human subjects”.²⁵ It was approved by the institutional review board at Kyoto Prefectural University of Medicine and is registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000023044). All patients provided written informed consent.

Results

Patients

Ten patients (seven males and three females) were enrolled in the study and included in efficacy and safety analysis. Patient characteristics are shown in Table 1. The mean \pm SD of the baseline ALT, HbA1c, and body mass index were 99.1 ± 52.6 U/L, $7.09 \pm 0.87\%$, and 29.09 ± 2.50 kg/m², respectively; age and duration of T2DM were 54.9 ± 12.9 and 4.64 ± 4.38 years, respectively. Fibrosis stages included stage 1 (n=5), stage 2 (n=3), and stage 3 (n=2).

Table 1 Patient demographics and characteristics

| | N or mean (SD) |
|------------------------------------|-----------------------|
| Sex | |
| Male | 7 |
| Female | 3 |
| Age (years) | 54.9 (12.9) |
| Duration of diabetes (years) | 4.64 (4.38) |
| HbA1c (%) | 7.09 (0.87) |
| Fasting plasma glucose (mg/dL) | 126.9 (29.8) |
| HOMA-IR | 4.10 (1.74) |
| Body weight (kg) | 77.51 (12.07) |
| BMI (kg/m ²) | 29.09 (2.50) |
| ALT (U/L) | 99.1 (52.6) |
| AST (U/L) | 69.9 (37.8) |
| γ -GTP (U/L) | 73.7 (41.1) |
| Platelet ($\times 10^3/\mu$ L) | 216.9 (67.7) |
| eGFR (mL/min/1.73 m ²) | 79.1 (16.0) |
| Complications | |
| Diabetic retinopathy | 1 |
| Hypertension | 6 |
| Dyslipidemia | 8 |
| Concomitant antidiabetic drugs | |
| No | 5 |
| Yes | 5 |
| Fibrosis stage | |
| Stage 1 | 5 |
| Stage 2 | 3 |
| Stage 3 | 2 |

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; eGFR, estimated glomerular filtration rate; γ -GTP, γ -glutamyl transpeptidase; HbA1c, hemoglobin A1c; HOMA-IR, homeostasis model of assessment-insulin resistance.

Efficacy

A decreasing trend in ALT levels was observed from week 4 (Figure 1A), and the mean change in ALT levels from baseline to week 12 (primary end point) was -23.9 U/L (95% CI -48.1 to 0.3 , $P=0.0526$) (Table 2), corresponding to the percent change of -19.94% (95% CI -40.40 to 0.53). AST, HbA1c, and body weight decreased from week 4 and were significantly reduced at week 12 (Figure 1B–D) (Table 2).

The other secondary end points, γ -GTP as a hepatic function-related marker, and platelets, type IV collagen 7S, the FIB-4 index and the FM-fibro index as hepatic fibrosis markers, were significantly changed at week 12. Other hepatic fibrosis markers including the NAFIC score and LSM/CAP evaluated using transient elastography did not change (Table 2).

Changes in body composition were assessed using a multifrequency impedance body composition analyzer; body fat mass was significantly decreased, and no significant changes in muscle mass or body water content were seen (Table 2). The percentage changes in each component are shown in Table S1.

Changes in ALT in each subject are shown in Figure 2; different trends in ALT-lowering effects between stage 1 and stage 2/3 subjects were observed. Post hoc analysis showed that in the stage 1 and stage 2/3 populations, the baseline ALT values were 109.6 ± 59.7 and 88.6 ± 49.0 U/L, respectively; changes in the ALT values from baseline to week 12 were -46.2 U/L (95% CI -84.0 to -8.4) and -1.6 U/L (95% CI -26.8 to 23.6), respectively.

Other secondary end points and exploratory end points are shown in Table S2. Among them, serum ferritin was significantly decreased at week 12. The SF-8 and DEBQ, indicators of quality of life and dietary habits, respectively, were unchanged due to the treatment (data not shown).

Safety

AEs occurred in five patients; however, no serious or liver-related AEs were reported (Table S3). The changes in laboratory values are shown in Table S4.

Discussion

This study examined the efficacy and safety of canagliflozin in T2DM patients with NASH; the treatment showed improvement in several hepatic function/fibrosis markers and metabolic parameters and was well-tolerated. Canagliflozin decreased ALT levels from baseline (-23.9 U/L); however, it was not statistically significant ($P=0.0526$).

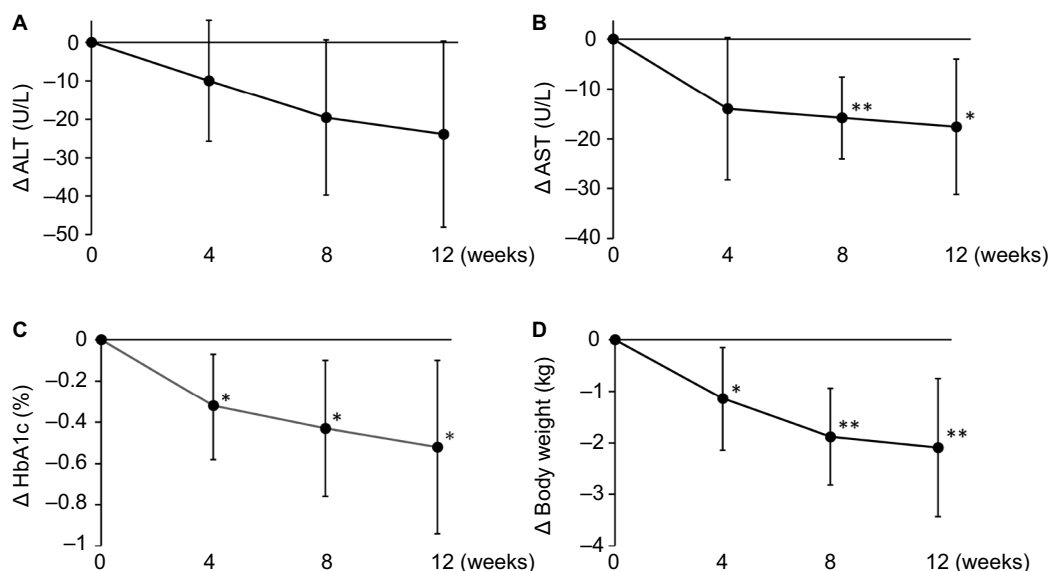


Figure 1 Changes in (A) ALT, (B) AST, (C) HbA1c, and (D) body weight over 12 weeks.

Note: * $P < 0.05$, ** $P < 0.01$ vs baseline (paired t-test).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; HbA1c, hemoglobin A1c.

Table 2 Effects of canagliflozin on hepatic function/fibrosis markers and metabolic parameters (n=10)

| | Week 0, Mean (SD) | Week 12, Mean (SD) | Changes from week 0 | | Paired t-test (P-value) |
|---------------------------------|----------------------|-----------------------|---------------------|--------------------|----------------------------|
| | | | Mean (SD) | 95% CI | |
| ALT (U/L) | 99.1 (52.6) | 75.2 (39.7) | -23.9 (33.9) | (-48.1 to 0.3) | 0.0526 |
| AST (U/L) | 69.9 (37.8) | 52.3 (26.2) | -17.6 (19.0) | (-31.2 to -4.0) | 0.0168 |
| γ -GTP (U/L) | 73.7 (41.1) | 57.7 (30.3) | -16.0 (15.4) | (-27.0 to -5.0) | 0.0094 |
| Platelet ($10^3/\mu\text{L}$) | 216.9 (67.7) | 240.3 (74.0) | 23.4 (21.3) | (8.1 to 38.7) | 0.0071 |
| Type IV collagen 7S (ng/mL) | 5.72 (1.36) | 5.13 (1.06) | -0.59 (0.64) | (-1.05 to -0.13) | 0.0176 |
| FIB-4 index | 2.077 (1.207) | 1.602 (0.981) | -0.475 (0.385) | (-0.750 to -0.200) | 0.0036 |
| FM-fibro index | 0.545 (0.133) | 0.491 (0.104) | -0.054 (0.070) | (-0.104 to -0.004) | 0.0376 |
| NAFIC score | 2.1 (1.4) | 1.9 (1.5) | -0.2 (0.8) | (-0.8 to 0.4) | 0.4433 |
| CAP (dB/m) | 327.4 (38.2) | 321.4 (36.4) | -6.0 (56.5) | (-46.4 to 34.4) | 0.7447 |
| LSM (kPa) | 8.85 (4.79) | 9.09 (7.39) | 0.24 (3.58) | (-2.32 to 2.80) | 0.8367 |
| HbA1c (%) | 7.09 (0.87) | 6.57 (0.55) | -0.52 (0.59) | (-0.94 to -0.10) | 0.0218 |
| Fasting plasma glucose (mg/dL) | 126.90 (29.85) | 115.40 (15.31) | -11.50 (18.17) | (-24.50 to 1.50) | 0.0763 |
| Body weight (kg) | 77.51 (12.07) | 75.42 (12.17) | -2.09 (1.87) | (-3.43 to -0.75) | 0.0064 |
| Body fat mass (kg) | 26.25 (4.80) | 24.60 (4.93) | -1.65 (1.65) | (-2.83 to -0.47) | 0.0116 |
| Skeletal muscle mass (kg) | 28.41 (6.92) | 28.21 (7.10) | -0.20 (0.57) | (-0.61 to 0.21) | 0.2972 |
| Total body water (L) | 37.76 (8.23) | 37.43 (8.42) | -0.33 (0.82) | (-0.92 to 0.26) | 0.2339 |
| BMI (kg/m^2) | 29.09 (2.50) | 28.28 (2.55) | -0.81 (0.70) | (-1.31 to -0.31) | 0.0052 |
| Uric acid (mg/dL) | 6.15 (1.27) | 5.09 (0.90) | -1.06 (0.75) | (-1.60 to -0.52) | 0.0016 |

Note: The laboratory reference range of each parameters: ALT (male) 10–42 U/L, ALT (female) 7–23 U/L, AST (male and female) 13–30 U/L; γ -GTP (male) 13–64 U/L, γ -GTP (female) 9–32 U/L.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; FIB-4 index, fibrosis-4 index; γ -GTP, γ -glutamyl transpeptidase; HbA1c, hemoglobin A1c; LSM, liver stiffness measurement.

This may have been due to a lack of statistical power. Canagliflozin significantly improved the hepatic fibrosis markers such as the FIB-4 index and the FM-fibro index, suggesting the possibility of improving hepatic fibrosis.

Conversely, NAFIC score, LSM, and CAP did not change; improvement in these parameters may not be detected during short-term treatments. Improvement in several hepatic function/fibrosis markers (ALT, AST, FIB-4 index) was

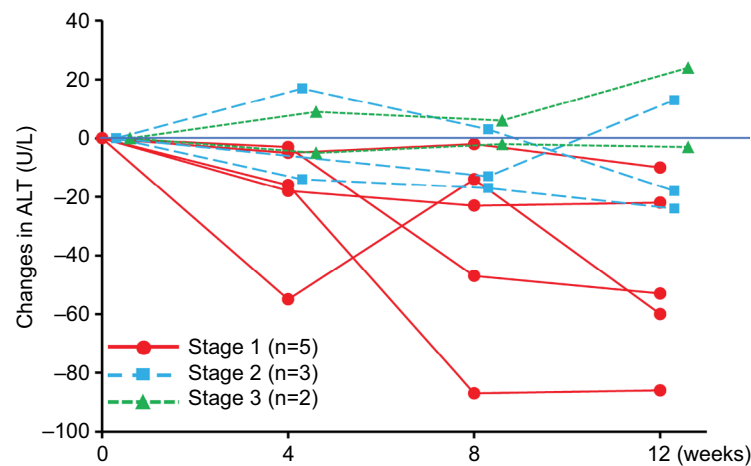


Figure 2 Time course of changes in ALT levels from baseline in individual subjects.

Note: The degree of ALT-lowering effect tended to be greater in the stage 1 group than in the stage 2/3 group.

Abbreviation: ALT, alanine aminotransferase.

consistent with previous reports involving SGLT2 inhibitors,^{20,21} except the FM-fibro index, a new fibrosis marker,²⁴ with the present study being the first reporting its use in drug evaluation. Although accuracy of FM-fibro index for diagnosing NASH fibrosis had been evaluated in a pilot²⁶ or multicenter studies,²⁷ the drug evaluation data is also important for characterization of FM-fibro index as a biomarker of NASH. In terms of hepatic fibrosis stage, decrease in ALT tended to be greater in the stage 1 group (-46.2 U/L) than in the stage 2/3 group (-1.6 U/L), suggesting greater improvement in hepatic function in the stage 1 group; this may indicate the significance of canagliflozin treatment in early-stage NASH patients.

In addition to reduction in ALT, improvement in HbA1c²⁸ and body weight reduction²⁹ are also important for preventing NASH progression. Adipose tissue inflammation is also associated with NASH progression³⁰; canagliflozin reduced fat mass, which may lead to inhibition of inflammatory cytokine release from adipocytes.³¹ The effects of SGLT2 inhibitors on these factors may contribute to prevention of NASH progression.

NAFLD/NASH with diabetes increases the risk of developing cardiovascular disease and serious hepatic diseases, particularly NASH, cirrhosis, and hepatocellular carcinoma.^{32,33} Moreover, malignant neoplasia is the leading cause of death and liver cancer is the second leading cause of cancer death in Japanese DM patients.³⁴ Therefore, interventions using SGLT2 inhibitors in T2DM patients with early-stage NASH may be important to prevent cardiovascular disease and liver cancer.

The limitations of this study were the small sample size, the short-term treatment period, and the lack of a control group.

Conclusion

Canagliflozin is effective and well-tolerated in patients with T2DM and NASH; canagliflozin may be especially useful for those in early stages of NASH. A long-term, large-scale, randomized control study is necessary to confirm the benefit of SGLT2 inhibitor treatment in patients with T2DM and NASH.

Data sharing statement

The datasets generated and/or analyzed during the current study are not publicly available because of lack of agreement for disclosing individual raw data in public but are available from the corresponding author on reasonable request.

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Author contributions

Y Seko, TN and YI contributed to the study design, data collection and analysis. Y Sumida, TO, MK, HI, and TH contributed to the study design. All authors contributed to data interpretation, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

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Supplementary materials

Table S1 Percent change of body composition after canagliflozin treatment at week 12 (n=10)

| | Percent changes from week 0 (%) | | Paired t-test (P-value) |
|----------------------|---------------------------------|-------------------|-------------------------|
| | Mean (SD) | 95% CI | |
| Body weight | -2.70 (2.60) | (-4.57 to -0.91) | 0.0081 |
| Body fat mass | -6.50 (7.20) | (-11.70 to -1.38) | 0.0186 |
| Skeletal muscle mass | -0.90 (2.10) | (-2.38 to 0.64) | 0.2235 |
| Total body water | -1.00 (2.30) | (-2.64 to 0.62) | 0.1950 |

Table S2 Effects of canagliflozin on other efficacy end points (n=10)

| | Week 0, Mean (SD) | Week 12, Mean (SD) | Changes from week 0 | | Paired t-test (P-value) |
|-------------------------------------|-------------------|--------------------|---------------------|---------------------|-------------------------|
| | | | Mean (SD) | 95% CI | |
| Secondary end points | | | | | |
| ALP (U/L) | 251.8 (42.2) | 243.7 (54.3) | -8.1 (30.8) | (-30.1 to 13.9) | 0.4274 |
| Hyaluronic acid (ng/mL) | 76.5 (52.0) | 67.1 (46.6) | -9.4 (25.4) | (-27.6 to 8.8) | 0.2725 |
| Fasting serum insulin (μ U/mL) | 13.675 (6.759) | 14.945 (6.626) | 1.270 (6.380) | (-3.294 to 5.834) | 0.5447 |
| Serum C-peptide (ng/mL) | 2.615 (0.581) | 2.709 (0.859) | 0.094 (0.769) | (-0.456 to 0.644) | 0.7081 |
| Total cholesterol (mg/dL) | 207.9 (31.9) | 205.6 (34.4) | -2.3 (17.0) | (-14.5 to 9.9) | 0.6792 |
| HDL cholesterol (mg/dL) | 49.6 (13.7) | 50.5 (12.0) | 0.9 (6.3) | (-3.6 to 5.4) | 0.6602 |
| LDL cholesterol (mg/dL) | 139.0 (33.7) | 136.3 (34.3) | -2.7 (15.2) | (-13.6 to 8.2) | 0.5877 |
| Triglyceride (mg/dL) | 133.0 (44.6) | 145.3 (50.9) | 12.3 (42.3) | (-18.0 to 42.6) | 0.3818 |
| Waist circumference (cm) | 98.75 (5.91) | 96.85 (5.82) | -1.90 (3.96) | (-4.74 to 0.94) | 0.1639 |
| Exploratory end points | | | | | |
| Ferritin (ng/mL) | 270.75 (243.54) | 157.58 (151.90) | -113.17 (103.43) | (-187.16 to -39.18) | 0.0072 |
| Glucagon (pg/mL) | 200.1 (61.9) | 255.3 (127.9) | 55.2 (77.0) | (0.1 to 110.3) | 0.0496 |

Abbreviations: ALP, alkaline phosphatase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table S3 Adverse events

| | n (%) |
|--|----------|
| All adverse events | 5 (50.0) |
| Gastrointestinal disorders | 1 (10.0) |
| Abdominal discomfort | 1 (10.0) |
| General disorders and administration site conditions | 2 (20.0) |
| Facial pain | 1 (10.0) |
| Oedema | 1 (10.0) |
| Pyrexia | 1 (10.0) |
| Renal and urinary disorders | 2 (20.0) |
| Haematuria | 1 (10.0) |
| Nocturia | 1 (10.0) |
| Pollakiuria | 1 (10.0) |
| Reproductive system and breast disorders | 1 (10.0) |
| Pruritus genital | 1 (10.0) |

Abbreviation: n, number of patients who experienced adverse events.

Table S4 Laboratory variables (n=10)

| | Week 0 | Week 12 | Changes from week 0 |
|---|---------------|----------------|----------------------------|
| SBP (mmHg) | 141.0 (15.9) | 130.8 (6.8) | -10.2 (15.7) |
| DBP (mmHg) | 85.8 (17.5) | 78.1 (9.0) | -7.7 (12.2) |
| Hb (g/dL) | 15.13 (1.55) | 15.84 (1.19) | 0.71 (0.89) |
| Ht (%) | 44.98 (4.46) | 47.73 (3.35) | 2.75 (2.93) |
| Blood ketone body fraction | | | |
| Acetoacetate ($\mu\text{mol/L}$) | 35.9 (20.5) | 41.8 (29.6) | 5.9 (27.5) |
| 3-Hydroxybutyric acid ($\mu\text{mol/L}$) | 69.9 (50.6) | 81.6 (60.2) | 11.7 (55.5) |
| Total ketone bodies ($\mu\text{mol/L}$) | 105.8 (70.5) | 123.4 (88.8) | 17.6 (81.7) |

Note: Data are expressed as mean (SD).

Abbreviations: Hb, hemoglobin; Ht, hematocrit.

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