ORIGINAL ARTICLE

Combination of tofogliflozin and pioglitazone for NAFLD: Extension to the ToPiND randomized controlled trial

Masato Yoneda[1] | **Takashi Kobayashi[1]** | **Yasushi Honda[1]** | **Yuji Ogawa[2]** |
Takaomi Kessoku[1] | **Kento Imajo[3]** | **Asako Nogami[1]** | **Masataka Taguri[4]** |
Hiroyuki Kirikoshi[5] | **Satoru Saito[1]** | **Atsushi Nakajima[1]**

[1]Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

[2]Gastroenterology Division, National Hospital Organization Yokohama Medical Center, Yokohama, Japan

[3]Department of Gastroenterology, Shin-yurigaoka General Hospital, Kawasaki, Japan

[4]Department of Data Science, Yokohama City University School of Data Science, Yokohama, Japan

[5]Laboratory of Physiology, Yokohama City University Hospital, Yokohama, Japan

Correspondence
Masato Yoneda, Department of Gastroenterology and Hepatology, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan.
Email: yoneda@yokohama-cu.ac.jp

Funding information
Kowa Company

Abstract

The incidence of nonalcoholic fatty liver disease (NAFLD) has recently increased and is related to obesity and the associated surge in type 2 diabetes mellitus (T2DM) and metabolic syndromes. This trial follows up on our previous work and forms part of the ToPiND study. We aimed to combine tofogliflozin and pioglitazone treatment for hepatic steatosis in patients with NAFLD and T2DM. In this open-label, prospective, single-center, randomized clinical trial, patients with NAFLD with T2DM and a hepatic fat fraction of ≥10% were assessed based on magnetic resonance imaging proton density fat fraction. Eligible patients received either 20 mg tofogliflozin or 15−30 mg pioglitazone orally, once daily for 24 weeks, followed by combination therapy with both medicines for an additional 24 weeks. The effects on diabetes mellitus and hepatic steatosis were examined at baseline and after the completion of monotherapy and combination therapy. Thirty-two eligible patients received the combination therapy of tofogliflozin and pioglitazone. The combination therapy showed additional improvement in glycated hemoglobin compared with each monotherapy group and showed improvement in steatosis, hepatic stiffness, and alanine aminotransferase levels compared with the tofogliflozin monotherapy group. Pioglitazone monotherapy–mediated increase in body weight decreased following concomitant use of tofogliflozin. The combination therapy resulted in lower triglyceride, higher high-density lipoprotein cholesterol, higher adiponectin, and higher ketone body levels. *Conclusion*: In addition to the additive effects of tofogliflozin and pioglitazone in patients with T2DM and NAFLD, combination therapy was suggested to reduce weight gain and induce cardioprotective effect. Further studies with more patients are needed to investigate the combination therapy of various drugs.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease with a prevalence rate of 25.2% globally, 29.6% in Asia, and 25.5% in Japan.[1–3] NAFLD is a reflection of adipose tissue dysfunction and insulin resistance and is associated with other risk factors for metabolic syndrome.[1,4–7]

NAFLD is a liver-specific disease and a mediator of several other diseases. Previous data support a bidirectional association between NAFLD and type 2 diabetes mellitus (T2DM). Several studies have consistently reported that individuals with NAFLD are at a higher risk of developing T2DM.[8,9] Additionally, patients with T2DM frequently acquire NAFLD and are at a higher risk of developing nonalcoholic steatohepatitis (NASH) than those without diabetes.[10]

In the last three decades, along with obesity and T2DM, NAFLD has been the only liver disease with a high prevalence rate.[11] The current cornerstones of NASH therapy include lifestyle interventions, such as caloric restriction and exercise, which can be difficult to achieve and maintain, underscoring the dire need for pharmacotherapy. However, there are no pharmacotherapies approved for NASH by the Federal Drug Administration or the European Medicines Agency.

The development of drugs for the treatment of NASH can broadly be divided into two approaches: One approach is to divert and use existing drugs approved for other diseases or adopt a drug repositioning/repurposing strategy in which clinical trial–tested drugs that are known to have a basic safety profile are used. Among them, antidiabetic drugs are the most studied because of the similarity in pathogenesis of diabetes with NAFLD.[12] The other approach is to initiate research and development of new drugs that can effectively treat NASH/NAFLD. Several drugs for the treatment of NASH/NAFLD are in phase 2 and 3 trials but are still far away from approval.[13]

Currently, international guidelines for NAFLD/NASH recommended pioglitazone, a peroxisome proliferator-activated receptor gamma agonist, as an antidiabetic agent for NASH associated with diabetes.[14,15] Glucagon-like peptide-1 receptor agonists and sodium-glucose co-transporter-2 (SGLT-2) inhibitors are actively being investigated as candidates for pharmacotherapy, as recently recommended by clinical practice guidelines from the Japan Society of Gastroenterology and the Japan Society of Hepatology.[16] The effect of the combination of different antidiabetic drugs on NAFLD is still unresolved. These combinations include peroxisome proliferator-activated receptor gamma agonists and glucagon-like peptide-1 receptor agonists; SGLT-2 inhibitors and glucagon-like peptide-1 receptor agonists; and glucagon-like peptide-1 receptor agonist and pioglitazone. In the present study, we investigated the effectiveness of combination therapy with tofogliflozin and pioglitazone, compared with that of monotherapy, for the treatment of NAFLD in patients with T2DM (ToPiND study). This study is part of the ToPiND study, in which we already investigated the effect of tofogliflozin and pioglitazone on NAFLD.[17,18] With this study, we are assessing whether the combination of the two drugs is more efficacious than either one on its own.

RESEARCH DESIGN AND METHODS

Study design and patients

The study design, study schedule, and outcomes of the ToPiND study have been previously described in detail.[17] A previous study compared the therapeutic effects of pioglitazone and tofogliflozin monotherapy in patients with NAFLD with T2DM within the first 24 weeks.[18] The current study constituting the second half of the ToPiND study was an open-label, prospective, single-center, randomized study that investigated the effectiveness of the combination of tofogliflozin and pioglitazone in treating hepatic steatosis in patients with NAFLD and T2DM for 24 weeks after the 24th week of monotherapy completion.[17] The study protocol complied with the principles of the Declaration of Helsinki and the Ethics Guidelines for Clinical Research published by the Ministry of Health, Labor, and Welfare of Japan. The study protocol was approved before the initiation of the study by the Yokohama City University Certified Institutional Review Board on October 19, 2017 (approval number: CRB318007). This trial has been registered in the Japan Registry of Clinical Trials (jRCTs031180159). Trial results were reported in accordance with the guidelines provided by the Consolidated Standards of Reporting Trials 2010.

The flowchart of the study is shown in Figure S1. Eligible patients were within the age group of 20–74 years with NAFLD and T2DM. Key inclusion criteria were as follows: glycated hemoglobin (HbA1c) levels ≥6.5%, alanine transaminase (ALT) levels beyond the institutional standard level (42 IU/L for men and 23 IU/L for women), and liver fat values ≥10% on magnetic resonance imaging proton density fat fraction (MRI-PDFF) at baseline. Patients were excluded if they used other medications like SGLT-2 inhibitors, thiazolidinedione, insulin, glucagon-like peptide-1 receptor agonists, or vitamin E. In principle, changes in the T2DM medication, like adding, discontinuing, or changing the dosage, were not allowed throughout the study period. The present open-label, prospective, randomized study included 40 adult patients from the Yokohama City University Hospital. The method for calculating the sample size was described in a previous paper.[18] Between March 30, 2018, and December 13, 2019, 42 patients were screened, and 40 eligible patients were randomly

assigned to receive tofogliflozin 20 mg (*n* = 21) or pioglitazone 15–30 mg (*n* = 19) as monotherapy for the first 24 weeks. Patients who completed 24 weeks of monotherapy and met the inclusion criteria were studied for an additional 24 weeks to determine the effects of the combination therapy. The combination therapy was initiated if HbA1c was 6.0% or higher (6.5% or higher if aged 65 years or older and on sulfonylurea) at the end of 24 weeks of monotherapy. Of the patients who received tofogliflozin and pioglitazone as the first monotherapy, 20 and 12 patients (total of 32 patients) received combination therapy, respectively. All patients provided written, informed consent before participating in the study.

Randomization

As previously stated, randomization was performed during the allocation of pioglitazone and tofogliflozin monotherapy in the first 24 weeks.[17,18] Briefly, the principal investigator or co-investigator completed the patient enrollment form for eligible patients (primary registration); then, patients with ≥10% liver fat content on MRI-PDFF were randomly assigned to the tofogliflozin or pioglitazone groups, stratified by HbA1c levels (<7%, ≥7.0%), ALT levels (<50 IU/L, ≥50 IU/L), and MRI-PDFF values (<20%, ≥20%) (definitive registration). Eligible patients were randomly assigned equally (1:1) to receive 20 mg tofogliflozin or 15–30 mg pioglitazone orally, once daily. Randomization was performed using a computer-generated, centrally administered procedure, using a minimization method. Patients were allocated to each treatment group through the central registration system, and both the physician and patient were aware of the allocated treatment. Patients with HbA1c ≥ 6.0% (≥6.5% in those older than 65 years, and those using sulfonylurea or rapid insulin secretagogue) after 24 weeks of monotherapy received combination of tofogliflozin and pioglitazone for another 24 weeks.

Procedures

After randomization, physical examinations and blood tests were performed (with fasting for 8 h) before and at 12, 24, 36, and 48 weeks after drug administration. The diet was standardized according to the guidelines provided by a comprehensive lifestyle approach.[14] MRI-PDFF and magnetic resonance elastography liver stiffness measurements (MRE-LSM) were performed at baseline, at 24 weeks (end of monotherapy), and at 48 weeks (end of combination therapy). MRI-PDFF and MRE-LSM results were interpreted by an independent, blinded, liver specialist according to a method reported previously.[19] Magnetic resonance imaging was performed using standardized instruments at high field strength (Discover MR750 3.0 T; GE Healthcare, Japan) without oral or intravenous contrast.

Outcomes

The primary endpoint of the change in hepatic steatosis was evaluated using MRI-PDFF at 0 weeks (before treatment), 24 weeks (end of monotherapy with tofogliflozin or pioglitazone), and 48 weeks (end of combination therapy with tofogliflozin and pioglitazone). The secondary endpoints were changes in MRI-LSM, HbA1c, fasting glucose, homeostasis model assessment–estimated insulin resistance, body weight, serum ALT levels, lipid panel (i.e., total cholesterol, low-density lipoprotein cholesterol, triglycerides, and high-density lipoprotein cholesterol), and hepatic fibrosis markers (type IV collagen 7S and Wisteria floribunda agglutinin–positive Mac-2 binding protein glycosylation isomers), cytokeratin-18 fragment M30, adiponectin, ketone body fraction (acetoacetic acid and 3-hydroxybutyric acid), and urinary 8-hydroxydeoxyguanosine.

Body weight, serum ALT, fasting glucose, and HbA1c levels were measured at 0, 12, 24, 36, and 48 weeks. The homeostasis model assessment–estimated insulin resistance, lipid panel, and uric acid levels were measured at the Yokohama City University Hospital at weeks 0, 24, 48. Fibrosis markers (type IV collagen 7S and Wisteria floribunda agglutinin–positive Mac-2 binding protein glycosylation isomers), urinary 8-hydroxydeoxyguanosine, adiponectin, and ketone body fraction (acetoacetic acid and 3-hydroxybutyric acid) were measured at a local laboratory (SRL, Tokyo, Japan) at weeks 0, 24, and 48.

Adverse events

Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. All AEs that occurred during the study were recorded in a case report form, which included information regarding the symptom/disease, its onset and end date, severity and seriousness, investigator's opinion regarding the association with tofogliflozin/pioglitazone treatment, action taken regarding tofogliflozin/pioglitazone use, treatment provided for AE, cause of the event, and resolution/outcome. AEs were followed up until normalization was achieved. In cases in which the AEs were irreversible, follow-up was performed until the symptoms were stable. If the investigator or co-investigator judged the AE as recovered or considered it to have no association with the study outcomes, it was not followed up and the reasons were mentioned in the medical records.

Criteria for discontinuation of treatment

Treatment was discontinued if the HbA1c level increased by >1.0% after initiation of medication, aspartate transaminase or ALT levels increased by >3-fold of that before medication, the patient became ineligible for the trial, medication compliance was <75%, continuous medical examination became challenging due to severe AEs, and in case of pregnancy. Treatment was also discontinued if the participant requested discontinuation, if the medication had to be combined with prohibited medicine because of deterioration of the primary disease or its complications, or if the doctor decided that discontinuation was appropriate for other reasons.

Statistical analyses

Per protocol, set analyses were performed to evaluate the primary and secondary endpoints. Those who seriously violated the research plan (e.g., those who did not provide consent and those with registration dates beyond the registration period) were excluded. During per protocol set analysis, we excluded patients with serious violations of inclusion and exclusion criteria, patients using prohibited drugs, and those with a compliance rate of >120% or <75%. Data from the remaining patients were analyzed.

The patients who had registered in the study started treatment as assigned, and those who underwent part of or all treatments comprised the target population for safety analysis. The statistical significance of the changes between the groups was evaluated for the endpoints. The chi-square test was used for comparisons between different groups, and the paired t test was used for comparisons between the same groups, with $p < 0.05$ considered significant. Medical statisticians developed a statistical analysis plan and specified the details of the statistical methods used for handling the data. The plan was prepared before data collection. John's Macintosh Project Pro version 15.0 was used for all analyses.

RESULTS

Patient baseline characteristics

Figure 1 shows an outline of the trial. Table 1 lists the baseline (before receiving monotherapy) characteristics of the 32 patients who eventually received the combination therapy. The baseline (0 week) mean ± SD of age, HbA1c, and body mass index (BMI) before monotherapy age were 58.5 ± 10.7 years, 7.27 ± 0.80%, and 30.3 ± 5.04 kg/m^2, respectively. The mean ± SD of HbA1c and BMI before combination therapy (24 weeks) were 6.78 ± 0.73% and 29.9 ± 5.07 kg/m^2, respectively.
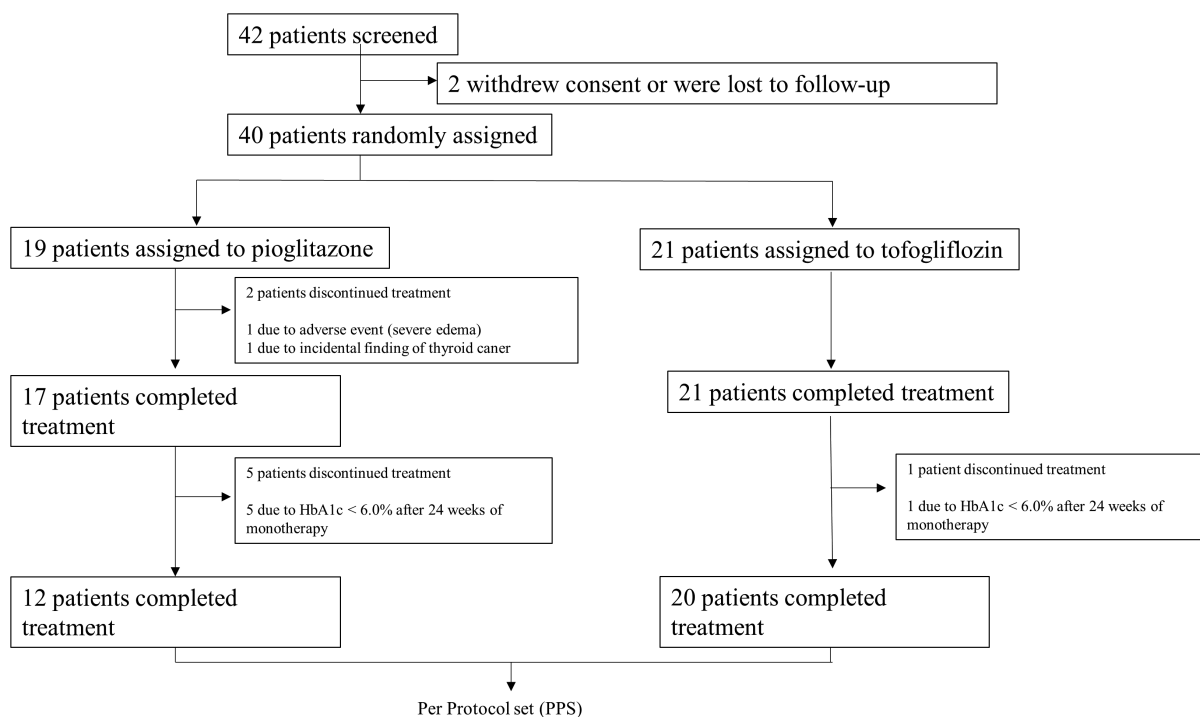
FIGURE 1   Outline of the trial

TABLE 1 Baseline characteristics of the patients

	Initial treatment before combination therapy		
	Tofogliflozin group (*n* = 20)	Pioglitazone group (*n* = 12)	*p* value
Age (years)	58.6±12.5	58.3±7.5	0.9305
Men, *n* (%)	12 (60)	6 (50)	0.5810
Weight (kg)	77.9±15.8	82.9±15.5	0.3885
BMI (kg/m^2)	29.6±4.9	31.5±5.3	0.3111
Waist circumference (cm)	100.8±10.3	103.7±11.4	0.4625
Albumin (g/dl)	4.45±0.26	4.46±0.22	0.8818
AST (IU/ml)	53.0±23.1	70.1±43.5	0.1556
ALT (IU/ml)	82.2±39.7	82.2±22.1	0.9989
ALP (IU/ml)	252.3±70.7	286.5±88.0	0.2356
GGT (IU/ml)	64.9±26.0	103.8±134.5	0.2145
CHE (IU/ml)	382.6±40.4	388.3±45.4	0.7106
Creatinine (mg/dl)	0.74±0.17	0.75±0.16	0.9009
eGFR (ml/min/1.73 m^2)	78.3±15.8	73.7±9.4	0.3625
FPG (mg/dl)	144.1±51.5	151.1±46.8	0.7016
HbA1c (%)	7.24±0.90	7.33±0.64	0.7434
HOMA-IR	6.53±4.32	7.26±5.06	0.6710
Total cholesterol (mg/dl)	190.6±33.0	190.8±27.0	0.9801
Triglycerides (mg/dl)	153.0±64.9	150.7±62.6	0.9213
HDL cholesterol (mg/dl)	56.7±12.3	56.7±16.8	0.9949
LDL cholesterol (mg/dl)	112.6±28.2	112.3±26.0	0.9829
Systolic blood pressure (mmHg)	133.2±10.8	143.0±23.5	0.1173
Diastolic blood pressure (mmHg)	84.5±11.4	88.3±15.5	0.4017
Uric acid (mg/dl)	5.74±1.06	5.62±0.94	0.7425
Platelet count (×10^3/μl)	251.2±61.0	238.8±72.5	0.6077
MRI-PDFF (%)	17.0±5.6	16.5±6.0	0.8347
MRE-LSM (kPa)	3.01±0.85	3.57±1.56	0.1974
WFA$^+$-M2BP (COI)	0.80±0.41	1.03±0.59	0.1989
Type IV collagen 7S (ng/ml)	4.36±1.03	4.17±1.47	0.6739
Uric 8-OHdG (ng/ml)	18.2±10.0	17.2±11.1	0.7893
Adiponectin (μg/ml)	5.08±3.26	7.03±3.04	0.1047
Acetoacetic acid (μmol/L)	32.5±22.9	35.6±21.0	0.7066
3-Hydroxybutyric acid (μmol/L)	64.3±50.2	67.8±70.1	0.8675
Ketone body (μmol/L)	96.8±72.6	103.4±88.7	0.8186
CK18 fragment M30 (U/L)	719.3±520.1	668.7±370.9	0.7511
Medication of diabetes (*n*)			
Metformin	8	4	
DPP-4 inhibitor	7	3	
Sulfonylurea	4	1	
Alpha-GI	1	0	

Note: Data are presented as mean±SD; *p<0.05.

Abbreviations: 8-OHdG, 8-hydroxy-deoxyguanosine; ALP, alkaline phosphatase; AST, aspartate transaminase; BMI, body mass index; CK-18, cytokeratin 18; CHE, cholinesterase; COI, cutoff index; DPP-4, dipeptidyl peptidase-4; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; GI, glucosidase inhibitor; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LSM, liver stiffness measurement.

Changes in intrahepatic fat content and liver tests

The effect in hepatic steatosis after 24 weeks of monotherapy with tofogliflozin or pioglitazone, and combination therapy with tofogliflozin and pioglitazone, were evaluated using MRI-PDFF at 0, 24, and 48 weeks (Table 2, Figure 2A). The 24-week tofogliflozin or pioglitazone monotherapy showed an improvement in MRI-PDFF of −3.38 ± 4.90% (p = 0.0061) and −5.56 ± 3.92% (p = 0.0005), respectively. The combination therapy with tofogliflozin and pioglitazone, 24 weeks after monotherapy, showed an improvement in MRI-PDFF of −5.98 ± 4.70% from baseline (p < 0.0001) (Figure 2A). The combination therapy further improved MRI-PDFF by −2.60% and −0.42%, when pioglitazone was added to initial treatment with tofogliflozin and when tofogliflozin was added to initial treatment with pioglitazone, respectively. When tofogliflozin was used as the first-line therapy and pioglitazone was subsequently added, the combination therapy showed a significant additional improvement over monotherapy (Figure 2B).

Changes in ALT levels were measured at 0, 12, and 24 weeks of the monotherapy, and at 36 and 48 weeks of the combination therapy. The tofogliflozin monotherapy, pioglitazone monotherapy, and combination therapy showed an improvement in ALT levels of −19.3 ± 34.5 IU/L (p = 0.0219), −34.0 ± 21.0 IU/L (p = 0.0002), and −35.7 ± 29.9 IU/L (p < 0.0001) from baseline, respectively (Figure 2C). When tofogliflozin was used as first-line therapy and pioglitazone was subsequently added, the combination therapy showed a significant additional improvement over monotherapy in ALT levels (Table 2, Figure 2D).

Changes in liver stiffness and hepatic fibrosis markers

The effect on liver stiffness measurement after 24 weeks of monotherapy with tofogliflozin or pioglitazone, and combination therapy with tofogliflozin and pioglitazone, were evaluated using magnetic resonance elastography at 0, 24, and 48 weeks (Table 2, Figure 3A,B). The 24-week tofogliflozin or pioglitazone monotherapy showed MRE-LSM of −0.11 ± 0.42 kPa (p = 0.2380) and −0.43 ± 0.61 kPa (p = 0.00364), respectively. The combination therapy of pioglitazone and tofogliflozin after 24 weeks that followed monotherapy showed MRE-LSM of −0.40 ± 0.54 kPa (p = 0.0002) (Table 2, Figure 3A). The monotherapy with tofogliflozin for 24 weeks did not significantly reduce MRE-LSM, while pioglitazone monotherapy or combination therapy with pioglitazone showed a significant reduction in MRE-LSM (Table 2, Figure 3B).

Wisteria floribunda agglutinin−positive Mac-2 binding protein showed significant improvement during monotherapy with tofogliflozin at 24 weeks, pioglitazone at 24 weeks, and combination therapy with pioglitazone and tofogliflozin (−0.09 ± 0.15, −0.20 ± 0.24, and −0.19 ± 0.18), respectively (Table 2, Figure 3C). Type IV collagen 7S showed no improvement during monotherapy with tofogliflozin or pioglitazone at 24 weeks but was significantly improved during the combination therapy with tofogliflozin and pioglitazone (−041 ± 0.95 ng/ml; p = 0.0193) (Table 2, Figure 3D).

Changes in body weight, glycemic and metabolic parameters

There was a contrast in weight change during treatments with tofogliflozin and pioglitazone. Compared with the baseline measurement, the body weight decreased significantly by −3.25 ± 3.34 kgs (−4.14% from baseline) in the tofogliflozin group (p = 0.0004) and increased significantly by 2.46 ± 3.67 kgs (+3.34% from baseline) in the pioglitazone group after 24 weeks of treatment (p = 0.0341) (Figure 3A). After the combination therapy with tofogliflozin and pioglitazone, the weight change was −0.79 ± 4.73 kg from baseline (−0.70 ± 5.91%; p = 0.2306) (Table 2, Figure 4A). The changes in weight every 3 months are shown in Figure 4B.

The main effect on glycemic control after 24 weeks of monotherapy with tofogliflozin or pioglitazone, and combination therapy with tofogliflozin and pioglitazone, were evaluated using HbA1c values. Twenty-four weeks of tofogliflozin or pioglitazone monotherapy showed HbA1c of −0.36 ± 0.47% (p = 0.0027) and −0.73 ± 0.75% (p = 0.0014), respectively. The combination therapy with tofogliflozin and pioglitazone after 24 weeks of monotherapy showed a change of −0.80 ± 0.71% from baseline (p < 0.0001) (Table 2, Figure 4C). The changes in HbA1c levels every 3 months are shown in Figure 4D. A decrease in homeostasis model assessment−estimated insulin resistance was observed in the combination therapy group, but no change in homeostasis model assessment−estimated insulin resistance was observed in the tofogliflozin or pioglitazone monotherapy groups (Table 2).

Triglyceride-lowering and high-density lipoprotein−raising effects were observed only in the pioglitazone-treated group, but no effect was observed in the tofogliflozin-treated group (Table 2). In the combination therapy group, triglyceride-lowering and high-density lipoprotein−increasing effects were observed. In contrast, uric acid−lowering effect was observed in the tofogliflozin and the combination therapy group, while

TABLE 2 Changes in several parameters from baseline

| | Monotherapy | | | | Combination therapy Pioglitazone 24 weeks and tofogliflozine 48 weeks (n = 20) Pioglitazone 48 weeks and tofogliflozine 24 weeks (n = 12) | |
| | Tofogliflozin | | Pioglitazone | | | |
	Changes from baseline	p Value	Changes from baseline	p Value	Changes from baseline	p Value
Weight (kg)	−3.25±3.34	0.0004*	2.46±3.67	0.0341*	−0.79±4.73	0.2306
Weight change/basal weight (%)	−4.14±4.33	—	3.34±5.38	—	−0.70±5.91	—
BMI (kg/m²)	−1.00±1.02	0.0003*	0.73±1.04	0.0338*	−0.36±1.32	0.2737
Waist circumference (cm)	−2.05±4.89	0.0762	0.54±5.82	0.3223	−1.08±5.32	0.1814
Albumin (g/dl)	0.14±0.18	0.0031*	0.07±0.17	0.2072	0.05±0.188	0.1207
AST (IU/ml)	−13.8±24.2	0.0195*	−31.2±34.9	0.0102*	−25.2±32.4	0.0001*
ALT (IU/ml)	−19.3±34.5	0.0219*	−34.0±21.0	0.0002*	−35.7±29.9	0.0001*
GGT (IU/ml)	−15.3±26.7	0.0189*	−50.5±94.9	0.0925	−37.4±74.7	0.0081*
CHE (IU/ml)	2.4±35.5	0.7706	−11.2±24.9	0.1482	−10.2±36.6	0.6364
Creatinine (mg/dl)	−0.0005±0.0733	0.9760	0.0133±0.0910	0.6217	0.0081±0.0873	0.6022
eGFR (ml/min/1.73 m²)	0.265±10.2	0.9085	−3.80±8.44	0.1473	−1.52±9.14	0.3513
FPG (mg/dl)	0.85±51.8	0.9423	−21.8±48.6	0.1497	−25.0±38.2	0.0008*
HbA1c (%)	−0.36±0.47	0.0027*	−0.73±0.75	0.0014*	−0.80±0.71	0.0001*
HOMA-IR	0.23±7.05	0.8925	−2.00±5.81	0.2810	−3.12±3.64	0.0002*
Total cholesterol (mg/dl)	8.6±39.3	0.3399	0.23±14.3	0.8436	9.47±35.2	0.1380
Triglycerides (mg/dl)	3.3±66.3	0.8262	−48.3±51.4	0.0077*	−24.5±48.2	0.0073*
HDL cholesterol (mg/dl)	2.90±6.46	0.0590	8.83±6.59	0.0007*	8.28±6.75	0.0001*
LDL cholesterol (mg/dl)	−0.50±33.4	0.9473	−3.08±11.2	0.3589	3.75±31.6	0.5078
Uric acid (mg/dl)	−0.90±0.98	0.0007*	−0.04±0.65	0.8287	−0.88±1.03	0.0001*
Platelet count (×10³/µl)	−1.6±24.5	0.7802	−10.6±18.9	0.0780	−6.3±20.8	0.0970
MRI-PDFF (%)	−3.38±4.90	0.0061*	−5.56±3.92	0.0005*	−5.98±4.70	0.0001*
MRE-LSM (kPa)	−0.11±0.42	0.2380	−0.43±0.61	0.0364*	−0.40±0.54	0.0002*
WFA⁺-M2BP (COI)	−0.09±0.15	0.0166*	−0.20±0.24	0.0136*	−0.19±0.18	0.0001*
Type IV collagen 7S (ng/ml)	−0.22±0.93	0.3048	−0.02±0.84	0.9461	−0.41±0.95	0.0193*
Uric 8-OHdG (ng/ml)	−8.93±9.58	0.0007*	−6.608±10.00	0.0428*	−4.86±12.00	0.0309*
Adiponectin (µg/ml)	0.40±0.63	0.0107*	7.21±5.12	0.0005*	5.45±3.90	0.0001*
Acetoacetic acid (µmol/L)	33.7±76.6	0.0643	−2.58±28.8	0.7620	17.63±40.3	0.0190*
3-Hydroxybutyric acid (µmol/L)	74.4±163.3	0.0559	6.91±73.3	0.7499	56.7±118.5	0.0109*
Ketone body (µmol/L)	108.0±239.1	0.0577	4.33±100.5	0.8840	74.3±157.5	0.0120*
CK18 fragment M30 (U/L)	−249.2±690.5	0.1442	−252.1±305.7	0.0156*	−377.5±508.2	0.0005*

Note: Data are presented as mean±SD.

*p<0.05.
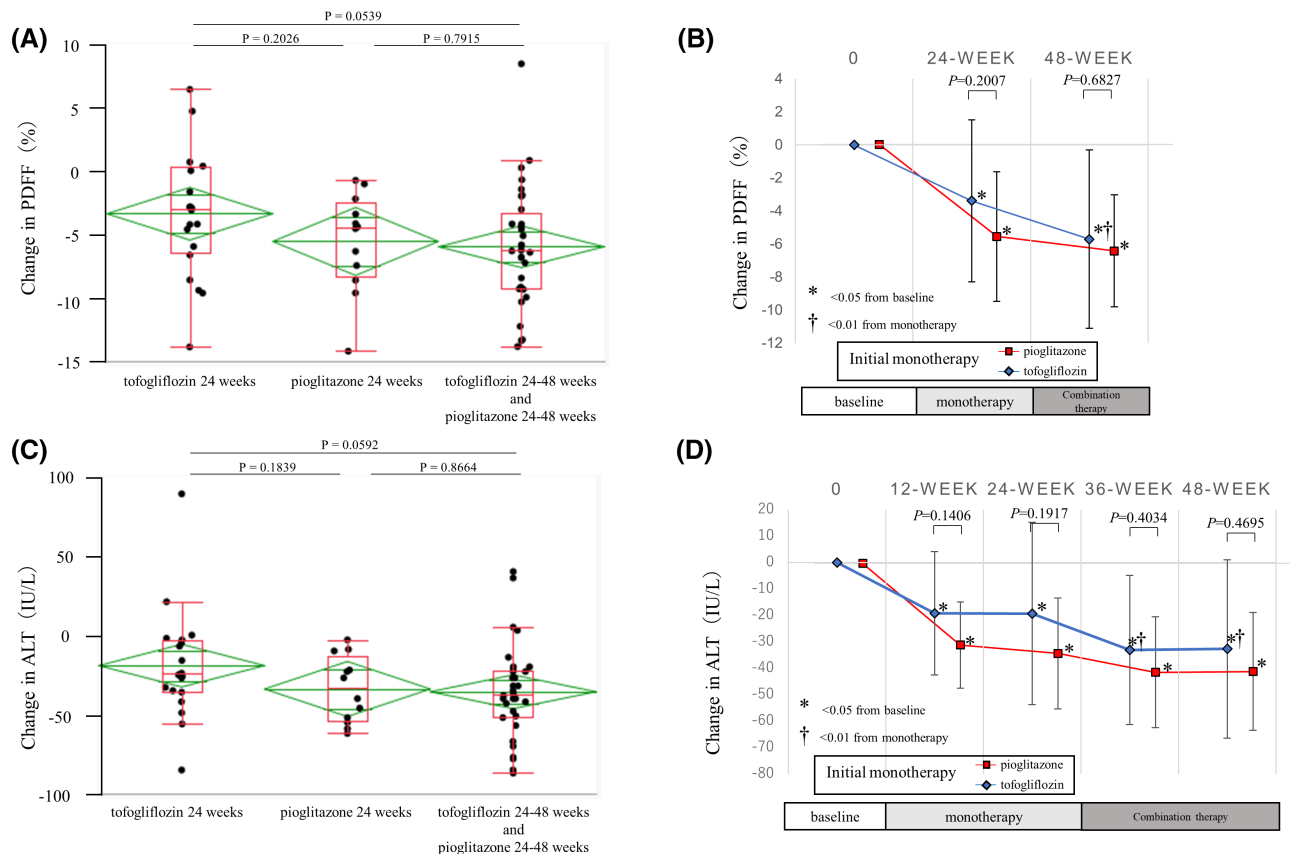
FIGURE 2 Changes in intrahepatic fat content and liver tests. (A) Magnetic resonance imaging proton density fat fraction (MRI-PDFF) changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination of tofogliflozin and pioglitazone. (B) Change in MRI-PDFF after 24 weeks and 48 weeks of monotherapy and combination therapy, listed by initial monotherapy. (C) Alanine transaminase (ALT) level changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination of tofogliflozin and pioglitazone. (D) Change in ALT levels after 12 weeks (monotherapy), 24 weeks (monotherapy), 36 weeks (combination therapy), and 48 weeks (combination therapy), listed by initial monotherapy

no therapeutic effect was observed in the pioglitazone group (Table 2).

Changes in oxidative stress, adiponectin, cytokeratin −18 fragment, and pro-ketogenic markers

The urinary 8-hydroxydeoxyguanosine level, a marker of oxidative stress, decreased significantly in both tofogliflozin and pioglitazone groups (−8.93 ng/ml and −6.61 ng/ml, respectively). In the combination therapy group, the 8-hydroxydeoxyguanosine levels were also significantly decreased (Table 2). Adiponectin, a key adipocytokine in metabolic syndrome, increased significantly in both tofogliflozin and pioglitazone groups (+0.40 and +7.21 mg/ml, respectively). An increase in adiponectin levels was also observed in the combination therapy group (Table 2).

The cytokeratin-18 fragment M30 antigen, which correlates with the magnitude of hepatocyte apoptosis and independently predicts the presence of NASH, decreased significantly in the pioglitazone group

(−252.1 U/L; p = 0.0156) and in the combination therapy group (Table 2).

The ketone bodies, acetoacetate, and 3-hydroxybutyrate levels tended to increase in the tofogliflozin-treated group, although the difference was not significant. These factors were significantly increased in the combination therapy group (Table 2).

Adverse events

In the present study, no AEs were reported during the 24-week combination therapy with tofogliflozin and pioglitazone.

DISCUSSION

In this prospective, randomized trial, we found that the combination therapy with tofogliflozin and pioglitazone could be a more effective and safer pharmacological therapy than monotherapy in patients with NAFLD. We previously reported a head-to-head study, comparing
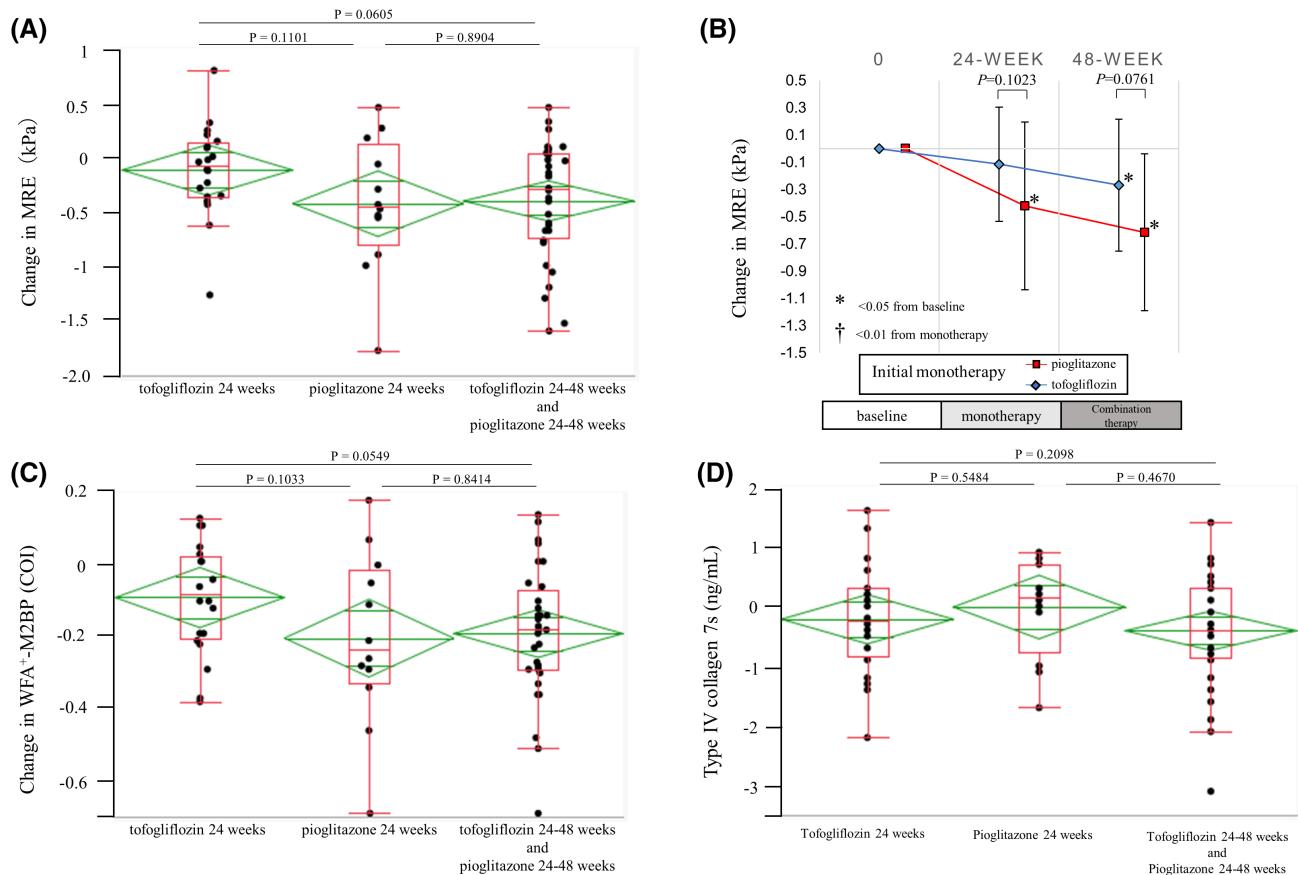
FIGURE 3 Changes in liver stiffness and hepatic fibrosis markers. (A) Magnetic resonance elastography liver stiffness measurements (MRE-LSM) changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination therapy of tofogliflozin and pioglitazone. (B) Change in MRE-LSM after 24 weeks and 48 weeks of monotherapy, and combination therapy, listed by initial monotherapy. (C) Wisteria floribunda agglutinin−positive Mac-2 binding protein (WFA+ −M2BP) changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination therapy of tofogliflozin and pioglitazone. (D) Type IV collagen 7S changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination of tofogliflozin and pioglitazone

the effects of pioglitazone versus tofogliflozin mono-therapies in patients with NAFLD with T2DM[18]; both tofogliflozin and pioglitazone 24-week monotherapies improved hepatic steatosis measured by MRI-PDFF, and no statistically significant difference was observed between the two treatments.[18] The effect of combination therapy with tofogliflozin and pioglitazone resulted in further improvement in HbA1c levels. In addition, pioglitazone was effective in improving hepatic steatosis, as evidenced by MRI-PDFF and decreasing ALT levels. Regarding liver fibrosis, both tofogliflozin and pioglitazone monotherapies showed an improvement in Wisteria floribunda agglutinin−positive Mac-2 binding protein, but no decrease in serum type IV collagen 7S domain. Furthermore, only the pioglitazone monotherapy group showed an improvement in MRE-LSM. In the combination therapy group, all fibrosis markers (Wisteria floribunda agglutinin−positive Mac-2 binding protein and type IV collagen 7S) and MRE-LSM significantly improved compared with those of the baseline. The combination therapy also reduced oxidative stress (urinary 8-hydroxydeoxyguanosine) and cytokeratin-18

fragments, which are thought to be involved in the pathogenesis of NASH. This result is clinically important because hepatic fibrosis is the most important predictor of clinical outcomes in patients with NASH.[20−26]

Pioglitazone is a peroxisome proliferator-activated receptor gamma agonist that is thought to improve NAFLD by reducing the size of hypertrophic adipocytes, improving insulin resistance, and inducing adiponectin expression. In a large randomized controlled trial of 24 weeks of treatment, pioglitazone was found to reduce serum liver enzymes, histological hepatic steatosis, hepatocyte ballooning, and inflammation.[27] At 3-year long-term follow-up, 58% of patients maintained improvement in NASH histologically, and 51% had resolution of NASH.[28] Therefore, the international guidelines for the treatment of NAFLD/NASH recommended pioglitazone as the first-line pharmacological treatment for NAFLD complicated with T2DM.[14,15] However, because of side effects such as weight gain, further verification is required.

SGLT-2 inhibitors inhibit glucose reabsorption in the proximal tubule, resulting in increased glucose
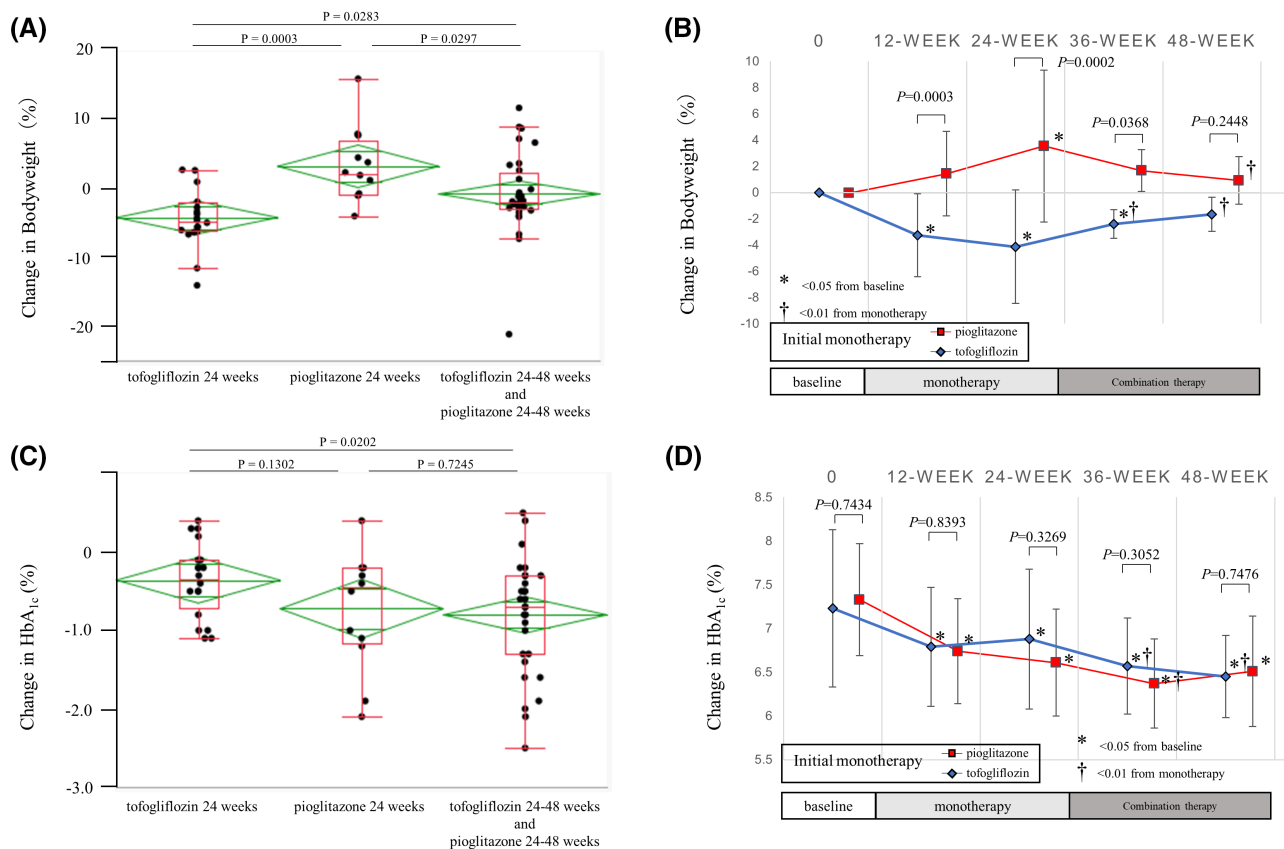
FIGURE 4 (A) Changes in body weight and glycated hemoglobin (HbA1c). Body weight changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination therapy of tofogliflozin and pioglitazone. (B) Changes in body weight after 12 weeks (monotherapy), 24 weeks (monotherapy), 36 weeks (combination therapy), and 48 weeks (combination therapy), listed by initial monotherapy. (C) HbA1c changes compared between baseline and tofogliflozin monotherapy, pioglitazone monotherapy, and combination of tofogliflozin and pioglitazone. (D) Change in HbA1c after 12 weeks (monotherapy), 24 weeks (monotherapy), 36 weeks (combination therapy), and 48 weeks (combination therapy), listed by initial monotherapy

excretion in the urine and decreased glucose levels in the plasma. The resultant glycemia-lowering effect is insulin-independent.[29] Although further research is needed to identify the mechanisms by which SGLT-2 inhibitors affect fatty liver and steatohepatitis, several trials in patients with T2DM have suggested that these drugs may be useful in treating both T2DM and NAFLD or NASH.[18,30–37] The proposed mechanism explaining the action of SGLT-2 inhibitors in NAFLD/NASH is based on the reduction in plasma glucose due to glucosuria, reversal of glucotoxicity, and reduction in circulating insulin and body weight. These collectively result in decreased peripheral and hepatic insulin resistance, and lead to the reduction of *de novo* lipogenesis in the liver.[38]

In this study, in addition to improving hepatic steatosis, fibrosis, and ALT levels, the combination of tofogliflozin and pioglitazone showed some interesting findings. The most important feature of the combination therapy is that the weight-gain effect of pioglitazone is suppressed by tofogliflozin. A recent preclinical study using a mouse model of T2DM showed that a combination of the SGLT-2 inhibitor ipragliflozin and

pioglitazone significantly improved multiple NASH parameters, including hyperglycemia, insulin resistance, hyperlipidemia, and liver injury (hepatic steatosis and fibrosis). Moreover, compared with the pioglitazone-treated group, mice in the combined treatment group showed decreased body and visceral fat weight without affecting food consumption.[39] The combination therapy of tofogliflozin and pioglitazone showed the following effects: lowering triglycerides; increasing high-density lipoprotein, which were significant only for pioglitazone; and lowering uric acid levels, which were observed only for tofogliflozin. In addition, the combination therapy of tofogliflozin and pioglitazone showed an increase in ketone bodies and an improvement in insulin resistance that were not observed or were not significantly different from those after monotherapy.

Cardiovascular disease has been reported as the most important cause of death, followed by non-liver malignancy, and complications of cirrhosis (along with hepatocellular carcinoma and liver transplantation) in patients with NAFLD.[21,40,41] SGLT-2 inhibitors have several other beneficial clinical effects, such as reducing the risk of cardiovascular and renal diseases,

in addition to reducing hyperglycemia.[42,43] The cardiovascular effect of SGLT-2 inhibitors is attributed to their early-onset hemodynamic effects, such as reduced blood pressure, decreased intravascular volume, and reduced aortic stiffness. Pioglitazone has also been shown to exert cardioprotective effects, albeit via mechanisms very different from those of SGLT-2 inhibitors.[44,45] Pioglitazone exerts a robust protective effect against atherosclerosis-driven cardiac and cerebrovascular events. In preclinical and clinical studies, pioglitazone reduced left ventricular systolic and diastolic dimensions and improved left systolic and diastolic functions. Pioglitazone also directly benefits cardiomyocyte electrophysiology, energy metabolism, ischemia–reperfusion injury, cardiac remodeling, neurohormonal activation, and pulmonary circulation. In the present study, the combination of tofogliflozin and pioglitazone showed multiple effects that may act to reduce the cardiovascular disease preventive effects, such as decreasing triglyceride, decreasing uric acid levels, increasing high-density lipoprotein cholesterol, increasing adiponectin, and increasing ketone bodies.[46,47] Thus, it is expected that this combination could result in additive benefits for the prevention of cardiovascular disease compared with monotherapy.

No serious side effects were observed in this study. The fact that no side effects such as edema occurred in 32 patients, 20 of whom were newly induced with pioglitazone, suggests that the side effects may have been offset by the diuretic effect of the SGLT-2 inhibitors.

One of the strengths of this study is that although there have been many articles on the effects of individual diabetes drugs, no studies have prospectively examined combination therapy. In particular, the combination of SGLT-2 inhibitors, glucagon-like peptide-1 analogs, and thiazolidinediones is clinically valuable. In addition, MRI-PDFF to assess fatty deposits in the liver and magnetic resonance elastography to assess fibrosis in the liver, which were used in this study, are the most reliable noninvasive diagnostic methods that currently exist.[19,26]

This study has several limitations. First, the sample size is relatively small. The sample size was originally calculated for a single-agent head-to-head trial, and the final sample size was 32, because of the exclusion of patients who did not require additional drugs when they were transferred to the combination therapy group. Indeed, the potential lack of diversity associated with small sample sizes may have affected our observations, and further research should establish whether our findings are applicable to other patient populations. Second, the duration of drug treatment itself is longer due to the addition of drugs to monotherapy regimens. Hence, it is impossible to distinguish the effect of the combination of drugs from the effect of long-term treatment. We should therefore emphasize that the reported observations are only reflecting the patient groups that have completed the combination therapy, and should be interpreted in this context when compared with our previous study that described the therapeutic effects of 24-week long pioglitazone and tofogliflozin monotherapy in patients with NAFLD with T2DM.[17] Third, because this was an open-label study without placebo, there is a possibility that factors, such as the Hawthorne effect, may have improved diabetes and NAFLD. Fourth, no liver biopsy or histological evaluation was performed in this study.

T2DM was reported to be the strongest contributor to cirrhosis and hepatocellular carcinoma in patients with NAFLD/NASH.[48] The co-existence of these two conditions is well known; the association is driven by a bidirectional pathological relationship whereby NAFLD increases the risk of T2DM, which contributes to, and accelerates the progression of, NAFLD. In the United States, 18.2 million people are estimated to have NAFLD with T2DM, and of those, 6.4 million (35%) have NASH. A recent study in the United States estimated that 65,000 liver transplants, 1,370,000 cardiovascular-related deaths, and 812,000 liver-related deaths will occur over the next 20 years.[49] The treatment of T2DM in these patients may reduce the anticipated clinical and economic burdens.

As NASH is a complex condition involving many factors, a single target may not provide sufficient therapeutic effects. Therefore, the use of multiple drugs with different mechanisms of action may provide further improvement not only in the liver but also in systemic diseases and may reduce the side effects. In this study, we investigated the efficacy of tofogliflozin and pioglitazone combination therapy in patients with NAFLD and T2DM. In addition to the additive effects of tofogliflozin and pioglitazone on T2DM and NAFLD, it was suggested that the combination therapy may reduce side effects, such as weight gain, and induce cardioprotective effect. Further studies with increased number of patients are needed to investigate the combination therapy of various drugs.

CONFLICT OF INTEREST
Nothing to report.

ORCID
Masato Yoneda https://orcid.org/0000-0001-7815-549X

Takashi Kobayashi 🄳 https://orcid.
org/0000-0002-7240-4851
Atsushi Nakajima 🄳 https://orcid.
org/0000-0002-6263-1436

REFERENCES

1. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73–84.

2. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999-2019: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2019;4:389–98.

3. Ito T, Ishigami M, Zou B, Tanaka T, Takahashi H, Kurosaki M, et al. The epidemiology of NAFLD and lean NAFLD in Japan: a meta-analysis with individual and forecasting analysis, 1995–2040. Hepatol Int. 2021;15:366–79.

4. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol. 2014;2:901–10.

5. Stefan N, Roden M. Diabetes and fatty liver. Exp Clin Endocrinol Diabetes. 2019;127:S93–6.

6. Stefan N, Häring HU, Cusi K. Non-alcoholic fatty liver disease: causes, diagnosis, cardiometabolic consequences, and treatment strategies. Lancet Diabetes Endocrinol. 2019;7:313–24.

7. Gastaldelli A, Cusi K. From NASH to diabetes and from diabetes to NASH: Mechanisms and treatment options. JHEP Rep. 2019;1:312–28.

8. Wargny M, Smati S, Pichelin M, Bigot-Corbel E, Authier C, Dierry V, et al. Fatty liver index is a strong predictor of changes in glycemic status in people with prediabetes: The IT-DIAB study. PLOS One. 2019;14:e0221524.

9. Mantovani A, Byrne CD, Bonora E, Targher G. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care. 2018;41:372–82.

10. Ciardullo S, Monti T, Perseghin G. High prevalence of advanced liver fibrosis assessed by transient elastography among U.S. adults with type 2 diabetes. Diabetes Care. 2021;44:519–25.

11. Younossi ZM, Stepanova M, Younossi Y, Golabi P, Mishra A, Rafiq N, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. Gut. 2020;69:564–8.

12. Cusi K. A diabetologist's perspective of non-alcoholic steatohepatitis (NASH): knowledge gaps and future directions. Liver Int. 2020;40:82–8.

13. Ratziu V, Friedman SL. Why do so many NASH trials fail? Gastroenterology. 2020;S0016-5085:30680-6.

14. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), and European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of nonalcoholic fatty liver diseases. J Hepatol. 2016;64: 1388–402.

15. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology. 2018;67:328–57.

16. Tokushige K, Ikejima K, Ono M, Eguchi Y, Kamada Y, Itoh Y, et al. Evidence-based clinical practice guidelines for nonalcoholic fatty liver disease/nonalcoholic steatohepatitis 2020. J Gastroenterol. 2021;56:951–63.

17. Ozaki A, Yoneda M, Kessoku T, Iwaki M, Kobayashi T, Honda Y, et al. Effect of tofogliflozin and pioglitazone on hepatic steatosis in non-alcoholic fatty liver disease patients with type 2 diabetes mellitus: a randomized, open-label pilot study (ToPiND study). Contemp Clin Trials Commun. 2020;17:100516.

18. Yoneda M, Honda Y, Ogawa Y, Kessoku T, Kobayashi T, Imajo K, et al. Comparing the effects of tofogliflozin and pioglitazone in non-alcoholic fatty liver disease patients with type 2 diabetes mellitus (ToPiND study): a randomized prospective open-label controlled trial. BMJ Open Diabetes Res Care. 2021;9:e001990.

19. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. Gastroenterology. 2016;150:626–37.

20. Sanyal AJ, Van Natta ML, Clark J, Neuschwander-Tetri BA, Diehl A, Dasarathy S, et al. Prospective study of outcomes in adults with nonalcoholic fatty liver disease. New Engl J Med. 2021;385:1559–69.

21. Angulo P, Kleiner DE, Dam-Larsen S, Adams LA, Bjornsson ES, Charatcharoenwitthaya P, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2015;149:389–97.

22. Taylor RS, Taylor RJ, Bayliss S, Hagström H, Nasr P, Schattenberg JM, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. Gastroenterology. 2020;158:1611–25.

23. Dulai PS, Singh S, Patel J, Soni M, Prokop LJ, Younossi Z, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology. 2017;65:1557–65.

24. Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stål P, Kechagias S, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology. 2015;61:1547–54.

25. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of pairedbiopsy studies. Clin Gastroenterol Hepatol. 2015;13:643–54.

26. Imajo K, Honda Y, Kobayashi T, Nagai K, Ozaki A, Iwaki M, et al. Direct comparison of us and mr elastography for staging liver fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2022;20:908–17.e11.

27. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355:2297–307.

28. Cusi K, Orsak B, Bril F, Lomonaco R, Hecht J, Ortiz-Lopez C, 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.

29. Kalyani RR. Glucose-lowering drugs to reduce cardiovascular risk in type 2 diabetes. N Engl J Med. 2021;384:1248–60.

30. Takahashi H, Kessoku T, Kawanaka M, Nonaka M, Hyogo H, Fujii H, et al. Ipragliflozin improves the hepatic outcomes of patients with diabetes with NAFLD. Hepatol Commun. 2022;6:120–32.

31. Akuta N, Kawamura Y, Watanabe C, Nishimura A, Okubo M, Mori Y, et al. Impact of sodium glucose cotransporter 2 inhibitor on histological features and glucose metabolism of non-alcoholic fatty liver disease complicated by diabetes mellitus. Hepatol Res. 2019;49:531–9.

32. Kahl S, Gancheva S, Straßburger K, Herder C, Machann J, Katsuyama H, et al. Empagliflozin effectively lowers liver fat content in well-controlled type 2 Diabetes: a randomized, double-blind, phase 4, placebo-controlled trial. Diabetes Care. 2020;43:298–305.

33. Eriksson JW, Lundkvist P, Jansson PA, Johansson L, Kvarnström M, Moris L, et al. Effects of dapagliflozin and n-3 carboxylic acids on non-alcoholic fatty liver disease in people

with type 2 diabetes: a double-blind randomised placebo-controlled study. Diabetologia. 2018;61:1923–34.

34. Latva-Rasku A, Honka MJ, Kullberg J, Mononen N, Lehtimäki T, Saltevo J, et al. The SGLT2 inhibitor dapagliflozin reduces liver fat but does not affect tissue insulin sensitivity: a randomized, double-blind, placebo-controlled study with 8-week treatment in type 2 diabetes patients. Diabetes Care. 2019;42:931–7.

35. Cusi K, Bril F, Barb D, Polidori D, Sha S, Ghosh A, et al. Effect of canagliflozin treatment on hepatic triglyceride content and glucose metabolism in patients with type 2 diabetes. Diabetes Obes Metab. 2019;21:812–21.

36. Ito D, Shimizu S, Inoue K, Saito D, Yanagisawa M, Inukai K, et al. Comparison of ipragliflozin and pioglitazone effects on nonalcoholic fatty liver disease in patients with type 2 diabetes: a randomized, 24-week, open-label, active-controlled trial. Diabetes Care. 2017;40:1364–72.

37. Sumida Y, Murotani K, Saito M, Tamasawa A, Osonoi Y, Yoneda M, et al. Effect of luseogliflozin on hepatic fat content in type 2 diabetes patients with non-alcoholic fatty liver disease: a prospective, single-arm trial (LEAD trial). Hepatol Res. 2019;49:64–71.

38. Gharaibeh NE, Rahhal MN, Rahimi L, Ismail-Beigi F. SGLT-2 inhibitors as promising therapeutics for non-alcoholic fatty liver disease: pathophysiology, clinical outcomes, and future directions. Diabetes Metab Syndr Obes. 2019;12:1001–12.

39. Tahara A, Takasu T. SGLT2 inhibitor ipragliflozin alone and combined with pioglitazone prevents progression of nonalcoholic steatohepatitis in a type 2 diabetes rodent model. Physiol Rep. 2019;7:e14286.

40. Hagström H, Nasr P, Ekstedt M, Hammar U, Stål P, Hultcrantz R, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol. 2017;67:1265–73.

41. Yoneda M, Yamamoto T, Honda Y, Imajo K, Ogawa Y, Kessoku T, et al. Risk of cardiovascular disease in patients with fatty liver disease as defined from the metabolic dysfunction associated fatty liver disease or nonalcoholic fatty liver disease point of view: a retrospective nationwide claims database study in Japan. J Gastroenterol. 2021;56:1022–32.

42. Neal B, Perkovic V, Mahaffey KW, De Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med. 2017;377:644–57.

43. Zinman B, Lachin JM, Inzucchi SE. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2016;374:1094.

44. Zhu J, Yu X, Zheng Y, Li J, Wang Y, Lin Y, et al. Association of glucose-lowering medications with cardiovascular outcomes: an umbrella review and evidence map. Lancet Diabetes Endocrinol. 2020;8:192–205.

45. Nesti L, Tricò D, Mengozzi A, Natali A. Rethinking pioglitazone as a cardioprotective agent: a new perspective on an overlooked drug. Cardiovasc Diabetol. 2021;20:1–7.

46. Kolb H, Kempf K, Röhling M, Lenzen-Schulte M, Schloot NC, Martin S. Ketone bodies: from enemy to friend and guardian angel. BMC Med. 2021;19:1–5.

47. Nakamura M, Odanovic N, Nakada Y, Dohi S, Zhai P, Ivessa A, et al. Dietary carbohydrates restriction inhibits the development of cardiac hypertrophy and heart failure. Cardiovasc Res. 2021;117:2365–76.

48. Alexander M, Loomis AK, Van Der Lei J, Duarte-Salles T, Prieto-Alhambra D, Ansell D, et al. Risks and clinical predictors of cirrhosis and hepatocellular carcinoma diagnose in adults with diagnosed NAFLD: real-world study of 18 million patients in four European cohorts. BMC Med. 2019;17:1–9.

49. Younossi ZM, Tampi RP, Racila A, Qiu Y, Burns L, Younossi I, et al. Economic and clinical burden of nonalcoholic steatohepatitis in patients with type 2 diabetes in the U.S. Diabetes Care. 2020;43:283–9.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.