Effects of a novel selective peroxisome proliferator-activated receptor-α modulator, pemafibrate, on hepatic and peripheral glucose uptake in patients with hypertriglyceridemia and insulin resistance

Ikuro Matsuba1*, Ren Matsuba2, Shun Ishibashi3, Shizuya Yamashita4, Hidenori Arai5, Koutaro Yokote7, Hideki Suganami8, Eiichi Araki9

1Matsuba Clinic, 2Department of Internal Medicine, Division of Metabolism and Endocrinology, St. Marianna University School of Medicine, Kanagawa, 3Division of Endocrinology and Metabolism, Department of Medicine, Jichi Medical University, Tochigi, 4Department of Community Medicine and Department of Cardiovascular Medicine, Osaka University Graduate School of Medicine, 5Rinku General Medical Center, Osaka, 6National Center for Geriatrics and Gerontology, Aichi, 7Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, 8Clinical Data Science Department, Kowa Company, Ltd., Tokyo, and 9Department of Metabolic Medicine, Faculty of Life Sciences, Kumamoto University, Kumamoto, Japan

Keywords
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*Correspondence
Ikuro Matsuba
Tel: +81-44-522-1678
Fax: +81-44-522-1698
E-mail address: ikuro@matsuba-web.com

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ABSTRACT
Aims/Introduction: Pemafibrate is a novel selective peroxisome proliferator-activated receptor-α modulator with potent triglyceride-lowering and high-density lipoprotein cholesterol-raising effects. We showed that pemafibrate decreased the homeostatic model assessment for insulin resistance in patients with dyslipidemia. To investigate how pemafibrate improves insulin sensitivity, we used a hyperinsulinemic-euglycemic clamp technique to determine the splanchnic and peripheral glucose uptake in patients with hypertriglyceridemia and insulin resistance.

Materials and Methods: A total of 27 patients with hypertriglyceridemia and insulin resistance were randomly assigned to receive pemafibrate (0.4 mg/day, b.i.d.) or placebo treatment for 12 weeks. The hyperinsulinemic-euglycemic clamp test combined with oral glucose loading was carried out at weeks 0 and 12 to evaluate the splanchnic and peripheral glucose uptake.

Results: Pemafibrate, but not the placebo, significantly increased the splanchnic glucose uptake rate from baseline (19.6 ± 5.9% with P = 0.005 and 2.1 ± 7.4% with P = 0.78, respectively), although no significant difference between the groups was observed (P = 0.084). Conversely, peripheral glucose uptake rate was not significantly altered. Pemafibrate, compared with the placebo, significantly decreased plasma triglycerides (−61.4 ± 16.4% vs −25 ± 41.4%, P = 0.001), free fatty acids (−24.8 ± 23.2% vs 2.0 ± 26.8%, P = 0.016) and gamma-glutamyl transpeptidase (−30 ± 46 vs 10 ± 19 U/L, P = 0.009) levels, and significantly increased fibroblast growth factor 21 (457.7 ± 402.1 vs −41.7 ± 37.4 pg/mL, P = 0.007) levels.

Conclusions: Pemafibrate increased splanchnic glucose uptake from baseline in patients with hypertriglyceridemia.

INTRODUCTION
Insulin resistance is an important risk factor for the development of type 2 diabetes mellitus and cardiovascular diseases1,2. The presence of insulin resistance is indicated by an elevated
homeostatic model assessment for insulin resistance (HOMA-IR) score, and is associated with high triglyceride (TG) and low high-density lipoprotein cholesterol (HDL-C) levels\(^{5,23}\).

Peroxisome proliferator-activated receptor (PPAR) agonists are used for the treatment of dyslipidemia or type 2 diabetes. PPARs are ligand-activated transcription factors of the nuclear hormone receptor superfamily, which is composed of three subtypes, including PPAR\(\alpha\), PPAR\(\delta\) and PPAR\(\gamma\). PPAR\(\gamma\) is highly expressed in adipose tissues, and PPAR\(\gamma\) agonists sensitize insulin action in both the liver and skeletal muscles, and lower plasma glucose in type 2 diabetes\(^{5-9}\). PPAR\(\delta\) is ubiquitously expressed in multiple tissues, including the liver and skeletal muscles\(^{9}\), and plays an essential role in mitochondrial oxidation capacity, including the liver, kidneys, heart, skeletal muscles and brown adipose tissues\(^{8}\). Additionally, PPAR\(\alpha\) agonists, also known as fibrates, stimulate FA oxidation, suppress FA and TG synthesis, and reduce plasma TG or TG-rich lipoprotein levels\(^{13}\). However, previous studies showed inconsistent results regarding the effects of PPAR\(\alpha\) agonists on glucose metabolism\(^{5,10,14-16}\).

Pemafibrate (K-877) has a more potent and selective stimulatory effect on PPAR\(\alpha\) than fenofibrate\(^{17,18}\). We have recently shown that pemafibrate treatment resulted in a significant plasma TG decrease and HDL-C increase in patients with dyslipidemia with less adverse effects, such as increased serum creatinine and liver enzyme\(^{19}\), which are commonly observed and associated with fenofibrate treatment\(^{20}\). Therefore, we designated pemafibrate as a selective PPAR\(\alpha\) modulator (SPPARM\(\alpha\)) with higher efficacy and safety than other agonists\(^{17,18}\). Interestingly, pemafibrate decreased fasting insulin levels and HOMA-IR in patients with hypertriglyceridemia\(^{19,21}\). It was unclear if their glucose tolerance was normal or not in the pemafibrate study; however, HOMA-IR was dependent on hepatic rather than peripheral insulin sensitivity in individuals with impaired fasting glucose/impaired glucose tolerance in another study\(^{22}\). Furthermore, liver enzyme levels significantly decreased with pemafibrate treatment\(^{19}\), suggesting its beneficial effects on the liver, which plays a key role in the homeostasis of blood glucose. Therefore, we carried out the hyperinsulinemic-euglycemic clamp study combined with oral glucose loading (OGL) to investigate how pemafibrate improves insulin sensitivity in patients with hypertriglyceridemia and insulin resistance. Plasma free fatty acid (FFA), adiponectin, and fibroblast growth factor 21 (FGF21) levels were examined to investigate whether they are correlated with the effects of pemafibrate on insulin sensitivity\(^{23-25}\).

METHODS

Study design and participants

The present study was an exploratory pharmacology clinical trial carried out in a randomized, parallel-group, placebo-controlled, double-blind design at Matsuba Clinic, Kanagawa, Japan, between December 2013 and March 2015, in accordance with the principles of the Declaration of Helsinki and in compliance with the Good Clinical Practice Guidelines issued by Japan’s Ministry of Health, Labor and Welfare (clinical trial registration ID: JapicCTI-142410, registered on 10 January 2014). The study protocol was approved by the institutional review board. All patients were fully informed of the nature of the study and provided written consent before their participation. Men aged ≥20 years and postmenopausal women with fasting TG levels between ≥200 and ≤500 mg/dL (≥2.26 and ≤5.65 mmol/L) and a HOMA-IR score between >1.6 and ≤4.0 were eligible. The following exclusion criteria applied: type 1 diabetes, poorly controlled type 2 diabetes (glycated hemoglobin ≥8.0%), poorly controlled hypertension (≥160 mmHg systolic blood pressure or ≥100 mmHg diastolic blood pressure), poorly controlled thyroid disorder, current or past history of hepatic impairment and aspartate aminotransferase or alanine aminotransferase (ALT) levels over threefold the upper limit of the normal range. The HOMA-IR upper limit for the eligibility was initially set at ≤3.0, because we were concerned about the possibility of underestimating splanchic glucose uptake (SGU), calculated using glucose infusion rates (GIR) described below, in patients with severe insulin resistance, as it was assumed difficult to maintain GIR >0 after OGL because of the potential impairment of SGU. However, we did not encounter such problems in the evaluation of patients with HOMA-IR of approximately 3.0. Hence, the upper limit was raised to 4.0 during the study.

The study comprised a washout period, a screening period of up to 8 weeks and a treatment period of 12 weeks. During the study, the use of therapeutic agents for dyslipidemia and those with possible effects on insulin sensitivity, such as antidiabetic agents, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, diuretics and \(\beta\)-blockers, were prohibited. Subsequently, eligible patients were randomly assigned to receive either pemafibrate (0.4 mg/day, b.i.d.) or placebo in a 2:1 ratio, to increase the probability of detecting the change in SGU rate from baseline, using a permuted block method with a block size of 6 after the screening period. An independent third party generated the random allocation sequence, confirmed that the study drugs were indistinguishable, and carried out the numbering and concealment of the drugs. All patients, investigators and the study sponsor were blinded to the treatment assignment during the study period. The study drugs were administered orally every morning and evening after meals for 12 weeks. The hyperinsulinemic-euglycemic clamp test was carried out at weeks 0 and 12, and laboratory examinations were carried out at weeks 0, 4, 8 and 12. Adverse events were recorded throughout the study period.

Clinical laboratory analysis

Fasting blood and urine samples were collected from patients at each attendance for clinical laboratory testing. The samples at weeks 0 and 12 were collected before the clamp test and the
administration of pemafibrate or placebo. Blood was also drawn before the OGL and after the clamp test. The lipoprotein cholesterol and apolipoprotein (Apo) levels were measured using direct enzymatic and immunoassay methods, respectively. Other common laboratory parameters were analyzed through standardized laboratory methods, except for FGF21, which was examined using an enzyme-linked immunosorbent assay with human FGF21 enzyme-linked immunosorbent assay (BioVendor R&D, Brno, Czech Republic). All measurements were carried out by the LSI Medience Corporation (Tokyo, Japan).

**Hyperinsulinemic-euglycemic clamp test combined with OGL**
The SGU and peripheral glucose uptake (PGU) rates were assessed through the hyperinsulinemic-euglycemic clamp technique combined with OGL. The hyperinsulinemic-euglycemic clamp technique, which was developed by DeFronzo et al.⁶,2⁶,2⁷, has been used as the gold standard method in the evaluation of insulin sensitivity. With this method, endogenous glucose production or hepatic glucose production (HGP) can be measured using 3-[³H]glucose, and hepatic glucose uptake can be measured in combination with OGL using a hepatic venous catheter. Kawamori et al.⁶,2⁸–3¹ developed a modified clamp method combined with OGL to non-invasively evaluate SGU, in addition to PGU, without the use of a tracer and hepatic venous catheterization on the basis that the liver plays a key role in the homeostasis of blood glucose in the post-absorptive state through insulin-mediated hepatic glucose uptake, as well as endogenous glucose production. The modified clamp test was used in the present study and carried out at weeks 0 and 12, after confirmation that the participants had been fasting for >10 h since their last meal. The clamp test at week 12 was commenced after the administration of the study drug in the morning. The insulin infusion rates and GIR were controlled using the artificial pancreas STG-55 (Nikkiso Co., Ltd., Shizuoka, Japan), an upgraded model of STG-22 that has been widely used in Japan since 1987. The plasma glucose levels during the clamp test were determined every minute through the continuous glucose monitoring system in the STG-55 at a sampling rate of 2 mL/h.

**PGU Evaluation**
A hyperinsulinemic state was achieved through primed-constant insulin infusion at a rate of 17.9 pmol/kg/min, which corresponds to approximately 695 pmol/m²/min, given the average Japanese physique. This was considered to be the rate to achieve a steady-state plasma insulin (SSPI) level of 1,390 pmol/L, which could almost completely suppress HGP, even in patients with insulin resistance. To attain the goal of maintaining a steady-state plasma glucose level of approximately 95 mg/dL (5.3 mmol/L), GIR was automatically controlled using STG-55, depending on the plasma glucose levels determined using its continuous glucose monitoring system.

After the achievement of a steady-state GIR, PGU was determined based on the mean GIR for 30 min, which was obtained approximately 90–120 min after the initiation of the insulin infusion. The mean GIR was divided by the SSPI and multiplied by 100 for convenience, and used as another PGU index to adjust for the variation in the individual SSPI level.

**SGU Evaluation**
After the measurement of the mean GIR, OGL was carried out to evaluate the SGU through oral administration of basically 0.3 g/kg glucose at weeks 0 and 12. After OGL, GIR was accordingly decreased to maintain the plasma glucose levels within a euglycemic range. The clamp test was continued until the GIR returned to its steady-state level before OGL, thus maintaining a steady-state plasma glucose level of approximately 5.3 mmol/L. The integrated GIR reduction, which reflects the glucose amount that was absorbed but not taken up by the liver, was subtracted from the amount of ingested glucose (OGL). The resulting values were divided by OGL and used as the SGU rate, that is: (OGL – ∑ΔGIR) / OGL × 100 (%).

**Statistical analysis**
The primary and secondary efficacy end-points were the changes from baseline in the SGU and PGU rates, respectively, measured at week 12. The primary safety end-points were the incidence rates of adverse events and adverse drug reactions. We analyzed the primary and secondary efficacy end-points using an analysis of covariance (ANCOVA) model with the baseline value as a covariate. Other laboratory test parameters were also analyzed using ANCOVA as post-hoc analysis. The continuous and categorical data of the baseline characteristics were analyzed using two-sample t-test and Fisher’s exact test, respectively. The reported P-values were two sided, and a value of 0.05 was considered to be significant. All randomized participants who received at least one dose of the study drug were included in the safety-analysis set, which was for primary safety analyses. Among them, those who had valid baseline and post-baseline measurements for the primary and secondary efficacy end-points without protocol deviations that can affect the efficacy evaluation were included in the per-protocol set, which was for primary efficacy analyses. The statistical power calculation was based on the results of a study that evaluated the effect of pioglitazone on insulin resistance in patients with non-insulin-dependent diabetes mellitus. In the pioglitazone study, the change from baseline in SGU rate was 30.8 ± 34.4% and –0.9 ± 21.0% in the pioglitazone and placebo group, respectively. Therefore, between-group difference in this parameter was assumed to be 30 ± 30%, or potentially 30 ± 20%, in the present study. Approximately 30 patients were considered as a feasible sample size for the present study. The estimated power was 60% with the difference in SGU rate of 30 ± 30%, but 91.1% with that of 30 ± 20%, in the setting with 24 patients composed of 16 in the pemafibrate and eight in the placebo group. Because the present study was an exploratory clinical pharmacology study, we set the target number of participants...
as 24 (16 in pемafibrate, 8 in placebo). All analyses were carried out using SAS version 9.3 software (SAS Institute Inc., Cary, NC, USA). The primary and secondary efficacy and primary safety end-points were analyzed in accordance with a pre-specified statistical analysis plan.

RESULTS

Recruitment and treatment of participants

Among 44 patients who provided informed consent, 27 patients were eligible and randomly assigned to either the placebo \((n = 8)\) or pемafibrate \((n = 19)\) group. All of the 27 patients were included in the safety-analysis set, and 18 patients (7 and 11 in the placebo and pемafibrate groups, respectively) were included in the per-protocol set because of protocol deviations in nine patients, such as consent withdrawal \((n = 1)\), discontinuation due to the occurrence of adverse events \((n = 2)\), extremely low TG levels at week 0 \((n = 1)\) or lack of paired measurements to calculate the change in glucose uptake rates due to failure of adhering to the hyperinsulinemic-euglycemic clamp test protocol \((n = 5)\).

Baseline characteristics

Tables 1 and 2 show the baseline characteristics and clinical laboratory test results, respectively. Although men, post-menopausal women and patients with type 2 diabetes were eligible for the study, all the patients were men without type 2 diabetes. The mean values for the baseline parameters were as follows: age 50 years; body mass index 25 kg/m\(^2\); fasting TG 3.30 mmol/L; fasting plasma glucose 5.66 mmol/L; and fasting insulin 83 pmol/L. All characteristics were comparable between the groups.

Glucose clamp test

The SSPI levels before OGL during the clamp test in the placebo and pемafibrate groups were 1,714 ± 438 and 1,741 ± 240 pmol/L, respectively, at week 0, and 1,928 ± 427 and 1,757 ± 397 pmol/L, respectively, at week 12. These were >1,900 pmol/L, the level at which the HGP can be almost completely suppressed, even in patients with insulin resistance. The SGU rates significantly increased from baseline in the pемafibrate group (19.6%, \(P = 0.005\)), but not in the placebo group (2.1%, \(P = 0.78\); Figure 1a). No significant difference between groups was observed \((P = 0.084)\). Furthermore, the changes in the mean GIR and GIR/SSPI from baseline and the differences between the groups were not significant (Figure 1b,c).

Major laboratory data

Table 2 and Figure 2 present the major laboratory test results. The TG, ApoCIII and FFA levels significantly decreased, whereas HDL-C, ApoAI and ApoAII levels significantly increased in the pемafibrate group compared with the placebo group. The glycemic parameters were not altered at week 12; however, the glycoalbumin level significantly decreased at weeks 4 and 8 in the pемafibrate group compared with the placebo group (Figure 2c). A similar trend was observed in the fasting plasma glucose and fasting insulin levels over the study period (Figure 2a,b). Further parameters showed significant changes at week 12: the FGF21 level increased (Figure 2e) and gamma-glutamyl transpeptidase level decreased throughout the study period (Figure 2f). The ALT level also decreased, although this change was not statistically significant. Several correlations between the SGU or PGU rate and laboratory parameters were found to be significant, but were not clinically meaningful (data not shown).

Adverse events

No serious adverse event was observed during the study period. The incidence rates of adverse events were 25.0% (2/8) and 10.5% (2/19) in the placebo and pемafibrate groups, respectively. Six adverse events were observed in four patients, which included arthropod sting and urticaria \((n = 1)\), increased blood creatine phosphokinase level, and abnormal liver function test \((n = 1)\) in the pемafibrate group, and hypoglycemia \((n = 1)\) and upper respiratory tract inflammation \((n = 1)\) in the placebo group. A causal relationship between pемafibrate treatment and increased creatine phosphokinase level and abnormal liver function test could not be excluded. Hypoglycemia observed in the placebo group was attributed to the hyperinsulinemic-euglycemic clamp test process. Participation in the study was discontinued for two patients in the pемafibrate group due to the occurrence of adverse events: increased creatine phosphokinase level and abnormal liver function tests \((n = 1)\), and urticaria \((n = 1)\). All patients who experienced adverse events recovered within 14 days.

| Table 1 | Baseline characteristics of the participants |
|---|---|---|
| | Placebo \((n = 7)\) | Pemafibrate \((n = 11)\) | \(P\)-value (vs placebo) |
| Age (years) | 46.4 ± 7.7 | 51.6 ± 10.4 | 0.27 |
| Body mass index (kg/m\(^2\)) | 25.8 ± 2.9 | 25.2 ± 2.3 | 0.60 |
| Waist circumference (cm) | 90.8 ± 6.5 | 89.0 ± 6.8 | 0.58 |
| Male | 7 (100) | 11 (100) | – |
| Current smoker | 3 (42.9) | 3 (27.3) | 0.23 |
| Hypertension | 0 | 2 (18.2) | 0.50 |

Data are presented as mean ± standard deviation and \(n\) (%) for continuous and categorical values, respectively.
### Table 2 | Laboratory test results

|                     | Placebo (n = 7) | Pemafibrate (n = 11) | P-value (vs placebo) |
|---------------------|----------------|----------------------|----------------------|
| **TG (mmol/L)**     |                |                      |                      |
| Baseline            | 3.07 ± 0.60    | 3.45 ± 1.10          |                      |
| Week 12             | 2.99 ± 1.34    | 1.24 ± 0.38          |                      |
| % Change            | −2.5 ± 41.4    | −61.4 ± 16.4         | 0.001                |
| **HDL-C (mmol/L)**  |                |                      |                      |
| Baseline            | 1.08 ± 0.12    | 1.08 ± 0.22          |                      |
| Week 12             | 1.07 ± 0.16    | 1.34 ± 0.31          |                      |
| % Change            | −0.4 ± 12.5    | 24.2 ± 15.6          | 0.004                |
| **Non-HDL-C (mmol/L)** |            |                      |                      |
| Baseline            | 4.86 ± 0.65    | 4.43 ± 1.08          |                      |
| Week 12             | 5.08 ± 0.89    | 3.92 ± 0.91          |                      |
| % Change            | 42 ± 7.3       | −96 ± 20.0           | 0.060                |
| **ApoA1 (mg/dL)**   |                |                      |                      |
| Baseline            | 123 ± 2        | 122 ± 18             |                      |
| Week 12             | 127 ± 15       | 143 ± 21             |                      |
| % Change            | 3.3 ± 9.7      | 17.9 ± 12.1          | 0.018                |
| **ApoAII (mg/dL)**  |                |                      |                      |
| Baseline            | 30.5 ± 2.5     | 28.6 ± 4.5           |                      |
| Week 12             | 29.9 ± 42.2    | 40.5 ± 5.5           |                      |
| % Change            | −2.1 ± 10.8    | 42.7 ± 13.6          | <0.001               |
| **ApoB (mg/dL)**    |                |                      |                      |
| Baseline            | 108 ± 16       | 95 ± 22              |                      |
| Week 12             | 110 ± 18       | 91 ± 19              |                      |
| % Change            | 1.6 ± 7.9      | −3.1 ± 16.8          | 0.238                |
| **ApoCIII (mg/dL)** |                |                      |                      |
| Baseline            | 15.8 ± 13.6    | 16.1 ± 5.8           |                      |
| Week 12             | 14.9 ± 44      | 8.8 ± 29             |                      |
| % Change            | −7.1 ± 15.6    | −42.3 ± 19.1         | 0.001                |
| **FFA (mEq/L)**     |                |                      |                      |
| Baseline            | 0.85 ± 0.30    | 0.69 ± 0.19          |                      |
| Week 12             | 0.84 ± 0.24    | 0.51 ± 0.15          |                      |
| % Change            | 2.0 ± 26.8     | −24.8 ± 23.2         | 0.016                |
| **HbA1c (%)**       |                |                      |                      |
| Baseline            | 5.6 ± 0.2      | 5.6 ± 0.2            |                      |
| Week 12             | 5.7 ± 0.3      | 5.8 ± 0.3            |                      |
| % Change            | 0.1 ± 0.1      | 0.2 ± 0.2            | 0.270                |
| **Glycoalbumin (%)**|                |                      |                      |
| Baseline            | 12.4 ± 1.2     | 12.8 ± 1.1           |                      |
| Week 12             | 12.4 ± 1.2     | 12.5 ± 1.3           |                      |
| % Change            | 0.0 ± 0.2      | −0.3 ± 0.5           | 0.233                |
| **FPG (mmol/L)**    |                |                      |                      |
| Baseline            | 5.58 ± 0.12    | 5.68 ± 0.49          |                      |
| Week 12             | 5.61 ± 0.24    | 5.65 ± 0.95          |                      |
| % Change            | 0.03 ± 0.18    | −0.03 ± 0.66         | 0.691                |
| **Fasting insulin (pmol/L)** |       |                      |                      |
| Baseline            | 85.0 ± 28.4    | 79.3 ± 38.5          |                      |
| Week 12             | 112.9 ± 76.4   | 105.7 ± 147.1        |                      |
| % Change            | 279.5 ± 55.6   | 264.6 ± 113.8        | 0.730                |
| **HOMA-IR**         |                |                      |                      |
| Baseline            | 3.1 ± 1.1      | 3.0 ± 1.8            |                      |
| Week 12             | 4.1 ± 2.8      | 4.6 ± 8.0            |                      |
| % Change            | 1.0 ± 2.0      | 1.6 ± 6.3            | 0.611                |

### Table 2 (Continued)

|                     | Placebo (n = 7) | Pemafibrate (n = 11) | P-value (vs placebo) |
|---------------------|----------------|----------------------|----------------------|
| **FGF21 (pg/mL)**   |                |                      |                      |
| Baseline            | 382.4 ± 140.6  | 409.1 ± 179.5        |                      |
| Week 12             | 340.7 ± 143.9  | 866.8 ± 451.0        |                      |
| % Change            | −41.7 ± 37.4   | 457.7 ± 402.1        | 0.007                |
| **Adiponectin (µg/mL)** |            |                      |                      |
| Baseline            | 2.71 ± 1.07    | 2.71 ± 1.08          |                      |
| Week 12             | 3.05 ± 1.15    | 2.65 ± 0.61          |                      |
| % Change            | 0.34 ± 0.62    | −0.06 ± 0.63         | 0.131                |
| **AST (U/L)**       |                |                      |                      |
| Baseline            | 27 ± 10        | 22 ± 7               |                      |
| Week 12             | 29 ± 10        | 26 ± 10              |                      |
| % Change            | 1 ± 6          | 4 ± 7                | 0.513                |
| **ALT (U/L)**       |                |                      |                      |
| Baseline            | 41 ± 24        | 28 ± 11              |                      |
| Week 12             | 42 ± 18        | 25 ± 12              |                      |
| % Change            | 1 ± 13         | −3 ± 11              | 0.138                |
| **GGT (U/L)**       |                |                      |                      |
| Baseline            | 83 ± 64        | 57 ± 55              |                      |
| Week 12             | 93 ± 79        | 27 ± 14              |                      |
| % Change            | 10 ± 19        | −30 ± 46             | 0.009                |

Data are presented as mean ± standard deviation. ALT, alanine aminotransferase; Apo, apolipoprotein; AST, aspartate aminotransferase; FFA, free fatty acid; FGF21, fibroblast growth factor 21; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; TG, triglyceride.

**DISCUSSION**

In patients with hypertriglyceridemia and insulin resistance, pemafibrate treatment (0.2 mg b.i.d.) for 12 weeks resulted in a significant increase in the SGU rate from baseline (P < 0.01), although the difference between the pemafibrate and placebo groups did not reach statistical significance (P = 0.084). The changes in the PGU rates were not statistically significant, and no clinically meaningful correlation was found between the SGU or PGU rate and laboratory parameters. Given the significant reduction in the plasma glycoalbumin level, we speculate that the study was slightly underpowered to detect the difference in the SGU and PGU rates and the correlations. Taken together, these findings indicate that pemafibrate might improve insulin resistance on SGU regulation.

The methodology used to measure the SGU rate in the present study has been well validated in other studies using similar techniques, with several assumptions that should be recognized: (i) ingested glucose was completely absorbed; (ii) HGP was completely suppressed; (iii) PGU was not affected by OGL; and (iv) endogenous insulin secretion was not affected by OGL.

Conclusive evidence regarding the effects of PPARα agonists on insulin sensitivity was not established. Bezafibrate decreased

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1327
the HOMA-IR and increased the PGU in patients with type 2 diabetes\textsuperscript{15,16}. This finding might be attributed to the low PPAR\textsubscript{a} selectivity and partial PPAR\textsubscript{c} agonistic activity, albeit weak, of bezafibrate\textsuperscript{34}. GFT-505, a PPAR\textsubscript{a}/\textsubscript{d} dual agonist, improved the insulin-stimulated HGP suppression and PGU in abdominally obese patients\textsuperscript{10}. This result could be associated with the PPAR\textsubscript{d} agonistic activity. In contrast, fenofibrate, which has higher PPAR\textsubscript{a} selectivity than bezafibrate, showed inconsistent effects on insulin sensitivity, and no clinical studies have shown improvement in the insulin-stimulated glucose uptake either in the liver or peripheral tissues\textsuperscript{5,14}. Therefore, the suggestion from the present study that pemafibrate, which has even higher PPAR\textsubscript{a} selectivity than fenofibrate, might increase SGU is interesting. A previous phase 2 study\textsuperscript{19} and an integrated analysis of phase 2/3 studies\textsuperscript{21} showed that the HOMA-IR decreased in patients with elevated HOMA-IR who were treated with pemafibrate in a dose-dependent manner. However, this result was not reproduced in the present study, probably due to the smaller sample size and lower baseline HOMA-IR compared with previous studies.

Next, we investigated the mechanism by which pemafibrate possibly increases SGU and ameliorates insulin resistance. The improvements in hepatic fat content, liver function tests, and plasma FFA and adiponectin levels have been postulated to mediate the effects of PPAR\textsubscript{c} agonists on insulin-stimulated glucose disposal and insulin-mediated HGP suppression\textsuperscript{5,9}. Although hepatic fat content was not measured in the present study, hepatic TG content decreased in Zucker fatty rats, and the \textit{b}-oxidation-related gene expression in the liver of Zucker fatty rats and mice, as well as primary human hepatocytes, increased after pemafibrate treatment\textsuperscript{18,35}. The gamma-glutamyl transpeptidase level significantly decreased and the ALT level decreased, although this change was not statistically significant, in the present study. Previous studies on the effects of pemafibrate reported significant decreases in both gamma-glutamyl transpeptidase and ALT levels\textsuperscript{19,36}. Patients with non-alcoholic fatty liver disease (NAFLD) have higher HOMA-IR and/or lower GIR levels than those without NAFLD\textsuperscript{37–39}. Therefore, pemafibrate can possibly ameliorate the potentially impaired liver function, which is a predisposing factor for NAFLD. However, further studies are required, as in the present study the diagnosis of NAFLD was not predetermined and hepatic fat content was not measured.
In a previous study, the SGU rate was reduced through exogenous lipid infusion to maintain elevated FFA levels during the hyperinsulinemic-euglycemic clamp test in patients with type 2 diabetes. Therefore, decreased plasma FFA as a result of pemafibrate treatment might contribute, at least in part, to the increase in the SGU rate. However, HGP was stimulated through lipid infusion in the relatively insulinopenic-hyperglycemic clamp method, but not in the hyperinsulinemic-euglycemic clamp method, where HGP was almost completely suppressed. Nevertheless, PGU was suppressed by the lipid infusion in these studies. Furthermore, FFA is well documented to compromise the insulin sensitivity in the skeletal muscles rather than that in the liver. Therefore, the decrease in the FFA level is unlikely to make a major contribution on the increase in the SGU on treatment with pemafibrate. In this regard, the effects of pemafibrate on plasma FGF21 are noteworthy.

FGF21 is a novel member of the FGF family with different metabolic effects, including decreased blood glucose and plasma lipid levels. FGF21 expression is induced through starvation and ketogenic diets through the PPARα signaling pathway. Exogenous FGF21 administration in ob/ob mice restored the insulin-mediated HGP suppression along with the increase in glucokinase activity and hepatic glycogen content, although the glucose 6-phosphatase activity remains unaffected, suggesting that the net flux of glucose in the liver favored glucose uptake. Pemafibrate induced FGF21 expression in Zucker fatty rats and humans more potently than fenofibrate. Therefore, a pemafibrate-induced increase in plasma FGF21 might play a role in the mediation of insulin-dependent hepatic glucose disposal. However, further studies are required to clarify whether the effects of FGF21 can still be observed under conditions with almost complete HGP suppression in a hyperinsulinemic state.
The adiponectin level did not increase on treatment with fenofibrate compared with the placebo in the present study. However, fenofibrate increased the adiponectin receptor expression and improved the adiponectin signaling in mice. GFT-505 improved the hepatic insulin sensitivity and liver function test, but reduced the adiponectin levels compared with the placebo. Therefore, the investigators suggested that GFT-505 might improve adiponectin signaling.

Plasma FFA and FGF21 also play a role in the mediation of peripheral insulin sensitivity. However, it remains to be established whether the significant changes in the FFA and FGF21 levels induced by pemafibrate had an impact on PGU.

The present study had several limitations. First, the number of patients in the per-protocol set was lower than the estimated number to have sufficient statistical power, because a considerable number of patients were excluded from 44 patients who provided informed consent; 17 were not eligible and nine were excluded from the per-protocol set as described above. Second, considering that the study participants were all Japanese and men, the results might not be generalizable to other populations. Third, HGP was not evaluated in the present study. However, according to the mean SSPI, residual HGP was assumed to be negligible in the calculation of the SGU rate. Fourth, even though the methodology used to measure the SGU rate in the present study has been well validated in similar techniques, there is no guarantee that all of the assumptions were well satisfied in the present study. Specifically, endogenous insulin secretion was not completely suppressed by exogenous insulin infusion and could be stimulated by OGTT even under the hyperinsulinemic clamp, which might have confounding effects on insulin-mediated glucose production and uptake in the liver.

In conclusion, pemafibrate increased SGU from baseline in patients with hypertriglyceridemia, possibly through the stimulation of FA β-oxidation and improvement of liver function. FGF21 might also be involved in this mechanism.

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