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[a]Weight reduction results are from PIONEER 4, a 52-week, double-blind, double-dummy trial in 711 adult patients with type 2 diabetes that compared the efficacy and safety of RYBELSUS® vs liraglutide and placebo.[4]

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ORIGINAL ARTICLE

Clinical Trials and Investigations

The tissue-specific metabolic effects of the PPARα agonist ciprofibrate in insulin-resistant male individuals: a double-blind, randomized, placebo-controlled crossover study

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Abstract

Objective: Insulin resistance is characterized by ectopic fat accumulation leading to cardiac diastolic dysfunction and nonalcoholic fatty liver disease. The objective of this study was to determine whether treatment with the peroxisome proliferator-activated receptor-α (PPARα) agonist ciprofibrate has direct effects on cardiac and hepatic metabolism and can improve insulin sensitivity and cardiac function in insulin-resistant volunteers.

Methods: Ten insulin-resistant male volunteers received 100 mg/d of ciprofibrate and placebo for 5 weeks in a randomized double-blind crossover study. Insulin-stimulated metabolic rate of glucose (MRgluc) was measured using dynamic ^{18}F-fluoro-deoxyglucose-positron emission tomography (^{18}F-FDG-PET). Additionally, cardiac function, whole-body insulin sensitivity, intrahepatic lipid content, skeletal muscle gene expression, 24-hour blood pressure, and substrate metabolism were measured.

Results: Whole-body insulin sensitivity, energy metabolism, and body composition were unchanged after ciprofibrate treatment. Ciprofibrate treatment decreased insulin-stimulated hepatic MRgluc and increased hepatic lipid content. Myocardial net MRgluc tended to decrease after ciprofibrate treatment, but ciprofibrate

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treatment had no effect on cardiac function and cardiac energy status. In addition, no changes in PPAR-related gene expression in muscle were found.

Conclusions: Ciprofibrate treatment increased hepatic lipid accumulation and lowered MRgluc, without affecting whole-body insulin sensitivity. Furthermore, parameters of cardiac function or cardiac energy status were not altered upon ciprofibrate treatment.

INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) is strongly increasing worldwide and is associated with many severe comorbidities and early mortality [1]. One of the metabolic changes during development of insulin resistance and diabetes is reduced oxidative capacity [2], which is typically associated with a blunted fat oxidation rate in the fasted state [3, 4]. In combination with the high availability of free fatty acids (FFAs) in obesity, this favors the accretion of fat accumulation ectopically (in skeletal muscle, heart, and liver). Therefore, enhancing mitochondrial oxidative capacity and stimulating oxidation of fat may be beneficial to put such a vicious cycle to a halt and to improve metabolic health. Many of the genes involved in oxidative metabolism are under the control of peroxisome proliferator-activated receptors (PPARs) [5]. Peroxisome proliferator-activated receptor-α (PPARα)-controlled genes are involved in lipid and lipoprotein metabolism and are abundantly expressed in tissues that require high rates of fatty acid oxidation, such as in the heart and liver parenchymal cells [5]. From previous studies in humans, it is known that PPARα stimulation in the liver results in increased uptake and oxidation of FFAs, increased triglyceride (TG) hydrolysis, and upregulation of apolipoproteins apoA1 and apoA2. The net effect is an increased fatty acid oxidation, a decrease in serum TGs, a rise in high-density lipoprotein (HDL) cholesterol levels, and an increase in cholesterol efflux [6, 7]. In a previous study in patients with a specific gene defect leading to blunted fatty acid-induced PPAR activation, bezafibrate increased whole-body and muscle-specific fatty acid oxidative capacity [8]. Based on these findings, we aimed to examine the tissue-specific effect of a specific PPARα agonist (ciprofibrate) on cardiac and hepatic metabolism in the insulin-resistant state because such data remain elusive in humans.

Insulin resistance is associated with an increased risk for cardiovascular disease [1] and nonalcoholic fatty liver disease (NAFLD) [9]. The underlying metabolic aberrations remain unclear. In animal studies, PPARα treatment has been shown to increase fatty acid oxidation with beneficial results on metabolism and cardiac function. In mice, PPARα prevented the development of NAFLD and cardiac dysfunction induced by a sucrose-enriched diet [10]. Therefore, it can be hypothesized that PPARα also stimulates tissue-specific fatty acid oxidation and lowers lipotoxicity, thereby impacting the development of T2DM and cardiovascular disease [11].

In humans, PPARα treatment is used to treat hypertriglyceridemia, but, contrary to the results in animals, it does not affect liver fat [12–14]. However, these studies have focused mainly on the TG-lowering effect of PPAR agonists and on cardiovascular outcomes. Mechanistic human studies investigating changes in metabolism on the whole body and at the tissue level upon PPARα stimulation are limited thus far. Therefore, further research on the effect of PPARα on metabolism in humans with compromised metabolic health is of utmost interest.

Study Importance

What is already known?

- Insulin resistance is characterized by high substrate availability and low FAO oxidative capacity, contributing to ectopic fat accumulation.
- Ectopic fat accumulation is associated with cardiac diastolic dysfunction and nonalcoholic fatty liver disease.
- Peroxisome proliferator-activated receptor-α (PPARα) treatment has been shown to impact fat metabolism and cardiac function.

What does this study add?

- Ciprofibrate leads to decreases in plasma total triglycerides, without changes in total cholesterol. There was a significant lowering of the triglyceride fraction in very low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein.
- Ciprofibrate treatment increased hepatic lipid accumulation and decreased insulin-stimulated metabolic rate of glucose in the liver.
- Similar tendencies were found in the heart, without changes in cardiac function or energy status and unaltered whole-body insulin sensitivity.
- Ciprofibrate did not alter muscle PPAR gene expression.

How might these results change the direction of research or the focus of clinical practice?

- Our study is one of the first, to our knowledge, in humans showing effects of ciprofibrate on hepatic and cardiac metabolism. Further studies are needed to determine its potential value in preventing the onset and treatment of type 2 diabetes and related comorbidities.

Ciprofibrate is a PPARα agonist and a registered drug for the treatment of hypertriglyceridemia. To further explore the effects of the PPARα agonist treatment on cardiac and hepatic metabolism in humans, we investigated the effects of ciprofibrate on the heart and liver. We hypothesize that ciprofibrate treatment will increase fatty acid oxidation rates, thereby lowering hepatic lipid content and improving tissue-specific insulin sensitivity and cardiac health. Because early changes in cardiac and hepatic metabolism in the insulin-resistant state precede the onset of T2DM, we studied these changes in metabolism in insulin-resistant volunteers by performing magnetic resonance spectroscopy (MRS) measurements with indirect calorimetry and dynamic positron emission tomography (PET) to quantify the metabolic rate of glucose (MRgluc) in a tissue-specific manner.

METHODS

Study design and participants

A double-blind, randomized, placebo-controlled crossover study was conducted at the Metabolic Research Unit Maastricht of Maastricht University (Maastricht, the Netherlands) between November 2018 and November 2020 (in the COVID-19 period). The Ethics Committee of Maastricht University approved the study, which was conducted in accordance with the Declaration of Helsinki. The study was registered at ClinialTrials.gov (NCT03662984). Upon written informed consent, participants were treated for 5 weeks with ciprofibrate (100 mg/d) and placebo (cellulose powder) in random order, with a washout period of 6 weeks. An overview of the study protocol can be found in Supporting Information Figure S3.

Study population

Ten insulin-resistant male volunteers between age 40 and 70 years (glucose clearance rate <360 mL/kg/min) with body mass index (BMI) in the range of 27 to 35 kg/m^2 were included in the study. The glucose clearance cutoff is based on the article by Mari et al. [15], in which a glucose clearance <360 mL/kg/min is characteristic for participants with impaired glucose metabolism. Exclusion criteria were presence of T2DM, unstable body weight (weight gain or loss >5 kg in the previous 3 months), presence of cardiac disease or unstable angina, impaired hepatic and/or kidney function, fibrate treatment, alcohol consumption of more than two glasses per day, and any contraindication to magnetic resonance imaging (MRI) scanning.

Sample size was based on a power calculation based on myocardial MRgluc, measured by ^{18}F-fluorodeoxyglucose (^{18}F-FDG)-PET-MRI with a power (π) of 80% and a significance level (α) of 0.05. Previous studies have found a difference of 19% among participants with normal and impaired glucose metabolism; therefore, we assumed the mean difference between treatments (μ) to be 19% [16] and an average myocardial MRgluc of 97.1 μmoL/100 g/min with a standard deviation (σ) of 12.8 [17]. A flowchart of the participant inclusion can be found in Supporting Information Figure S1.

Blood and tissue sampling

On days 0, 7, 14, 21, 28, and 35 of both the ciprofibrate and placebo period, fasting blood samples were drawn for analysis of general safety parameters (creatinine). In addition, glucose, C-reactive protein, FFAs, cholesterol, and TGs were analyzed. On day 35, a muscle biopsy was taken from the vastus lateralis in the fasting state. In muscle, the gene expression of carnitine O-palmitoyltransferase 1 (CPT-1), pyruvate dehydrogenase kinase, isozyme 4 (PDK4), and PPAR gamma coactivator 1-alpha (PGC1α) was measured. For additional information, see Supporting Information.

Ambulatory blood pressure

Ambulatory blood pressure was measured (Mobil-O-Graph, IEM GmbH) during week 4 of each intervention for 24 hours. Mean systolic and diastolic blood pressure during daytime and nighttime, as well as nighttime dipping, was calculated as previously described [18]. Night was defined as the time between 23:00 until 07:00.

Magnetic resonance spectroscopy

On day 33 of each intervention, participants underwent MRS at 08:00 after a standardized dinner on day 32 followed by an overnight fast. In addition, participants were asked to refrain for at least 48 hours from strenuous physical activity. Proton MRS (^{1}H-MRS) was used to quantify intrahepatic lipid content and composition. Phosphorus MRS (^{31}P-MRS) was used for assessment of the phosphocreatine (PCr) to ATP ratio as a marker of cardiac energy status. All measurements were performed on a 3.0-T whole-body scanner (Achieva Tx, Philips Healthcare). For the scan protocol, see Supporting Information.

Whole-body maximum aerobic capacity

Maximal aerobic capacity (VO_2max) and maximal performance (Wmax) were assessed during an incremental cycling test on an electronically braked cycle ergometer (Lode Excalibur) [19] on day 33 of the intervention period. For the full protocol, see Supporting Information.

Postprandial metabolism and overnight whole-body energy metabolism

On day 34 of the intervention period, an intravenous cannula was placed, and a fasted blood sample was drawn ($T = 0$). At 17:00, participants were asked to consume a standardized meal within 15 minutes.

This meal was similar for all participants and it was the same in both interventions. Subsequently, participants entered a metabolic chamber at 17:30 and remained fasted until the next morning. Blood samples were drawn 15, 30, 45, 60, 90, 120, 180, and 240 minutes after dinner. Participants went to sleep at 23:00 at the latest and they were woken up at 06:00 the next morning. In the metabolic chamber, oxygen consumption and carbohydrate oxidation were measured continuously in sampled room air, and sleeping metabolic rate was assessed as the lowest average 3-hour energy expenditure during the sleeping period. Calculations of energy expenditure were performed with the Weir equation [20], assuming a 12.4% protein oxidation of total energy expenditure. Glucose oxidation and fat oxidation rates were calculated according to Pérronet et al. [21].

Body composition

Body composition was determined on day 35 of each intervention at 06:00 after an overnight fast. Body mass and body volume were assessed using air-displacement plethysmography using a Bod Pod device (Cosmed) according to the manufacturer's instructions [22]. Thoracic gas volume was predicted on equations included in the Bod Pod software. From these data, body composition (including body fat percentage) was calculated as described by Siri et al. [23].

Cardiac function and structure

On day 34 of each intervention period at 16:00, participants underwent echocardiography by an experienced echocardiographist. Cardiac function measurements were done by two-dimensional and (tissue) Doppler echocardiography (Philips IE 33/Philips Epiq CVx/Philips Affiniti 70, Philips Healthcare) with a X5 xMATRIX array transducer. Results were interpreted according to the current European Society of Cardiology (ESC) and American Heart Association (AHA) guidelines [24].

On day 35 of each intervention, at 07:00 after an overnight fast and before the start of the hyperinsulinemic euglycemic clamp and at 11:00 during the steady state of the hyperinsulinemic euglycemic clamp, participants underwent Cine MRI imaging to determine cardiac function on a 3-T Biograph mMR scanner (Siemens Healthineers) using a body coil and a spine coil. The second measurement (at 11:00) took place during insulin stimulation (during the hyperinsulenemic euglycemic clamp). For the imaging protocol, see Supporting Information.

^{18}F-FDG-PET-MRI and measurements of insulin sensitivity

To determine insulin sensitivity and insulin-stimulated MRgluc of the heart and liver, thoracic PET images were obtained on day 35 during a hyperinsulinemic euglycemic clamp [25]. The clamp was started after an overnight fast outside the scanning room. Insulin was infused starting at 8:30 at 40 mIU/m^2/min for 2 hours to measure whole-body glucose disposal (Rd), as previously described [26]. A total of 20% glucose was co-infused at a variable rate to maintain euglycemia (~6.0 mmoL/L). During 60 minutes in the steady state, blood samples were collected, and MRgluc and cardiac function were measured using ^{18}F-FDG-PET-MRI. Participants were resting on a bed throughout the entire procedure. For the full protocol, see online Supporting Information, including Supporting Information Figure S2 (delineation of the whole left ventricle).

Statistical analysis

Participant characteristics and results are reported as mean (standard error of the mean [SEM]). Data are presented for $n = 10$, unless otherwise indicated. Differences among interventions were analyzed with a two-tailed paired t test. The Kolmogorov–Smirnov normality was used to evaluate normality of the distribution; all data were normally distributed. Differences among the different time points of the postprandial blood sampling were analyzed with a repeated-measures ANOVA test. All statistical tests were performed two-sided, and $p < 0.05$ was considered statistically significant. Missing data were not replaced. Statistical analyses were performed with the use of SPSS Statistics for Mac, version 23 (IBM Corp.).

RESULTS

Participant population and study compliance

Ten insulin-resistant male volunteers (mean [SEM], age 62 [3] years; BMI 29.1 [0.9] kg/m^2; glucose clearance rate 307 [16] mL/kg/min) completed the study. Ciprofibrate at 100 mg/d was well tolerated, and no adverse events were reported. Compliance in both periods was 100%. Participants were instructed to maintain their habitual diet and physical activity pattern throughout the study. Body mass was stable at 92.4 [4.0] kg after 5 weeks of placebo compared with 92.9 [4.3] kg after 5 weeks of ciprofibrate treatment ($p = 0.344$). Furthermore, body composition and VO_2max were comparable after both placebo and ciprofibrate interventions. Participant characteristics are reported in Table 1.

Substrate kinetics and muscle tissue analysis

Ciprofibrate treatment lowered TG concentrations in plasma ($p < 0.001$) and in the very low-density lipoprotein (VLDL) fraction (<0.05). TG content in the low-density lipoprotein (LDL) fraction tended to be lower ($p = 0.068$; Table 2). Total cholesterol was unchanged, although the cholesterol content in the VLDL fraction was significantly lower after ciprofibrate treatment, with unchanged cholesterol content in the LDL and HDL fractions (Table 2 and Figure 1). Furthermore, ciprofibrate treatment tended to lower C-reactive protein ($p = 0.074$). However, ciprofibrate did not affect fasting plasma glucose or FFAs (Table 2).

TABLE 1 Volunteer characteristics ($n = 10$) at day 35 in the two intervention arms

Parameter	Placebo	Ciprofibrate	*p* value
Age (y)	62 ± 3	62 ± 3	0.591
Body weight (kg)	92.4 ± 4.0	92.9 ± 4.3	0.344
BMI (kg/m^2)	29.1 ± 0.9	29.3 ± 1.0	0.340
Fat mass (kg)	32.0 ± 2.4	33.1 ± 2.7	0.309
Fat free mass (kg)	60.4 ± 2.5	59.9 ± 7.8	0.394
Fat percentage (%)	34.4 ± 1.6	35.3 ± 1.8	0.340
Fat free percentage (%)	65.6 ± 1.6	64.7 ± 1.8	0.328
VO_2max (mL/min/kg)[a]	29.3 ± 2.0	29.3 ± 2.0	0.796

Note: Data are presented as mean ± SEM.
Abbreviation: VO_2max, maximal aerobic capacity.
[a]VO_2max was obtained in only eight participants.

Muscle biopsies were taken from the vastus lateralis, and the downstream targets of PPARα were measured. There were no significant changes in muscle PGC1α, PDK4, or CPT-1 mRNA expression upon ciprofibrate treatment (data not shown).

Whole-body insulin sensitivity

The effects of ciprofibrate on whole-body insulin sensitivity were assessed by a one-step hyperinsulinemic euglycemic clamp. Whole-body insulin-stimulated glucose uptake, as expressed by the M-value, was not different between ciprofibrate and placebo (M-value: placebo, 21.8 ± 4.1 vs. ciprofibrate, 20.0 ± 3.6 µmol/kg/min; $p = 0.402$). Plasma FFA concentrations were suppressed by insulin to a similar extent between ciprofibrate and placebo (placebo, −366 ± 99 vs. ciprofibrate, −356 ± 83 µmol/L; $p = 0.900$), suggesting similar insulin sensitivity of adipose tissue.

Cardiac and hepatic insulin sensitivity measured by ^{18}F-FDG-PET-MRI

Ciprofibrate treatment did not alter insulin-stimulated cardiac MRgluc (mean [SEM]: placebo, 32.5 [18.3] vs. ciprofibrate, 27.2 [11.6] µmol/100 mL tissue/min; $p = 0.22$; Figure 2 and Table 3). However, plasma insulin levels during the steady state were variable among participants (placebo, 122.3 [35.58] vs. ciprofibrate, 131.85 [36.49] mIU/mL; $p = 0.12$), with stable plasma glucose (placebo, 6.0 [3.0] vs. ciprofibrate, 5.9 [0.5] mmol/L; $p = 0.89$). Because plasma insulin levels do affect cardiac metabolic rate, the net influx rate constant (Ki) and MRgluc were corrected for plasma insulin levels. Here, we did find a tendency toward a decreased rate constant of glucose after ciprofibrate treatment (corrected Ki, 0.49 [0.30] vs. 0.40 [0.24] mL/100 mL tissue/min/mIU insulin; $p = 0.098$). Furthermore, ciprofibrate tended to decrease insulin-stimulated net hepatic MRgluc (placebo, 5.2 [1.4] vs. ciprofibrate, 4.1 [1.4] µmol/100 mL tissue/min; $p = 0.064$). The rate constant of glucose in the liver was significantly lower upon ciprofibrate treatment (Figure 2 and Table 3; $p = 0.043$). Insulin-stimulated MRgluc and Ki for visceral adipose tissue were unchanged upon ciprofibrate treatment both with and without correction for plasma insulin levels (Table 3 and Figure 2).

TABLE 2 Blood biochemistry ($n = 10$) and ^{1}H-MRS and ^{31}P-MRS after intervention

	Placebo	Ciprofibrate	*p* value
Plasma parameters			
Fasting glucose (mmol/L)	5.7 ± 0.2	5.5 ± 0.1	0.376
Total cholesterol (mmol/L)	4.9 ± 0.7	4.6 ± 0.7	0.100
VLDL (mmol/L)	0.77 ± 0.41	0.56 ± 0.37	<0.01
LDL (mmol/L)	2.69 ± 0.43	2.55 ± 0.46	0.32
HDL (mmol/L)	1.41 ± 0.36	1.45 ± 0.32	0.20
True TGs (mmol/L)	1.66 ± 1.04	1.15 ± 0.78	<0.01
VLDL (mmol/L)	1.10 ± 0.77	0.71 ± 0.62	<0.01
LDL (mmol/L)	0.41 ± 0.19	0.34 ± 0.13	0.068
HDL (mmol/L)	0.16 ± 0.10	0.12 ± 0.06	0.039
FFAs (µmol/L)	605 ± 71	566 ± 51	0.558
CRP (mg/L)	1.7 ± 0.4	0.9 ± 0.1	0.074
^{1}H-MRS and ^{31}P-MRS			
Intrahepatic lipid content (%)	7.1 ± 2.2	10.4 ± 3.2	0.021
SFA (%) ($n = 8$)	41.3 ± 1.8	41.6 ± 1.9	0.419
PUFA (%) ($n = 7$)	15.1 ± 1.3	14.6 ± 1.1	0.758
MUFA (%) ($n = 7$)	43.6 ± 2.1	44.9 ± 1.9	0.925
Liver volume (L; $n = 8$)	1.7 ± 0.1	1.8 ± 0.2	0.598
Cardiac PCr/ATP ($n = 9$)	1.76 ± 0.25	1.72 ± 0.25	0.920

Note: Data are presented as mean ± SEM. Owing to analytical problems, not all participants could be included in the analysis, as described in the Table.
Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; ^{31}P-MRS, phosphorus magnetic resonance spectroscopy; CRP, C-reactive protein; FFA, free fatty acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MUFA, monounsaturated fatty acid; PCr, phosphocreatine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein.

Ambulatory blood pressure

A significant time–intervention interaction effect was found on 24-hour diastolic blood pressure, indicating higher diastolic blood pressure levels over time ($p < 0.001$) after ciprofibrate treatment (Figure 3B). However, no intervention effect of ciprofibrate was found on 24-hour systolic blood pressure and heart rate ($p = 0.519$ and 0.879, respectively; Figure 3A,C). Separate analyses of daytime and nighttime measurements revealed a stimulating effect of ciprofibrate on the average heart rate during the night (ciprofibrate, 68 ± 4 vs. placebo, 63 ± 4 beats/min; $p = 0.017$). In addition, nighttime dipping of the heart rate was not detectable upon ciprofibrate treatment ($p = 0.037$), whereas it was present upon placebo.

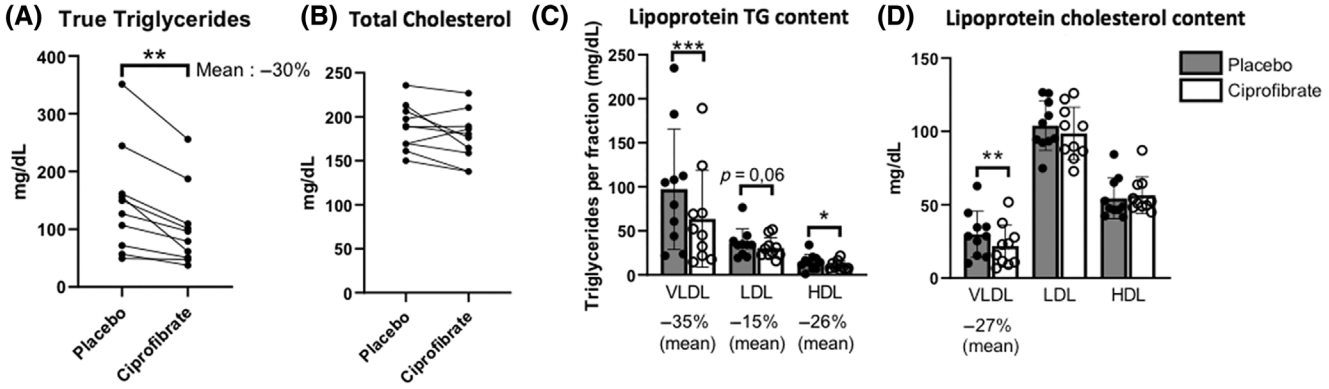

FIGURE 1 Effect of ciprofibrate treatment on (A) plasma TGs, (B) total cholesterol, and (C,D) lipoprotein TGs and distribution profiles. Ciprofibrate treatment decreased plasma TGs without affecting total cholesterol. Furthermore, ciprofibrate lowered TG content in the VLDL and, to a lesser extent, in the LDL fractions. Total cholesterol was not significantly altered by ciprofibrate, but cholesterol content in the VLDL fraction decreased significantly. $^{**}p < 0.01$, $^{***}p < 0.001$. HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; VLDL, very low-density lipoprotein

Magnetic resonance spectroscopy

Intrahepatic lipid content, measured by ^{1}H-MRS, was higher after ciprofibrate treatment (mean [SEM]: placebo, 7.1% [2.2%] vs. ciprofibrate, 10.4% [3.20%]; $p = 0.021$; Table 2; values are given as T2 corrected fat fraction [$CH_2/(CH_2 + H_2O)$], expressed as percentage), but no effect was detected in hepatic lipid composition, as depicted in Table 2. Furthermore, liver volume was not different between the two interventions.

Cardiac energy status (phosphocreatine/ATP ratio), measured by ^{31}P-MRS, was not affected by ciprofibrate (placebo, 1.76 [0.25] vs. ciprofibrate, 1.72 [0.25]; $p = 0.920$).

Postprandial metabolism and substrate kinetics

FFA and glucose plasma concentrations were not significantly different after the meal test between ciprofibrate and placebo (area under the curve: $p = 0.756$ and $p = 0.283$ for FFAs and glucose, respectively); however, TGs were lower in the fasted state and they remained lower, also after the meal, upon ciprofibrate treatment ($p = 0.006$; Figure 4).

Overnight whole-body energy metabolism

Owing to technical reasons and the COVID-19 pandemic, overnight energy metabolism in our whole-body respiration chamber could be performed only in seven volunteers. In those volunteers, fat oxidation seemed higher ($p = 0.12$) and carbohydrate oxidation seemed lower ($p = 0.15$) upon ciprofibrate treatment, although this did not reach statistical significance (Figure 5). Because higher TGs are associated with a higher amount of liver fat, ciprofibrate may have stronger effects in these volunteers. Interestingly, explorative analysis shows that, in all volunteers with a fatty liver (liver fat >5%; albeit only $n = 4$), an increase in fat oxidation ($p = 0.01$) and a decrease in the respiratory exchange ratio ($p = 0.04$) were observed (Supporting Information Figure S4).

Cardiac function and structure

Echocardiography and MRI revealed no effect of ciprofibrate on systolic and diastolic cardiac function and structure (Supporting Information Tables S1 and S2). During the insulin-stimulated phase, contraction was stimulated but to a similar extent in the placebo and ciprofibrate conditions (Supporting Information Table S2). Therefore, the known inotropic effect of insulin (Δ in Supporting Information Table S2) on the heart [27] was not affected by ciprofibrate treatment.

DISCUSSION

Early changes in cardiac and hepatic metabolism in insulin resistance and T2DM encompass reductions in mitochondrial function contributing to ectopic fat accumulation, insulin resistance, and cardiac diastolic dysfunction. The PPARα agonist ciprofibrate is known to stimulate fat metabolism in animal studies, including increased fatty acid mitochondrial oxidative capacity, uptake, and oxidation. Large cohort studies have already proven its safety and demonstrated some positive effects of the PPARα treatment on plasma TGs and cardiovascular outcomes [28–30]; however, the direct metabolic effects that parallel the TG decrease have not yet been properly investigated [31]. Therefore, in this study, we assessed tissue-specific metabolic effects of 5 weeks of treatment with the PPARα agonist ciprofibrate in insulin-resistant volunteers.

In contrast to our hypothesis, we found a tendency toward a decrease in insulin-stimulated myocardial MRgluc upon ciprofibrate treatment. In animal studies, cardiac-specific overexpression of PPARα also induces a reduction in cardiac glucose oxidation and an increased rate of FFA uptake and oxidation [32, 33]. Decreased

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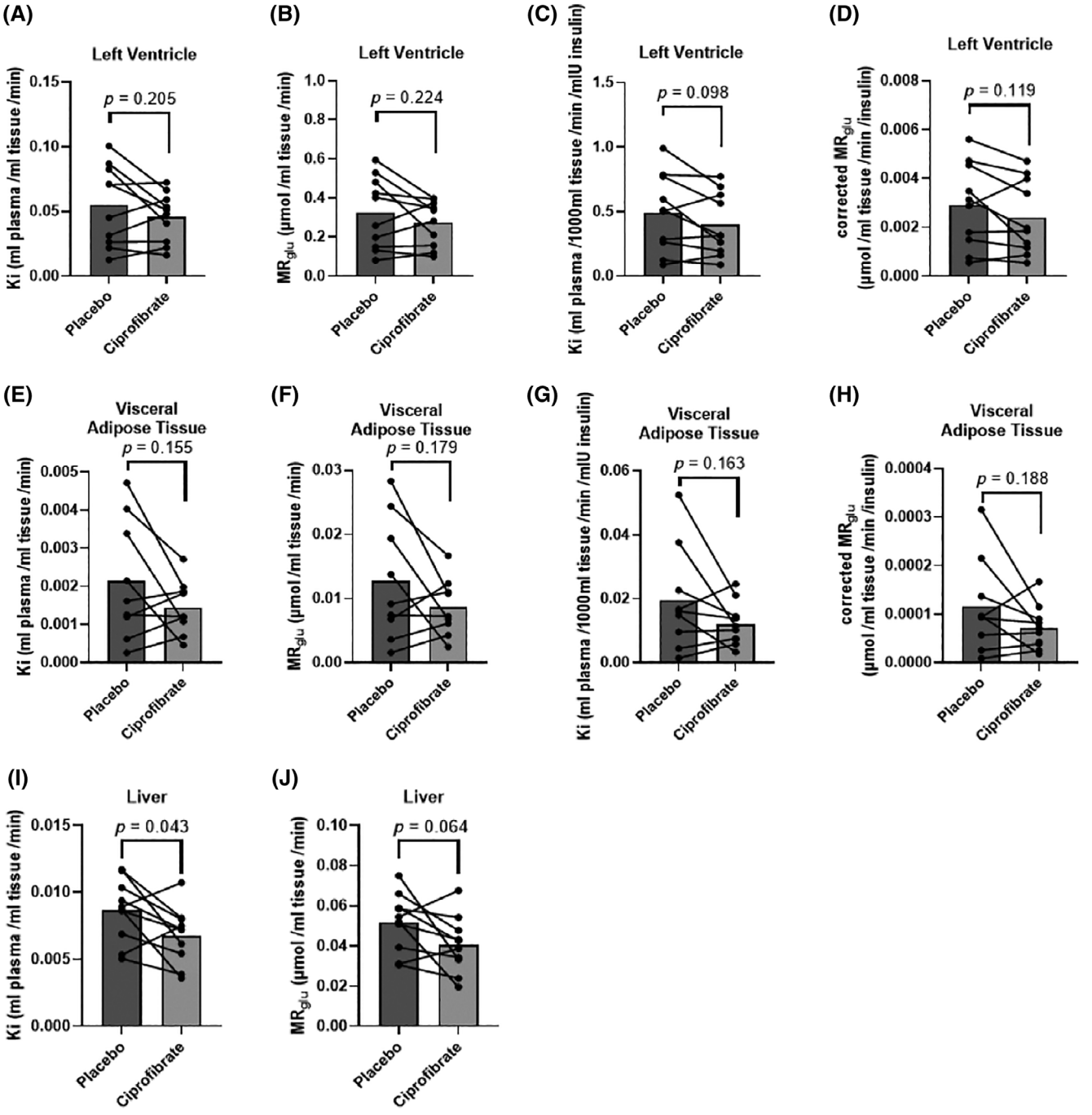

FIGURE 2 Insulin-stimulated Ki and MRgluc ($n = 10$) at day 35 in the two intervention arms. Ciprofibrate did not alter (A) Ki of FDG cardiac function and (B) MRgluc measured in the LV. However, when corrected for plasma glucose, there was a tendency toward (C) a lower Ki ($p = 0.098$) but not (D) corrected MRgluc ($p = 0.119$) in the LV. Ciprofibrate did not affect the (E,F) insulin-stimulated Ki or MRgluc for visceral adipose tissue, not even after (G,H) correction for plasma insulin. Ciprofibrate treatment decreased insulin-stimulated hepatic Ki of FDG ($p = 0.043$) and tended to decrease insulin-stimulated hepatic MRgluc ($p = 0.064$). Data are presented as mean ± SEM. FDG, fluorodeoxyglucose; Ki, net influx rate constant; LV, left ventricle; MRgluc, metabolic rate of glucose

myocardial MRgluc has been linked to overabundance of substrate (glucose and fatty acids) and has been associated with myocardial dysfunction [34, 35]. However, in our insulin-resistant volunteers, the tendency toward a lower myocardial MRgluc upon ciprofibrate treatment was not triggered by overabundance. Although 24-hour diastolic blood pressure levels were higher after ciprofibrate treatment, no negative effect on cardiac function was observed, which is in line with large cohort studies such as the Action to Control Cardiovascular Risk in Diabetes (ACCORD) and Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) trials [28, 29] and a study of Peterson et al., which did not find a change in diastolic cardiac function after longer-term PPARα treatment [31].

TABLE 3 Plasma values of insulin and glucose during the dynamic PET and Ki and MRgluc for liver, VAT, and LV

	Placebo	Ciprofibrate	*p* value
Plasma glucose levels (mmol/L)	6.0 ± 0.3	5.9 ± 0.5	0.89
Steady-state plasma insulin levels (mIU/mL)	122.3 ± 35.6	131.85 ± 36.5	0.12
Liver			
Ki (mL/100 mL/min)	0.87 ± 0.23	0.68 ± 0.21	**0.043**
MRgluc (μmoL/100 mL/min)	5.2 ± 1.4	4.1 ± 1.4	**0.064**
VAT			
Ki (mL/100 mL/min)	0.21 ± 0.16	0.14 ± 0.10	0.155
MRgluc (μmoL/100 mL/min)	1.3 ± 0.9	0.84 ± 0.42	0.179
Insulin-corrected Ki (mL/100 mL/min/mIU insulin)	0.0019 ± 0.0016	0.00120 ± 0.0007	0.163
Insulin-corrected MRgluc (μmoL/100 mL/min/mIU insulin)	0.0116 ± 0.0096	0.0071 ± 0.0045	0.188
LV			
Ki (mL/100 mL/min)	5.5 ± 3.1	4.5 ± 1.9	0.205
MRgluc (μmol/100 mL/min)	32.5 ± 18.3	27.2 ± 11.6	0.224
Insulin-corrected Ki (mL/100 mL/min/mIU insulin)	0.049 ± 0.030	0.040 ± 0.024	**0.098**
Insulin-corrected MRgluc (μmoL/100 mL/min/mIU insulin)	0.29 ± 0.17	0.24 ± −0.15	0.119

Note: Data are presented as mean ± SEM. *P* values < 0.1 are indicated with bold numbers.
Abbreviations: Ki, net influx rate constant; LV, left ventricle; MRgluc, metabolic rate of glucose; PET, positron emission tomography; VAT, visceral adipose tissue.

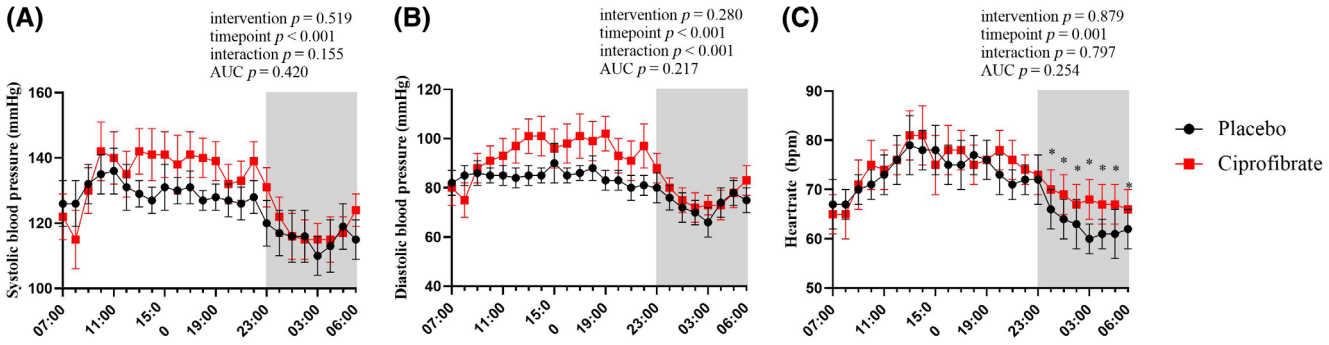

FIGURE 3 Ambulatory blood pressure measurement ($n = 9$). Twenty-four-hour diastolic blood pressure levels were significantly higher over time after ciprofibrate treatment. No intervention effect of ciprofibrate was found on 24-hour systolic blood pressure and heart rate, but, in the night, heart rate was significantly higher after ciprofibrate treatment. $p < 0.05$. AUC, area under the curve [Color figure can be viewed at wileyonlinelibrary.com]

In the current study, fasting TG concentrations in plasma were significantly lower after ciprofibrate treatment, as was anticipated, indicating efficacy of the drug. In line, TGs in the VLDL and, to a lesser extent, in the LDL and HDL fractions were significantly reduced, which was accompanied by a decrease in VLDL cholesterol levels, whereas plasma total cholesterol levels were unchanged. TG plasma concentrations remained lower in the postprandial state after ciprofibrate treatment compared with placebo. Surprisingly, liver fat content increased, without any changes in liver fat composition and liver volume. This is surprising because previous studies have shown no change in liver fat in both healthy and insulin-resistant volunteers [12–14]. Ciprofibrate may reduce TGs by acting on the production of plasma TG-controlling apolipoproteins such as by reducing apoC3 and increasing apoA5, together with an induction of VLDL receptor expression in the liver, thereby enhancing remnant uptake by the liver, which may increase liver fat content [36]. Although we did not measure apoC3 in this study, its plasma levels are well known to correlate closely with plasma TGs. Whether the increase in liver fat in this study also led to a decrease in hepatic insulin sensitivity could not be determined, but we did not find a decrease in whole-body insulin sensitivity. On the other hand, we found that ciprofibrate decreased insulin-stimulated hepatic MRgluc. This might be a result of a change in substrate partitioning. One could speculate that hepatic steatosis, together with decreased insulin-stimulated MRgluc of the liver, can be signs of substrate overabundance and insulin resistance, although, under such conditions, hepatic steatosis is associated with high instead of low TG concentrations.

Although, in the postprandial state, PPARα activation is expected to promote *de novo* lipogenesis, PPARα shifts its activity to promote

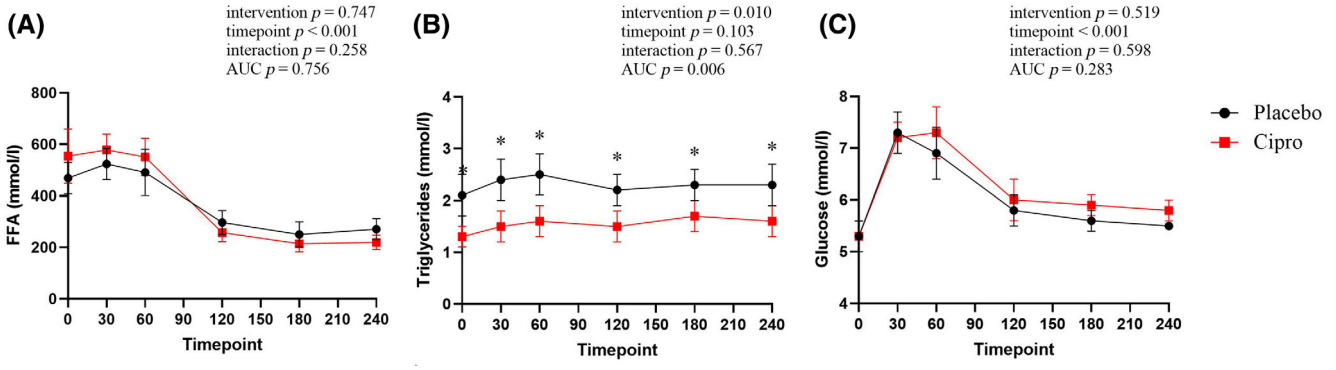

FIGURE 4 Response of FFAs, TGs, and glucose on a standardized dinner ($n = 9$). Substrate kinetics 4 hours after the consumption of a meal showed no effect of ciprofibrate on FFAs and glucose, although TGs remained lower after ciprofibrate treatment. *$p < 0.05$. AUC, area under the curve; FFA, free fatty acid; TG, triglyceride [Color figure can be viewed at wileyonlinelibrary.com]

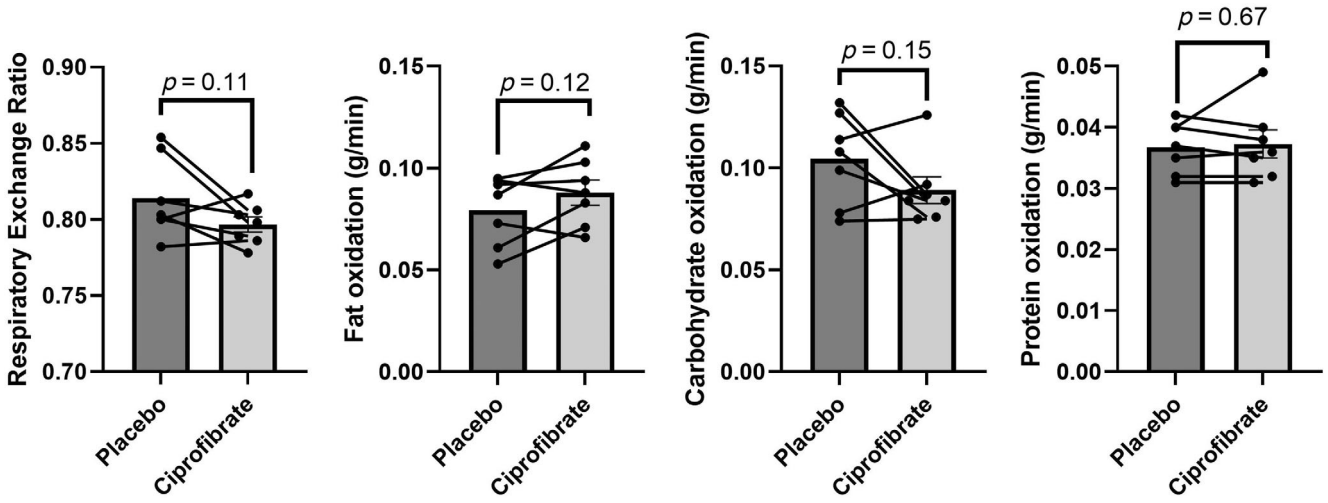

FIGURE 5 Measurements of substrate oxidation during the night ($n = 7$)

both fatty acid uptake and oxidation in the fasted state [37, 38]. Other studies have shown that, in conditions of lipid excess, mitochondrial oxidative capacity is also increased, and it could be speculated that lipid-induced activation of PPAR is involved [39, 40]. Although herein we could not determine mitochondrial capacity or tissue-specific fat oxidation, we did find a numerical increase in fatty acid oxidation rates as measured in our respiration chamber overnight, which was more prominent in the NAFLD subpopulation. However, combined with the increase in hepatic fat, it could be speculated that, also in humans, PPARα promotes both fatty acid uptake and oxidation in liver.

To our knowledge, this is the first study performed in humans addressing the tissue-specific metabolic effects of PPARα agonists in liver and heart. Our results showed a tendency toward a decreased myocardial MRgluc, which was not associated with decreased cardiac function. The idea that this increase in liver fat is different from hepatic fat accumulation in obesity and T2DM is supported by the finding that PPARα treatment did not decrease whole-body insulin sensitivity and numerically increased whole-body fat oxidation. Ciprofibrate did result in a further reduction of hepatic MRgluc, which could be interpreted as a reduction in hepatic insulin sensitivity, consistent with the elevated hepatic fat content. Alternatively, reduced hepatic MRgluc can also be seen as an increased reliance on fatty acid oxidation and can be regarded as a reflection of altered substrate competition and not necessarily a reflection of reduced hepatic insulin sensitivity. This would be in line with previous studies that have shown that PPARα agonists do not influence glucose homeostasis in humans [14, 41]. In concert, these findings have suggested that, upon PPARα treatment, a redistribution of substrate occurs in the liver and the heart toward increased fatty acid uptake and oxidation. Similarly, the tendency toward decreased myocardial MRgluc in our study was not associated with decreased cardiac function. Again, PPARα-induced changes in substrate metabolism may be metabolically different from obesity-induced insulin resistance in the heart, where there is increased fatty acid availability without concomitant changes in fat oxidation. In concert, these findings may suggest that, upon PPARα treatment, a redistribution of substrate occurs in the liver and the heart toward increased fatty acid uptake and oxidation. However, this needs to be further investigated.

To minimize participant heterogeneity, and because PPARα and estrogen compete [42], we chose to include only male individuals in

this study. However, we know from literature that male and female individuals with T2DM may differ in cardiac function. Therefore, the metabolic effects of treatment in the female population remain to be explored. Second, our volunteers had a marginally elevated lipid profile, with average TG levels of 2.15 mmoL/L. Patients with markedly elevated baseline TG levels may show greater treatment effects based on lowering of the plasma TG levels [43], but the sample size of the current study is too small to conclude whether baseline TG levels had any effect on the metabolic outcomes in our study and/or whether larger effects can be expected in patients with markedly elevated TG levels. In addition, we gave ciprofibrate for a period of 5 weeks, which is enough to change plasma levels of TGs and PPARα expression [44, 45]. However, the long-term effects on metabolism still need to be established. Finally, our insulin-resistant volunteers had normal diastolic cardiac function, implying that the window for improvement of cardiac function after ciprofibrate treatment was very small.

Some studies have suggested that fibrates may increase muscle fatty acid oxidation rates [46]. This may also contribute to a lowering of plasma TG levels. However, the muscle-specific effects of fatty acid oxidation upon ciprofibrate remain debatable [47, 48]. Fatty acid oxidation in muscle upon ciprofibrate treatment has not been investigated in humans. In our study, we also did not measure fatty acid oxidation rates in muscle. This is a limitation. However, we also did not find any effects on the downstream targets of PPARα (PDK4, CTP-1, and PGC1a). Therefore, an upregulation of fatty acid oxidation rates in muscle is less likely.

Last, a limitation of this study is that dietary intake was not registered. Participants were instructed to maintain stable dietary habits. However, ciprofibrate could have affected appetite. This was not measured. However, the weight of the participants during the study was stable, and body composition was unaltered. Therefore, we do conclude that, if there were any effects, these effects were probably minor and could not have affected the change in fat distribution with an increase in hepatic TGs.

CONCLUSION

Five weeks of treatment with the PPARα agonist ciprofibrate decreased insulin-stimulated MRgluc in the liver, with a similar tendency in the heart. Furthermore, PPARα agonist treatment increased liver fat, which was not associated with a decline in whole-body insulin sensitivity or with reduced cardiac function parameters or cardiac energy status. The mechanisms behind these effects remain speculative and they need further investigation. One direction may be to investigate whether a redistribution of substrate metabolism occurs in the liver and the heart, i.e., toward increased fatty acid uptake and oxidation.O

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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SUPPORTING INFORMATION

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