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Fenofibrate administration does not affect muscle triglyceride concentration or insulin sensitivity in humans

Leigh Perreault, Bryan C. Bergman, Devon M. Hunerdosse, David J. Howard, Robert H. Eckel*

Division of Endocrinology, Metabolism and Diabetes, University of Colorado Denver School of Medicine, Aurora, CO 80045, USA

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ABSTRACT

Animal data suggest that males, in particular, rely on peroxisome proliferator activated receptor–α activity to maintain normal muscle triglyceride metabolism. We sought to examine whether this was also true in men vs women and its relationship to insulin sensitivity (Si). Normolipidemic obese men (n = 9) and women (n = 9) underwent an assessment of Si (intravenous glucose tolerance test) and intramuscular triglyceride (IMTG) metabolism (gas chromatography/mass spectrometry and gas chromatography–combustion isotope ratio mass spectrometry from plasma and muscle biopsies taken after infusion of [U-^{13}C]palmitate) before and after 12 weeks of fenofibrate treatment. Women were more insulin sensitive (Si: 5.2 ± 0.7 vs 2.4 ± 0.4 ×10^{-4}/ μU/mL, W vs M, $P < .01$) at baseline despite similar IMTG concentration (41.9 ± 15.5 vs 30.8 ± 5.1 μg/mg dry weight, W vs M, $P = .43$) and IMTG fractional synthesis rate (FSR) (0.27%/h ± 0.07%/h vs 0.35%/h ± 0.06%/h, W vs M, $P = .41$) as men. Fenofibrate enhanced FSR in men (0.35 ± 0.06 to 0.54 ± 0.06, $P = .05$), with no such change seen in women (0.27 ± 0.07 to 0.32 ± 0.13, $P = .73$) and no change in IMTG concentration in either group (23.0 ± 3.9 in M, $P = .26$ vs baseline; 36.3 ± 12.0 in W, $P = .79$ vs baseline). Insulin sensitivity was unaffected by fenofibrate ($P \geq .68$). Lower percentage saturation of IMTG in women vs men before (29.1% ± 2.3% vs 35.2% ± 1.7%, $P = .06$) and after (27.3% ± 2.8% vs 35.1% ± 1.9%, $P = .04$) fenofibrate most closely related to their greater Si ($R^2 = 0.34$, $P = .10$) and was largely unchanged by the drug. Peroxisome proliferator activated receptor–α agonist therapy had little effect on IMTG metabolism in men or women. Intramuscular triglyceride saturation, rather than IMTG concentration or FSR, most closely (but not significantly) related to Si and was unchanged by fenofibrate administration.

1. Introduction

Skeletal muscle is the major tissue responsible for insulin action on peripheral glucose uptake and therefore has been implicated as a primary site for the development of insulin resistance and type 2 diabetes mellitus [1,2]. Considerable attention has been paid to examining the role of intramuscular triglyceride (IMTG), in particular, in this process. Repeated observations using different techniques have noted a positive linear relationship between IMTG concentration and insulin resistance [3-6] in both men and women. Nevertheless, when men and women are directly compared as first-degree relatives of people with diabetes [6], by age [7], or by glucose tolerance status [8], women are generally more

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* *Corresponding author*. University of Colorado Denver, PO Box 6511, MS F8106, Aurora, CO 80045, USA. Tel.: +1 303 724 3947; fax: +1 303 724 3920.
E-mail address: leigh.perreault@ucdenver.edu (L. Perreault).

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insulin sensitive than men despite as great or greater concentration of IMTG. Together, these findings have led to speculation about the importance of composition and/or turnover of the IMTG pool, rather than its total size [9]. Recently, Bergman et al [9,10] demonstrated the significance of low IMTG synthesis rate (a component of IMTG turnover) and high saturation of IMTG to insulin resistance. However, these data were predominantly collected in men. Because women generally have a higher concentration of IMTG compared with men, it is possible that higher rates of IMTG synthesis and/or lower percentage saturation in IMTG helps explain sex differences in insulin sensitivity (Si) and in the progression from obesity to diabetes [8].

Of the vast array of possible regulators of IMTG metabolism, there is reason to believe that peroxisome proliferator activated receptor–α (PPAR- α) is particularly critical to maintain normal lipid trafficking and insulin action in male animal models. For example, etomoxir (a carnitine palmitoyltransferase–1 [CPT-1] inhibitor, CPT-1 being a known target gene of PPAR-α) is universally lethal when given to male, but not female, PPAR-α knockout mice [11]. Furthermore, the male mice can be rescued when pretreated with estradiol. In addition, the use of fenofibrate (PPAR-α agonist) has been shown to specifically reduce IMTG and improve Si proportionally in several male animal models [12-14]. Whether fenofibrate administration could favorably alter IMTG dynamics and insulin action in obese men vs women had not been previously explored and hence was the aim of the current study. We hypothesized that men would have lower baseline PPAR-α protein expression in muscle commensurate with lower IMTG fractional synthesis rate (FSR)—as a surrogate measure of IMTG turnover—and lower Si and, furthermore, that we could enhance IMTG FSR and Si with fenofibrate administration.

2. Methods

2.1. Subjects

Overweight or obese nonsmoking men (n = 9) and postmenopausal women (n = 9) between the ages of 45 and 70 years were studied. All subjects were free of diabetes, but were at high risk for the disease by virtue of their age, body mass index, and having a first-degree relative with type 2 diabetes mellitus. Volunteers were sedentary (<90 min/wk planned activity) and were deemed healthy by history, physical examination, and screening blood tests. Subjects were excluded for the following: fasting glucose greater than 6.9 mmol/L, glucose 2 hours post–75-g oral glucose load greater than 11.1 mmol/L, thyroid-stimulating hormone less than 50 or greater than 500 mU/L, fasting triglycerides (TGs) greater than 2.26 mmol/L, creatinine greater than 130 μmol/L, elevated liver function tests (>2× normal), hematocrit less than 38%, or white blood cell count less than 3.0×10^3. Use of medications for lipid and/or glucose lowering also excluded enrollees. Approval for this study was obtained by the Colorado Multiple Institutional Review Board before its commencement. All volunteers provided their informed consent.

2.2. Prestudy measures

Body composition was estimated from dual-energy x-ray absorptiometry.

2.3. Intravenous glucose tolerance test

A modified frequently sampled intravenous glucose tolerance test (IVGTT) was performed as previously described by Bergman et al [15]. Insulin sensitivity and secretion (acute insulin response (AIR) and the disposition index (Si × AIR = DI)) were calculated using the MINMOD computer program (Millennium version; MINMOD, Los Angeles, CA).

2.4. Prestudy diet control

Subjects were fed a control diet for 3 days before admission to the General Clinical Research Center (GCRC) for pre- and postintervention study days. The control diet was isocaloric (calculated as 1.4 × [372 + (23.9 × fat-free mass)] calories per day), using the fat-free mass measured by dual-energy x-ray absorptiometry. The diet composition was standardized as 30% fat (saturated, polyunsaturated, and monounsaturated fats in a 1:1:1 ratio), 15% protein, and 55% carbohydrate.

2.4.1. Study day

Subjects were fasted overnight (~12 hours) and were admitted to the GCRC at 7:30 AM on the day of the IMTG turnover study. Upon admission, an intravenous catheter was placed in an antecubital vein for infusion; and sampling catheter was placed in a dorsal hand vein of the contralateral arm. For all blood samples, the heated hand technique was used to arterialize the blood. Background sampling began 30 minutes after sampling catheters had been placed. A baseline blood sample was drawn for determination of circulating hormone and substrate concentrations (catecholamines, insulin, glucose, c-peptide, glucagon, free fatty acids [FFAs], glycerol, and lactate). Following the baseline blood draw, a continuous infusion of [U-^{13}C]palmitate (Isotec, Miamisburg, OH) bound to human albumin was initiated at 0.0174 μmol/(kg min) and continued throughout the study. Subjects remained semirecumbent for 4 hours to allow for tracer incorporation into the intramuscular lipid pools. Blood samples were taken for hormone and substrate concentrations (as above) during the final 30 minutes of the 4-hour rest period. Indirect calorimetry was performed before blood sampling. Following the rest period, a vastus lateralis skeletal muscle biopsy was taken using the Bergstrom technique [16]. Muscle was immediately flash frozen in liquid nitrogen and stored at –80°C until dissection and analysis.

2.4.2. Intervention

After completion of the study day, all subjects received open-label fenofibrate (Tricor, Abbott Laboratories, Abbott Park, IL) 145 mg po qd × 12 weeks. Participants were counseled at length about possible adverse effects and allergic reactions. Information on tolerability was solicited weekly, and medication compliance was assessed by final pill count. Subjects were asked to remain weight stable throughout the intervention period. The diet was not controlled except for the 3 days before the postintervention study day.

2.4.3. *Postintervention study day*

Twenty-four hours after ingestion of the final dose of fenofibrate, subjects returned to the GCRC, at which time the study day (described above) was repeated.

2.5. *Methods*

2.5.1. *Circulating hormone and substrate concentrations*

All samples were stored at –80°C until analysis. Radioimmunoassay was used to determine insulin and glucagon (Linco Research, St Louis, MO), as well as c-peptide (Gamma counter; Diagnostic Products, Los Angeles, CA), concentrations. Standard enzymatic assays were used to measure glucose (COBA-Mira Plus; Roche Diagnostics, Mannheim, Germany), screening lipid panel (Beckman Coulter, Fort Collins, CO), lactate (Kit 826; Sigma, St Louis, MO), glycerol (Boehringer Mannheim Diagnostics, Mannheim, Germany), and FFAs (NEFA Kit, Wako, TX). Epinephrine and norepinephrine concentrations were measured using high-performance liquid chromatography (Dionex, Sunnyvale, CA).

2.5.2. *Whole-body substrate oxidation*

Whole-body substrate oxidation was measured using indirect calorimetry. Oxygen consumption and carbon dioxide production were used to calculate metabolic rate, as well as the oxidation of carbohydrate and fat using standard equations.

2.5.3. *Muscle lipid analysis*

Skeletal muscle samples were dissected free of extramuscular fat on ice as described by Guo et al [17]. Muscle (~70 mg) was lyophilized, added to 1 mL iced MeOH along with internal standards of tripentadecanoic acid and dipentadecanoic acid, and homogenized (Omni TH; Omni International, Marietta, GA). Total lipids were extracted [18] and then added to solid phase extraction columns (Supelclean LC-NH_2, 3 mL; Supelco Analytical, Bellefonte, PA) to isolate FFAs and IMTG as described by Kaluzny et al [19]. The FFA fraction was methylated using 0.5 mL 2% sulfuric acid and heated at 100°C for 1.5 hours. The IMTG fraction was converted to fatty acid methyl ester by transmethylation using sodium methoxide. Stable isotope ratios of ^{13}C in fatty acid methyl esters were measured using a gas chromatography (GC)–combustion isotope ratio mass spectrometer system (Thermo Electron, Bremen, Germany). Enrichment was calculated based on a standard curve of known enrichments and corrected for variations in abundance [20]. Concentration and composition analysis was performed on an HP 6890 GC (Hewlett-Packard, Palo Alto, CA) with a 30-m DB-23 capillary column, connected to an HP 5973 mass spectrometer (MS). Peak identities were determined by retention time, and mass spectra were compared with standards of known composition.

2.5.4. *Whole-body palmitate oxidation*

Two milliliters of breath CO_2 was transferred into a 20-mL Exetainer for the measurement of $^{13}CO_2/^{12}CO_2$ with continuous flow isotope ratio mass spectrometry (Delta V, Thermo Electron, Labco Ltd, High Wycombe, Buckinghamshire, England). Each sample was injected (1.2 μL per injection) in duplicate for isotope ratio analyses, with an average standard deviation for all injections of 0.0001 atom percent.

2.5.5. *Plasma palmitate enrichment and TG composition*

Methylation and extraction of plasma palmitate were performed as previously described [21]. Plasma TG was isolated as described by Agren et al [22] and methylated using sodium methoxide as previously published [9]. Samples were run on an HP 6890 GC with a 30-m DB-23 capillary column, connected to a HP 5973 MS. Enrichments were calculated based on a standard curve of known enrichments and corrected for variations in abundance [20]. Peak identities were determined by retention time, and mass spectra were compared with standards of known composition.

2.5.6. *Western blotting*

Frozen skeletal muscle samples were weighed and homogenized on ice using a Kontes glass homogenizer (Kimble/Kontes, Vineland, NJ) in buffer [10]. Protein was extracted, concentration was measured (Calbiochem, San Diego, CA), and 40 μg of sample protein and an internal standard were run on an sodium dodecyl sulfate polyacrylamide gel electrophoresis 8% Bis-Tris gel (Invitrogen, Carlsbad, CA) using standard methods previously described [10]. Anti-human myosin A4.840 and A4.74 antibodies were purchased from the University of Iowa Hybridoma Bank (Iowa City, IA); anti-rabbit succinate dehydrogenase (SDH) and PPAR-α (Santa Cruz Biotechnology, Santa Cruz, CA), mitogen-activated protein kinase kinase kinase kinase 4 (Abgent, San Diego, CA), IRS-1ser^{636} and IRS-1total (Cell Signaling Technology, Danvers, MA), protein kinase C isoform ε (Cell Signaling Technology, Beverly, MA), and CPT-1 (Alpha Diagnostics International, San Antonio, TX) antibodies were commercially available. The rabbit anti–4-hydroxynonenal antibody was a generous gift from Dr Dennis Peterson (University of Colorado Denver). Secondary antibodies were from Bio-Rad (Hercules, CA).

2.5.7. *Calculations*

The IMTG FSR was calculated as previously described by our laboratory [10].

$$\%\ \text{Saturation of IMTG} = (\text{laurate} + \text{myristate} + \text{palmitate} + \text{stearate}) / \left(\sum \text{FFA species}\right) \times 100,$$

where FFAs represent concentration of individual FFA species in IMTG after transmethylation.

Palmitate rate of disappearance (Rd) and palmitate rate of oxidation were calculated using steady-state kinetics and a whole-body estimate of carbon label retention as previously described [23]. Calculation of palmitate oxidation rates was made using published values for the acetate recovery factor in obese humans at rest [24]. Nonplasma fatty acid oxidation was calculated as the difference between whole-body fat oxidation and whole-body palmitate oxidation.

2.6. *Statistical analysis*

Prespecified primary outcome data were Si and IMTG FSR, with secondary outcomes specified as IMTG concentration and saturation. Because of the small sample size, data were not corrected for multiple comparisons. Testing of the data

revealed a nonnormal distribution; therefore, analyses were conducted on log-transformed values. Comparisons between groups were made using analysis of variance for continuous variables and χ^2 (Fisher exact where appropriate) for categorical variables (SPSS, Chicago, IL). All data are presented as mean ± SEM. Overall significance was set at $P < .05$.

3. Results

3.1. Subject demographics

Men and women were of similar age (59 ± 1.8 vs 60 ± 0.9 years, respectively; $P = .43$). Body mass index was higher in men (32 ± 1.4 vs 28 ± 0.7 kg/m^2, $P = .03$ vs W), whereas body fat was higher in women (41% ± 1.4% vs 32% ± 1.4%, $P < .01$ vs M). Total cholesterol was higher in women (6.0 ± 0.2 vs 5.1 ± 0.2 mmol/L, W vs M, $P = .01$), in part because of higher high-density lipoprotein cholesterol (1.4 ± 0.1 vs 1.0 ± 0.1 mmol/L, W vs M, $P < .01$). Otherwise, baseline low-density lipoprotein cholesterol (3.9 ± 0.3 vs 3.3 ± 0.2 mmol/L, W vs M, $P = .19$) and plasma TGs (1.6 ± 0.3 vs 1.6 ± 0.2 mmol/L, W vs M, $P = 1.0$) were similar between the sexes on the screening (no preceding diet control) lipid panel.

3.2. Hormone and substrate concentrations

Pre- and postintervention hormone and substrate concentrations are summarized in Table 1. There were no sex differences in hormone and substrate concentrations at baseline except slightly higher norepinephrine in women vs men ($P = .04$). Fenofibrate lowered fasting glucose in both groups ($P < .01$) and also insulin concentration in women ($P = .05$) such that it was lower than in men postintervention ($P = .03$).

3.3. Insulin action and secretion

Insulin action (Si) was greater in women vs men before ($P = .004$) and after ($P = .004$) fenofibrate and was unchanged in either group ($P \geq .68$; Fig. 1). Measures of insulin secretion (AIR and DI) were not different before (AIR: 337 ± 40 vs 265 ± 71 mU/[mL min], M vs W, $P = .64$; DI: 649 ± 241 vs 1313 ± 366 × 10^{-4}/min, M vs W, $P = .16$) or after ($P \geq .35$) fenofibrate and also were unchanged by the drug ($P \geq .63$).

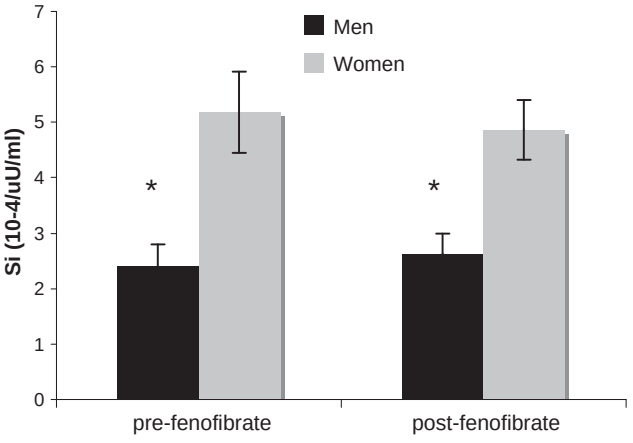

Fig. 1 – Insulin sensitivity pre- and postfenofibrate in men and women. *P < .05 men vs women.

3.4. Whole-body substrate use

Respiratory exchange ratio (RER), a measure of whole-body substrate use, was similar between groups at baseline (0.78 ± 0.02 vs 0.75 ± 0.01, M vs W, $P = .21$). Following fenofibrate administration, there was a trend for an increase in RER in the men ($P = .09$) such that the RER was higher than that in women (0.83 ± 0.02 vs 0.77 ± 0.01, M vs W, $P = .004$).

3.5. IMTG concentration, composition, and synthesis rate

Intramuscular triglyceride concentration was similar at baseline ($P = .43$) and after fenofibrate ($P = .22$; Fig. 2A). Intramuscular triglyceride FSR (a measure of IMTG turnover) increased in men (pre- vs posttreatment, $P = .05$), with no change observed in women ($P = .73$; Fig. 2B). A trend for lower IMTG saturation seen in women pretreatment ($P = .06$) became significant posttreatment ($P = .04$; Fig. 2C) despite little change in either group ($P \geq .65$). A nonsignificant trend relating IMTG saturation to Si ($R^2 = 0.34$, $P = .10$) was observed in the cohort as a whole. In examining the individual fatty acid species in IMTG, women had more oleic acid before ($P = .01$) and after ($P = .02$) fenofibrate, whereas the men had more palmitic ($P = .04$)

Table 1 – Hormone and substrate concentrations

Prefenofibrate	Glucose (mmol/L)	Insulin (pmol/L)	Glucagon (ng/L)	FFA (μmol/L)	Glycerol (μmol/L)	Lactate (mmol/L)	Epi (pmol/L)	Norepi (nmol/L)
Men	5.7 ± 0.2	88 ± 15	78 ± 11	559 ± 41	87 ± 5.5	0.69 ± 0.07	153 ± 17	1.3 ± 0.1 *
Women	5.3 ± 0.2	57 ± 11	60 ± 5.3	663 ± 63	112 ± 12.2	0.54 ± 0.08	137 ± 17	1.9 ± 0.2
Postfenofibrate	**Glucose (mg/dL)**	**Insulin (μU/mL)**	**Glucagon (pg/mL)**	**FFA (μmol/L)**	**Glycerol (μmol/L)**	**Lactate (mmol/L)**	**Epi (pg/mL)**	**Norepi (pg/mL)**
Men	4.8 ± 0.1 †	73 ± 17	74 ± 10	639 ± 74	84 ± 9.2	0.56 ± 0.06	126 ± 12	1.3 ± 0.1
Women	4.6 ± 0.1 †	31 ± 5 *,†	57 ± 4.5	725 ± 62	104 ± 11.4	0.43 ± 0.10	153 ± 16	1.7 ± 0.2

Epi indicates epinephrine; Norepi, norepinephrine.
* $P < .05$ men vs women.
† $P < .05$ pre- vs postintervention.

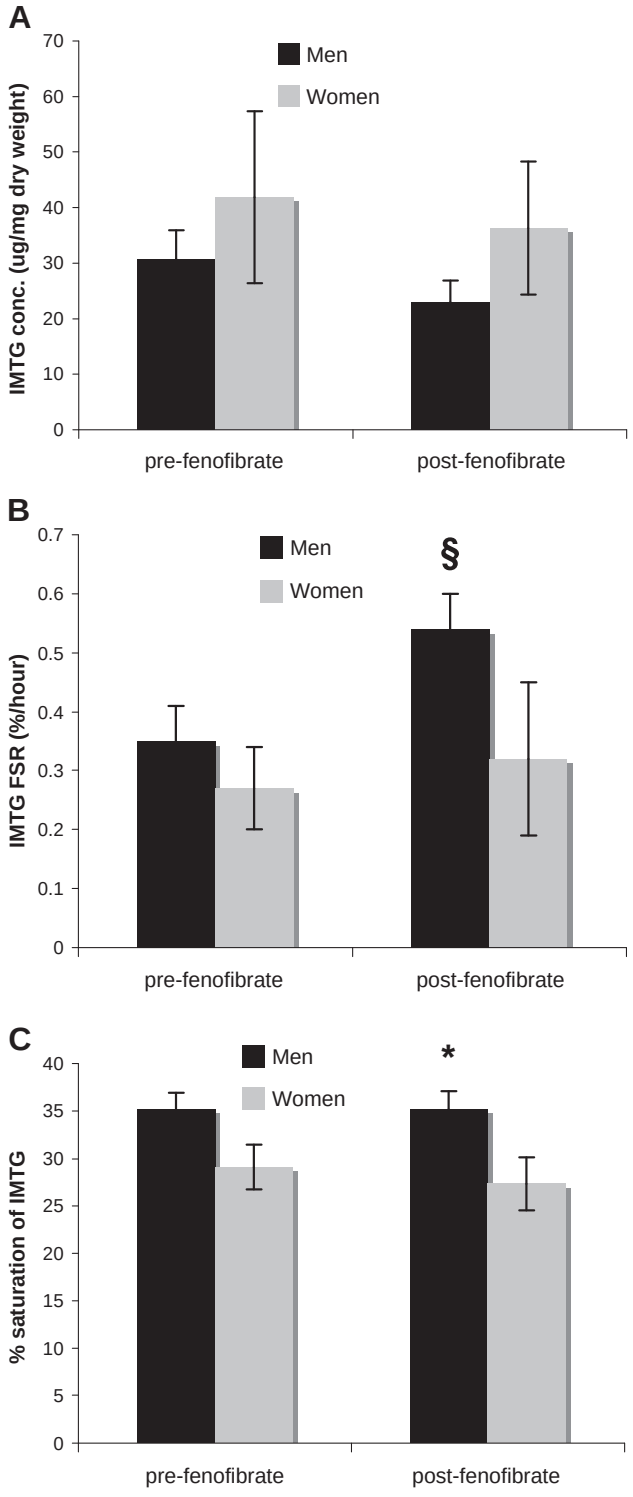

Fig. 2 – Intramuscular TG concentration (A), FSR (B), and saturation (C) pre- and postfenofibrate in men and women. *P < .05 men vs women; §P < .05 pre- vs postintervention.

and stearic (P = .03) acid, as well as lower palmitoleic acid (P = .02), vs women postfenofibrate (Table 2). Ratios of 16:1/16:0 (0.25 ± 0.06 vs 0.13 ± 0.01, W vs M posttreatment, P = .02) and 18:1/18:0 (11.4 ± 2.6 vs 5.1 ± 0.9, W vs M posttreatment, P = .02), a surrogate for stearyl CoA desaturase–1 (SCD-1) activity [25], were both significantly higher in women posttreatment but not different between groups pretreatment. Palmitate Rd was similar between men and women predrug (3.44 ± 0.78 vs 3.53 ± 0.46 μmol/[kg min], M vs W, P = .92); yet a marked decline in Rd in men was seen postdrug, such that men had a significantly lower palmitate disposal posttreatment (1.85 ± 0.30 vs 4.22 ± 0.51 μ/[kg min], M vs W, P = .003). No differences were noted between the sexes with respect to palmitate incorporation or oxidation before or after fenofibrate (data not shown).

3.6. *Plasma TG concentration and composition*

Plasma TG concentration (assessed by GC/MS post–diet control) trended to be higher in men (1.37 ± 0.08 mmol/L) vs women (1.04 ± 0.15 mmol/L, P = .09) at baseline. However, fenofibrate significantly decreased total plasma TG in men (P = .03; not women, P = .28) by preferentially decreasing unsaturated fatty acids such as oleic acid (P = .01), linoleate (P = .01), and linolenic acid (P = .02) (Table 2). Men tended to have more overall saturated plasma TG at baseline and following fenofibrate (P = .06 for both). Women had less myristic acid (P = .02) and stearic acid (P = .02), as well as more arachidonic acid (P = .03), at baseline, whereas no such differences were noted posttreatment (Table 2).

3.7. *Protein expression*

Enzymes influencing IMTG metabolism were examined by Western blot [10]. Skeletal muscle oxidative capacity, assessed by SDH, was lower in men (0.75 ± 0.06 AU) compared with women (1.30 ± 0.24 AU, P = .02) posttreatment, but not pretreatment (P = .17). Fenofibrate also lowered CPT-1 in men (1.19 ± 0.10 vs 0.81 ± 0.11 AU, P = .03). Otherwise, protein abundance for myosin A4.840 (type 1 muscle fibers), myosin A4.74 (type 2 muscle fibers), mitogen-activated protein kinase kinase kinase kinase 4, 4-hydroxynonenal, insulin receptor substrate–1 serine phosphorylated at position 636 (IRS-1ser^{636}), ratio of IRS-1ser^{636} to IRS-1total, PPAR-α, and protein kinase C isoform ε was not different between groups (data not shown).

3.8. *Medication tolerability and compliance*

Fenofibrate at 145 mg orally per day × 12 weeks was well tolerated in all subjects. There were no dropouts and no serious adverse events, and compliance was greater than 95% (no difference between men and women). There was no significant change in weight in either group (0.58 ± 0.36 vs 0.63 ± 0.25 kg weight change, M vs W, P = .92).

4. Discussion

Increasing evidence suggests that obese men and women may develop diabetes differently. Specifically, we have previously shown that altered IMTG metabolism relates to diminished insulin action in men, but not women, in the progression to diabetes [8]. Specifically, lower Si, higher IMTG concentration, and lower IMTG FSR cluster in men with prediabetes (vs simple obesity), with no such differences observed in women

Table 2 – Composition of plasma and IMTG pre- and postfenofibrate

Plasma	Myristic (mmol/L)	Palmitic (mmol/L)	Palmitoleic (mmol/L)	Stearic (mmol/L)	Oleic (mmol/L)	Linoeate (mmol/L)	γ-Linolenic (mmol/L)	α-Linolenic (mmol/L)	Arachidonic (mmol/L)
Prefenofibrate									
Men	0.12 ± 0.02 *	1.04 ± 0.08	0.12 ± 0.01	0.40 ± 0.04 *	1.19 ± 0.07 †	1.01 ± 0.10 †	0.03 ± 0.00	0.07 ± 0.01 †	0.11 ± 0.01 *
Women	0.06 ± 0.01	0.83 ± 0.12	0.13 ± 0.03	0.27 ± 0.03	1.11 ± 0.19	0.91 ± 0.11	0.02 ± 0.00	0.07 ± 0.01	0.08 ± 0.01
Postfenofibrate									
Men	0.07 ± 0.01	0.79 ± 0.09	0.11 ± 0.02	0.27 ± 0.05	0.90 ± 0.07	0.66 ± 0.05	0.03 ± 0.00	0.04 ± 0.01	0.11 ± 0.01
Women	0.04 ± 0.01	0.63 ± 0.10	0.11 ± 0.02	0.20 ± 0.03	0.86 ± 0.15	0.62 ± 0.12	0.02 ± 0.00	0.04 ± 0.01	0.09 ± 0.01

Muscle	Myristic (μ/mg dry wt)	Palmitic (μ/mg dry wt)	Palmitoleic (μg/mg dry wt)	Stearic (μg/mg dry wt)	Oleic (μg/mg dry wt)	Linoeate (μg/mg dry wt)	Linolenic (μg/mg dry wt)	Arachidonic (μg/mg dry wt)
Prefenofibrate								
Men	1.84 ± 0.21	24.19 ± 0.62	4.51 ± 0.57	9.14 ± 1.52	33.56 ± 3.29 *,†	16.13 ± 0.33	0.84 ± 0.05	0.85 ± 0.11
Women	2.24 ± 0.16	23.88 ± 0.86	5.00 ± 0.35	6.23 ± 0.59	45.89 ± 1.68	15.21 ± 0.40	0.86 ± 0.05	0.68 ± 0.10
Postfenofibrate								
Men	2.23 ± 0.20	25.46 ± 0.69 *	3.40 ± 0.31 *	9.35 ± 1.03 *	42.19 ± 1.36 *	15.02 ± 0.06	0.91 ± 0.04	0.89 ± 0.08
Women	1.78 ± 0.31	21.62 ± 1.62	5.25 ± 0.74	5.26 ± 1.24	49.40 ± 2.39	14.89 ± 0.05	0.85 ± 0.04	0.73 ± 0.09

Wt indicates weight.
* $P < .05$ men vs women.
† $P < .05$ pre- vs postfenofbrate.

at the same stages of glucose intolerance. Peroxisome proliferator activated receptor–α is a known mediator of intramuscular lipid trafficking, particularly requisite in male animal models [11]. The significance of PPAR-α in skeletal muscle of male humans at risk for diabetes had not been previously explored and was the aim of the current study. Major findings from this study demonstrated that fenofibrate (a PPAR-α agonist) administration (1) increased IMTG FSR in men but (2) did not change IMTG concentration or saturation in either sex and (3) ultimately did not impact Si in men or women. Notably, IMTG saturation, rather than concentration or FSR, most closely (but not significantly) related to insulin resistance in the cohort as a whole. Fenofibrate lowered total plasma TG concentration in the normolipidemic men in this study, but did so at the expense of unsaturated fatty acids. In contrast, presumed enhancement of SCD-1 activity further desaturated IMTG in women and may have contributed to their greater Si.

Reports examining the effect of fenofibrate on Si are mixed, largely because of differences in PPAR-α distribution in insulin-sensitive tissues that vary widely between species. For example, improved Si post–fibrate treatment has been universally observed in rodent models [13,26,27], but virtually never in human studies [28-30], including ours. We did observe a decrease in both circulating glucose and insulin concentration in women, as well as a lower circulating glucose concentration in men, postfenofibrate; but these subtle changes did not significantly impact the index of whole-body Si derived by the IVGTT. Furthermore, markers of insulin signaling in muscle from the Western blots, although noninsulin stimulated, were unchanged posttreatment in either sex. Together, these data serve as the first to demonstrate that PPAR-α agonists have no major effect on insulin action in human skeletal muscle in vivo.

Increasing evidence suggests that IMTG concentration and Si can be dissociated [31-33]. Our results suggest that the same may be true for IMTG FSR and Si. Collectively, these observations underscore the complexity and continued interest in the link between IMTG dynamics and insulin action [34,35]. Recent studies in both animals and humans have concluded that high IMTG concentration may not be deleterious to Si as long as IMTG flux is preserved [31-33]. Nevertheless, the current study increased IMTG FSR in men a priori and did not observe the expected improvement in Si. Of note, in the basal state, IMTG FSR approximates IMTG degradation and thus serves as a surrogate marker of turnover in the IMTG pool. It is possible that fenofibrate had mixed actions on IMTG metabolism in men that obscured an improvement in Si from increased FSR. For example, markers of lipid uptake (palmitate Rd and CPT-1) and oxidation (SDH) were suppressed by fenofibrate. Unchanged IMTG concentration amidst an increase in IMTG FSR would suggest that the degradation rate of IMTG also increased to match the synthesis rate. Taken together, the FFAs liberated during IMTG degradation likely were used for IMTG resynthesis, rather than oxidized. These findings support the notion that PPAR-α activity has distinct effects on tissue lipid partitioning in men, and these are independent of insulin action.

In contrast to the changes observed in men, fenofibrate administration appeared to have virtually no effect in women. Women were more insulin sensitive than men both before and after the intervention, as has been previously reported in carefully matched men and women [8,36,37]. Interestingly, degree of saturated IMTG, rather than IMTG concentration or FSR, most closely (although not significantly) related to Si in this cohort, as in others [9,10]. Our women trended toward having less saturated IMTG before treatment that became significantly lower after treatment. This may have been due to an increase in skeletal muscle SCD-1 activity, as suggested by an increase in 16:1/16:0 and 18:1/18:0 ratios [25]. Similar to skeletal muscle, plasma TG also tended to be less saturated in women before and after treatment. Understanding how women maintain a state of less saturated TG and how fenofibrate may have influenced this may prove a novel pathway for insulin sensitization.

The observed changes in IMTG composition and FSR were presumed to be due to PPAR-α activation in skeletal muscle by fenofibrate. Most surprising was the lack of change in skeletal muscle PPAR-α protein expression following treatment. Interestingly, PPAR-α is expressed most prominently in human skeletal muscle [38]; but its activity appears to be far greater in liver [39,40]. Specifically, fenofibrate accelerates the clearance of TG-rich lipoproteins [30], likely affecting the delivery of FFA to muscle for uptake into IMTG. Thus, we speculate that changes in IMTG metabolism may have been indirect, through the enhanced action of PPAR-α in the liver, affecting downstream delivery of substrate to muscle, not altered PPAR-α activity in muscle itself. This speculation cannot be confirmed, as we did not measure PPAR-α protein expression in liver. However, the decline in plasma TG in the men is consistent with this contention. In short, much work is still needed to elucidate the sexual dimorphism in response to PPAR-α agonists noted here and in recent clinic trials [41].

There are several limitations of the current study worth noting. First, a published, not individually measured, acetate recovery factor was used in the calculation of palmitate oxidation. This was done because IMTG dynamics, not palmitate oxidation, was the primary outcome of interest. Second, all subjects received open-label fenofibrate; and none received placebo. Thus, day-to-day variation in our outcome measures was not captured; and additional studies will be needed to confirm our findings. It is also possible that use of the hyperinsulinemic/euglycemic clamp may have been a more precise measure of Si, but numerous validation studies have demonstrated close correlation between Si (from the IVGTT) and glucose infusion rate (from the clamp). Lastly, our small sample size likely reduced the ability to detect subtle differences in some parameters of interest. It should also be noted that the data are not corrected for multiple comparisons and thus should be interpreted with caution, generating hypotheses rather than being fully conclusive.

In summary, the current study examined the role of PPAR-α as a possible mediator for the sex differences observed in intramuscular lipid metabolism in obese people at risk for diabetes. We observed an increase in IMTG FSR in men, a change that has been previously shown to relate to enhanced Si in men [8]; however, no change in Si was observed in the current study. Insulin sensitivity appears more closely related to the degree of IMTG saturation than to its concentration or synthesis rate [9,10]. Lower TG saturation in plasma and/or muscle of women may be vital in maintaining their higher Si in this study and others. These data suggest that changing plasma and/or IMTG saturation may be an avenue for future therapies to enhance Si and prevent the development of diabetes.

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