Activation of Peroxisome Proliferator–Activated Receptor (PPAR)δ Promotes Reversal of Multiple Metabolic Abnormalities, Reduces Oxidative Stress, and Increases Fatty Acid Oxidation in Moderately Obese Men

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OBJECTIVE—Pharmacological use of peroxisome proliferator–activated receptor (PPAR)δ agonists and transgenic overexpression of PPARδ in mice suggest amelioration of features of the metabolic syndrome through enhanced fat oxidation in skeletal muscle. We hypothesize a similar mechanism operates in humans.

RESEARCH DESIGN AND METHODS—The PPARδ agonist (10 mg o.d. GW501516), a comparator PPARα agonist (20 μg o.d. GW590735), and placebo were given in a double-blind, randomized, three-parallel group, 2-week study to six healthy moderately overweight subjects in each group. Metabolic evaluation was made before and after treatment including liver fat quantification, fasting blood samples, a 6-h meal tolerance test with stable isotope fatty acids, skeletal muscle biopsy for gene expression, and urinary isoprostanes for global oxidative stress.

RESULTS—Treatment with GW501516 showed statistically significant reductions in fasting plasma triglycerides (−30%), apolipoprotein B (−26%), LDL cholesterol (−23%), and insulin (−11%), whereas HDL cholesterol was unchanged. A 20% reduction in liver fat content ($P < 0.05$) and 30% reduction in urinary isoprostanes ($P = 0.01$) were also observed. Except for a lowering of triglycerides (−30%, $P < 0.05$), none of these changes were observed in response to GW590735. The relative proportion of exhaled CO_2 directly originating from the fat content of the meal was increased ($P < 0.05$) in response to GW501516, and skeletal muscle expression of carnitine palmitoyl-transferase 1b (*CPT1b*) was also significantly increased.

CONCLUSIONS—The PPARδ agonist GW501516 reverses multiple abnormalities associated with the metabolic syndrome without increasing oxidative stress. The effect is probably caused by increased fat oxidation in skeletal muscle. ***Diabetes* 57: 332–339, 2008**

Hypertriglyceridemia and abdominal obesity are key components of the metabolic syndrome. They may result from an inability of adipose tissue to sequester fatty acids appropriately for storage (1). Instead, fatty acids are deposited as ectopic fat in skeletal muscle (2), liver (3), and other organs (4). It is thought that such fat accumulation is linked to impaired metabolic function of the tissue in question (5,6).

Genes under transcriptional control of peroxisomal proliferator-activated receptors (PPARs), including the subtypes PPARγ, PPARα, and PPARδ, encode a range of proteins and enzymes regulating fatty acid metabolism. These systems are already targets for pharmacological intervention in the treatment of type 2 diabetes or hyperlipidemia. For example, the thiazolidinedione PPARγ agonists are antidiabetes agents that lower plasma glucose (7), reduce insulin resistance (8–11), reduce ectopic fat accumulation in liver (9,12), and delay onset of type 2 diabetes (13). Agonists to PPARα, fibrates, have been used for many years and primarily lower plasma triglycerides. However, fibrates are not known to ameliorate insulin resistance when evaluated with the hyperinsulinemic normoglycemic clamp technique (14), and effects on ectopic fat deposition have not been studied in humans. The triglyceride-lowering effect of fibrates is mediated both by induction of lipoprotein lipase (LPL), the key enzyme for triglyceride removal from blood (15–19), and by reduced expression of apolipoprotein (apo)CIII (20–22), an inhibitor of the action of LPL (23). Fibrates also induce apoAII expression (24), which alters HDL composition and often gives rise to a moderate increase in HDL cholesterol.

Considerably less is known about the function of the

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Received for publication 14 September 2007 and accepted in revised form 10 November 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 16 November 2007. DOI: 10.2337/db07-1318.

U.R. and D.S. contributed equally to this work.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-1318.

Apo, apolipoprotein; AST, aspartate aminotransferase; AUC, area under the curve; γGT, γ-glutamyltransferase; LCM, laser capture microdissection; LPL, lipoprotein lipase; MRI, magnetic resonance imaging; NEFA, nonesterified fatty acid; TTR, tracer-to-tracee ratio.

PPARδ system. Rodent studies suggest that a key feature of PPARδ activation is induction of skeletal muscle fatty acid oxidation (25–27). On activation of PPARδ in skeletal muscle in mice, the fiber composition changes toward the oxidative type I with induction of fatty acid oxidation (28). Interestingly, this type of adaptation is identical to that seen in response to physical exercise, and indeed, mice with transgenic overexpression of PPARδ exhibit increased running endurance (28). The effect of PPARδ on insulin and glucose homeostasis is somewhat controversial. Rhesus monkeys treated with a high dose of the PPARδ agonist GW501516 exhibited reduced fasting insulin concentrations but maintained normal fasting glucose concentrations (29), whereas cultured human myoblasts exposed to the same drug did not show any enhancement of insulin-mediated glucose uptake (30). PPARδ knockout mice appear to be insulin resistant (31). A recent report showed that GW501516 given to lean healthy subjects during metabolic ward conditions for 2 weeks resulted in a modest lowering of diurnal triglyceride concentrations and a lesser lowering of HDL cholesterol concentrations than in controls (32).

To test the hypothesis that lipid lowering can be achieved by an agonist to PPARδ through enhancement of fatty acid oxidation without adverse effects on oxidative stress, we studied the effect of a 2-week exposure to GW501516 on metabolic function in humans. To increase the understanding of the potential lipid-lowering effect of the PPARδ agonist, we also made a comparison with a novel and potent PPARα agonist. The data constitute the first evidence for amelioration of multiple metabolic syndrome components by activation of PPARδ in humans.

RESEARCH DESIGN AND METHODS

This was a double-blind, randomized, three-parallel group, 2-week study of effects on metabolic characteristics and gene expression in response to a PPARδ agonist (10 mg o.d. GW501516) (29), a PPARα agonist (20 μg o.d. GW590735) (33), and placebo. The doses of GW501516 and GW590735 were based on toxicity studies in rodents, dogs, and humans performed within GlaxoSmithKline, where maximal safe doses were 20 μg o.d. for GW590735 and 10 mg o.d. for GW501516. It was also known that these doses resulted in reductions of fasting plasma triglycerides of the same magnitude as that associated with fenofibrate. In addition, in a pilot study on the effects of skeletal muscle transcriptional changes, it had been noted that 2.5 mg o.d. of GW501516 provided at best a marginal change in carnitine palmitoyl-transferase 1b (*CPT1b*) mRNA content. To keep within the limits of the preclinical toxicity range and to offer the best opportunity for finding pharmacodynamic effects, the top doses were chosen for each compound.

Six Caucasian male subjects were randomized to each treatment. All subjects gave written consent. The study protocol was approved by the local Oxford research ethics committee. All subjects (n = 18) were apparently healthy but abdominally obese with moderate dyslipidemia. Subjects were recruited after responding to an advertisement. Inclusion criteria were BMI >27 kg/m^2, waist girth >95 cm, age 18–50 years, fasting plasma triglycerides 100–350 mg/dl, and HDL cholesterol <50 mg/dl. Exclusion criteria were a history of diabetes, cardiovascular disease, or thyroid dysfunction; presence of fasting hyperglycemia (<110 mg/dl), smoking, or any clinically relevant abnormality of a routine 12-lead electrocardiogram; regular intense exercise (defined as sporting at a competitive level or >3 h of accumulated leisure activity aerobic exercise per week); or more than 1.5× ULN elevation of liver function tests. Use of antihypertensive, hypolipidemic, or antidiabetes medication was also an exclusion criterion.

Metabolic parameters were evaluated during two visits before commencing the treatment (day −6 to −2 and day −1) and two visits after 12–14 days of treatment (days 12 and 14). At the first investigation, liver fat content was quantified by magnetic resonance imaging (MRI), and a skeletal muscle biopsy was taken. At the second visit, a metabolic evaluation was made using a standardized meal test, with subsequent blood sampling. Subjects were resident within a clinical research organization (Richmond Pharmaceuticals, London, U.K.) where continuous safety monitoring was conducted. This also allowed for provision of standardized diets for all participants before and during the entire study period. Physical exercise and alcohol intake were not allowed. The study days were conducted in the clinical research unit in the Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, U.K., and the MRI scanning was performed in the MRI Centre at the John Radcliffe Hospital in Oxford, U.K.

Muscle biopsies were taken from the vastus lateralis after infiltration of the biopsy area with 1% lignocaine. The piece of muscle was snap-frozen in liquid nitrogen for later extraction of mRNA. RNA quality and quantity were assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) and RiboGreen RNA quantitation reagent (Molecular Probes, Eugene, OR), respectively. In addition, optical densities at 260 and 280 nm were measured. All samples possessed 18S and 28S rRNA bands with minimal signs of RNA degradation. Briefly, single-stranded cDNA was synthesized from each total RNA sample. Quantitative analysis to confirm the mRNA content was performed by a Taqman lightcycler, and data were normalized for β-actin as a housekeeping gene.

After these studies were complete, remaining biopsy tissue from the end of both the PPARδ agonist and placebo treatment periods were embedded in OCT and sectioned using cryotome. Tissue sections were stained on ice with RNase inhibitor (Ambion) in staining solution for protection of the integrity of RNA. An AutoPix 100e (Arcturus) laser capture microdissection (LCM) instrument was used to isolate slow fiber skeletal myocytes (stained with anti-myosin heavy-chain slow-isoform antibodies) and fast myocytes (unstained) (~300 cells per type). RNA was prepared from each type using a Picopure RNA purification kit (Arcturus) and subsequently converted to cDNA by reverse transcription using reagent from Applied Biosystems. Amplified DNA (Applied Biosystems) was incorporated into real-time RT-PCR using an ABI 7900HT Sequence Detector System in a 10-ul reaction volume within a 384-well plate according to manufacturer specifications.

Liver fat content was determined by MRI scanning using T1 weighted transverse images of the liver and compared with subcutaneous adipose tissue (maximum fat content). This procedure has previously been evaluated against fat content in liver biopsies (34).

For the standardized meal test, subjects arrived in the clinical research unit after an overnight fast. An indwelling catheter was put in the antecubital vein, from which blood samples were drawn at −30, 0, 15, 30, 60, 90, 120, 180, 240, 300, and 360 min. Breath samples were collected hourly by expiration into a closed bag. A mixed meal consisting of 40 g fat and 40 g carbohydrate (chocolate-flavored fat emulsion, skimmed milk, and Rice Krispies) was given at time 0. The fat consisted of 40 g olive oil and 100 mg [U^{13}C]-palmitate. Blood samples were taken in precooled heparinized syringes (Sarstedt, Leicester, U.K.), and plasma was immediately separated at 4°C and frozen for later analysis. Subjects were resting in a semirecumbent position throughout the sampling period.

Plasma glucose, triglycerides, and nonesterified fatty acids (NEFAs), HDL cholesterol, apoB, and β-OH-butyrate (Randox, RANBUT, RB1007) concentrations were determined enzymatically using an ILab 600 Multianalyser (Instrumentation Laboratory, Warrington, U.K.). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyltransferase (γGT) were analyzed by Richmond Pharmacology (www.richmondpharmacology.com). Insulin levels were determined by radioimmunoassay using a commercially available kit (Linco Research, St. Charles, MO). To determine fatty acid composition and isotopic enrichment, total lipids were extracted from plasma and methyl esters prepared from NEFA and triglyceride fractions as previously described (35). Fatty acid compositions (measured as micromoles per 100 μmol total fatty acids) in these fractions were determined by gas chromatography. [U^{13}C]palmitate enrichments were determined by gas chromatography–mass spectrometry using a 5890 GC coupled to a 5973N MSD (Agilent Technologies). The GC was equipped with a DB-Wax 30-m capillary column (0.25 mm, film thickness 0.25 μm; Agilent), and ions with mass-to-charge ratios (m/z) of 270 (M + 0) and 286 (M + 16) were determined by selected ion monitoring. Dwell time was 100 ms. Tracer-to-tracee ratios (TTRs) for [U^{13}C]palmitate (M + 16)/(M + 0) were multiplied by the corresponding palmitate NEFA or palmitate triglyceride concentrations to give plasma tracer concentrations.

As a marker of systemic oxidative stress (36,37), we measured urinary concentrations of free 8-iso-prostaglandin-F$_{2\alpha}$. F$_2$-isoprostanes appear to be the most reliable and clinically relevant markers of global oxidative stress in vivo in humans (36,37). The content of F$_2$-isoprostanes was determined by using a validated radioimmunoassay with a specific antibody raised against F$_2$-isoprostanes, as previously described (38). The radioimmunoassay had a detection limit of about 23 pmol/l. Intra- and interassay coefficients of variation were 4.5 and 7.5%, respectively. Urinary contents of F$_2$-isoprostanes are presented adjusted to urinary creatinine concentrations.

Statistics. Data are expressed as means ± SD or in the figures as SEs. Variables with skewed distributions were logarithmic transformed before analyses. Nonparametric tests were used if data were not normally distributed

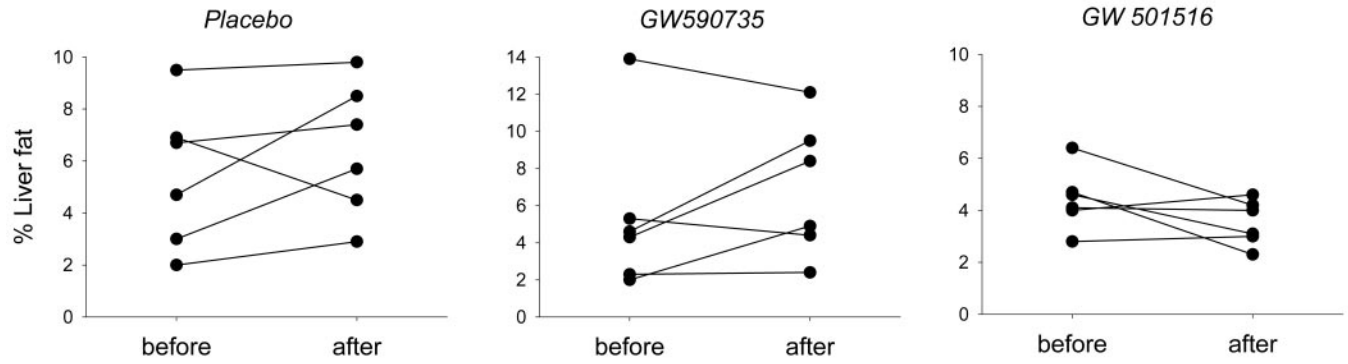

FIG. 1. Individual changes in percentage liver fat content measured before and after drug treatment. There was a statistically significant reduction in liver fat content in response to GW501516 treatment when the individual, before-treatment liver fat content was used as covariate ($P = 0.04$).

after logarithmic transformation. A paired t test was used for within-group effects from baseline. Differences between groups from baseline to 2 weeks were initially assessed using an overall test (ANOVA) or a nonparametric test. In case of a significant overall test, an unpaired t test or Mann-Whitney's nonparametric test was used for differences between two groups. ANCOVA was used to test the differences between groups using the baseline value as a covariate. Pearson's or Spearman's correlation coefficient was determined. All tests were two tailed. $P < 0.05$ was regarded as statistically significant. For variables with multiple measurements on the same day, area under the curve (AUC) was calculated to give a summary variable or repeated-measures ANOVA was used. For calculation of the treatment effect of liver fat content, baseline fat content was used as a covariate. JMP software was used for statistical analyses (SAS Institute, Cary, NC).

RESULTS

There were no significant differences between the three groups after randomization. Thus, age, blood pressure, total cholesterol, apoB, HDL cholesterol, and triglycerides were balanced between groups (Table 1). There was no difference in baseline ALT and AST concentrations between the groups (Table 1). Three of six subjects in the GW501516- and GW590735-treated groups fulfilled the The National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome (39), whereas two of six fulfilled the criteria in the placebo group.

Tolerability. There were no significant symptomatic side effects, and the results of liver (AST and ALT), hematology (blood cell counts), and renal function (creatinine) tests were unchanged in all groups. The concentration of γGT was unchanged in the placebo and GW590735 groups, but a 23% ($P < 0.05$) lowering was seen in response to GW501516 treatment (Table 2).

One subject developed a skin rash within 24 h after the first dose of GW590735, leading to withdrawal from the study. Treatment allocation for this patient remained concealed after this event, and a new subject was recruited to continue the study with the same blinded treatment allocation. Body weights did not change in placebo and GW590735-treated subjects, whereas subjects treated with GW501516 tended to lose weight (-1.7 ± 0.7 kg, $P = 0.05$) over the 2-week treatment period.

TABLE 1
Baseline characteristics of anthropometry, plasma biochemistry, liver enzymes, liver fat content, and urinary isoprostanes in the groups after randomization

	Placebo	GW590735	GW501516	Normal range*	P (ANOVA)
n	6	6	6		
BMI (kg/m^2)	32.6 ± 3.8	30.7 ± 2.7	30.5 ± 2.3	NA	0.44
Waist girth (cm)	107 ± 10	104 ± 6	102 ± 6	NA	0.50
Plasma glucose (mmol/l)	4.9 ± 0.1	5.0 ± 0.4	5.1 ± 0.5	4.4–6.0	0.80
Plasma insulin (mU/l)	17 ± 6	17 ± 6	15 ± 6	4–19	0.83
HOMA-IR	3.6 ± 1.2	3.7 ± 1.6	3.4 ± 1.5	0.8–5.1	0.92
Plasma NEFA (μmol/l)	418 ± 192	608 ± 172	605 ± 84	211–980	0.09
Triglycerides (mmol/l)	1.4 ± 0.4	1.5 ± 0.7	1.6 ± 0.4	0.52–2.69	0.85
Plasma cholesterol (mmol/l)	4.5 ± 0.3	4.7 ± 0.8	5.1 ± 0.8	3.9–7.1	0.35
LDL cholesterol (mmol/l)	3.4 ± 0.2	3.5 ± 0.7	3.9 ± 0.7	2.2–5.0	0.35
HDL cholesterol (mmol/l)	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9–2.0	0.49
Plasma apoB (g/l)	0.88 ± 0.09	0.89 ± 0.10	0.96 ± 0.05	0.52–1.23	0.47
β-OH-butyrate (μmol/l)	33 ± 14	62 ± 49	62 ± 26	19–281	0.24
Liver fat (%)	5.5 ± 2.8	5.3 ± 4.4	4.4 ± 1.2	NA	0.83
Urinary F_2-isoprostanes (nmol · l^{-1} · mmol^{-1} creatinine)	0.38 ± 0.06	0.31 ± 0.08	0.35 ± 0.08	0.11–0.62	0.28
γ-GT (IU/l)	28 ± 10	25 ± 6	30 ± 5	9–40	0.58
AST (IU/l)	28 ± 5	27 ± 9	26 ± 6	15–40	0.88
ALT (IU/l)	43 ± 13	37 ± 15	45 ± 15	10–50	0.65

Data are means ± SD or ranges unless otherwise indicated. ANOVA describes the overall statistical significance between the groups. The ranges for β-OH-butyrate and urinary isoprostanes are based on subsets ($n = 110$ and $n = 30$, respectively). The ranges for the liver enzymes were provided by Richmond Pharmacology (www.richmondpharmacology.com). *5th and 95th percentile of a local healthy population using the same laboratory methods ($n = 1,263$). NA, not applicable.

TABLE 2
Change from baseline to 14 days in fasting plasma biochemistry, liver enzymes, and urinary isoprostanes

	Placebo	GW590735	GW501516	*P* (ANOVA)
n	6	6	6	
Glucose (mmol/l)	−0.1 ± 0.1*	−0.3 ± 0.2*	−0.3 ± 0.2*	0.25
Insulin (mU/l)	1.2 ± 1.1*	1.0 ± 1.9	−1.7 ± 2.2*†‡	0.01
HOMA-IR	0.17 ± 0.18	−0.02 ± 0.40	−0.58 ± 0.53*†‡	0.006
NEFA (μmol/l)	22 ± 212	−62 ± 90	−247 ± 101†§¶	0.01
Triglycerides (mmol/l)	0.1 ± 0.7	−0.4 ± 0.6	−0.5 ± 0.4*	0.16
Total cholesterol (mmol/l)	−0.3 ± 0.5	−0.6 ± 0.4*	−1.0 ± 0.7*	0.13
LDL cholesterol (mmol/l)	−0.3 ± 0.4	−0.6 ± 0.4*	−0.9 ± 0.6*	0.13
HDL cholesterol (mmol/l)	−0.05 ± 0.08	0.07 ± 0.07*‖	0.003 ± 0.07	0.049
ApoB (g/l)	−0.06 ± 0.09	−0.12 ± 0.06¶	−0.20 ± 0.15*	0.06
β-OH butyrate (μmol/l)	8 ± 27	−10 ± 37	−19 ± 23*	0.14
Urinary F_2-isoprostanes (nmol · l^{-1} · $mmol^{-1}$ creatinine)	−0.02 ± 0.08	0.05 ± 0.10	−0.10 ± 0.05§¶	0.01
γ-GT (U/l)	−3 ± 6	−5 ± 3*	−8 ± 6¶	0.11
AST (U/l)	−1 ± 6	1 ± 2	0 ± 4	0.50
ALT (U/l)	−3 ± 14	−4 ± 14	−13 ± 13	0.44

Data are means ± SD. *P* (ANOVA) describes the overall *P* value between groups. **P* < 0.05, within-group change from baseline (paired *t* test); †*P* < 0.01, different from placebo (unpaired *t* test); ‡*P* < 0.01, different from PPARα (unpaired *t* test); §*P* < 0.05, different from PPARα (unpaired *t* test); ¶*P* < 0.01, within-group change from baseline (paired *t* test); ‖*P* < 0.05, different from placebo (unpaired *t* test).

Fasting plasma metabolic characteristics. Total and LDL cholesterol were reduced by 20 and 23%, respectively (both $P < 0.05$), accompanied by a 21% lowering of apoB ($P < 0.05$) in subjects receiving GW501516 (Table 2). HDL cholesterol was unchanged. Triglycerides were lowered by 31% ($P < 0.05$). There was a very distinct lowering of fasting plasma NEFA (−40%, $P < 0.01$). The lowering of NEFA was supported by significant lowering (−25%, $P < 0.05$) of fasting plasma glycerol (data not shown). Treatment with GW590735 resulted in a similar reduction in fasting plasma triglycerides (−27%, $P < 0.05$) but no change in NEFA (Table 2) or glycerol (data not shown). LDL cholesterol did not change significantly, whereas a modest reduction in apoB (−13%, $P < 0.05$) was observed with GW590735. HDL cholesterol tended to increase. Corresponding changes in lipids and lipoproteins in placebo-treated subjects were small and not statistically significant.

Fasting plasma insulin concentration was slightly reduced in response to GW501516 together with a small reduction in fasting plasma glucose ($P < 0.05$). This led to a significantly improved insulin sensitivity, calculated as homeostasis model assessment of insulin resistance (40), which was not seen in the GW590735 and placebo groups (Table 2).

Liver fat. There was no statistically significant difference in liver fat content between the groups at baseline. The GW501516-treated group exhibited a 20% reduction in liver fat that was statistically significant after adjustment for baseline fat content (Fig. 1). Liver fat content in both GW590735-treated and placebo subjects tended to increase during the treatment period; neither of these changes were statistically significant.

To explore the possible biological significance of alteration in liver fat content, correlations were sought among changes in γGT and liver fat content and changes in apoB and plasma triglycerides (markers of lipoprotein production). Change in liver fat content was positively correlated with change in γGT ($r = 0.72$, $P = 0.0007$), indicating that subjects whose liver fat content reduced also had a proportional reduction in systemic concentrations of γGT (Fig. 2). Conversely, positive correlations were found between change in liver fat and change in triglycerides

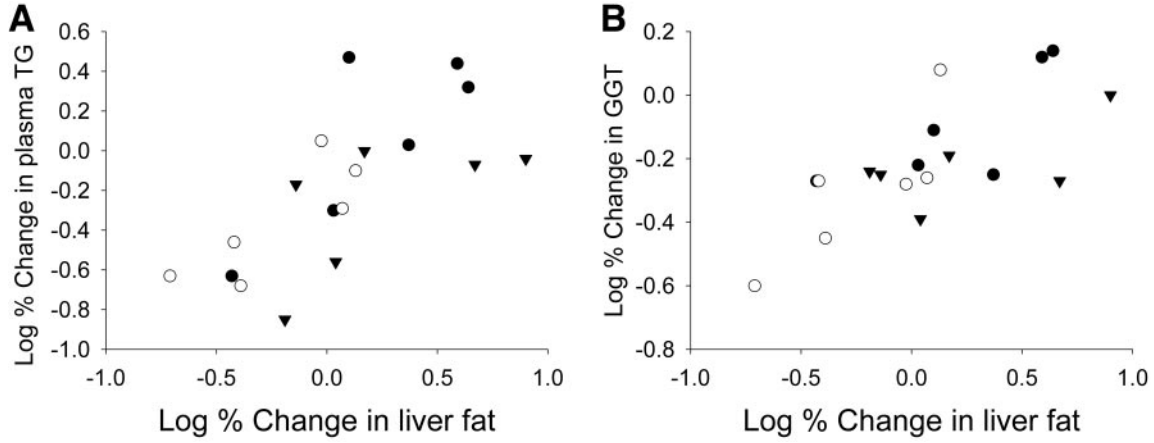

FIG. 2. Correlations between change in liver fat content and change in fasting plasma triglyceride (TG) concentration ($r = 0.75$, $P = 0.006$) (*A*) and change in γGT (GGT) ($r = 0.72$, $P = 0.007$) (*B*) before and after drug treatment. The Pearson correlation coefficient includes all subjects. ●, placebo; ○, GW501516 treatment; ▲, GW590735 treatment.

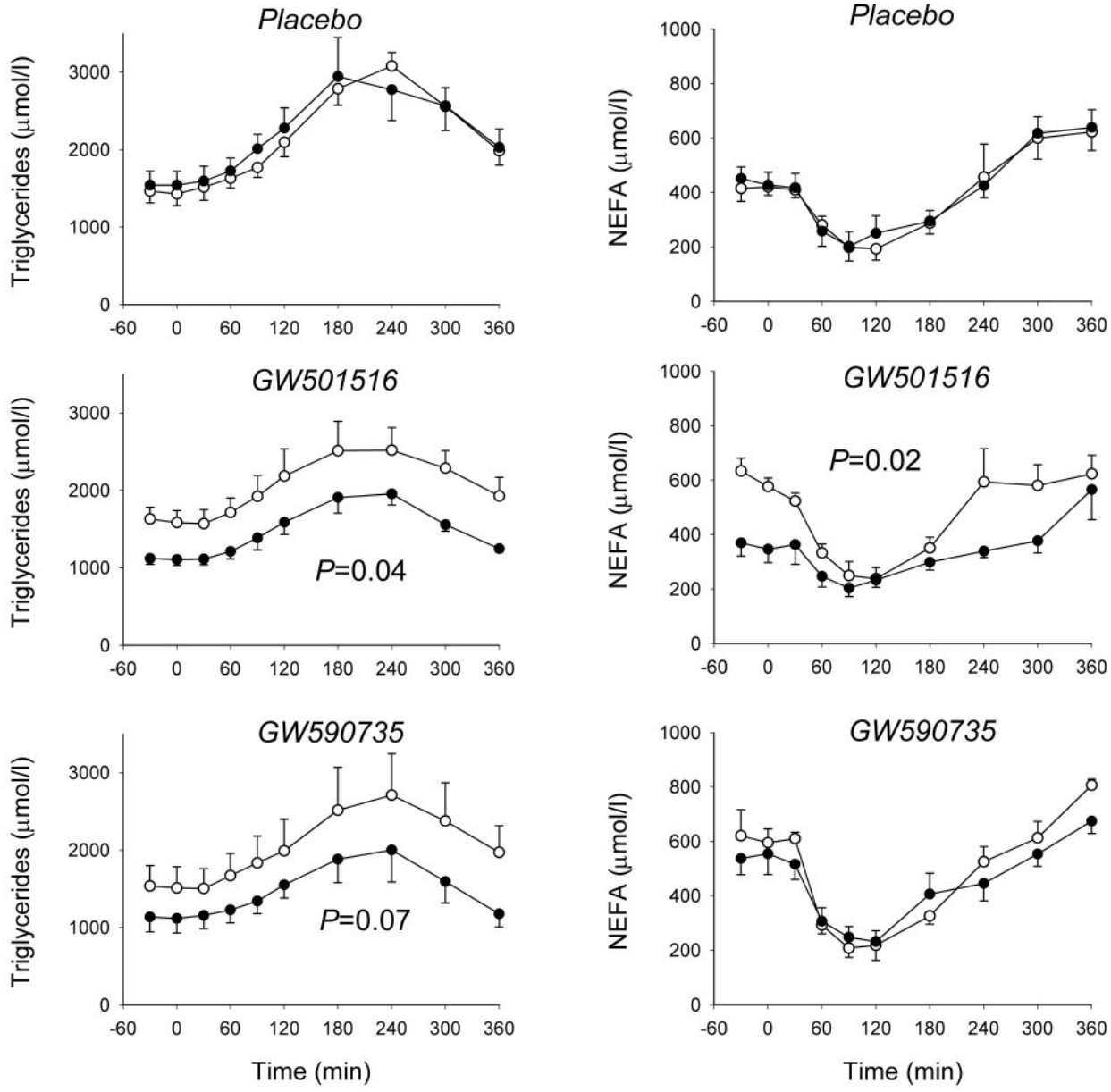

FIG. 3. Fasting and postprandial plasma triglyceride and NEFA concentrations before (○) and after (●) drug treatment. A mixed meal was given at time 0.

($r = 0.75$, $P = 0.0006$) (Fig. 2) and change in plasma apoB ($r = 0.74$, $P = 0.001$).

Meal tolerance test. The postprandial concentrations of triglycerides were substantially reduced in response to both GW501516 and GW590735 treatment, with no change in the placebo group (Fig. 3). Individual responses are shown in an online appendix (available at http://dx.doi.org/10.2337/db07-1318). There were small differences in the postprandial excursions of plasma glucose, with the only statistically significant difference (a 2% AUC decrease) seen in response to GW501516 treatment ($P = 0.046$). There were no significant changes in AUC of postprandial insulin concentrations in any of the groups.

The lowering of fasting NEFA concentrations was replicated in the postprandial state in the GW501516 group; the postprandial AUC for NEFA was reduced by 27% ($P = 0.02$) (Fig. 3). The postprandial NEFA AUCs were unchanged in response to GW590735 treatment (−5%, $P = 0.59$), and no difference was seen in the placebo group (2%, $P = 0.80$). Individual responses are shown in the online appendix. The fasting and postprandial concentrations of β-OH-butyrate appeared to reflect NEFA concentrations in all groups; the ratios of β-OH-butyrate to NEFA concentrations were unchanged in response to treatment (data not shown).

The meal contained ^{13}C-labeled palmitate, which appeared in plasma as triglycerides (incorporated into chylomicrons) and as NEFA (lipolytic products of LPL-mediated hydrolysis of chylomicron triglycerides). The postprandial concentration of ^{13}C-palmitate in plasma triglycerides was reduced in response to both GW501516 and GW590735 treatment (−37%, $P = 0.06$ and −62%, $P = 0.08$) but with no effect in the placebo group (−2%, $P = 0.94$). The effect was particularly striking in response to GW590735 treatment, where relative abundance of ^{13}C-palmitate in triglycerides (TTR) was also reduced ($P =$

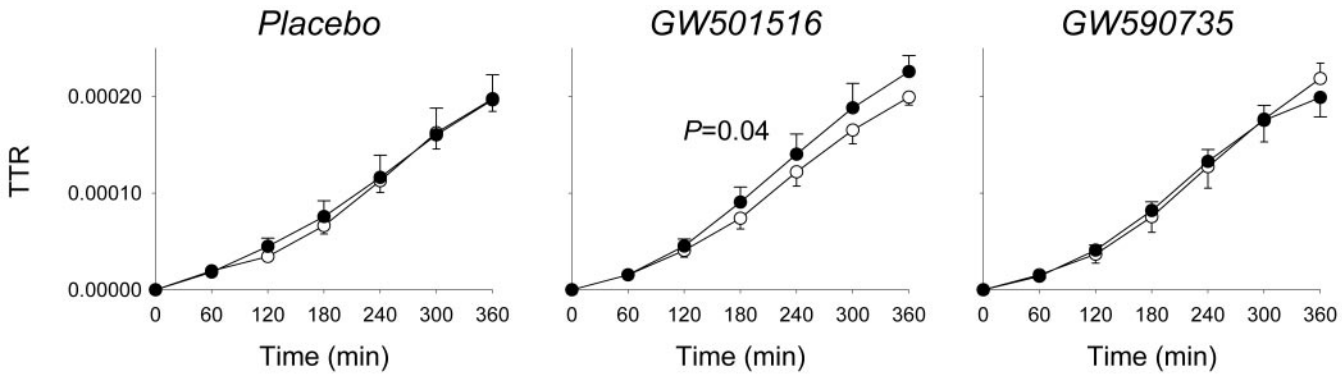

FIG. 4. The relative enrichment (TTR) of ^{13}C in CO_2 captured in expired breath after a standardized meal containing ^{13}C-palmitate. The increased ratio seen in the GW501516 group indicates that a greater proportion of the meal-derived fatty acids have undergone oxidation in the postprandial state. ○, before treatment; ●, after treatment.

0.03). The appearance of ^{13}C-palmitate in the NEFA fraction was significantly reduced only in response to GW501516 treatment (−26%, $P = 0.001$), but there was no difference in the relative abundance of ^{13}C-palmitate (TTR). The relative abundance of $^{13}CO_2$ in expired breath was increased in response to GW501516 treatment ($P = 0.03$) but unchanged in the GW590735 and placebo groups (Fig. 4). Individual responses are shown in the online appendix.

Oxidative stress. There was no baseline difference between the groups for urinary F_2-isoprostanes (Table 2). Treatment with GW501516 significantly reduced the urinary content of F_2-isoprostanes (−30%, $P < 0.01$), with no effect in the placebo and GW590735 groups. Individual responses are shown in the online appendix. The change in urinary F_2-isoprostanes was related to change in liver fat content ($r = 0.51$, $P = 0.03$; $n = 18$).

Muscle biopsy. The mRNA content of several genes involved in skeletal muscle fatty acid handling was specifically studied. This involved *CPT1b*, carnitine acyltransferase (*CRAT*), acyl-CoA carboxylase 2 (*ACAB2*), and hydroxyl Co-enzyme dehydrogenase (*HADHA*) together with *PPARD*. The changes in response to treatment were uniformly small in all groups, and only *CPT1b* showed a significant increase in response to GW501516 treatment (Table 3). This was further corroborated when placebo ($n = 5$) was compared to GW501516 treatment ($n = 3$) using LCM. In cells identified as fast and slow fibers and isolated by LCM via myosin staining, the expression of *CPT1b* mRNA was 2.7- and 3.0-fold greater, respectively, in samples from subjects treated with GW501516 compared with that in samples from placebo subjects. In contrast, mRNA expression of the housekeeping genes *GAPDH*, *ACTB*, and *B2M* remained stable in the same samples regardless of treatment regimen.

DISCUSSION

PPARδ activation for 2 weeks led to lowering of fasting and postprandial plasma triglycerides, LDL cholesterol, and apoB, without any sign of adverse reactions. Reductions were also seen in liver fat content and urinary isoprostanes, the latter a marker of whole-body oxidative stress. The HDL cholesterol concentration did not change. The reduction of fasting triglycerides was of the same magnitude as that seen for the novel PPARα agonist tested here. The lowering of postprandial triglycerides was also very similar. There was also a striking reduction of fasting plasma NEFA, which was seen in the context of increased relative incorporation of labeled CO_2 originating from the fat content in the experimental meal, suggesting increased fat oxidation. The pattern of change in mRNA content in skeletal muscle suggested that pathways for fat oxidation, in both fast and slow fiber types, were specifically upregulated in response to the PPARδ drug, which is in line with observations from studies in rodents and human cells (32). Importantly, the increased fat oxidation was seen without any signs of deterioration of glucose/insulin homeostasis.

The triglyceride-lowering effect of the PPARδ agonist is similar to that of the PPARα agonist but probably brought about by different mechanisms. Of note, the PPARα agonist fenofibrate has no effect on plasma NEFA concentrations or on turnover of NEFAs in plasma (41). However, whereas PPARα agonists (fibrates) are known to increase catabolism of triglyceride-rich lipoproteins (42), it is likely that some of the reduction of triglycerides in response to the PPARδ agonist is due to reduced production from the liver. A lower influx of NEFA to the liver, due to the lower systemic concentrations of NEFA, together with the lowering of liver fat content, is a plausible background for reduced triglyceride production from the liver. The prom-

TABLE 3
Ratio (after/before) of *CPT1B*, *ACAB*, and *PPARD* mRNA content in skeletal muscle in response to placebo, GW590735, and GW501516 treatment

Transcript	Placebo	GW590735	GW501516	ANOVA
n	6	6	6	
CPT1B	0.85 ± 0.08	0.92 ± 0.07	1.19 ± 0.07	0.03
ACAB	1.21 ± 0.09	1.09 ± 0.25	1.18 ± 0.14	0.71
PPARD	1.11 ± 0.19	1.12 ± 0.25	0.95 ± 0.17	0.85

Data are means ± SE. Level of significance was calculated using ANOVA.

inent lowering of apoB would also support this reasoning, but kinetic studies of lipoproteins would be needed to study this phenomenon in detail. It is tempting to speculate that an increased fat oxidation in skeletal muscle drives the reduction of NEFA and that this effect is instrumental for the global metabolic effects of the PPARδ agonist. LDL cholesterol showed a substantial lowering by the PPARδ agonist, which is also in line with the reduction in apoB. An additional possible mechanism for this could be intestinally mediated by the reduction of the Niemann-pick C1 like-1 (NPC1L1) protein by PPARδ agonists demonstrated in mice (43). The inhibition of NPC1L1 would lead to reduced cholesterol absorption.

The reduction in liver fat was surprising and could be of clinical interest. Nonalcoholic liver fatty liver disease is closely associated with obesity and insulin resistance (44), but there are few effective treatment strategies. Although treatment with PPARγ agonists has consistently shown reduction in liver fat content (12,45), the mechanism for the beneficial effect of PPARγ agonists in this respect is not entirely clear. A possible mechanism is reduced flux of fatty acids to the liver in response to PPARγ agonist treatment. However, in humans, fasting plasma NEFAs are only moderately reduced by thiazolidinediones, typically by up to 10–30%, and these changes are not always statistically significant (8–10,46,47). Tan et al. (47) showed a reduction of postprandial NEFA concentrations. These effects have been attributed to increased trapping of fatty acids in adipose tissue. Here, the lower systemic NEFA concentrations observed in response to the PPARδ agonist will also reduce fatty acid supply to the liver, but as already outlined, the mechanism might involve increased utilization of fatty acids by skeletal muscle. The change in liver fat observed in all subjects in this study taken together showed strong relationships both with the concentration of the liver enzyme γGT and with the change in fasting plasma triglycerides and apoB. The latter correlation also accords with recent kinetic data suggesting a link between liver fat and hepatic VLDL production (48).

There is little evidence from this study that the PPARδ agonist directly altered either adipose or liver fatty acid handling substantially. We used the plasma concentration of β-OH-butyrate to monitor hepatic fatty acid oxidation. The ratio of plasma NEFA concentrations to β-OH-butyrate was similar between groups, and there was no apparent change in response to treatment in any of the groups, indicating that the same proportion of NEFA is being oxidized to β-OH-butyrate. In adipose tissue, the efficiency of the uptake of fatty acids from chylomicrons in the postprandial state was monitored by quantifying the appearance of labeled fatty acids appearing in the NEFA fraction. A decreased proportion of these fatty acids appearing in the NEFA fraction would indicate that adipose tissue is becoming more efficient in trapping fatty acids. We recently described such a phenomenon in response to treatment with the PPARγ agonist rosiglitazone in adipose tissue of type 2 diabetic patients (47). In the present study, there was no difference in the relative proportion of labeled fatty acids appearing in the NEFA fraction, indicating that this pathway was unaltered in response to both GW501516 and GW590735 treatment.

The limitations of the present study are the short treatment period and the restricted living conditions of the participants compared with free-living individuals. The effect of GW501516 over a longer period of time is yet to be determined.

In summary, the PPARδ agonist GW501516 attenuated multiple metabolic abnormalities normally associated with the metabolic syndrome in humans, and this was probably due to an increase in skeletal muscle fatty acid oxidation. Presently, the individual components of the metabolic syndrome are treated separately; i.e., statins are used for elevated cholesterol, fibrates are used to reduce triglycerides, and metformin and thiazolidinediones are used for hyperglycemia. The wide range of beneficial effects suggested by the response to GW501516 calls for a larger study in patients to evaluate the clinical efficacy of PPARδ agonists for the treatment of hyperlipidemia, liver fat accumulation, obesity, and insulin resistance.

ACKNOWLEDGMENTS

This study was funded through a research grant to the University of Oxford made available by GlaxoSmithKline. F.K. is a Wellcome Trust Senior Clinical Fellow. U.R. was funded by Henning and Johan Throne Holst Foundation and Stiftelsen för Vetenskapligt Arbete inom Diabetologi.

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