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Tesaglitazar, a dual PPARα/γ agonist, ameliorates glucose and lipid intolerance in obese Zucker rats

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Oakes, Nicholas D., Pia Thalen, Therese Hultstrand, Severina Jacinto, Germán Camejo, Boel Wallin, and Bengt Ljung. Tesaglitazar, a dual PPARα/γ agonist, ameliorates glucose and lipid intolerance in obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 289: R938–R946, 2005; doi:10.1152/ajpregu.00252.2005.—Insulin resistance, impaired glucose tolerance, high circulating levels of free fatty acids (FFA), and postprandial hyperlipidemia are associated with the metabolic syndrome, which has been linked to increased risk of cardiovascular disease. We studied the metabolic responses to an oral glucose/triglyceride (TG) (1.7/2.0 g/kg lean body mass) load in three groups of conscious 7-h fasted Zucker rats: lean healthy controls, obese insulin-resistant/dyslipidemic controls, and obese rats treated with the dual peroxisome proliferator-activated receptor α/γ agonist, tesaglitazar, 3 μmol·kg⁻¹·day⁻¹ for 4 wk. Untreated obese Zucker rats displayed marked insulin resistance, as well as glucose and lipid intolerance in response to the glucose/TG load. The 2-h postload area under the curve values were greater for glucose (+19%), insulin (+849%), FFA (+53%), and TG (+413%) compared with untreated lean controls. Treatment with tesaglitazar lowered fasting plasma glucose, improved glucose tolerance, substantially reduced fasting and postload insulin levels, and markedly lowered fasting TG and improved lipid tolerance. Fasting FFA were not affected, but postprandial FFA suppression was restored to levels seen in lean controls. Mechanisms of tesaglitazar-induced lowering of plasma TG were studied separately using the Triton WR1339 method. In anesthetized, 5-h fasted, obese Zucker rats, tesaglitazar reduced hepatic TG secretion by 47%, increased plasma TG clearance by 490%, and reduced very low-density lipoprotein (VLDL) apolipoprotein CIII content by 86%, compared with obese controls. In conclusion, the glucose/lipid tolerance test in obese Zucker rats appears to be a useful model of the metabolic syndrome that can be used to evaluate therapeutic effects on impaired postprandial glucose and lipid metabolism. The present work demonstrates that tesaglitazar ameliorates these abnormalities and enhances insulin sensitivity in this animal model.

in vivo; metabolism; liver; triglyceride

ABNORMALITIES IN BOTH GLUCOSE and lipid metabolism in patients with the metabolic syndrome, including those with type 2 diabetes, are thought to be critical in the pathogenesis of cardiovascular disease. Hyperglycemia, resulting from the combination of insulin resistance and inadequate insulin secretion, is involved in microvascular complications (1). Dyslipidemia appears to have a critical role in the pathogenesis of macrovascular disease (atherogenesis), the major cause of early mortality in patients with metabolic syndrome, including those with type 2 diabetes (4). The development of dyslipidemia is complex and probably multifactorial. However, disturbances in fatty acid transfer have been implicated as key underlying events. Thus, an oversupply of free fatty acid (FFA) to the liver, leading to increased hepatic triglyceride (TG) production and hypertriglyceridemia, is thought to play a critical role in the generation of the “atherogenic lipoprotein profile” (34, 44). Decreased plasma TG clearance also contributes to the hypertriglyceridemia in patients with metabolic syndrome, and important factors in this defect are likely to be reduced lipoprotein lipase (LPL) (18), perhaps a consequence of insulin resistance (16), as well as raised levels of apolipoprotein (apo) C-III (43), an inhibitor of LPL.

For the majority of relatively sedentary people in Western societies, the regular consumption of mixed meals containing fat and carbohydrates represent a major challenge to metabolic control. Even in healthy individuals, consumption of a meal containing fat produces postprandial TG excursions that persist for many hours and can impair the ability of insulin to regulate glucose metabolism in the postprandial phase of the subsequent meal (13, 41). Exacerbated postprandial hypertriglyceridemia, associated with insulin resistance, is evident in patients with newly diagnosed hypertension (20) and patients with type 2 diabetes (33), including their first-degree relatives (3). This manifestation of the metabolic syndrome may be especially important in light of evidence linking exaggerated postprandial hyperlipidemia and coronary artery disease (22, 40). In spite of this, and the current eating practices in Western societies (the predominance of fat-containing meals and that a major fraction of life spent in the postprandial state), metabolic assessments of the metabolic syndrome are most often made in the fasting state or in response to a pure glucose challenge.

We report here studies in the obese fa/fa Zucker rat, a widely used animal model of glucose metabolic insulin resistance and dyslipidemia. The metabolic responses in these animals to a combined oral glucose and TG load were characterized. The results demonstrate metabolic defects, which although exaggerated, closely parallel the defects in postprandial handling of plasma glucose and lipids in patients with metabolic syndrome. We were also interested in evaluating the effect of tesaglitazar (formerly AZ 242) on these metabolic defects. Tesaglitazar binds and activates peroxisome proliferator-activated receptor (PPAR)α and PPARγ (10) and is currently under clinical
investigation to assess its ability to correct disorders of glucose and lipid metabolism associated with type 2 diabetes and the metabolic syndrome. The present results demonstrate that tespaglitazar is effective in enhancing the ability of insulin to suppress plasma FFA, as well as ameliorating both the glucose and lipid intolerance in the obese Zucker rat. The improved lipid tolerance involves decreased hepatic TG secretion and accelerated TG clearance associated with reduced apoC-III content of TG rich lipoproteins.

MATERIALS AND METHODS

Animals and General Procedures

Experimental procedures were approved by the Local Ethics Review Committee on Animal Experiments (Göteborg region). Male, 8-wk-old Zucker rats (Charles River Wiga GmbH, Suffield, Germany) were housed in a temperature- (20–22°C) and humidity-controlled (40–60% relative humidity) facility with a facility with a 12:12 h light-dark cycle (lights on 0600) and had free access to rodent chow (R3, Laktamin AB, Stockholm, Sweden) and tap water.

Study 1: Handling of an Oral Combined Glucose and Lipid Load in Conscious Rats

Treatment groups. Lean (Fa/Fa or Fa/fa) and obese (fa/fa) Zucker rats were studied in three groups: lean untreated controls (Lean), obese untreated controls (Obese), and obese rats treated with tespaglitazar (AstraZeneca R&D, Möln达尔, Sweden) 3 μmol·kg⁻¹·day⁻¹ (Tespaglitazar). Treated rats were dosed at 1300 for 3–4 wk by gastric gavage. Untreated rats were dosed with an equal volume of vehicle (0.5% carboxymethyl cellulose, 2.5 ml/kg). Animals were not dosed on the day of the acute experiment. During the entire treatment period, food consumption and body weight were recorded daily.

Conscious, chronically catheterized rat preparation. One week before the acute study, rats were fitted with a jugular vein catheter under isoflurane anesthesia. Prophylactic antibiotics were administered subcutaneously both the day before and on the day of surgery (ampicillin 150 mg/kg, Doktacillin, AstraZeneca, Sweden). The cannula was exteriorized via a small cutaneous incision at the nape of the neck. Animals had free access to water for the duration of the experiment.

Study 2: Hepatic VLDL Triglyceride Production and Plasma Clearance of Triglyceride in Anesthetized Rats

Treatment groups. Two groups of obese Zucker rats were studied: obese untreated controls (Obese), and obese treated with tespaglitazar 3 μmol·kg⁻¹·day⁻¹ (Tespaglitazar). Treated rats were dosed daily at 1300 for 3–4 wk by gastric gavage. Untreated control rats were gavaged with an equal volume of vehicle (0.5% carboxymethyl cellulose, 2.5 ml/kg). Animals were not dosed on the day of the acute experiment.

Anesthetized rat preparation. In the morning of the day of the experiment, food was withdrawn at 0700. Rats were anesthetized at 1000 (Na-thiobutabarbitol, Inactin, RBI, Natick, MA; lean 120 mg/kg, obese 180 mg/kg). Body temperature was monitored using a rectal probe and maintained between 37.5 and 38.0°C throughout the experiment. Animals were tracheotomized, and catheters were placed in the right jugular vein (for top-up dosing of anesthetic, as required, and infusion of Triton WR1339, as detailed below) and left carotid artery (for blood sampling). Arterial catheter patency was maintained throughout the experiment by continuous infusion (10 μl/min) of a sterile saline solution containing sodium citrate (20.6 mM). The acute experimental protocol for determination of plasma TG kinetics (see below) began at 1400 after a 1.5-h postsurgery stabilization period.

Acute Study. The Triton method (38) was used to assess plasma TG kinetics. In principle, this agent blocks the clearance of TG from plasma, allowing hepatic TG secretion to be calculated from the subsequent accumulation of TG in the plasma (see Calculations below). Two groups of anesthetized, 7-h fasted rats were studied: Obese and Tesaglitazar (treated for 3 wk). After the 90-min postsurgery stabilization period, basal arterial blood samples were collected. Rats then received an intravenous dose of 20% (wt/wt) Triton WR1339 (200 mg/kg, Tyloxapol; Sigma, St. Louis, MO) in normal saline. Arterial blood samples (0.2 ml) were collected 30, 60, 90, and 120 min after Triton administration. Plasma TG determinations were made in the basal, 30-, 60-, 90-, and 120-min samples. Plasma lipoprotein profiles (see below) were only determined in the basal (Pre-Triton) sample and the 120-min (Post-Triton) samples.

Analysis of Plasma and Tissue Samples

Plasma lipids, glucose, insulin, and C-peptide. Colorimetric kit methods were used to measure plasma FFA (NEFA C, Wako, Richmond, VA), and glucose (Glucose HK, Roche, Stockholm, Sweden). Plasma TG was determined also using a colorimetric method by measuring the glycerol released by complete enzymatic hydrolysis of triglycerides (Triacylglycerides/GB, Böhringer Mannheim, Indianapolis,
IN). This method explicitly excludes free glycerol. Colorimetric measurements were performed on a centrifugal analyzer (Cobas Bio, F. Hoffmann-La Roche, Basel, Switzerland). Radioimmunoassays were used to measure plasma concentrations of insulin (Rat Insulin RIA Kit, Linco Research, St. Charles, MO) and C-peptide (Rat C-peptide RIA Kit, Linco Research).

**Lipoprotein profiles.** Cholesterol distribution profiles were measured in 10-μl plasma samples using a size exclusion HPLC system, with a Superox 6 PC 3.2/30 column (Amersham Pharmacia Biotec, Uppsala, Sweden), as previously described (42). The various peaks in the profiles were designated VLDL, “LDL,” and “HDL” for simplicity, by analogy to the nomenclature used for the human profile.

**ApoC-III content of VLDL.** Rat plasma very low density lipoproteins (VLDL, density = 1.006–1.019 g/ml) were prepared from EDTA-plasma by flocculation with ultracentrifugation in 10 mM HEPES/D_2O buffer, pH 7.2 in a volume of 1.0 ml using a Beckman TLX ultracentrifuge (Beckman, Palo Alto, CA) at 100,000 rpm for 3 h (37). VLDL apolipoproteins were separated by SDS-PAGE using Novex 16% tricine gels (Invitrogen Carlsbad, CA). Two bands, corresponding to different glycosylated isoforms of the sequences described for rat apo-CIII (SWISS-PROT database, PO6759) were identified by peptide mapping fingerprinting by MALDI-TOF-MS (Voyager STR, Perceptive Biosystems, Framingham, MA) and electrospray MS/MS (Q-ToF, Micromass, Manchester, UK), essentially as previously described (12, 26). Protein bands were stained by SYPRO Ruby ( Molecular Probes Europe, Leiden, The Netherlands). For quantification, images were acquired using Molecular Image FX (Bio-Rad Laboratories, Hercules, CA). Samples were analyzed using Quantity One software (Bio-Rad). All samples were normalized against the mean of the control samples. VLDL total apolipoprotein was measured using the BCA method (Pierce, Rockford, IL). VLDL triglycerides were measured using a commercial kit (Trig/GB, Roche Diagnostics, Stockholm, Sweden). Samples were analyzed using a centrifugal analyzer (Cobas Bio, F. Hoffmann-La Roche AG, Basel, Switzerland) for enzymatic colorimetric determination of triglyceride concentration.

**Calculations**

**Estimation of lean body mass.** Lean body mass (LBW) was estimated from the following regression formulas derived from conventional body composition analysis, performed in-house, of a large series of lean and obese Zucker rats, respectively, relating LBW (g) to body weight (BW, g): lean Zucker: LBW = BW × 0.77 + 31 and obese Zucker: LBW = BW × 0.5 + 59.

**Estimation of hepatic triglyceride output.** Triton WR1339 effectively blocks the clearance of plasma TG. When applied in the postabsorptive state, the rate of hepatic TG output (HTGO) can be calculated from the linear rate of TG accumulation in plasma (38) and by assuming a lipoprotein distribution space of 4% of lean body mass, equivalent to plasma volume (27). Plasma TG clearance rate (KTG), an index of the combined ability of the tissues to remove TG from the circulation, was calculated as the ratio HTGO/KTG, where KTG refers to the basal plasma TG immediately before Triton administration.

**Statistics**

Analysis of group data was based on comparisons to specifically test the principal a priori questions of the study: Lean vs. Obese (obesity effect) and Tesaglitazar vs. Obese (treatment effect). Statistical significance of the differences were evaluated on the basis of F-tests. Between-group comparisons of time-dependent effects were evaluated on the basis of ANOVA with repeated measures. Statistical tests were performed using the program SPSS (SPSS, Chicago, IL). Results are reported as means ± SE. P < 0.05 was considered statistically significant.

**RESULTS**

**Study 1: Metabolic Responses to an Oral Combined Glucose and Lipid Load in Conscious Rats**

Obese animals were hyperphagic and had increased body weight gain compared with Lean. Over the study period, average daily food intake was Obese 38.2 ± 1.1 g/day vs. Lean 22.5 ± 0.4 g/day, while average daily body weight gain was Obese 6.8 ± 0.3 vs. Lean 2.8 ± 0.3 g/day. These values yield average food utilization factors (body weight gain/food intake × 100) of Obese 17.8 ± 0.5 vs. Lean 12.5 ± 1.3%. In the basal 7-h fasting state, just before receiving the mixed oral load, Obese rats had marked hyperinsulinemia associated with a mild elevation of plasma glucose, substantially elevated C-peptide levels, hypertriglyceridemia, and elevated plasma FFA (Table 1), compared with Lean. Compared with the Obese group, the Tesaglitazar group showed increased daily body weight gain (8.9 ± 0.3 g/day), which could be explained by increased food intake (47.0 ± 1.5 g/day), as the food utilization factor was not altered (19.0 ± 0.3%). Tesaglitazar treatment of the obese rats for 4 wk lowered basal insulin, C-peptide, glucose, and TG. Tesaglitazar did not alter basal FFA levels.

Plasma level responses to the oral combined glucose/TG load are shown in Fig. 1. Glucose, insulin and C-peptide levels rose rapidly in all groups, peaking at 10 min following the load; earlier and more frequent blood sampling confirmed the timing of these peaks (results not shown). Plasma FFA also responded rapidly, falling to minimum levels by 15–20 min. In contrast with the other plasma variables, TG responded slowly in all three groups, with levels rising to a broad peak between 60 and 180 min following the oral load.

The Obese group displayed marked impairments in metabolic responses to the mixed glucose/TG load. Compared with the Lean group, Obese animals exhibited much larger and more
prolonged excursions in plasma glucose, insulin and C-peptide (Fig. 1), leading to substantially greater areas under the curve (AUCs) for all these factors (Table 1). In the Obese, compared with the Lean animals, AUC for plasma FFA was higher during the postload period, in spite of very high insulin levels (Fig. 1 and Table 1).

In addition to glucose intolerance, insulin hypersecretion and elevated FFA levels, TG AUCs were greater in the Obese group compared with the Lean group (Table 1). Most of this latter difference probably reflects genuine TG intolerance, although, a smaller component could be due to accelerated absorption of the TG load. Thus the fractional absorption of the $^3$H-vitamin A spike 4 h after the mixed glucose/TG load was greater in Obese compared with Lean animals (88 ± 3 vs. 67 ± 4%; $P < 0.01$), consistent with previously reported accelerated gastric emptying in obese Zucker rats (14). This suggests a greater level of absorption of the TG component of the mixed-meal Obese compared with Lean animals, since the absorption of vitamin A, like dietary TG, requires incorporation into chylomicrons (5).

Tesaglitazar substantially improved the glucoregulatory response to the mixed TG/glucose load in the obese Zucker rats. Although the initial glucose excursions were similar in both groups of obese rats (Fig. 1), the return toward basal was more rapid in the Tesaglitazar group, as indicated by a smaller deviation from basal already at the 30-min time point (repeated-measures ANOVA, $P < 0.01$). Over the 0- to 2-h period of plasma glucose perturbation, glucose AUC was reduced by tesaglitazar (Table 1). The tesaglitazar-induced improvement in glucose tolerance was achieved in spite of an apparent large reduction in insulin secretion, consistent with a treatment-induced enhancement in insulin action. Thus post-

Table 1. Plasma factor responses to an oral combined glucose/TG load in Lean, Obese and Tesaglitazar groups of Zucker rats

<table>
<thead>
<tr>
<th></th>
<th>Lean Control</th>
<th>Obese Control</th>
<th>Obese Tesaglitazar</th>
</tr>
</thead>
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<tr>
<td>Body weight, g</td>
<td>341 ± 10</td>
<td>550 ± 16$^c$</td>
<td>607 ± 18$^d$</td>
</tr>
<tr>
<td>Plasma Glucose</td>
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<tr>
<td>Basal (mM)</td>
<td>6.7 ± 0.2</td>
<td>7.7 ± 0.4$^a$</td>
<td>6.9 ± 0.2$^a$</td>
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<td>AUC(2 h), mM/min</td>
<td>954 ± 30</td>
<td>1,137 ± 47$^b$</td>
<td>1019 ± 31$^d$</td>
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<tr>
<td>AUC(4 h), mM/min</td>
<td>1,779 ± 42</td>
<td>1,929 ± 55$^a$</td>
<td>1,890 ± 51</td>
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<tr>
<td>Plasma Insulin</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Basal, nM</td>
<td>0.3 ± 0.1</td>
<td>3.0 ± 0.3$^c$</td>
<td>0.7 ± 0.1$^a$</td>
</tr>
<tr>
<td>AUC(2 h), nM/min</td>
<td>70 ± 7</td>
<td>664 ± 27$^a$</td>
<td>184 ± 18$^a$</td>
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<tr>
<td>AUC(4 h), nM/min</td>
<td>119 ± 14</td>
<td>1,089 ± 56$^b$</td>
<td>276 ± 24$^a$</td>
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<tr>
<td>Plasma C-Peptide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal, nM</td>
<td>0.5 ± 0.1</td>
<td>4.6 ± 0.2$^a$</td>
<td>2.4 ± 0.2$^a$</td>
</tr>
<tr>
<td>AUC(2 h), nM/min</td>
<td>45 ± 2</td>
<td>186 ± 14$^a$</td>
<td>115 ± 12$^c$</td>
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<tr>
<td>AUC(4 h), nM/min</td>
<td>129 ± 20</td>
<td>459 ± 33$^a$</td>
<td>177 ± 34$^a$</td>
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<tr>
<td>Plasma FFA</td>
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<tr>
<td>Basal, mM</td>
<td>0.67 ± 0.05</td>
<td>0.95 ± 0.07$^b$</td>
<td>0.90 ± 0.06</td>
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<td>AUC(2 h), mM/min</td>
<td>60 ± 4</td>
<td>92 ± 5$^c$</td>
<td>65 ± 2$^c$</td>
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<td>AUC(4 h), mM/min</td>
<td>132 ± 9</td>
<td>221 ± 13$^c$</td>
<td>154 ± 6$^a$</td>
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<tr>
<td>Plasma TG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal, mM</td>
<td>0.8 ± 0.1</td>
<td>3.4 ± 0.4$^c$</td>
<td>1.1 ± 0.2$^c$</td>
</tr>
<tr>
<td>AUC(2 h), mM/min</td>
<td>128 ± 15</td>
<td>657 ± 56$^c$</td>
<td>192 ± 20$^c$</td>
</tr>
<tr>
<td>AUC(4 h), mM/min</td>
<td>263 ± 34</td>
<td>1,438 ± 114$^a$</td>
<td>392 ± 47$^c$</td>
</tr>
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</table>

Results are expressed as means ± SE (n = 9 or 10). Basal values were obtained just before giving the glucose/triglyceride (TG) load. Plasma responses to the load are expressed as areas under the curve to 120 min, and 240 min, AUC(2 h) and AUC(4 h), respectively. $^aP < 0.05$, $^bP < 0.01$, $^cP < 0.001$ vs. Lean; $^dP < 0.05$, $^eP < 0.001$ vs. Obese.

Fig. 1. Plasma factor responses to an oral combined glucose/TG load in Lean, Obese and Tesaglitazar groups of Zucker rats. Data points represent means ± SE (n = 9–10).
load insulin and C-peptide levels, expressed as either total AUCs (Table 1) or incremental areas above basal (results not shown), were much lower in tesaglitazar-treated animals compared with Obese animals. In addition to improving glucose-regulation, tesaglitazar markedly reduced plasma FFA exposure over the postload period (Table 1). Suppression of FFA levels, particularly in response to the initial insulin peak, was much greater and more persistent in the Tesaglitazar than in the Obese group (Fig. 1). The fractional absorption of the 3H-vitamin A spike 4 h after the mixed glucose/TG load was similar in the Obese and Tesaglitazar groups, 88 ± 3 vs. 86 ± 5%. Despite an apparently equivalent intestinal TG absorption in Tesaglitazar and Obese animals alike, plasma TG excursions and total plasma TG exposures over the 4-h postmeal period were dramatically reduced in the tesaglitazar-treated compared with Obese animals (Table 1), demonstrating a substantial enhancement in TG tolerance with tesaglitazar.

Study 2: Hepatic VLDL Triglyceride Production and Plasma Clearance of Triglyceride in Anesthetized Rats

The results are summarized in Table 2. The basal (pre-Triton) TG levels in these Obese animals were higher than the levels seen in the Obese animals in basal state in Study 1. This can be attributed to interbatch variation (which in our experience is large in obese animals of this age) or to interstudy differences in experimental conditions (e.g., duration of fasting). Importantly, in both studies, there was a clear tesaglitazar-induced reduction in basal plasma TG to low absolute levels (compare Tables 1 and 2). In response to Triton WR 1339 administration, plasma TG rose linearly with time in both treated and untreated groups, with no tendency to plateau over the 120-min post-Triton period. Rates of HTGO and plasma TG clearance (K_{TG}) for Obese and Tesaglitazar groups are presented in Table 2. The marked decrease of basal plasma TG was due to a substantial, approximately six-fold enhancement in K_{TG} and a marked reduction in HTGO.

Lipoprotein profiles. Plasma lipoprotein profiles (Fig. 2) were assessed by continuous total cholesterol measurements of the elution profiles based on size exclusion chromatography. This analysis was performed in samples taken immediately before and 2 h after Triton WR 1339 administration (labeled Pre-Triton and Post-Triton, respectively, in Fig. 2). Pre-Triton, tesaglitazar treatment virtually eliminated the substantial VLDL-C peak. The virtual absence of a pre-Triton VLDL-C peak in the tesaglitazar-treated animals reflects a very rapid clearance of newly secreted VLDL from the plasma by the action of LPL. This is confirmed by the finding that after Triton administration and the subsequent blockade of LPL action, VLDL-C accumulated in the plasma (Post-Triton sample, Fig. 2).

Hepatic apoC-III mRNA and apoC-III content of VLDL. ApoC-III is a physiologically important regulator (inhibitor) of plasma TG clearance, which is synthesized in the liver. Previous studies have shown that agonists selective for PPARα, reduce hepatic apoC-III mRNA levels, while PPARγ agonists do not (8, 11). Consistent with significant PPARα activation in vivo, tesaglitazar induced a substantial reduction in liver apoC-III mRNA levels (Fig. 3). That the downregulation in hepatic apoC-III mRNA level induced by tesaglitazar translated into an alteration in protein expression was confirmed by the observed marked reduction in apoC-III content of VLDL (isolated by ultracentrifugation) by 86% (P < 0.05) as shown in Fig. 3.

Hepatic triglyceride content. Tesaglitazar-induced liver enlargement: liver weight was 22.8 ± 1.0 g in the Obese group and 27.0 ± 0.9 g in the Tesaglitazar group (P < 0.01). This is an expected effect of hepatic PPARα activation in rodents (28). Previous studies have shown that obese Zucker rats exhibit substantial accumulation of triglyceride stores in nonadipose tissues, including the liver, and have furthermore implicated tissue lipids in the accumulation of various key clinically significant manifestations of the insulin resistance syndrome (46). Treatment of the obese rats with tesaglitazar induced a substantial reduction in both hepatic TG concentration (to 50% of Control, P < 0.01) and total hepatic content (from 19.3 ± 0.3 g/rat in Control to 1.1 g/rat in Tesaglitazar, P < 0.05).

DISCUSSION

The present studies examined a widely used animal model of the metabolic syndrome, the nonobese obese fa/fa Zucker rat. A novel approach was used to characterize the responses of conscious, chronically catheterized animals to an oral mixed glucose and TG load. In terms of glucose regulation, responses to the mixed load in the untreated controls agreed with previously reported results of oral glucose tolerance tests (21): marked glucose intolerance and insulin hyper-secretion was seen in the obese animals (Table 1, Fig. 1). In addition to these disturbances in glucose control, the mixed-load evaluation revealed a substantial TG intolerance, as well as inadequate postprandial suppression of plasma FFA levels. These defects parallel postprandial disturbances in FFA and TG metabolism in humans, which are seen in association with insulin resistance (6), and are thought to play an important role in atherogenesis (22). Overall, the responses in the obese Zucker rat to a mixed oral load qualitatively reproduced the spectrum of associated metabolic disturbances seen in patients with the metabolic syndrome.

A major aim of this study was to examine the ability of tesaglitazar, a dual-PPARα/γ agonist, to correct these metabolic disturbances. Oral tesaglitazar treatment of obese rats for 4 wk improved glucose control by reducing fasting plasma glucose levels and ameliorating glucose intolerance. Treatment also lowered both fasting and postprandial insulin, as well as C-peptide levels, confirming our previous finding of a substantial enhancement in whole body insulin sensitivity and a reduction in insulin secretory burden (30). Enhanced insulin sensitivity at the level of adipose tissue was strongly indicated...
Figure 2. Cholesterol distribution profiles in Obese and Tesaglitazar groups of Zucker rats. Cholesterol concentration (y-axis), given in units of the spectrophotometer output (in millivolts), is plotted against elution time (x-axis). Profiles for each animal were obtained before (left) and 120 min after (right) Triton WR 1339 administration. Peaks have been labeled VLDL, “LDL,” and “HDL,” analogous to the human lipoprotein profile.

Figure 3. Hepatic apolipoprotein CIII (apoC-III) mRNA level (left) and plasma VLDL content of apoC-III expressed relative to total VLDL protein content (right) of Obese and Tesaglitazar groups of Zucker rats. Values are normalized to average levels in the Obese group. Data are means ± SE. †P < 0.05 vs. Obese.

Fig. 3. Hepatic apolipoprotein CIII (apoC-III) mRNA level (left) and plasma VLDL content of apoC-III expressed relative to total VLDL protein content (right) of Obese and Tesaglitazar groups of Zucker rats. Values are normalized to average levels in the Obese group. Data are means ± SE. †P < 0.05 vs. Obese.
by the treatment-induced restoration of the ability of insulin to suppress FFA. Tesaglitazar treatment exerted remarkable effects on the metabolism of TG-rich lipoproteins, markedly reducing the hypertriglyceridemia of the fasting state, as well as the alimentary hypertriglyceridemia in response to the mixed TG/glucose load. Triton WR1339 (38) studies, performed in anesthetized animals, revealed the kinetic mechanisms responsible for the effective antihypertriglyceridemic action of tesaglitazar. The treatment-induced lowering of the fasting hypertriglyceridemia involved two distinct effects. First, an accelerated rate of TG stripping from newly secreted VLDL was implied by the augmented plasma TG clearance (Table 2), as well as the virtual disappearance of VLDL-C from the lipoprotein profile (see Pre-Triton state, Fig. 2). Secondly, tesaglitazar lowered the VLDL TG secretion rate (Table 2). Both the enhanced plasma TG clearance and the reduced TG production are likely to have contributed to the observed tesaglitazar-induced enhancement in oral TG tolerance (Fig. 1) because chylomicrons and VLDL compete for a common lipolytic clearance process (7, 23).

PPARγ activation by tesaglitazar is likely to have contributed to the effects on FFA availability and TG metabolism. Thus in obese Zucker rats, selective PPARγ agonists lower FFA availability and decrease hepatic TG production (36). PPARγ agonists enhance insulin-mediated suppression of systemic FFA release (as was the case for tesaglitazar, Fig. 1) and traffic FFA into adipose tissue. This latter effect results from a PPARγ agonist-induced increase in the ability of adipose tissue to take up and store FFA (36), an effect that we have not seen with selective PPARα agonists (unpublished observations). Stimulation of hepatic PPARα could also be involved in the lowering of hepatic TG content, as well as output. Thus numerous studies provide evidence that PPARα stimulation augments hepatic fatty acid oxidation capacity via upregulation of enzymatic machinery involved in β-oxidation e.g., (9), although direct demonstration of increased hepatic FFA oxidation in vivo is still lacking. Although expression levels of β-oxidation enzymes were not assessed in the current experiment, the downregulation of both hepatic apoC-III, as well as the hepatic enlargement, are consistent with intense hepatic PPARα stimulation at the dose of tesaglitazar used in the current study (see below). The treatment-induced reduction in fatty acid availability may well be responsible for metabolic improvements beyond lowering of hepatic TG output. Thus a compelling body of evidence suggests that fatty acid overload is the central causative factor in glucoregulatory disorders; skeletal muscle, and hepatic insulin resistance, as well as, insulin secretory defects (32). We propose that the reversal of lipid overload in nonadipose tissues by PPARα/γ activation provides the basis for the tesaglitazar-induced improvements in glucose tolerance and insulin sensitivity in the obese Zucker rats. Overall, available evidence supports the view that PPAR activation reduces muscle tissue lipid overload (e.g., Refs. 19, 24, 25, 48). One study with tesaglitazar has addressed this issue, and it was found that treatment lowered skeletal muscle diacylglycerides and long-chain acyl CoA in rats fed a high-fat diet (17). There are, however, studies showing no reduction or even increased lipid loading with PPAR agonists (e.g., Refs. 29, 31, 35). Improved methodology for localization and characterization of key intramyocellular lipids may help to resolve these apparently contradictory findings.

In vitro, tesaglitazar is a PPARα and PPARγ agonist, as studied at the levels of ligand binding, coactivator recruitment, and in reporter gene assays (10, 30). In vivo, it is difficult to ascribe specific metabolic effects to distinct activation of PPARα vs. PPARγ, as the effect patterns resulting from stimulation with selective agonists show considerable overlap in rodents (15, 48). However, the present finding of increased body weight gain associated with increased food intake is an expected consequence of PPARγ activation in obese Zucker rats (47). On the other hand, liver enlargement and apoC-III suppression can likely be attributed to PPARα activation. Thus in the obese Zucker rat, selective PPARα agonists consistently induce robust increases in liver size (39), whereas selective PPARγ agonists induce modest reductions in liver size (36). In terms of apoC-III regulation in this animal model, fenofibrate (a selective PPARα agonist) has been reported to downregulate hepatic mRNA expression, while rosiglitazone, even at high doses, does not reduce expression (8, 11).

A combination of effects could explain the tesaglitazar-induced enhancement of plasma TG clearance. First, the observed reduction in the apoC-III content of VLDL would be expected to promote delivery of TG-rich lipoproteins to LPL by increasing binding to the cell surface glycosaminoglycan matrix, as well as increasing LPL activity (43). Second, combined PPARα/γ agonism is expected to increase LPL mass via upregulation of LPL gene transcription in the liver via PPARα activation and in adipose tissue via PPARγ activation (2). Third, the reduction of VLDL TG secretion associated with the assembly of small particles more susceptible to LPL could theoretically increase plasma TG clearance (45).

In conclusion, the results of this study demonstrate that a mixed-load evaluation provides a simultaneous and integrative assessment of glucose and lipid metabolism. When applied in the obese Zucker rat, the method confirmed impairments in glucose control and glucose metabolic insulin resistance and revealed TG intolerance, as well as FFA metabolic insulin resistance. These findings extend the degree of parallelism between this animal model and human conditions of the metabolic syndrome. Tesaglitazar, a PPARα/γ agonist, was highly effective in correcting both TG intolerance and insulin resistance in this animal model. Assuming translation to the clinical setting, these findings suggest that tesaglitazar may be useful for improving glucose regulation and correcting dyslipidemia in patients with metabolic syndrome, including those with type 2 diabetes.

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DISCLOSURES

Tesaglitazar, the agent discussed in this article, is being developed by the employer of the authors (AstraZeneca) as a new commercially available medicine.

REFERENCES

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