Tissue-specific postprandial clearance is the major determinant of PPARγ-induced triglyceride lowering in the rat

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Laplante M, Festuccia WT, Soucy G, Blanchard PG, Renaud A, Berger JP, Olivercrona G, Deshaies Y. Tissue-specific postprandial clearance is the major determinant of PPARγ-induced triglyceride lowering in the rat. Am J Physiol Regul Integr Comp Physiol 296: R57–R66, 2009. First published October 29, 2008; doi:10.1152/ajpregu.90552.2008.—Peroxisome proliferator-activated receptor-γ (PPARγ) agonism potently reduces circulating triglycerides (TG) in rodents (16, 26, 27), whereas others observed decreased (−30%) in refed rats. In contrast, postprandial VLDL-TG clearance was increased 2.6-fold by COOH, which concomitantly stimulated adipose tissue TG-derived lipid uptake and one of its major determinants, lipoprotein lipase (LPL) activity, in a highly depot-specific manner. TG-derived lipid uptake and LPL were indeed strongly increased in subcutaneous inguinal white adipose tissue and in brown adipose tissue, independently of the nutritional state, whereas of the three visceral fat depots examined (epididymal, retroperitoneal, mesenteric) only the latter responded consistently to COOH. Robust correlations (0.5 < r < 0.9) were observed between TG-derived lipid uptake and LPL in adipose tissues. The agonist did not increase LPL in muscle, and its enhancing action on postprandial muscle lipid uptake appeared to be mediated by post-LPL processes involving expressed fatty acid binding/transport proteins (aP2, likely in infiltrated adipocytes, FAT/CD36, and FATP-1). The study establishes in a diet-induced obesity model the major contribution of lipid uptake by specific, metabolically safe adipose depots to the postprandial hypotriglyceridemic action of PPARγ agonism, and suggests a key role for LPL therein.

PPARγ agonist; white adipose tissue; brown adipose tissue; triglyceride secretion; triglyceride clearance

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-activated nuclear receptor that is highly expressed in adipose tissue, in which it regulates the expression of numerous genes involved in lipid and glucose metabolism (3). PPARγ agonists of the thiazolidinedione (TZD) class are currently used for the treatment of insulin resistance and type 2 diabetes. Beyond insulin sensitization, PPARγ agonism robustly reduces circulating triglycerides (TG) in rodents (16, 26, 36, 37) and tends to do so more modestly in humans as shown in some (1, 12, 46) but not all (13, 20, 42) studies. Such interspecies difference may be associated with the capacity for adipose mass expansion and the presence in rodents of a relatively large amount of brown adipose tissue (BAT), which switches from a thermogenic to a major lipid-storing fat depot in response to PPARγ agonism (44).

Triglyceridemia represents the balance between gut and liver-derived TG-rich lipoprotein secretion and lipoprotein lipase (LPL)-mediated clearance in extrahepatic tissues. PPARγ agonists are known to lower TG partly by favoring the intravascular hydrolysis of TG-rich lipoproteins through increased LPL expression/activity and subsequent fatty acid uptake in white adipose tissue (WAT) (26, 27, 30). We have further established the important contribution of BAT to the short-term PPARγ-mediated acceleration of TG clearance, as well as the depot specificity (subcutaneous vs. visceral) of WAT therein (25). With regard to the contribution of VLDL-TG secretion, several studies have reported a lack of effect of PPARγ agonists (18, 25, 27), whereas others observed reduced secretion in insulin-resistant rodent models (6, 36). Discrepancies among the above studies are likely the result of the use of different animal models and variations in treatment procedures. Specifically, the nutritional status is an obvious powerful modulator of intravascular TG metabolism that has been shown to influence PPARγ action on adipose tissue fatty acid metabolism (36). Yet the impact of the nutritional status on the modulation of the major determinants of triglyceridemia by PPARγ agonism has not been systematically addressed to date.

Our previous study (25) established adipose depot-specific TG clearance as the mechanism of the very short-term (3–5 day) hypolipidemic action of PPARγ agonism in rats fed a low-fat diet and studied in the fed state, using a dose of agonist that favors fat accretion in both subcutaneous and visceral adipose depots (11). Importantly, however, TZD treatment of humans stimulates fat deposition specifically in subcutaneous fat while maintaining or even reducing visceral WAT (31, 32, 45), such redistribution being considered important in the amelioration of the metabolic profile (17, 21, 23). Fat redistribution, such as that seen in humans, can be achieved in rats with higher doses of PPARγ agonists (24, 26). Whether the respective contribution of subcutaneous and visceral fat to TG clearance is altered in conditions of fat redistribution remains unknown. In addition, individual depots (mesenteric, epididy-
mal, retroperitoneal) within the visceral compartment exhibit metabolic specificities that distinguish them from each other (4); however, their relative contribution to TG clearance has not been established.

The present study was designed to address the above issues. Accordingly, we assessed the contribution of VLDL-TG secretion vs. tissue- and adipose depot-specific TG clearance to the hypotriglyceridemic action of chronic PPARγ agonism, in both the fasted and fed states, in rats fed an obesity-promoting diet and in which treatment elicited depot-specific fat redistribution.

RESEARCH DESIGN AND METHODS

Animals and treatments. Male Sprague-Dawley rats initially weighing 90–100 g (n = 24–32 per protocol, 3 protocols) were purchased from Charles River Laboratories (St. Constant, QC, Canada) and housed individually in stainless steel cages in a room kept at 23 ± 1°C with a 10:14-h light-dark cycle (lights on at 12:00, off at 22:00). The animals were cared for and handled in conformance with the “Canadian Guide for the Care and Use of Laboratory Animals,” and the protocols were approved by our institutional animal care committee. Upon arrival, rats had free access to tap water and a ground stock diet (Charles River Rodent Diet cat. no. 5075; Ralston Products, Woodstock, ON, Canada). Rats were then fed a purified high-sucrose, high-fat diet that favors obesity and insulin resistance, to maximize the effect of PPARγ agonism on lipid flux and to mimic some of the physiological features that characterize the human population targeted for PPARγ agonist treatment. The composition of the obesity-promoting diet is detailed elsewhere (40). For 23 days, half of the animals were given the non-TZD PPARγ full agonist COOH [2-[2-(4-phenoxycarbonyl)ethyl]indole-5-acetic acid]. The metabolic signature of COOH, including insulin sensitization, lipid lowering, and depot-specific adipose tissue accretion, is very similar to that of the TZD rosiglitazone, as determined previously (16). In addition, COOH and rosiglitazone display analogous pharmacological interactions with the PPARγ receptor (9). COOH was given as an adjunct to the diet at a dose of 30 mg·kg⁻¹·day⁻¹, EDTA-containing serum, after which rats were injected through the cannula with 2 ml/kg Triton WR1339 (300 mg/ml saline; Sigma-Aldrich, St-Louis, MO), a detergent that prevents intravascular TG catabolism (38). Blood samples (0.15 ml) were taken 20, 40, and 60 min after the injection. Rats were then injected with a lethal dose of ketamine-xylazine. Blood samples were centrifuged at 60 g for 10 min, and plasma was stored at −20°C for later plasma TG quantification.

The rate of VLDL-TG secretion into the circulation was determined from regression analysis of TG accumulation in plasma vs. time. Secretion rate was calculated by multiplying the slope of the regression line by plasma volume estimated from body weight and expressed as micromoles TG per minute. The fat-free refeeding paradigm avoided intestinal chylomicron formation, and TG secretion was therefore considered as originating mainly from the liver. Assuming steady-state plasma TG levels in the fasted state and 6 h into refeeding the fat-free diet (26), the endogenous TG clearance rate (KTC) was calculated as the ratio of TG secretion rate-to-basal plasma TG at t = 0 before Triton administration (36).

Serum and tissue collection for other assays. To obtain blood and other tissues free of radiolabel and detergent, a third protocol identical to the above was performed with 24 rats (n = 6 per group) not subjected to surgery. At the end of the 23-day treatment, and following the fasting-refeeding protocol described above in protocol 1, rats were killed by decapitation. Trunk blood was centrifuged (1,500 g, 15 min, 4°C) and serum was stored at −70°C until later quantification of metabolites and insulin. Samples of WAT, BAT, and muscles were quick-frozen and stored at −70°C for later RNA isolation and analysis.

LPL activity. One hundred microliters of thawed tissue homogenates were incubated under gentle agitation for 1 h at 28°C with 100 μl of a substrate mixture consisting of 0.2 mol/l Tris·HCl buffer, pH 8.6, which contained 10 MBq/l [14C]-[1-14C]tripalmitin (Amersham, Oakville, Canada) and 2.52 mmol/l cold triolein emulsified in 50 g/l gum arabic, as well as 20 g/l fatty acid-free bovine serum albumin, 10% porcine serum as a source of apolipoprotein C-II, and either 0.2 or 2 M NaCl. Free oleate released by LPL was then separated from total radiolabel and detergent, and sample [14C] radioactivity was determined in a final NaCl concentration of 1 M (non-LPL activity) from total lipolytic activity measured in a final NaCl concentration of 0.1 M. LPL activity was expressed as micromoles (1 μM = 1 μmol nonesterified fatty acids (NEFA) released per hour of incubation at 28°C). The activity of LPL is expressed per total adipose depot to reflect the relative contribution of specific depots to TG kinetics.

DNA isolation and analysis. Total RNA was isolated from the soleus of fasted and refed rats (pooled because there was no effect of nutritional status on gene expression) using Qiazol and the RNeasy...
Adipose-derived fatty acids, insulin, and liver lipid content are all potential determinants of hepatic VLDL-TG production and secretion. As depicted in Table 3, refeeding rats the fat-free diet brought about the expected increase in serum glucose and insulin concentrations, along with a reduction in serum NEFA and glycerol levels. Chronic COOH treatment did not affect serum glucose but markedly reduced fasting insulinemia as well as its postprandial excursion (−75% and −25%, respectively). In parallel, COOH reduced fasting (−26%) and postprandial NEFA (−36%), which led to a robust reduction in the NEFA/glycerol ratio, an index of fatty acid reesterification within adipose tissue. The above nutritional status and COOH treatment effects on plasma variables were replicated in two separate protocols. Whereas liver TG concentration was unaffected by the nutritional status, it was robustly reduced by COOH, to less than half of control levels.

The impact of chronic PPARγ agonism on triglyceridemia and its major determinants were next evaluated. Serum TG of control rats did not rise upon refeeding the fat-free diet (Fig. 1A). Whereas COOH treatment had no impact on fasting TG, it reduced postprandial TG by 68% relative to fasted levels. Refeeding untreated rats the fat-free diet did not alter the rate of VLDL-TG secretion, but modestly decreased secretion rate (−30%) in COOH-treated rats (Fig. 1B). Actual clearance rates of endogenous TG were calculated from secretion rates and pre-Triton TG levels. As shown in Fig. 1C, refeeding did not alter clearance of VLDL-TG in untreated rats, nor did COOH treatment affect clearance in fasted rats. In refed rats, however, COOH greatly increased (2.6-fold) the rate of clearance of endogenous VLDL-TG. We next assessed the impact of COOH on the global capacity, or potential, for TG clearance by quantifying the rate of disappearance of an intravenously-administered labeled chylomicron-like TG emulsion. As expected, the emulsion was cleared from the circulation within minutes (Fig. 1D). Whereas the nutritional status remained without effect, COOH greatly increased the capacity for TG clearance independently of the latter. In fact, acceleration of clearance was such that even when log transformed, the clearance-time relationship was nonlinear and absolute rates could not be calculated.

TG-derived lipid uptake (from the labeled emulsion) by various adipose depots and skeletal muscle was next assessed.

Effect of nutritional state. As expected, refeeding increased lipid uptake in several adipose depots, including iWAT (overall nutritional state effect by ANOVA [N]: P = 0.003), rWAT (N: P = 0.007), and mWAT (N: P = 0.001), but not significantly so in BAT and eWAT (Fig. 2). Refeeding decreased lipid uptake in the soleus of control rats, whereas this effect was blunted in COOH-treated animals (N × T interaction: P <

Table 1. Primers used for mRNA quantification by real-time RT-PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>5’ Primer (5’3’)</th>
<th>3’ Primer (5’3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>36B4</td>
<td>NM_022402</td>
<td>TAAAGACTGGAGACAAAGTG</td>
<td>GTGATCTCACTCCACGAGA</td>
</tr>
<tr>
<td>αP2</td>
<td>NM_053365</td>
<td>ATGTTTGGACTTTTGGG</td>
<td>CCCAGTTTGAAGGAAATCTC</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>NM_031561</td>
<td>AGTAACTCAAATGTTACGTG</td>
<td>GTGCAAGCAGACTGAAATGATA</td>
</tr>
<tr>
<td>FATP-1</td>
<td>NM_053580</td>
<td>TCTGCCGCGCTTGGGCTTAT</td>
<td>TTGTTGGGGTCTTGCAGTA</td>
</tr>
<tr>
<td>hFABP</td>
<td>NM_024162</td>
<td>ACATGAACTCAACTGGTGG</td>
<td>AGATGCAGATGGGATGAGT</td>
</tr>
</tbody>
</table>
Radiolabel uptake only in the fasted state (4-fold, T: 0.0001). These findings are in sharp contrast with what occurred in visceral WAT depots. Indeed, no treatment effect was noted significant trend was noted in the diaphragm. The marked elevation in TG-derived lipid uptake by the liver and spleen, in which resident macrophages are responsible for the LPL-independent clearance of a large fraction of synthetic lipid emulsions (14, 15).

Of note, TG-derived lipid uptake of whole adipose depots correlated strongly with uptake expressed on a unit weight (per gram tissue) basis (WAT depots, 0.82 ± 0.05; BAT, 0.46 ± 0.03; NS, not significant).

Effect of COOH treatment. COOH strongly increased lipid uptake in iWAT of fasted (14-fold) and refed (3-fold) rats (overall treatment effect by ANOVA [T]: P < 0.0001). The most robust changes in lipid uptake were found in BAT, in which COOH increased TG-derived lipid uptake 44-fold and ninefold in the fasted and refed states, respectively (T: P < 0.0001). These findings are in sharp contrast with what occurred in visceral WAT depots. Indeed, no treatment effect was observed in eWAT, whereas in rWAT, the agonist increased radiolabel uptake only in the fasted state (4-fold, T: P = 0.001). Visceral mWAT displayed increased lipid uptake in response to COOH in both fasted (4-fold) and refed (3-fold) states. In the soleus muscle, as noted above, COOH affected lipid uptake only in refed rats (4-fold), counteracting the refeeding-induced reduction in uptake seen in untreated rats. A similar, nonsignificant trend was noted in the diaphragm. Lipid uptake by the heart was not affected by COOH, either in the fasted or fed state (not shown), and the heart was not further explored. The marked elevation in TG-derived lipid uptake by some adipose tissues occurred at the expense of radiolabel uptake by the liver and spleen, in which resident macrophages are responsible for the LPL-independent clearance of a large fraction of synthetic lipid emulsions (14, 15).

Table 2. Food intake, energy balance determinants, and adipose tissue weights of rats treated or not with COOH for 23 days

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>COOH</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight, g</td>
<td>334 ± 7</td>
<td>350 ± 5</td>
<td>(0.08)</td>
</tr>
<tr>
<td>Body weight gain, g</td>
<td>182 ± 5</td>
<td>196 ± 4</td>
<td>0.04</td>
</tr>
<tr>
<td>Food intake, MJ</td>
<td>8.3 ± 0.2</td>
<td>8.7 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>Food efficiency, MJ/MJ</td>
<td>22.0 ± 0.5</td>
<td>22.6 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Last 6-h food intake, g</td>
<td>13.2 ± 0.6</td>
<td>15.4 ± 0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>iWAT, g</td>
<td>8.0 ± 0.5</td>
<td>11.6 ± 0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>rWAT, g</td>
<td>6.6 ± 0.4</td>
<td>3.8 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>eWAT, g</td>
<td>6.9 ± 0.5</td>
<td>4.5 ± 0.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mWAT, g</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>BAT, g</td>
<td>0.46 ± 0.03</td>
<td>3.41 ± 0.41</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 11–16 rats. iWAT, inguinal white adipose tissue; rWAT, retroperitoneal WAT; eWAT, epididymal WAT; mWAT, mesenteric WAT; BAT, brown adipose tissue; NS, not significant.

Table 3. Serum metabolite and hormone concentrations and liver triglyceride concentration in rats treated or not with COOH for 23 days and studied after a 10-h fast or a 17-h fast followed by a 6-h refeeding period with a fat-free diet

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>COOH</th>
<th>N</th>
<th>T</th>
<th>N × T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mM</td>
<td>8.1 ± 0.2</td>
<td>7.9 ± 0.3</td>
<td>9.9 ± 0.3</td>
<td>9.4 ± 0.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>337 ± 66</td>
<td>85 ± 22</td>
<td>729 ± 95</td>
<td>545 ± 72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NEFA, mM</td>
<td>0.66 ± 0.05</td>
<td>0.49 ± 0.03</td>
<td>0.22 ± 0.06</td>
<td>0.07 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Glycerol, mM</td>
<td>0.20 ± 0.03</td>
<td>0.22 ± 0.02</td>
<td>0.11 ± 0.01</td>
<td>0.07 ± 0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NEFA/glycerol ratio</td>
<td>3.4 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>1.9 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Liver TG, µmol/g</td>
<td>44 ± 2.7</td>
<td>18 ± 3</td>
<td>37 ± 7</td>
<td>13 ± 2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 4–6 rats. NEFA, xxx; TG, triglycerides. ANOVA values represent the level of significance of the nutritional state (N) with 2 levels (Control, COOH), and their interaction (N × T).
Lipoprotein lipase, the rate-limiting enzyme in clearance of circulating TG, is a key modulator of postprandial triglyceridemia (7). To assess its contribution to the COOH-mediated increase in TG clearance and tissue uptake, LPL activity was measured in several quantitatively important tissues (Fig. 3).

**Effect of nutritional state.** As expected, the activity of LPL was strongly increased upon refeeding in iWAT (N: \( P < 0.0003 \)), BAT (N: \( P = 0.0002 \)), rWAT (N: \( P = 0.02 \)), and mWAT (N: \( P < 0.0001 \)), but no significant effect was observed either in eWAT or in soleus and diaphragm muscles.

**Effect of COOH treatment.** In response to COOH, LPL activity was increased in iWAT (T: \( P = 0.004 \)), BAT (T: \( P < 0.0001 \)), and mWAT (T: \( P = 0.02 \)), and slightly reduced in eWAT (T: \( P = 0.03 \)) and rWAT (T: \( P = 0.07 \)). The activity of LPL was reduced in the diaphragm of COOH-treated rats but no effect was observed in the soleus.

Because of the potential importance of LPL in tissue-specific TG-derived lipid uptake, correlative relationships between those variables were deemed of particular interest. As depicted in Fig. 4, robust correlations were found between LPL activity and TG-derived lipid uptake by adipose tissues, strongly suggesting that in the latter clearance of the emulsion and tissue uptake of radiolabeled lipids was mainly determined by LPL-mediated hydrolysis of TG. In muscle, no such correlation was observed, as COOH treatment was associated with higher uptake in refed rats in the absence of any positive change in LPL activity. The COOH-mediated increase in TG-derived lipid uptake by skeletal muscle therefore appears independent from modulation of LPL availability per se. Post-LPL events can, however, modulate the process; indeed, facilitated NEFA uptake cannot only accelerate lipid entry into the cell but also increase LPL efficiency through attenuating product inhibition of its activity. Notably, COOH significantly increased muscle expression of the genes encoding FAT/CD36 and fatty acid transporter-1 (FATP-1) (Fig. 5). The agonist also increased (from virtually nil) adipocyte fatty acid binding protein (aP2) expression, confirming local adipocyte proliferation and suggesting their possible contribution to lipid uptake.

In an attempt to explore whether the metabolic effects of COOH are PPAR\(\gamma\) mediated rather than molecule-specific, we...
qualitatively compared the present findings with those of a separate study on the impact of the TZD rosiglitazone on relevant end points. As depicted in Fig. S1, under conditions relatively similar (dose of agonist, treatment duration) but not identical (diet, nutritional state at time of tissue harvesting) to those of the present study, rosiglitazone impacted triglyceridemia, adipose remodeling, and LPL activity in a pattern corresponding to that of COOH. The effects of rosiglitazone included plasma TG lowering (\(-46\%\)), increased iWAT mass (\(-2\)-fold) and total LPL activity (\(-6\)-fold), reduced rWAT mass (\(-38\%\)), and unchanged LPL, as well as increased BAT mass (7-fold) and LPL (8-fold).

**DISCUSSION**

The present fasting-refeeding paradigm aimed to address the as yet unexplored impact of the nutritional state on the mechanisms, whereby PPAR\(\gamma\) affects TG handling in a rat model of diet-induced obesity. It was found that triglyceridemia was not lowered by COOH after fasting for 10 h, confirming an earlier study of longer-term (24 h) fasting (26). The global TG clearance potential at the whole body level (represented by the rate of clearance of an exogenous, chylomicron-like TG load) was, however, greatly increased in COOH-treated rats relative to controls, due to the maintenance of an elevated lipid uptake capacity of specific WAT depots and BAT. These data are best interpreted as meaning that, in the fasted state, such increased clearance capacity in some adipose depots does not reduce plasma VLDL-TG of endogenous origin below a certain threshold, likely because intravascular LPL-lipoprotein interactions are optimal at that steady-state TG level. Clearly, it is in the fed state that the hypotriglyceridemic action of PPAR\(\gamma\) agonism was fully expressed. Indeed, whereas as previously shown (28), refeeding the fat-free diet did not alter plasma TG in untreated rats, their levels were dramatically reduced by COOH to less than one-third of fasting values. This reduction was due to the combination of a modest (30%) decrease in endogenous VLDL-TG secretion and a marked, 2.6-fold increase in their estimated clearance from the circulation.

The fasting (fat-free) refeeding transition affected neither triglyceridemia nor its major kinetic determinants in untreated rats. Thus the increase seen in WAT LPL was not sufficient [or perhaps compensated by transient, opposite changes in muscle tissues (41)] to increase endogenous VLDL-TG clearance or the global capacity to clear exogenous chylomicron-derived TG. In contrast, refeeding COOH-treated rats resulted in a large reduction in endogenous TG that coincided with a robust increase in their estimated clearance and with amplification of the feeding-induced elevation in LPL activity in several adipose depots (iWAT, mWAT, and BAT). Notably, this was not reflected in the kinetics of the chylomicron-like emulsion, likely because the rapid clearance/uptake rates of these particles with high affinity for LPL were already maximally stimulated by COOH in the fasted state. It is therefore reasonable to suggest that the tracer likely underestimates the magnitude of the impact of COOH on postprandial TG kinetics and tissue-specific uptake, as well as the strength of their relationship with LPL activity.

In a previous study aimed at investigating the very early (3–5-days) effect of PPAR\(\gamma\) agonism on lipemia, it was found that the rapid reduction in postprandial TG was entirely due to
enhanced clearance (25), with no contribution of rates of TG appearance in the circulation. Because PPARγ agonism has been reported to reduce fasting VLDL-TG secretion in the Zucker rat model of extreme obesity (36), and because of the very low expression level of PPARγ in the liver, we hypothesized that long-term treatment may elicit secondary changes that could, in turn, reduce VLDL-TG secretion, e.g., liver lipid content, or adiponectin, which partly mediates the effects of PPARγ agonism on liver glucose metabolism and insulin sensitivity (34). In addition, in that previous study, rats were refed their habitual low-fat diet, such that TG appearance rates represented the sum of exogenous and endogenous lipid input. Refeeding a fat-free diet here allowed examination of endogenous VLDL-TG kinetics in isolation. The present findings clearly show that, despite its strong antisteatotic action (Table 3) and robust effect on adiponectin, PPARγ agonism had little impact on hepatic VLDL-TG secretion in vivo. The slight reduction observed postprandially may be related to lesser NEFA provision from adipose tissue and increased sensitivity of VLDL secretion to the inhibitory action of insulin. In any case, the study shows that TG clearance remains the major mechanism, whereby long-term PPARγ agonism reduces triglyceridemia in the rat.

With regard to TG clearance, we wished to extend our previous study (25) by selecting treatment conditions (long-term, high-dose) that elicit adipose tissue redistribution, by studying several visceral adipose depots, and by assessing lipid uptake in vivo. The findings indicate that the general pattern of the tissue specificity of action of PPARγ on TG uptake by WAT is maintained long-term and is not altered by remodeling of adipose depots. Indeed, subcutaneous WAT and BAT remained the major targets of PPARγ agonism in terms of TG-derived lipid uptake and concomitant increase in LPL activity. The study further revealed, however, that the depot specificity (subcutaneous vs. visceral) of action of PPARγ agonism in WAT in terms of lipid uptake extends to structurally distinct visceral adipose depots, as mWAT, contrary to eWAT and rWAT, was found to contribute to some extent to...
the COOH-mediated increase in uptake. This could, in turn, explain why mWAT mass was not reduced by chronic COOH treatment as was the case for the other visceral depots. Although mWAT appeared to be as potent as iWAT to clear TG-derived lipid on a per unit weight basis, its global contribution to TG clearance from plasma was lesser than that of iWAT because of its smaller mass.

The PPARγ-mediated increase in adipose tissue LPL activity (26, 27, 30) is thought to be mediated by the presence of a functional peroxisome proliferator response element in the promoter of LPL (43). In close agreement with lipid uptake discussed above, PPARγ agonism was previously shown to increase LPL activity in an adipose depot-specific manner, i.e., in subcutaneous fat (iWAT), and BAT but not in visceral rWAT (26). The present results extend those observations to several structurally distinct visceral fat depots by showing that only mWAT displayed a very modest increase in LPL activity in response to COOH. The precise molecular mechanisms leading to the depot specificity of LPL activation by PPARγ agonism remain to be established but likely act at the transcriptional level. As discussed earlier (24, 26), receptor expression per se is unlikely to be involved. PPARγ activity is, however, highly regulated by multiple nuclear coactivators and corepressors (3, 39), the depot-specific recruitment of which might conceivably explain the depot-specific actions of PPARγ agonism. Finally, COOH tended to exert a neutral or negative effect on muscle LPL, emphasizing the tissue-specific nature of its action.

Uptake of NEFA following LPL-mediated hydrolysis is facilitated within the tissue microenvironment by binding/transport proteins that promote NEFA cellular uptake, thereby relieving product inhibition of LPL action (10). Increased expression of genes involved in NEFA uptake, binding, and retention in adipose tissue is a well-established effect of PPARγ agonists (3, 39) and likely contribute to their stimulation of lipid uptake from the circulation. As suggested earlier (24), such facilitation may explain, for instance, the increased fasting lipid uptake in the face of unchanged LPL, seen here in rWAT and the stimulation of lipid uptake by COOH in mWAT, despite modest LPL activation. In our previous study (24), expression levels of the transport proteins FAT/CD36 and FATP-1 were found to be increased in visceral fat by short-term rosiglitazone treatment. Adipose expression of transport proteins was not assessed here, a limitation of the present study; whether COOH shares this effect with TZDs and whether it is adipose depot specific, therefore, remains unresolved. Future assessment of the contribution of post-LPL modulation of NEFA uptake by individual transport proteins to the depot specificity of action of COOH and other PPARγ agonists on TG-derived lipid uptake is clearly warranted to broaden our knowledge of their modes of action. The strong correlations between LPL activity and TG-derived lipid uptake by adipose tissues strongly suggest, however, that globally the latter primarily depends upon LPL-mediated hydrolysis of TG. Contrastingly, in muscle, in which the major effect of COOH was to prevent the postprandial decrease in lipid uptake, no such correlation was observed, suggesting that the extent of muscle TG-derived lipid uptake was independent from changes in LPL availability per se. Notably, COOH increased muscle expression of FAT/CD36 and FATP-1, which may have facilitated postlipolytic NEFA uptake by muscle cells (47). In addition, increased expression of aP2, a fatty acid-binding protein recognized as a marker of adipocyte differentiation (48), suggests intramuscular adipocyte proliferation [perhaps from satellite cells (8, 22)], consistent with increased extramyocellular lipid content in muscle of humans treated with rosiglitazone (29). The respective contribution of myocytes (47) and intermyofibrillar adipocytes to the PPARγ-induced increase in lipid uptake by skeletal muscle remains to be determined. It should be noted that postprandial muscle lipid uptake was modest relative to that of adipose tissues; however, skeletal muscle mass as a whole may make for a nonnegligible contribution to overall TG clearance.

Another limitation of the present study is that we have not determined whether COOH action on TG clearance is mediated by its activation of PPARγ or might instead be related to some particular pharmacologic action of the COOH molecule unrelated to its effects on PPARγ activity. In an attempt to indirectly address the issue, we have determined in a separate study whether the effects of the TZD rosiglitazone (same dose and treatment duration as COOH in the present study, but in different nutritional conditions) on triglyceridemia, adipose tissue remodeling and depot-specific LPL activity qualitatively recapitulated those of COOH. The similarity of the metabolic signatures of these two chemically distinct molecules supports the notion that their actions are PPARγ-mediated. Given the importance role of LPL shown here, it can reasonably be suggested that such similarity of action between COOH and rosiglitazone would extend to TG-derived lipid uptake, although this as well as the direct involvement of the PPARγ receptor remain to be established experimentally.

In summary, this study demonstrates that long-term treatment of diet-induced obese rats with a full PPARγ agonist brings about a marked reduction in triglyceridemia that is limited to the fed state and is associated with a robust amplification of adipose depot-specific TG clearance. The agonist strongly increased in vivo TG-derived lipid uptake and LPL activity in metabolically safe subcutaneous fat (iWAT) and BAT, whereas of the three visceral fat depots examined, only mWAT showed a consistent increase in TG-derived lipid uptake.
uptake in response to treatment. Robust correlations were observed between lipid uptake and LPL activity, suggesting a key role of the latter in determining the extent of the former in adipose tissue. In skeletal muscle, the postprandial enhancement of TG-derived lipid uptake was apparently mediated by postlipolytic processes independent of LPL. The impact of PPARγ agonism on VLDL-TG secretion was modest, suggesting a relatively minor contribution to PPARγ-induced TG lowering. These findings establish in a dietary model of obesity that PPARγ agonism affects TG metabolism in a nutritional status-dependent manner, mainly through clearance mechanisms, and reveal the depot-specific nature of PPARγ action on lipid uptake by visceral fat.

**Perspectives and Significance**

This study highlights the remarkable flexibility of the TG clearance system and its powerful potential to modulate triglyceridemia. In addition to being a major target of PPARγ agonism, as illustrated here, TG clearance has been shown to also mediate the profound impact on triglyceridemia of physiological interventions such as acute exercise (TG lowering) and short-term, severe inflammation (hyper-TG). Liver VLDL-TG secretion is a key determinant of triglyceridemia in a number of physiological conditions; however, the profound changes in hepatic energy substrate metabolism brought about by exposure to PPARγ agonists [e.g., resolution of steatosis (2)] do not appear to greatly impact triglyceridemia. Although reducing circulating lipids (NEFA and TG) generally has obvious beneficial metabolic consequences in terms of health risk, the handling of lipids by the organs involved in uptake is at least equally important. The present study shows that PPARγ agonism quantitatively directs TG-derived fatty acids mainly towards metabolically safe adipose depots, which tend to retain fatty acids and to prevent their recycling to the circulation and to other organs. The metabolic consequences of the modest increase in lipid uptake by visceral mWAT (and rWAT in the fasted state) is apparently benign, as suggested by low plasma NEFA levels. Notably, PPARγ agonism also favors extra- and intracellular lipid accumulation in skeletal muscle (33), a condition usually associated with insulin resistance. Fortunately, however, PPARγ agonism, not unlike high-intensity exercise training, favors accumulation of metabolically neutral TG and reduces the formation of insulin resistance-promoting lipid intermediates such as ceramide. With regard to species specificity, the hypolipidemic action of PPARγ agonism is clearly more marked in rodents than in humans. The rodent model, however, supports the recently formulated concept that, beyond obesity per se, expansion of the lipid storage capacity of metabolically safe adipose tissue can be regarded as a viable strategy for the organism to maintain whole-body lipid homeostasis under conditions of energy surfeit (19). Relevance of the rodent model is also supported by the recent identification of significant amounts of BAT in humans (35).

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