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

Mathieu Laplante¹, William T. Festuccia¹, Geneviève Soucy¹, Pierre-Gilles Blanchard¹, Alexandra Renaud¹, Joel P. Berger², Gunilla Olivecrona³, Yves Deshaies¹

¹ Laval Hospital Research Center and Department of Anatomy and Physiology, Faculty of Medicine, Laval University, Québec, QC, Canada, G1V 4G5
² Department of Metabolic Disorders, Merck Research Laboratories, Rahway, NJ, USA, 07065-900
³ Department of Medical Biosciences, Umeå University, Umeå, Sweden

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Address correspondence to: Dr. Yves Deshaies
Laval Hospital Research Center
Faculty of Medicine, Laval University,
2725 Ch Sainte-Foy, Québec, QC, Canada G1V 4G5
Phone: 418-656-8711 # 3738  • Fax: 418-656-4942
E-mail: yves.deshaies@phs.ulaval.ca
ABSTRACT

Peroxisome proliferator-activated receptor (PPAR)γ agonism potently reduces circulating triglycerides (TG) in rodents and more modestly so in humans. This study aimed to quantify in vivo the relative contribution of hepatic VLDL-TG secretion and tissue-specific TG clearance to such action. Rats were fed an obesogenic diet, treated with the PPARγ full agonist COOH (30 mg/kg/day) for 3 weeks, and studied in both the fasted and refed (fat-free) states. Hepatic VLDL-TG secretion rate was not affected by chronic COOH in the fasted state and was only modestly 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. Triglyceride-derived lipid uptake and LPL were indeed strongly increased in subcutaneous inguinal white adipose tissue (iWAT) and in brown adipose tissue (BAT), 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 increased expression of 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.

Keywords: PPAR-gamma agonist; White adipose tissue; Brown adipose tissue; Triglyceride secretion; Triglyceride clearance
INTRODUCTION

Peroxisome proliferator-activated receptor (PPAR) \( \gamma \) 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). PPAR\( \gamma \) agonists of the thiazolidinedione (TZD) class are currently used for the treatment of insulin resistance and type 2 diabetes. Beyond insulin sensitization, PPAR\( \gamma \) 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 inter-species 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 PPAR\( \gamma \) agonism (44).

Triglyceridemia represents the balance between gut/liver-derived TG-rich lipoprotein secretion and lipoprotein lipase (LPL)-mediated clearance in extrahepatic tissues. PPAR\( \gamma \) 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 PPAR\( \gamma \)-mediated acceleration of TG clearance, as well as the depot specificity (subcutaneous vs. visceral) of WAT therein (25). With regards to the contribution of VLDL-TG secretion, several studies have reported a lack of effect of PPAR\( \gamma \) agonists (18, 25, 27) whereas others did observe 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 PPAR\( \gamma \) 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, epididymal, 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 noon, 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 #5075; Ralston Products, Woodstock, ON, Canada). Rats were then fed a purified high-sucrose, high-fat diet (HSHF) that favors obesity and insulin resistance, to maximize the effect of PPARγ agonism on lipid flux and to mimick 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-phenoxy-2-propylphenoxy)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 physico-chemical interactions with the PPARγ receptor (9). COOH was given as an adjunct to the diet at a dose of 30 mg/kg/day, previously shown to bring about a frank redistribution of fat in the rat (24, 26). The amount of COOH was adjusted every other day to body weight and food consumption. All 3 protocols were of the same 2 × 2 factorial design, the factors being Nutritional Status (N) with two levels (Fasted, Refed) and Treatment (T) with two levels (Control, COOH). Each of the 4 groups thus formed included 6-8 animals.
Exogenous triglyceride clearance rate and tissue uptake. In protocol 1, at day 19 into the 23-day treatment period, control and COOH-treated rats were cannulated into the jugular vein under isoflurane anesthesia. After 3 days of recovery during which treatment was continued, rats of the Fasted groups had their food removed for 10 h (from 23:00 to 09:00). Rats of the Refed groups were first fasted for 17 h (from noon to 05:00, that is during the whole lighted phase and part of the dark phase) and were then refed for 6 h their usual diet without the fat components. This refeeding paradigm allowed the study of plasma lipid clearance and tissue uptake in postprandial conditions (including feeding-induced hyperinsulinemia) without overt changes in triglyceridemia and tracer specific activity relative to the fasted state. Following the fasting and refeeding periods, the animals were injected through the jugular catheter with 0.15 ml/kg of 10% Intralipid containing $^3$H-9,10-labeled trioleylglycerol (570 dpm/nmol fatty acid) diluted 1:6 with 20% Intralipid (165 mg/kg TG were injected) and prepared as described previously (14). The integrity of the emulsion was verified, and >99% of label was found in the non-polar fraction, with only trace amounts of radioactivity in the polar (nonesterified fatty acids) fraction. Blood samples (0.15 ml) were then collected with an EDTA-containing syringe 1, 2, 5, 10 and 20 min after the injection. Twenty minutes after injection, rats were killed by ketamine-xylazine injection. Blood, inguinal (iWAT), mesenteric (mWAT), retroperitoneal (rWAT) and epididymal (eWAT) white adipose depots, interscapular BAT, soleus and diaphragm muscles, spleen, and a liver sample were quickly excised, weighed, and frozen at –70°C until later use. The radioactivity content of tissues, including blood, was quantified as described previously (14). Clearance of the emulsion from blood was visualized by plotting log-transformed plasma radioactivity against time, with the 0 time point estimated from the amount of label administered. Tissue lipid uptake is expressed as % injected dose. Additional samples of
WAT, BAT and muscles were homogenized exactly as previously described (5) and homogenates were frozen at –70°C for later determination of LPL activity, as described below.

**Endogenous VLDL-triglyceride secretion and clearance rates.** In protocol 2, at day 19 into the 23-day treatment period, control and COOH-treated rats were cannulated into the jugular vein under isoflurane anesthesia. After 3 days of recovery during which treatment was continued, and following the fasting-refeeding protocol described above in protocol 1, an initial blood sample (0.15 ml) was withdrawn into an EDTA-containing syringe, 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 was expressed as µmol TG/min. 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 (K_{TG}) 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 (1500 × 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.

**Lipoprotein lipase activity.** One hundred μl 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 [carboxyl-14C]triolein (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 intact triolein, and sample 14C radioactivity was determined in a scintillation counter and adjusted for the presence of 3H. LPL activity was calculated by subtracting lipolytic activity 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 microunits (1 µU = 1 µmol 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.

**RNA isolation and analysis.** Total RNA was isolated from the soleus of fasted and refeed rats (pooled because there was no effect of nutritional status on gene expression) using QIAzol and the RNeasy Lipid Tissue Kit (QIAGEN, Mississauga, ON, Canada). For cDNA synthesis, Expand Reverse Transcriptase (Roche Diagnostics, Montreal, QC, Canada) was used following manufacturer’s instructions and cDNA was diluted in DNase-free water (1:25) before quantification by real-time quantitative PCR (qPCR). mRNA transcript levels were measured in duplicate samples using a Rotor Gene 3000 system (Montreal Biotech, Montreal, QC, Canada). The primers used for the PCR reactions are presented in Table 1. Chemical detection of the PCR
products was achieved with SYBR Green I (Molecular Probes, Willamette Valley, OR). At the end of each run, melt curve analyses were performed and a few samples representative of each experimental group were run on agarose gel to verify specificity of the amplification. Data were normalized by calculating the ratio between the expression of the target gene and the housekeeping gene 36B4. Results are expressed as relative expression of candidate genes in COOH-treated rats vs. control rats.

**Serum/plasma determinations.** Serum glucose concentrations were measured by the glucose oxidase method with the YSI 2300 STAT plus glucose analyzer. Serum insulin was determined by radioimmunoassay (Linco Research, St. Charles, MO) with rat insulin as standard. Serum/plasma TG (Roche Diagnostics, Montreal, QC, Canada), NEFA (NEFA C test kit, Wako Pure Chemical Industries, Richmond, VA) and glycerol (Sigma, Oakville, ON, Canada) were measured enzymatically.

**Statistical analysis.** Data are presented as means ± SEM. Data from fasted and refed groups were pooled for analysis of variables related to food intake, body and tissue weights by Student’s unpaired t test. All other end points were analyzed according to a 2 × 2 factorial design, the factors being Nutritional Status (N) with two levels (Fasted, Refed) and Treatment (T) with two levels (Control, COOH). Pairwise between-group comparisons were analyzed by the post hoc Tukey-Kramer test. A P < 0.05 was considered significant. The Spearman correlation test was used to analyze relationships between end points.
RESULTS

Chronic treatment with COOH slightly increased, as expected, cumulative food intake (5%), final body weight (5%) and body weight gain (8%) (Table 2). The agonist did not affect food efficiency (g weight gain per MJ energy ingested). Upon refeeding for 6 h after 17 h of fasting (intake of ~75% of daily ad libitum intake), PPARγ-treated rats ingested significantly more food (17%) than controls. Although changes in body weight were relatively modest, COOH led to profound modifications of fat distribution. The agonist significantly increased subcutaneous iWAT (35%), whereas weights of the visceral rWAT and eWAT depots were both reduced by the agonist (~42% and ~35% respectively). The weight of the third visceral depot examined, mWAT, was not affected by COOH. Brown adipose tissue is a major target of PPARγ agonists in rodents, as confirmed here, as COOH increased BAT weight more than 7-fold. As expected, COOH led to cardiac hypertrophy, however, kidney, skeletal muscle and liver weights remained unaffected (data not shown).

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 i.v.-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.

Triglyceride-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: \( P < 0.05 \)). Similar, non-significant trends were noted in the diaphragm. **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 9-fold 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, non-significant 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).

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, \( 0.82 < r < 0.95 \); BAT, \( r = 0.71 \); \( P < 0.0001 \)), indicating that changes in lipid uptake were not solely due to fat mass redistribution among depots. Because COOH treatment increased TG-derived lipid uptake in subcutaneous iWAT and in the most responding visceral depot (mWAT) in the face of divergent effects on their mass (increased iWAT and unchanged mWAT weight), it was deemed of interest to further
compare lipid uptake per unit weight to evaluate the respective potential of these depots for TG-derived lipid uptake and responsiveness to treatment. As shown in Electronic Supplementary Figure S1, iWAT and mWAT had a similar capacity for lipid uptake per g tissue in basal conditions as well as in response to refeeding and to COOH, with a somewhat more robust response of mWAT to the agonist in the postprandial state.

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. **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 can not 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 PPARγ-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 Electronic Supplementary 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 triglyceride 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 PPARγ 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 mesenteric WAT, 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 lipids 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 PPRE 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 micro-environment 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 post-lipolytic 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 non negligible 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 that 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 important 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 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 post-lipolytic 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 (hyperTG). 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 regards 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 brown adipose tissue in humans (35).
AKNOWLEDGMENTS

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REFERENCES


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

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession #</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>TAAAGACTGGAGACAAGGGTG</td>
<td>GTGTAGTCAGTCCACACAGA</td>
</tr>
<tr>
<td>aP2</td>
<td>NM_053365</td>
<td>ATGTGTGATCCCTTTGTGGG</td>
<td>CCCAGTTTGAGGAAATCTC</td>
</tr>
<tr>
<td>FAT/CD36</td>
<td>NM_031561</td>
<td>AGTAATCTCAAATAACTGTACGTCG</td>
<td>CTGCAAGCACAGTATGAAATCATAA</td>
</tr>
<tr>
<td>FATP-1</td>
<td>NM_053580</td>
<td>TCTCGGCGCTCGATGGCTAT</td>
<td>TTGTGGGGGTCTGCAATGGC</td>
</tr>
<tr>
<td>hFABP</td>
<td>NM_024162</td>
<td>ACATGAAAGTCTCGGTGTG</td>
<td>ACATGGCCCATGGGTGAGAGT</td>
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</tbody>
</table>
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 (g/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 mean ±SEM 11-16 rats. 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>Fasted Control</th>
<th>Fasted COOH</th>
<th>Refed Control</th>
<th>Refed COOH</th>
<th>ANOVA N</th>
<th>ANOVA T</th>
<th>ANOVA 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>
<td>NS</td>
<td>NS</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>
<td>0.01</td>
<td>NS</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>
<td>0.002</td>
<td>NS</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>
<td>NS</td>
<td>NS</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>
<td>0.0002</td>
<td>NS</td>
</tr>
<tr>
<td>Liver TG (µmol/g)</td>
<td>44±7</td>
<td>18±3</td>
<td>37±7</td>
<td>13±2</td>
<td>NS</td>
<td>0.0002</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are means ±SEM 4-6 rats. NS, not significant. The ANOVA values represent the level of significance of the Nutritional state (N) with 2 levels (Fasted, Refed) and agonist Treatment (T) with 2 levels (Control, COOH), and their interaction (N×T).
FIGURE LEGENDS

Figure 1 – Plasma triglyceride (TG) concentration (A), rate of VLDL-TG secretion (B), calculated rate of VLDL-TG clearance (C), and TG clearance of a synthetic chylomicron-like TG emulsion in fasted (10 h) or refed (6 h) rats treated or not with COOH for 23 days. Each point represents the mean ± SEM of 4-6 rats. * $P < 0.05$ vs. untreated rats in same nutritional state.

Figure 2 – Triglyceride-derived lipid uptake by tissues 20 min following the injection of a chylomicron-like TG emulsion in fasted (10 h) or refed (6 h) rats treated or not with COOH for 23 days. Each column represents the mean ± SEM of 4-6 rats. * $P < 0.05$ vs. untreated rats in the same nutritional state; † $P < 0.05$ vs. fasted rats of the same treatment group. iWAT, inguinal white adipose tissue; BAT, brown adipose tissue; eWAT, epididymal WAT; rWAT, retroperitoneal WAT; mWAT, mesenteric WAT.

Figure 3 – Lipoprotein lipase activity in tissues of fasted (10 h) or refed (6 h after 17-h fast) rats treated or not with COOH for 23 days. Each column represents the mean ± SEM of 4-6 rats. * $P < 0.05$ vs. untreated rats in the same nutritional state; † $P < 0.05$ vs. fasted rats of the same treatment group. See legend to Figure 2 for significance of abbreviations.

Figure 4 – Correlations between lipoprotein lipase (LPL) activity and triglyceride (TG)-derived lipid uptake in tissues of fasted (10 h) or refed (6 h after 17-h fast) rats treated or not with COOH for 23 days. Each point represents an individual animal. See legend to Figure 2 for significance of abbreviations.
Figure 5 – Quantitative RT-PCR analysis of mRNA expression in soleus of rats treated or not with COOH for 23 days. Data are expressed as relative expression of candidate genes in COOH-treated rats vs. control rats. Each column represents the mean ± SEM of 10-12 rats. * $P < 0.05$ vs. untreated rats.
Figure 3

Graphs showing the effect of fasting and refeeding on the levels of iWAT, eWAT, rWAT, mWAT, Soleus, and Diaphragm. The graphs compare the levels in control (open bars) and COOH (filled bars) conditions. The data points are marked with asterisks (*) and double daggers (†) to indicate significant differences.
Figure 4

- **iWAT uptake** vs. **iWAT LPL**: $r = 0.69$, $p = 0.0006$
- **BAT uptake** vs. **BAT LPL**: $r = 0.61$, $p = 0.002$
- **eWAT uptake** vs. **eWAT LPL**: $r = 0.53$, $p = 0.01$
- **rWAT uptake** vs. **rWAT LPL**: $r = 0.56$, $p = 0.0006$
- **mWAT uptake** vs. **mWAT LPL**: $r = 0.70$, $p = 0.0006$
- **Soleus uptake** vs. **Soleus LPL**: $r = 0.19$, $p = 0.4$
- **Diaphragm uptake** vs. **Diaphragm LPL**: $r = -0.16$, $p = 0.5$
Figure 5

![Bar chart showing relative mRNA expression for 36B4, hFABP, FAT/CD36, FATP-1, and aP2.](image)