Involvement of adipose tissues in the early hypolipidemic action of PPARγ agonism in the rat

Mathieu Laplante, William T. Festuccia, Geneviève Soucy, Yves Gélinas, Josée Lalonde, and Yves Deshaies
Laval Hospital Research Center, Faculty of Medicine, Laval University, Quebec, Canada

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Laplante M, Festuccia WT, Soucy G, Gélinas Y, Lalonde J, Deshaies Y. Involvement of adipose tissues in the early hypolipidemic action of PPARγ agonism in the rat. Am J Physiol Regul Integr Comp Physiol 292: R1408 –R1417, 2007. First published December 14, 2006; doi:10.1152/ajpregu.00761.2006.—Agonists of the peroxisome proliferator-activated receptor γ (PPAR-γ) are insulin sensitizers that potently improve lipemia in rodents. This study aimed to determine the contribution of lipid secretion vs. clearance and the involvement of white adipose tissue (WAT) and brown adipose tissue (BAT) in the rapid hypolipidemic action of PPARγ agonism. Male rats were treated with rosiglitazone (RSG; 15 mg·kg⁻¹·day⁻¹) for 1 to 4 days, and determinants of lipid metabolism were assessed postprandially. Serum triglycerides (TG) were lowered (−54%) after 3 days of RSG treatment, due to accelerated clearance from blood without contribution of changes in secretion rates. Both BAT and WAT were the major sites of RSG action on TG clearance, the increase in TG-derived fatty acid (FA) uptake reaching threefold in BAT and 60–90% in WAT depots. Accelerated TG clearance was associated with increased lipoprotein lipase (LPL) activity mostly in BAT. Serum nonesterified FA were lowered (−20%) by a single dose of RSG, an effect associated with increased expression levels of FA binding/transport (fatty acid binding protein-4), esterification (diacylglycerol acyltransferase-1), and recycling glycerol kinase and phosphoenolpyruvate carboxykinase enzymes in BAT and WAT, suggesting FA trapping. After 4 days of RSG treatment, nonesterified fatty acid (NEFA) uptake was also stimulated in both BAT (2.5-fold) and WAT (40%). These findings demonstrate the causal involvement of increased efficiency of LPL-mediated TG clearance and reveal the important contribution of TG-derived and albumin-bound FA uptake by BAT in the rapid hypolipidemic action of PPARγ agonism in the rat.

triglycerides; triglyceride secretion; triglyceride clearance; lipoprotein lipase; nonesterified fatty acids

POREXISOME PROLIFERATOR-ACTIVATED RECEPTOR γ (PPARγ) is a ligand-activated nuclear receptor that is highly expressed in white adipose tissue (WAT). PPARγ agonists of the thiazolidinedione (TZD) class are used clinically for the treatment of insulin resistance and type 2 diabetes. In addition to improving insulin sensitivity, TZDs tend to reduce circulating nonesterified fatty acid (NEFA) and, more modestly, triglycerides (TG) in humans but do so very robustly and within a few days in rodents (19, 31, 48). The precise mechanisms whereby TZD affect the metabolism of circulating lipids are not completely understood.

Plasma TG levels represent the balance between gut- and liver-derived, TG-rich lipoprotein secretion and lipoprotein lipase (LPL)-mediated clearance in various extrahepatic tissues. The TZD rosiglitazone (RSG) given for 3 wk to insulin-resistant hamsters was shown to reduce plasma TG by decreasing hepatic very-low-density lipoprotein (VLDL)-TG secretion, without any apparent change in clearance (25), whereas a similar long-term treatment in obese Zucker rats reduced TG secretion but also increased their clearance (32). Thiazolidinediones strongly induce the expression of genes involved in lipid uptake, trafficking, and esterification in WAT (2). Consequent lipid retention in WAT is thought to reduce ectopic fat storage and protect the liver and muscle from fatty acid-induced insulin resistance (32, 49). PPARγ-induction alterations in WAT metabolism could also influence circulating lipids through changes in adipokine production (e.g., adiponectin), provision of precursor NEFA to the liver for TG production/secretion, or uptake of lipoprotein TG-derived fatty acid (FA). The contribution of these various processes to the rapid hypolipidemic action of PPARγ agonism has not been addressed.

PPARγ agonism leads to a depot-specific increase in adiposity (10, 30). In rodents, brown adipose tissue (BAT) undergoes remarkable morphologic and metabolic changes, turning off its energy-dissipating activity to become a lipid storage organ (3, 5, 23). As in some WAT depots, PPARγ agonism induces cell proliferation (4), differentiation (41), and lipid accretion in BAT (47). This occurs despite a robust increase in the thermogenic effector uncoupling protein-1 (UCP-1) (6). PPARγ agonism, therefore, renders BAT quite similar to its WAT counterpart; however, its contribution to the modulation of plasma lipids by PPARγ agonists remains unknown.

The hypolipemic action of PPARγ agonism is much more marked and more rapidly established in rodents (days) than in humans (weeks). Brown adipose tissue is virtually absent in humans but constitutes a major target organ of PPARγ agonism in rodents in terms of fat accretion. This suggests the possibility of the involvement of adipose tissue, in general, and of BAT in particular, in the action of PPARγ agonism on the metabolism of circulating lipids. The present study was carried out to determine the contribution of lipid secretion vs. clearance, as well as that of WAT and BAT, to the rapid and robust hypolipidemic effect of PPARγ agonism in the rat. These objectives were pursued by treating rats for 1 to 4 days with the PPARγ agonist RSG, and by assessing whole-body as well as BAT and WAT determinants of lipid metabolism. Because long-term PPARγ agonism may exert depot-specific actions on lipid metabolism and fat accretion (22, 23), inguinal (iWAT) and epididymal WAT (eWAT) were studied as representative of subcutaneous and visceral WAT, respectively.

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MATERIALS AND METHODS

Animals and Treatments

Male Sprague-Dawley rats (200–225 g) 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 12:12-h light-dark cycle (lights on at 1600). 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 No. 5075; Ralston Products, Woodstock, ON, Canada; digestible energy content: 12.9 kJ/g). In each of the protocols described below, half of the rats were given rosiglitazone (RSG) purchased as AVANDIA at a local pharmacy) as an adjunct to the diet at a dose of 15 mg·kg⁻¹·day⁻¹. Rats were treated for a period of 1 to 5 days as indicated in the individual protocols below. To maximize the impact of treatment on TG metabolism, rats were studied in the postprandial state (23), standardized as follows: The day before the end of treatment, after normal access to food during the dark period, food was removed at 1600, and rats were given a dose of RSG (15 mg/kg) in 0.5% methylcellulose (vehicle alone to controls) by gavage to ensure exposure to RSG during the fasting period. At 0800 on the next morning, food (with or without RSG) was reintroduced, and rats were allowed to eat ad libitum for 6 h, after which time the various procedures described below were performed.

Triglyceride Appearance Rate

Eight control and 8 RSG-treated rats were used to assess the rate of appearance of TG into the circulation. Because rats were in the postprandial state, the procedure estimates the combined appearance of chylomicron- and VLDL-TG. Rats were treated with RSG for 5 days as described above. Triglyceride appearance rate was measured at day 5 to cover the whole treatment period so as to obtain a conservative estimate of the impact of short-term RSG treatment on TG appearance. Three days after the onset of treatment, rats were cannulated into the jugular vein under isoflurane anaesthesia. Two days later and following the refeeding procedure described in Animals and Treatments, rats were injected through the jugular catheter with 300 mg/kg of Triton WR-1339, a detergent that prevents intravascular TG catabolism. Blood samples (0.15 ml) were taken with an EDTA-containing syringe before (time 0), 20, 40, and 60 min after Triton injection. The rate of TG appearance into the circulation was determined from regression analysis of TG accumulation in plasma vs. time, with correction for plasma volume calculated from body weight (34).

Triglyceride Clearance Rate and Tissue Uptake

Fourteen rats were cannulated into the jugular vein as described in Triglyceride Appearance Rate. Two days after surgery, treatment with RSG was initiated in half of the animals and continued for 3 days. Triglyceride clearance rate and tissue uptake were assessed after 3 days of treatment, the time at which TG began to be significantly reduced by the agonist. At the end of the refeeding procedure, rats were injected through the jugular catheter with 0.15 ml/kg of 10% Intralipid containing 1H-9,10-labeled trioleoylglycerol (570 dpm/ mmol FA) diluted 1:6 with 20% Intralipid (165 mg/kg of TG were injected). The labeled TG emulsion was kindly provided by Drs. Thomas and Gunilla Olivecrona (Umeå University, Sweden) and prepared, as described previously (18). Blood samples (0.15 ml) were collected with an EDTA-containing syringe 1, 2, 3, 5, and 10 min after the injection. Rats were then killed by ketamine-xylazine injection, and various tissues were collected. Measurement of radioactivity in blood and in tissues was performed as described by Hultin et al. (18). Clearance rate of TG from plasma was calculated from the slope of disappearance of label from plasma. A significant proportion of artificial TG emulsions is cleared from the circulation through LPL-independent pathways, with a large contribution from the liver (18). Triglyceride-derived NEFA uptake by tissues was therefore expressed as % injected dose minus liver uptake. The latter was not affected by RSG treatment (not shown).

In Vivo Rates of FA Synthesis

Rats treated for 7 days with RSG exactly as described in Animals and Treatments and in the fed state were injected intraperitoneally with 3H2O (3 mCi in 0.5 ml saline). One hour later, they were killed by decapitation; blood samples were collected for determination of serum water-specific radioactivity, and eWAT and BAT were rapidly removed for measurement of label incorporation into triglycerides. Tissue lipid extraction, isolation of triglyceride-fatty acids, radioactivity counting, and plasma water-specific radioactivity determination were carried out exactly as previously described (21). Lipogenesis was expressed as nanomoles of fatty acids per gram of tissue per minute.

Serum and Tissue Sampling

Rats were killed by decapitation. Trunk blood was centrifuged (1500 g, 15 min, 4°C), and serum was stored at −70°C until later for biochemical measurements. Adipose tissue samples destined for quantification of LPL activity were homogenized and processed exactly as described earlier (36) and stored at −70°C until assayed.

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 radioimmunooassay (Linco Research, St. Charles, MO) with rat insulin as a standard. Serum/plasma TG (Roche Diagnostics, Montreal, QC, Canada) and NEFA C test kit (Wako, Richmond, VA) were measured enzymatically.

Adipose Tissue Lipoprotein Lipase Activity

Enzyme activity in adipose tissues was determined exactly as described (27). Briefly, tissue homogenates were incubated with a substrate mixture containing [carboxyl-14C] triolein, and NEFA released by LPL were separated and counted. LPL activity was expressed as micromoles (1 μU = 1 μmol NEFA released per hour of incubation at 28°C). The interassay coefficient of variation was 11.2% and was determined using bovine skim milk as a standard source of LPL. Enzyme activity is expressed per total adipose depot to illustrate its global tissue availability in relation to lipid uptake.

Adipose Tissue Total TG Content

Adipose TG content was determined enzymatically as above in total lipid extracts prepared by the method of Folch (14).

Brown Adipocyte Morphology by Light Microscopy

BAT samples from six control and six RSG-treated (4 days) rats were fixed in 0.1 mmol/l PBS (pH 7.3) containing 4% paraformaldehyde and embedded in paraffin. Thin sections were mounted on glass slides and dried with hematoxylin/eosin. At least five digital images of each tissue were captured using an Olympus BX60 microscope equipped with a Sony RT Slider Spot Camera (Camsen Group, Markham, ON, Canada) at a magnification of ×40. Quantification was achieved using Image Pro Plus 5.0 (MediaCybernetics, Silver Spring, MD). Briefly, on blinded samples, the lipid surface of five different slides per depot was measured using the Measure-Count/Size tool, and an average was calculated for each rat.
Ex Vivo NEFA Uptake

Fat pads from rats treated or not for 1 or 4 days with RSG were removed immediately after decapitation. Fat explants of eWAT and iWAT (20–25 mg) and BAT (10–15 mg) were weighed and preincubated for 10 min in 1 ml Krebs-Ringer bicarbonate (KRB) buffer of the following composition (in mM): 118 NaCl, 4.8 KCl, 1.25 CaCl₂, 1.2 KH₂PO₄, 1.2 MgSO₄, 25 NaHCO₃, 5 glucose, supplemented with 2.5% fatty acid-free BSA (Sigma, Oakville, Canada), pH 7.4. The tissue explants were then transferred into 24-well plates, each well containing 1 ml KRB supplemented with ³H-labeled oleic acid (0.8 μCi/well). Expanses were exposed or not to insulin (375 pM, which approximates postprandial insulinemia); however, insulin did not impact NEFA uptake, and data from nonexposed explants are presented herein. Fat explants were incubated for 2 h in a humidified atmosphere of 5% CO₂-95% O₂ at 37°C. At the end of the incubation, tissue pieces were washed twice in cold saline and extracted overnight at room temperature with 1 ml of heptane-isopropanol (2:3). The organic solvent was evaporated, total ³H-labeled lipids were dissolved in scintillation liquid and counted.

RESULTS

Ex Vivo NEFA Uptake

R1410 SHORT-TERM PPARγ AGONISM AND LIPEMIA

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RNA Isolation and Analysis

Total RNA was isolated from adipose depots using Qiazol and the RNasy Lipid Tissue Kit (Qiagen, Mississauga, ON, Canada). For cDNA synthesis, Expand Reverse Transcriptase (Roche Diagnostics, Montreal, QC, Canada) was used following the 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 are expressed as the ratio between the expression of the target gene and the housekeeping gene L27.

Statistical analysis. Data are expressed as means ± SE. When appropriate, variables were first analyzed by factorial ANOVA to establish the individual and interactive effects of PPARγ agonist treatment, with two levels (control, RSG), and time of treatment, with four levels (1, 2, 3, or 4 days). Individual pairwise between-group comparisons were then made using Fisher’s protected least significant difference test. Some data were log transformed before analysis to ensure homogeneity of variance. Significance was set at P < 0.05.

RESULTS

Treatment with RSG for 1 to 4 days did not affect body weight or food intake, nor did it alter postprandial serum levels of glucose and insulin (Tables 2 and 3). The impact of RSG on postprandial serum TG and NEFA is depicted in Fig. 1. Circulating TGs were significantly higher in RSG-treated rats after the first day of treatment; however, this effect disappeared on the following day (Fig. 1A). RSG strongly reduced circulating TG at days 3 and 4 (−54% and −59% relative to control, respectively). The agonist also rapidly lowered NEFA (Fig. 1B), the effect being significant after only 1 day of treatment (−20%) and maintained thereafter (−26 to −38% below control) until the end of the 4-day study.

Triglyceride clearance was quantified after 3 days of treatment, the time at which triglyceridemia became significantly reduced by the agonist (Fig. 1A). As shown in Fig. 2A, the rate of disappearance of plasma-labeled TG was significantly accelerated by RSG. In contrast, the rate of appearance of TG into the circulation, measured in another rat cohort after 5 days of treatment with RSG, remained unaltered (Fig. 2B), indicating that the rapid RSG-induced lowering of triglyceridemia was entirely due to an amelioration of TG clearance. We next assessed which tissues were responsible for faster TG clearance in RSG-treated rats. The agonist significantly increased TG-derived NEFA uptake in all adipose tissues examined (Table 4). Of note is the fact that the relative magnitude (% over control) of such an increase was much more pronounced in BAT than in iWAT and eWAT. In fact, whole tissue lipid uptake by BAT of RSG-treated rats reached the level of uptake seen in WAT of untreated rats. The agonist had no significant effect on TG-derived NEFA uptake by the nonadipose tissues examined.

Because RSG improved triglyceridemia by rapidly increasing adipose tissue TG clearance, the activity of LPL, the rate-limiting enzyme in intravascular TG hydrolysis, was assessed in BAT and eWAT. The activity of LPL is expressed per total adipose depot to reflect the contribution of the whole tissue to the TG clearance potential. As depicted in Fig. 3A, the agonist progressively increased total LPL activity in BAT from day 2 to day 4, such increase reaching its peak at day 3 (234% over control). A more modest increase in LPL activity was also observed in iWAT from day 3 of treatment; such an increase reached significance at day 4 (Fig. 3B). Rosiglitazone did not affect LPL activity in eWAT (Fig. 3C).

Treatment with PPARγ agonists decrease both serum TG and albumin-bound NEFA, as confirmed here. To determine the potential of adipose depots to take up albumin-bound NEFA, fat explants from rats treated during 1 or 4 days with RSG were

<table>
<thead>
<tr>
<th>Table 1. Primers used for qPCR</th>
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<tr>
<td><strong>Gene</strong></td>
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<tr>
<td>ADRP</td>
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<tr>
<td>CD36</td>
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<tr>
<td>DGAT-1</td>
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<tr>
<td>FABP-4</td>
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<td>FAS</td>
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<td>GlyK</td>
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<tr>
<td>L27</td>
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<tr>
<td>PEPCCK</td>
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<td>UCP-1</td>
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ADRP, adipose differentiation-related protein; CD36 is also known as fatty acid translocase; FABP-4, adipose fatty acid binding protein 4 (also known as aP2); DGAT-1, diacylglycerol acyltransferase 1; FATP-1, fatty acid transport protein 1; FAS, fatty acid synthase; GlyK, glycerol kinase; L27, ribosomal protein L27; PEPCCK, phosphoenolpyruvate carboxykinase; UCP-1, uncoupling protein-1.

AJP-Regul Integr Comp Physiol • VOL 292 • APRIL 2007 • www.ajpregu.org
The RSG-induced relative increase in BAT oleate uptake increased 3H-labeled oleate uptake in BAT, iWAT, and eWAT (Fig. 1). Incubation with 3H-labeled oleate. The agonist significantly increased 3H-labeled oleate uptake in BAT, iWAT, and eWAT (Fig. 4). In BAT, FA uptake was not changed after the first day of treatment but was robustly increased at day 4. As in the case of TG-derived NEFA, the level of oleate uptake by BAT at day 4 was similar to the uptake by iWAT and eWAT of untreated rats (BAT: 2.9 ± 0.3, iWAT: 3.4 ± 0.5, eWAT: 3.8 ± 0.6). The RSG-induced relative increase in BAT oleate uptake (237%) was by far the largest observed among adipose tissues, the increase in iWAT and eWAT being 38% and 41% over control, respectively, at day 4. Of note, the stimulation of NEFA uptake in eWAT was observed after a single administration of RSG, suggesting that this depot may have contributed to the first-day effect of RSG on the reduction in circulating NEFA.

The impact of the RSG-induced increase in TG-derived and albumin-bound NEFA uptake by adipose tissues on tissue weight and TG content was next assessed. A single administration of RSG resulted in a 39% increase in BAT weight (Fig. 5A). This effect was maintained throughout the study and reached a maximum of 102% at day 3. As expected, the increase in BAT weight was associated with higher TG accretion (Fig. 5, B and C). There was no effect of RSG on weight, TG content, or cell morphology of WAT depots (not shown). To establish that the RSG-induced increase in BAT TG content was due to higher lipid uptake and esterification rather than de novo biosynthesis of lipids, the expression level of fatty acid synthase (FAS), an index of lipogenesis, was assessed. Treatment with RSG did not significantly increase the expression of FAS in BAT on day 1 (Table 5). The 4-day treatment tended to increase FAS in BAT and did so significantly in WAT. However, direct assessment of de novo lipogenesis in vivo performed in a separate study in which rats were treated for 7 days with RSG indicated that this process does not contribute at all to TG deposition in BAT and WAT within this time frame (BAT/Ctrl, 50 ± 17 nmol FA·g⁻¹·min⁻¹ vs. RSG, 46 ± 5; eWAT/Ctrl, 51 ± 17 vs. RSG, 63 ± 17).

The reduction in circulating NEFA in RSG-treated rats depicted in Fig. 1B was tightly associated with the increase in BAT weight. As shown in Fig. 6A, a strong correlation was observed between BAT weight and blood NEFA (r² = 0.53, P < 0.0001). This robust association was confirmed in a separate experiment (not shown). The link between circulating NEFA and the weight of iWAT (Fig. 6B, r² = 0.30, P < 0.0001) and eWAT (Fig. 6C, r² = 0.27, P < 0.0002) was significant but not as robust as that observed for BAT (Fig. 5, A and C).

Table 2. Body weight, food intake, serum glucose, and insulin of control and RSG-treated rats after 1, 2, 3, and 4 days of treatment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
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<td></td>
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<td>Day 2</td>
<td>Day 3</td>
<td>Day 4</td>
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<td>Cumulative FI, g</td>
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<td>48±4</td>
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<td>Insulin, pmol/l</td>
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<td>346±28</td>
<td>361±20</td>
<td>451±40</td>
<td>341±27</td>
<td>333±36</td>
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Table 3. Statistical analysis of treatment and duration effects and their interaction on body weight, food intake, glucose, and insulin

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<tr>
<td>Final BW</td>
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<td>Cumulative FI</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Insulin</td>
<td></td>
<td>NS</td>
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Data are means ± SE of 6 rats. BW, body weight; FI, food intake; RSG, rosiglitazone; NS, not significant.
B and C). Similar but weaker relationships were noted between triglyceridemia and adipose tissue weight (BAT: $r^2 = 0.20$, $P < 0.002$, $n = 47$; iWAT: $r^2 = 0.20$, $P < 0.003$, $n = 43$; eWAT: $r^2 = 0.10$, $P < 0.04$, $n = 48$).

Obesity-associated hyperlipidemia constitutes strong evidence that increased lipid uptake by adipose tissues is not necessarily sufficient in itself to lower plasma lipids. Hence PPARγ agonism has been shown to impact several processes involved in adipose tissue lipid retention following their uptake from the circulation. To assess the tissue-specific contribution of these processes to the hypolipidemic action of RSG, genes coding for proteins involved in FA uptake/esterification and storage were assessed in BAT and WAT. Because no marked metabolic differences were noted between iWAT and eWAT, gene expression was measured only in eWAT as representative of WAT. To confirm the well-established impact of RSG on gene expression, UCP1 mRNA levels were quantitated in this tissue and were found to be elevated from the first day of treatment (Table 5). Rosiglitazone increased the mRNA levels of CD36, a membrane-bound FA transporter that facilitates LPL-mediated TG clearance (15) (Table 5). The effect was observed earlier in BAT than in WAT. The expression of fatty acid transport protein-1 (FATP-1), which also favors FA uptake at the cell membrane, was induced only in WAT, and after 4 days of treatment. The PPARγ agonist also increased the expression of adipocyte fatty acid binding protein 4 (FABP-4, or aP2, a major adipose PPARγ target involved in adipogenesis, long-chain FA uptake and retention) in BAT (threefold) and in WAT (twofold) from the first day of treatment. Phosphoenolpyruvate carboxykinase (PEPCK) and glycerol kinase (GyK) contribute to FA trapping and TG synthesis by favoring glycerol-3-phosphate synthesis, to which FAs are esterified to form TG. The agonist increased PEPCK expression seven- and 25-fold in BAT and by six- and 22-fold in WAT on days 1 and 4, respectively. The increase in GyK mRNA expression occurred from day 1 on and reached threefold in BAT and 14-fold in WAT on day 4 of treatment. The mRNA level of diacylglycerol acyltransferase-1, which catalyzes the committed step in TG synthesis (40), was also increased by a single administration of RSG (approximately twofold), an effect that was slightly amplified at day 4. The expression of adipocyte differentiation related protein (ADRP), which is expressed early during adipocyte differentiation and which plays an important role in the maintenance of intracellular lipid droplets (26), was increased twofold in BAT and three- to fourfold in WAT from the first day until the end of the 4-day treatment.

**DISCUSSION**

This study was designed to assess the contribution of TG secretion and clearance, as well as the role of BAT and WAT in the rapid and marked improvement in the serum lipid profile brought in the rat by PPARγ agonism. The findings show that, at a time when the rate of appearance of TG into the circulation was not altered, short-term RSG treatment increased intravascular LPL-mediated TG clearance, as well as NEFA uptake, retention, and lipid deposition in adipose tissues through increasing the activity/expression of key enzymes genes involved in these processes. The acute effects of RSG on albumin-bound NEFA and TG-derived NEFA uptake were relatively stronger in BAT than in WAT. The study underlines the key role of adipose tissues and highlights the previously unrecognized contribution of BAT in the rapid hypolipidemic effect of PPARγ agonism.

The rapid reduction in circulating TG is in line with previous work in which obese rats treated with PPARγ agonists showed lower circulating TG after only 2 days of treatment (19). PPARγ agonists given for a longer period of time (weeks) have been shown to decrease hepatic VLDL-TG secretion in the obese Zucker rat (32) and fructose-fed hamster models (7), suggesting a role for this process in the reduction in lipemia. Such longer-term treatment with PPARγ agonists is able to robustly decrease liver lipid content even in the lean, chow-fed rat model used here (M. Laplante and Y. Deshaies, unpublished observations). In the present study, RSG given for 5 days did not affect the rate of TG appearance into the circula-

**Table 4.** Distribution of radiolabeled lipids following the injection of a $^3$H-labeled triglyceride emulsion into rats treated or not with RSG for 3 days

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% of Injected Dose</th>
<th>% of Change</th>
<th>P Value</th>
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<tbody>
<tr>
<td>BAT</td>
<td>0.87±0.15</td>
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<td>eWAT</td>
<td>4.66±0.23</td>
<td>+60</td>
<td>0.002</td>
</tr>
<tr>
<td>iWAT</td>
<td>3.08±0.57</td>
<td>+92</td>
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<td>Gastrocnemius</td>
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<td>0.01</td>
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<tr>
<td>Soleus</td>
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<td>0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>1.14±0.13</td>
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<td>0.01</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1.20±0.44</td>
<td>+92</td>
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Data are expressed as means ± SE of 6 rats. *Different from control, $P < 0.05$. BAT, brown adipose tissue; eWAT, epididymal white adipose tissue; iWAT, inguinal WAT.
tion, suggesting that the impact of PPARγ agonism on liver TG metabolism is delayed. Because of the low level of expression of PPARγ in the liver, the action of its agonists on hepatic lipid metabolism is thought to be indirect and may derive from modifications in WAT metabolism, including a reduced provision of NEFA as precursors for TG synthesis and an increase in adiponectin secretion.

The absence of a short-term effect of RSG on TG secretion implies that accelerated intravascular catabolism of TG represents the sole mechanism by which short-term PPARγ agonism lowered triglyceridemia in the present model. The finding of a more rapid rate of disappearance of a labeled TG emulsion from blood in RSG-treated rats relative to controls confirms this notion. It was further established that the major sites of the RSG-induced increase in TG clearance are BAT and WAT. These findings confirm our previous study (22) in establishing that PPARγ agonism stimulates the uptake by WAT not only of NEFA (9) but also of lipoprotein TG-derived FA as well. The study further extends these findings by identifying BAT as a site of the RSG-induced increase in TG-derived lipid uptake. Indeed, in control conditions, BAT took up 3–4 times less lipid than WAT, whereas after 3 days of RSG treatment BAT took up as much lipid as did control WAT. Hence RSG increased uptake 60–90% in WAT but nearly threefold in BAT. Such robust action on uptake, which extends to albumin-bound NEFA (see below), appears to explain most of the twofold increase in BAT TG content, given that short-term RSG did not affect de novo lipogenesis. The higher number of competent brown fat cells resulting from increased adipogenesis (4, 41) by PPARγ stimulation likely contributed toward increasing the ability of BAT to take up and store lipids.

Lipoprotein lipase, considered a key modulator of postprandial triglyceridemia (8), is increased by PPARγ agonists in adipose tissue of rodents and humans (23, 24, 44). In the present study, RSG increased LPL activity more rapidly and more strongly in BAT than in WAT. In fact, RSG had no effect on LPL activity in eWAT, confirming our previous observation that LPL of visceral fat is far less responsive to PPARγ agonism than that of subcutaneous fat (22, 23). Of note, the induction of LPL activity in BAT preceded the reduction in circulating TG, further supporting the involvement of BAT LPL in the short-term hypotriglyceridemic action of RSG. Although TG-derived NEFA uptake correlated well with LPL activity, discrepancies were noted. For instance, after 3 days of RSG treatment, TG-derived lipid uptake by eWAT was significantly increased in the absence of change in LPL. Uptake of TG-derived NEFA is not a completely efficient process (29, 33, 43), and the stimulation of LPL activity in BAT preceded the reduction in circulating TG, further supporting the involvement of BAT LPL in the short-term hypotriglyceridemic action of RSG. Among the sites of TG metabolism is considered as one important mechanism by which PPARγ agonists reduce circulating albumin-bound NEFA levels, which is decreased by PPARγ agonism in rodents and humans (23, 24, 44). Consistent with previous studies (19, 32), RSG rapidly reduced circulating albumin-bound NEFA levels, which is decreased by PPARγ agonism in rodents and humans (23, 24, 44). Consistent with previous studies (19, 32), RSG rapidly reduced circulating albumin-bound NEFA levels, which is decreased by PPARγ agonism in rodents and humans (23, 24, 44). Long-term treatment with PPARγ agonists reduces circulating NEFA by increasing the potential of WAT to take up, esterify, recycle, and store NEFA as TG (2, 9, 22), such processes also leading to a reduction in the output by WAT of the products of intracellular lipolysis (12, 32). The present study extends this notion in several ways. First, the involvement of BAT in NEFA trapping, in parallel with that of WAT, was established. Although at the functional level the RSG-induced stimulation of the uptake of albumin-bound NEFA appeared earlier in eWAT than in BAT, the latter displayed the largest relative increase over time. No systematic relationship was observed here between functional oleate uptake and expression levels of the major transport proteins CD36 and FATP-1. Uptake of NEFA through the cell membrane is a complex process involving passive diffusion and possibly several transporters (17, 20, 37), and further work is clearly needed to unravel the precise mechanisms of action of PPARγ agonism thereupon. Second, the study showed that a single dose of RSG, which reduces serum NEFA, increased in both WAT and BAT the expression of genes involved in FA binding, esterification, and recycling, as previously shown for longer-term treatment with PPARγ agonists (16, 22, 45, 46). Such a trapping effect likely contributed to the increase in FA uptake (28). Third, the study revealed a rapid induction of the expression of ADRP, which along with perilipin plays an important role in the stabilization of storage lipid droplets (26). Finally, it is noteworthy that a
relationship was established between the reduction in circulating NEFA and the increase in BAT weight and that of WAT to a lesser extent. In the absence of change in de novo lipogenesis, such a relationship suggests that BAT is a site of albumin-bound NEFA uptake and retention, as well as a significant contributor, probably along with decreased NEFA release by WAT, to the rapid RSG-induced reduction in circulating NEFA. Similar relationships were also noted between serum TG and adipose tissue accretion, but these were weaker, likely because of the delay in the LPL-mediated TG response to RSG treatment.

Intriguingly, the rapid modification by PPARγ agonism of the BAT phenotype from a thermogenic to a lipid storage organ, which is a component of the expansion of whole-body adiposity, occurs despite a marked increase in the expression of the thermogenic gene UCP-1 (6, 13, 39, 42), and there is no translation of such an increased thermogenic potential into functional heat production (39). Therefore, the so-called thrifty nature of PPARγ agonism clearly extends to a fundamental change in function of the major thermogenic organ in rodents, which becomes a significant lipid storage site.

Fig. 4. Ex vivo oleate uptake by BAT (A), iWAT (B), and eWAT (C) explants from rats treated or not with RSG for 1 or 4 days. Each bar represents the mean ± SE of 6 rats. *P < 0.05 vs. control on the same day.

Fig. 5. Weight (A) and total TG content (B) of BAT isolated from rats treated or not with RSG for 1 to 4 days. Each bar represents the mean ± SE of 6 rats. *P < 0.05 vs. control on the same day; †P < 0.05 vs. day 1 of same treatment group. C: representative micrographs of BAT adipocytes from rats treated or not with rosiglitazone for 4 days.
Table 5. mRNA expression in BAT and WAT after 1 and 4 days of treatment with RSG

<table>
<thead>
<tr>
<th>Gene</th>
<th>Day 1 Control</th>
<th>Day 1 RSG</th>
<th>Day 4 Control</th>
<th>Day 4 RSG</th>
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<tr>
<td>BAT</td>
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<td>FAS</td>
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<td>509±31*</td>
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<td>DGAT-1</td>
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<tr>
<td>ADRP</td>
<td>20±2</td>
<td>45±3*</td>
<td>50±5*</td>
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</table>

Data are expressed as arbitrary units mRNA relative to the housekeeping gene L27 and represent means ± SE of 6 rats. *Different from control on the same day, P < 0.05.

In summary, the present study showed that acute treatment with RSG leads to profound modifications in BAT metabolism that contribute to the rapid improvement in lipemia in the rat. The reduction in triglyceridemia by RSG was synchronized with an increase in adipose LPL activity and TG-derived FA uptake that were particularly marked in BAT. Also, the expression of genes involved in FA retention, previously known to be increased in WAT and thought to mediate the effect of PPARγ agonism on circulating NEFA, were similarly increased in BAT. A strong link between the reduction in serum NEFA and the increase in BAT weight was observed, without alteration of de novo lipogenesis, suggesting the involvement of BAT in the reduction in circulating NEFA levels. Without underestimating the importance of WAT, the present findings highlight the previously unrecognized contribution of BAT in the early hypolipidemic effects of PPARγ agonists in the rat and suggest one possible explanation for the species specificity of the magnitude and chronology of the hypolipidemic action of PPARγ agonism.

**Fig. 6.** Correlation between serum NEFA concentration and the weight of BAT (A), iWAT (B), and eWAT (C) of rats treated with RSG or not (CTRL) for 1 to 4 days. Each symbol represents one individual rat.

**Perspectives**

In the context of understanding the mechanisms of action of PPARγ agonism on lipid metabolism and insulin sensitivity, the present study lends strong support to the so-called “lipid steal” scenario, which suggests that lipid trapping by adipose tissue constitutes an important component of the metabolic actions of these compounds. The study extends this concept to BAT, which under PPARγ agonism turns from a relatively modest contributor to lipid clearance from the circulation to a significant site of uptake. Investigation of the mechanisms of action of PPARγ agonism on lipid metabolism in the context of whole body rodent physiology should therefore consider the contribution of BAT. Obviously, there is little or no BAT in humans; however, PPARγ agonism does tend to promote the development of a brown adipocyte phenotype in human white adipose tissue, as evidenced by the appearance of UCP1 and PGC1α expression. The present findings may therefore apply to a subpopulation of adipocytes found in humans under...
PPARγ agonist treatment. Finally, because brown adipocytes develop many characteristics of white adipocytes in response to PPARγ agonism, rodent BAT provides a relevant model for the study of adipocyte plasticity.

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