PPAR-γ Activation Mediates Adipose Depot−Specific Effects on Gene Expression and Lipoprotein Lipase Activity

Mechanisms for Modulation of Postprandial Lipemia and Differential Adipose Accretion

Mathieu Laplante,[1] Henrike Sell,[1] Karen L. MacNaul,[2] Denis Richard,[1] Joel P. Berger,[2] and Yves Deshaies[1]

This study sought to determine whether the adipose depot−specific (subcutaneous [SF] vs. visceral [VF]) action of peroxisome proliferator−activated receptor-γ (PPAR-γ) agonists on fat deposition extends to the expression of lipoprotein lipase (LPL) and other key adipose lipid metabolism genes, and whether changes in LPL impact triglyceridemia. Rats were fed a standard diet or an obesity-promoting diet for 3 weeks, with or without treatment with COOH, a nonthiazolidinedione PPAR-γ agonist. Treatment effects were essentially similar in both dietary cohorts. COOH did not affect weight gain, but increased SF (inguinal) fat mass twofold and reduced VF (retroperitoneal) accretion by half. Corresponding depot-specific alterations were observed in mRNA levels of the glucocorticoid-activating enzyme 11β-hydroxysteroid dehydrogenase 1 (11β-HSD-1) and the thermogenic modulator uncoupling protein 1 (UCP-1). COOH increased brown adipose tissue (BAT) weight and LPL availability by five- to eightfold. In rats refed standard diet after a 24-h fast, COOH reduced the insulin excursion by half. The agonist increased SF LPL activity and mRNA levels, but had no effect on VF LPL. The two- to threefold postprandial increase in plasma triglycerides (TGs) was abrogated in COOH-treated rats, likely in part because of increased LPL in SF and BAT. Thus PPAR-γ agonist treatment had a powerful, site-specific effect on adipose metabolism and lipid deposition, and greatly impacted the postprandial handling of TG-rich lipoproteins. These depot-specific effects may be mediated by differential regulation of key metabolic genes, including LPL, 11β-HSD-1, and UCP-1. *Diabetes* 52:291–299, 2003

From the [1]Department of Anatomy and Physiology, Laval Hospital Research Center, School of Medicine, Laval University, Québec, Canada; and [2]Department of Metabolic Disorders, Merck Research Laboratories, Rahway, New Jersey.

Address correspondence and reprint requests to Dr. Yves Deshaies, Department of Anatomy and Physiology, School of Medicine, Laval University, Québec, QC, Canada G1K 7P4. E-mail: yves.deshaies@phs.ulaval.ca.

BAT, brown adipose tissue; 11β-HSD-1, 11β-hydroxysteroid dehydrogenase 1; HSHF, high sucrose, high fat; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; PLSD, protected least squares difference; PPAR-γ, peroxisome proliferator−activated receptor-γ; RIA, radioimmunoassay; SF, subcutaneous fat; TG, triglyceride; TZD, thiazolidinedione; UCP-1, uncoupling protein 1; VF, visceral fat; WAT, white adipose tissue.

Elevated postprandial triglyceridemia is considered to be highly atherogenic and is frequently associated with obesity, particularly its visceral form (1–3). Endothelium-bound lipoprotein lipase (LPL; EC 3.1.1.34) hydrolyzes circulating triglycerides (TGs) (4) and is therefore a key modulator of postprandial triglyceridemia (5). Recently, it was shown that white adipose tissue (WAT) LPL activity becomes transiently resistant to insulin, the necessary and sufficient postprandial activator of adipose LPL (6), in both a genetic and a dietary model of the resistance of glucose metabolism to insulin (insulin resistance) (7,8). In the dietary model, the blunted postprandial increase in adipose LPL has been associated with hypertriglyceridemia without changes in intravascular TG input, which would therefore suggest a causal role for LPL.

Peroxisome proliferator−activated receptor-γ (PPAR-γ) is a ligand-activated nuclear receptor whose γ_2 form is highly expressed in WAT, where it regulates the expression of a number of genes involved in lipid metabolism, including LPL. The net result of PPAR-γ activation is a remodeling of WAT with a larger number of smaller adipocytes (9–12). PPAR-γ activation also efficaciously ameliorates whole-body and muscle insulin resistance (9,13,14) and hypertriglyceridemia (10,15). Synthetic thiazolidinedione (TZD) PPAR-γ ligands are used clinically to treat insulin resistance and type 2 diabetes (14,16). At least part of the insulin-sensitizing action of PPAR-γ agonists is thought to derive from an enhanced lipid retention by WAT that reduces exposure of insulin-sensitive tissues to fatty acids.

Because PPAR-γ agonists positively modulate WAT LPL expression, the first objective of the present study was to assess whether increased transcription of the LPL gene by a PPAR-γ agonist would influence LPL activity and thereby impact postprandial triglyceridemia. PPAR-γ agonists have been reported to exert a depot-specific action on lipid accumulation in subcutaneous fat (SF) and visceral fat (VF) in humans (17–20). Because LPL acts as a gateway for the entry of TG-derived fatty acids into WAT, the second objective of the study was to verify whether there existed

TABLE 1
Primer/probe sets used for amplification

Gene	5′ Primer (5′-3′)	3′ Primer (5′-3′)	Probe (5′-3′)
LPL	CTAAACTATGTACTTCAGGCTTACCTTGAA	ATGGTTATCAAGCTCCCAGCAC	TCTCAACCATCCTGCCTTGGCTTCCT
11β-HSD-1	GAAACAGAGCAATGGCAGCAT	CAAACTTGCTTGCAGAGTAGGAAG	CTCCATGGCTGGGAAAATGACCCAA
UCP-1	CATCACCTTCCCGCTGGA	CTAATAGTACTGGAAGCCTGGCCT	CAAAGTCCGCCTTCAGATCCAAGGTG

some depot specificity in the PPAR-γ−mediated modulation of LPL expression, as well as that of other PPAR-γ−responsive enzymes and modulators of adipose lipid metabolism (21,22). These included 11β-hydroxysteroid dehydrogenase 1 (11β-HSD-1), which converts inactive glucocorticoids into potent glucocorticoid receptor agonists in WAT, and uncoupling protein 1 (UCP-1), which dissipates energy from fatty acid oxidation as heat in brown adipose tissue (BAT).

The above objectives were pursued by chronically treating rats with the non-TZD PPAR-γ agonist COOH, which bears a carboxylic acid pharmacophore in place of the TZD moiety found in glitazones. As shown earlier (23), this non-TZD compound is a potent and highly selective PPAR-γ full agonist with many features (except structure) similar to rosiglitazone. The response of WAT LPL to refeeding in 24-h–fasted, insulin-sensitive, and diet-induced obese, insulin-resistant rats (7,24–27) was quantified. The length of fasting was selected for its ability to reduce both insulinemia and adipose LPL of obese rats to that of lean animals (7), thereby allowing assessment of LPL responsiveness to food intake against similar fasting backgrounds. Pelleted standard diet was given to both dietary cohorts during refeeding to allow comparison of the LPL response to an identical nutritional stimulus.

RESEARCH DESIGN AND METHODS

Animals and treatments. Male SD rats ($n = 96$; 90–100 g) were purchased from Charles River Laboratories (St. Constant, Canada) and housed individually in stainless steel cages in a room kept at $23 \pm 1°C$ with a 12:12 h light-dark cycle (lights on at 20:00 h). 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. For the first 2 days, rats had free access to tap water and a stock diet (Charles River Rodent Diet #5075; Ralston Products, Woodstock, Canada; digestible energy content: 12.9 kJ/g). Then rats were divided into two groups: 48 rats were given the ground stock diet (standard diet) and the remaining 48 animals were fed a purified high-sucrose, high-fat (HSHF) diet, the composition of which is detailed elsewhere (7). The HSHF diet rapidly induces insulin resistance in peripheral tissues, including adipose tissue and muscles (24,28). Half of the animals in each dietary cohort were given the non-TZD PPAR-γ agonist COOH [2-(2-(4-phenoxy-2-propylphenoxy)ethyl)indole-5-acetic acid] as an adjunct to their diet at a dosage of $30 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for the entire feeding period of 3 weeks. The amount of COOH was adjusted twice weekly to the average consumption of each diet. At the end of the treatment, the animals were killed 1 h after the beginning of the lighted period, after either a 24-h fast or a 24-h fast followed by 1, 3, or 6 h of refeeding, but within the same 3-h period of the day to allow comparison of groups at the same point in the circadian glucocorticoid rhythm. All rats were refed pelleted standard diet, without the agonist, to allow comparison of treatment-related changes independent of meal size and composition (7,8).

Plasma and tissue sampling. Rats were killed by decapitation. Their blood was centrifuged (1,500g, 15 min at 4°C) and the plasma was stored at −70°C until later biochemical measurements. Inguinal (SF) and retroperitoneal (VF) WAT and interscapular BAT were excised and weighed. Tissue samples were homogenized and processed exactly as described earlier (6), and were stored at −70°C until measurement of LPL activity.

Plasma determinations. Plasma glucose concentrations were measured by the glucose oxidase method with the Beckman glucose analyzer. Insulin and corticosterone levels were determined by radioimmunoassay (RIA; Linco Research, St. Charles, MO) with rat insulin and corticosterone as standards,

respectively. Plasma TGs were measured by an enzymatic method (Roche Diagnostics, Montreal, Canada), as were plasma nonesterified fatty acids (NEFAs; Wako Pure Chemical Industries, Richmond, VA).

Tissue lipoprotein lipase activity. Enzyme activity in adipose tissues was determined exactly as described (6). Briefly, tissue homogenates were incubated with a substrate mixture containing [carboxyl-14C]triolein, and NEFAs released by LPL were separated and counted. LPL activity was expressed as microunits ($1 \text{ μU} = 1 \text{ μmol}$ NEFA released per hour of incubation at 28°C). The interassay coefficient of variation was 4.1%, and was determined using bovine skim milk as a standard source of LPL. To account for diet- and agonist-induced differences in tissue TG content, data are expressed as LPL activity per gram of total tissue protein (29). LPL activity per total adipose depot is depicted for some time points to illustrate its global tissue availability.

RNA isolation and analysis. Total RNA was prepared from WAT of rats chronically fed the standard diet, both in the fasted state and after refeeding for 6 h, using the Ultraspec Total RNA Isolation Reagent (Biotecx, Houston, TX) and the RNAeasy 96 total RNA isolation kit (Qiagen, Valencia, CA) according to the manufacturers' protocols. RNA concentration was estimated from absorbance at 260 nm. The expression level of specific mRNAs was quantitated using quantitative fluorescent real-time PCR. RNA was first reverse transcribed using random hexamers according to the manufacturer's protocol (PE Applied Biosystems, Foster City, CA). Amplification of each target cDNA was then performed with TaqMan PCR Reagent Kits in the ABI Prism 7700 Sequence Detection System according to the manufacturer's protocols (PE Applied Biosystems). Primer/probe sets were selected using the Primer Express program (PE Applied Biosystems) and were synthesized by the same company. The levels of mRNA were normalized to the amount of 18 S ribosomal RNA (primers and probes from PE Applied Biosystems) detected in each sample. Results are expressed as target mRNA/18 S mRNA. The primer/probe sets in Table 1 were used for the amplification step.

Statistical analysis. Data are presented as means ± SE. The main and interactive effects of the chronic diet and agonist on variables related to food intake and body and organ weight were analyzed by a 2 × 2 (standard diet/HSHF diet and control/COOH) factorial ANOVA. The main and interactive effects of the chronic diet, agonist, and nutritional status on variables measured at various times of refeeding were analyzed separately for the effect of diet and the agonist. The effect of diet was analyzed by a 2 × 4 (standard diet/HSHF diet and 0/1/3/6 h of refeeding) factorial ANOVA in the control and COOH-treated cohorts separately. Likewise, the effect of agonist treatment was analyzed by a 2 × 4 (control/COOH and 0/1/3/6 h of refeeding) factorial ANOVA in the standard diet−fed and HSHF diet–fed cohorts separately. When justified by a significant treatment interaction, differences between individual group means were analyzed by Fisher's protected least squares difference (PLSD) test. The effects of agonist treatment (control, COOH) and dietary status (fasted, 6-h refed) on mRNA levels in adipose depots (inguinal, retroperitoneal) were compared according to a 2 × 2 × 2 factorial ANOVA. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Cumulative energy intake was larger (17%) in the HSHF diet–fed than in the standard diet−fed cohort, and treatment with the PPAR-γ agonist slightly increased food intake (7%) independent of diet (Table 2). Final body weight and weight gain were proportionally increased (gain of 15%) by the HSHF diet, but not by the agonist, which only tended to increase body weight gain (5%; $P = 0.06$). Food efficiency (grams of weight gain per megajoule energy ingested) was not affected by diet or COOH. Upon refeeding, the HSHF diet–fed rats unexpectedly ingested less diet (–3.5 g) than did those chronically fed the standard diet, whereas PPAR-γ agonist−treated rats ingested more food acutely (+2.5 g). Over 6 h of refeeding

TABLE 2
Food intake and body weight variables in rats chronically fed standard or HSHF diet and treated or not with the PPAR-γ agonist COOH for 3 weeks

| | Standard diet | | HSHF diet | | ANOVA | | |
	Placebo	COOH	Placebo	COOH	Diet	Agonist	Diet $\times$ agonist
Food intake (MJ)	6.2 ± 0.1	6.8 ± 0.1	7.4 ± 0.2	7.7 ± 0.1	<0.0001	0.0002	NS
Body weight (g)	294 ± 5	302 ± 4	316 ± 5	323 ± 5	<0.0001	NS	NS
Body weight gain (g)	136 ± 5	144 ± 4	157 ± 4	165 ± 4	<0.0001	NS	NS
Food efficiency (g/MJ)	22.1 ± 0.6	21.2 ± 0.5	21.2 ± 0.4	21.4 ± 0.4	NS	NS	NS
6-h refeeding intake (g)	15.4 ± 1.1	17.9 ± 0.9	12.1 ± 0.8	14.4 ± 1.4	0.005	0.04	NS

Data are means ± SE of 24 animals. The ANOVA columns represent the level of significance of the effects of diet with two levels (standard and HSHF diets) and agonist (placebo and COOH) and their interaction. Food efficiency represents grams of body weight gain per megajoule of ingested food. NS, not significant.

standard diet, rats ingested ~67% of their daily ad libitum food intake.

As depicted in Table 3, the inguinal depot weight was increased 61% in the HSHF diet cohort compared to in the standard diet−fed cohort, whereas COOH treatment increased inguinal weight by 46% independent of diet. Total protein content of the depot was increased 21% by diet, and was doubled by PPAR-γ agonist treatment in both dietary cohorts. Therefore, the metabolic (protein) mass of the tissue was increased by both the HSHF diet and the agonist, but more so by the latter, with these effects being additive. In sharp contrast, diet and the agonist had opposite effects on retroperitoneal adipose depot weight. Retroperitoneal weight was roughly doubled by ingestion of the HSHF diet compared to standard diet, whereas COOH treatment reduced retroperitoneal pad accretion by 28% in standard diet−fed rats, and even more so (65%) in HSHF diet rats (diet $\times$ agonist interaction). Total protein content of VF was increased ~60% by the HSHF diet in both control and treated rats, and protein content was increased by the PPAR-γ agonist by the same amount in both dietary cohorts. Diet and agonist effects were fully additive. Inasmuch as the inguinal and retroperitoneal fat depots are representative of SF and VF, respectively, in general, the sum of the weight of the two depots and their weight ratio were computed to obtain a crude representation of total body fat and distribution. As is shown in Table 3, chronic consumption of the HSHF diet roughly doubled the sum of the two depots, whereas the PPAR-γ agonist did not affect this sum. The inguinal/retroperitoneal weight

ratio was the same in both dietary cohorts, but was doubled in COOH-treated animals compared to their untreated counterparts regardless of diet. Therefore, the HSHF diet increased fat deposition equally in both SF and VF, whereas the PPAR-γ agonist brought about a redistribution of fat deposition toward SF at the expense of VF. Diet and agonist treatment interacted with BAT and protein content. The HSHF diet did not increase BAT weight and protein in untreated rats, but did in COOH-treated animals (weight, +41%; protein, +18%). Similarly, COOH increased BAT weight in both dietary cohorts, but slightly more so in HSHF diet–fed (weight: 7.7-fold; protein: 5.1-fold) than in standard diet−fed animals (weight, 6.4-fold; protein, 4.6-fold).

As shown in Fig. 1, diet exerted a significant effect ($P < 0.002$) on glycemia, with glucose being higher in HSHF diet–fed than in standard diet−fed animals, but there was no diet-related difference in glucose levels in COOH-treated rats (Fig. 1A). Overall, the PPAR-γ agonist slightly raised plasma glucose levels ($P < 0.02$) in standard diet−fed rats, but no between-group difference was significant at any individual time point (including fasting). The agonist did not affect plasma glucose in HSHF diet–fed rats. The nature of the chronic diet did not influence fasting or postprandial plasma insulin levels in either control or COOH-treated rats (Fig. 1B). The PPAR-γ agonist did not affect fasting insulinemia in either dietary cohort, but greatly reduced the postprandial insulin rise in both groups ($0.03 > P < 0.0001$).

The postprandial increase in TG upon refeeding with the

TABLE 3
Weight and protein content of inguinal and retroperitoneal WAT and interscapular BAT of rats chronically fed standard or HSHF diet and treated or not with the PPAR-γ agonist COOH for 3 weeks

| | Standard diet | | HSHF diet | | ANOVA | | |
	Control	COOH	Control	COOH	Diet	Agonist	Diet $\times$ agonist
Ing weight	1.16 ± 0.05	1.80 ± 0.07	1.98 ± 0.10	2.80 ± 0.11	<0.0001	<0.0001	NS
Ing protein (mg)	32 ± 2	66 ± 4	40 ± 4	81 ± 6	0.007	<0.0001	NS
Ret weight (g)	1.06 ± 0.07[a]	0.76 ± 0.05[b]	2.36 ± 0.10[c]	1.43 ± 0.09[d]	<0.0001	<0.0001	0.0001
Ret protein (mg)	14 ± 1	24 ± 1	24 ± 1	37 ± 2	<0.0001	<0.0001	NS
Sum Ing + Ret weight (g)	2.2 ± 0.1	2.6 ± 0.1	4.3 ± 0.2	4.2 ± 0.2	<0.0001	NS	NS
Ing/Ret weight ratio	1.1 ± 0.1	2.5 ± 0.1	0.9 ± 0.0	2.1 ± 0.1	0.0009	<0.0001	NS
BAT weight (g)	0.29 ± 0.02[a]	1.86 ± 0.12[b]	0.36 ± 0.02[a]	2.76 ± 0.15[c]	<0.0001	<0.0001	<0.0001
BAT protein (mg)	33 ± 2[a]	153 ± 8†	35 ± 2[a]	180 ± 9[c]	0.02	<0.0001	0.05

Data are means ± SE of 24 animals. The ANOVA columns represent the level of significance of the effects of diet with two levels (standard and HSHF diets) and agonist (placebo and COOH) and their interaction. When a treatment interaction was detected by ANOVA, post hoc individual between-group comparisons (Fisher's PLSD) were performed. For the latter variables, means not sharing a common superscript are significantly different from each other, $P < 0.05$. Ing, inguinal; Ret, retroperitoneal.
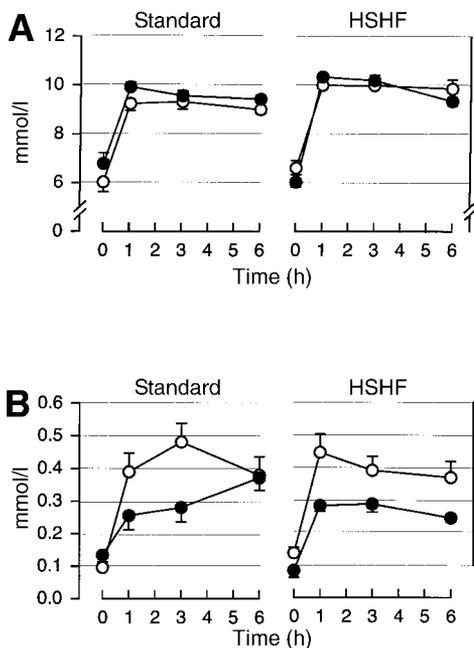
FIG. 1. Plasma glucose (*A*) and insulin (*B*) concentrations after a 24-h fast (0-h time point) and after 1, 3, or 6 h of refeeding with standard diet ad libitum, in rats chronically fed standard or HSHF diet and treated (●) or not (○) with the PPAR-γ agonist COOH for 3 weeks. Each point represents the average ± SE of six animals. The 2 × 2 ANOVA results that follow represent the level of significance (*P* value) of the main effects of diet (D) and time (T) and their interaction (D × T) in untreated and COOH-treated rats, and that of the main effects of agonist (A) and time and their interaction (A × T) in standard diet–fed and HSHF diet–fed rats. Glucose: in untreated (controls), D = 0.002, T < 0.0001, D × T = NS; in COOH-treated, D = NS, T < 0.0001, D × T = NS; in standard diet–fed, A = 0.02, T < 0.0001, A × T = NS; in HSHF diet–fed, A = NS, T < 0.0001, A × T = NS. Insulin: in untreated, D = NS, T < 0.0001, D × T = NS; in COOH-treated, D = NS, T < 0.0001, D × T = NS; in standard diet–fed, A = 0.03, T < 0.0001, A × T = 0.05; in HSHF diet–fed, A < 0.0001, T < 0.0001, A × T = NS.

FIG. 2. Plasma TG (*A*) and NEFA (*B*) concentrations after a 24-h fast (0-h time point) and after 1, 3, or 6 h of refeeding with standard diet ad libitum in rats chronically fed standard or HSHF diet and treated (●) or not (○) with the PPAR-γ agonist COOH for 3 weeks. Each point represents the average ± SE of six animals. The 2 × 2 ANOVA results that follow represent the level of significance (*P* value) of the main effects of diet (D) and time (T) and their interaction (D × T) in untreated and COOH-treated rats, and that of the main effects of agonist (A) and time and their interaction (A × T) in standard diet–fed and HSHF diet–fed rats. TGs: in untreated, D = 0.008, T < 0.0001, D × T = NS; in COOH-treated, D = 0.002, T = 0.002, D × T = NS; in standard diet–fed, A < 0.0001, T = 0.0003, A × T < 0.0001; in HSHF diet–fed, A = 0.002, T < 0.0001, A × T = NS. NEFAs: in untreated, D = NS, T < 0.0001, D × T = 0.008; in COOH-treated, D < 0.0001, T < 0.0001, D × T = 0.02; in standard diet–fed, A = NS, T < 0.0001, A × T = NS; in HSHF diet–fed, A = 0.04, T < 0.0001, A × T = 0.006.

standard diet was of a lesser magnitude in rats fed the HSHF diet long term than in those on the standard diet (Fig. 2*A*). COOH abrogated the postprandial TG increase in both dietary cohorts, and even reduced postprandial TG levels below fasting values in the cohort on the standard diet (agonist × time interaction). As expected, plasma NEFA levels were largely reduced upon refeeding, but less so in HSHF diet–fed than in standard diet–fed rats with or without COOH (diet × time interaction, 0.02 > *P* < 0.008) (Fig. 2*B*). The postprandial fall in plasma NEFA levels was not affected by COOH in standard diet–fed rats, but was potentiated by the agonist in HSHF diet–fed rats (agonist × time interaction in HSHF diet–fed rats, *P* < 0.006).

The typical adipocyte remodeling elicited by PPAR-γ agonist treatment was confirmed in the present study, as both inguinal and retroperitoneal depots displayed an increased number of small adipocytes and fewer large adipocytes (data not shown). In the standard diet–fed cohort, PPAR-γ agonist treatment increased inguinal LPL activity at all time points, including in the fasted state, whereas in the HSHF diet–fed rats, COOH prolonged LPL activation until the 6-h time point without affecting enzyme activity earlier during the refeeding period (Fig. 3*A*). Diet interacted with time on retroperitoneal LPL activity, as evidenced by the fact that LPL was increased twofold by standard diet refeeding in rats fed that diet chronically, but was only minimally affected in HSHF diet–fed animals,

thereby indicating insulin resistance (Fig. 3*B*). COOH had no effect on retroperitoneal LPL regardless of the chronic diet. Such depot specificity was independent of the time that elapsed after COOH was last ingested, as it was identical to that observed in fasted and postprandial rats treated short-term with COOH (not shown). To illustrate the diet and agonist effects on global LPL availability, LPL activity per total depot in the fasted state is depicted in Fig. 3*C*. As expected after the 24-h fast, total inguinal LPL activity was identical in both dietary cohorts. PPAR-γ agonist treatment increased total inguinal LPL nearly fourfold independently of diet. In the retroperitoneal depot, total LPL activity remained unaffected by diet (*P* = 0.06) and COOH treatment.

As is shown in Fig. 4*A*, refeeding did not alter BAT LPL regardless of the chronic diet, but COOH slightly increased postprandial LPL in standard diet–fed rats (*P* < 0.02). However, fasting enzyme activity per total depot was enhanced five- to eightfold by the PPAR-γ agonist in both dietary cohorts (Fig. 4*B*). In the present study, diet and agonist effects on skeletal muscle (soleus and vastus lateralis) LPL activity were minor and not significant (data not shown).

Refeeding decreased corticosterone levels in all groups (Fig. 5). Fasting corticosterone levels in standard diet–fed rats were not significantly affected by COOH, whereas the fasting hypercorticosteronemia present in rats chronically

FIG. 3. Relative (per unit total protein) LPL activity in inguinal (*A*) and retroperitoneal (*B*) depots after a 24-h fast (0-h time point) and after 1, 3, or 6 h of refeeding with standard diet ad libitum in rats chronically fed standard (STD) or HSHF diet and treated (●) or not (○) with the PPAR-γ agonist COOH for 3 weeks. The 2 × 2 ANOVA results that follow represent level of significance (*P* value) of the main effects of diet (D) and time (T) and their interaction (D × T) in untreated and COOH-treated rats, and that of the main effects of agonist (A) and time and their interaction (A × T) in standard diet–fed and HSHF diet–fed rats. Inguinal relative LPL: in untreated, D = NS, T = 0.002, D × T = NS; in COOH-treated, D = 0.05, T < 0.0001, D × T = NS; in standard diet–fed, A = 0.0002, T = 0.0005, A × T = NS; in HSHF diet–fed, A = 0.03, T = 0.0008, A × T = 0.03. Retroperitoneal relative LPL: in untreated, D = 0.002, T < 0.0001, D × T = 0.007; in COOH-treated, D < 0.0001, T < 0.0001, D × T = 0.02; in standard diet–fed, A = NS, T < 0.0001, A × T = NS; in HSHF diet–fed, A = NS, T < 0.04, A × T = NS. *C*: Total LPL activity per depot in the fasted state. *Different from control in corresponding dietary cohort, *P* < 0.004. Each point or column represents the average ± SE of six animals. ▨, control; ■, COOH.

FIG. 4. Relative (per unit total protein) LPL activity in interscapular BAT (*A*) after a 24-h fast (0-h time point) and after 1, 3, or 6 h of refeeding with standard diet ad libitum in rats chronically fed standard or HSHF diet and treated (●) or not (○) with the PPAR-γ agonist COOH for 3 weeks. The 2 × 2 ANOVA results that follow represent level of significance (*P* value) of the main effects of diet (D) and time (T) and their interaction (D × T) in untreated and COOH-treated rats, and that of the main effects of agonist (A) and time and their interaction (A × T) in standard diet–fed and HSHF diet–fed rats. Relative LPL: in untreated, D = NS, T = NS, D × T = NS; in COOH-treated, D = NS, T = NS, D × T = NS; in standard diet–fed, A = 0.02, T = NS, A × T = NS; in HSHF diet–fed, A = NS, T = NS, A × T = NS. *B*: Total LPL activity per depot in the fasted state. *Different from control in corresponding dietary cohort, *P* < 0.0001. Each point or column represents the average ± SE of six animals. ▨, control; ■, COOH.

fed the HSHF diet was abrogated by chronic PPAR-γ agonist treatment. Overall, the PPAR-γ agonist potentiated the refeeding-induced reduction in corticosterone (agonist × time interaction, 0.02 > *P* < 0.003).

Chronic COOH treatment increased LPL mRNA in inguinal SF, but not in retroperitoneal VF (agonist × depot interaction, *P* < 0.004) regardless of the nutritional status, which, as expected, also had no effect (Fig. 6*A*). In sharp contrast, the PPAR-γ agonist did not affect 11β-HSD-1 mRNA expression in SF, but greatly decreased it in VF (agonist × depot interaction, *P* < 0.02), again independently of nutritional status (Fig. 6*B*). UCP-1 mRNA was affected by adipose depot location, nutritional status, and COOH treatment, and all factors interacted with each other (depot × nutrition × agonist interaction, *P* < 0.01) (Fig. 6*C*). Refeeding increased UCP-1 mRNA more robustly in rats previously treated with the PPAR-γ agonist

than in untreated rats, whereas in both the fasted and fed states, COOH treatment increased UCP-1 mRNA to a much higher level in VF than in SF.

DISCUSSION

This study demonstrated that activation of PPAR-γ abrogates the postprandial rise in TG levels seen in rats chronically fed diets that maintain either a low or high lipid flux. In association with this change in TG levels, COOH increased LPL expression and activity in an adipose depot–specific manner. Also, PPAR-γ activation increased subcutaneous fat deposition at the expense of visceral fat. Such remodeling was accompanied by a congruent depot specificity of changes in the mRNA levels of proteins associated with increased (LPL and 11β-HSD-1) or reduced (UCP-1) lipid accumulation.

We have previously shown that rats fed the HSHF diet are frankly hypertriglyceridemic and hyperinsulinemic compared to standard diet–fed rats after a short-term (overnight) fast as well as after being refed their habitual diet (24). In the present study, based on our previous work on the insulin resistance of adipose LPL (7,8), rats were fasted for 24 h and refed standard diet. This, along with a slightly lower food intake upon refeeding, explains the weaker TG response in HSHF diet–fed compared to standard diet–fed rats (liver lipogenesis from carbohydrate precursors is lowered by chronic high-fat feeding) (30) and their similar postprandial rise in insulin (glucose-mediated insulin secretion is hampered in high-fat–fed rats) (31). These observations should not be interpreted as an absence of glucose metabolism insulin resistance in HSHF diet rats, which has been amply demonstrated (7,24–27),
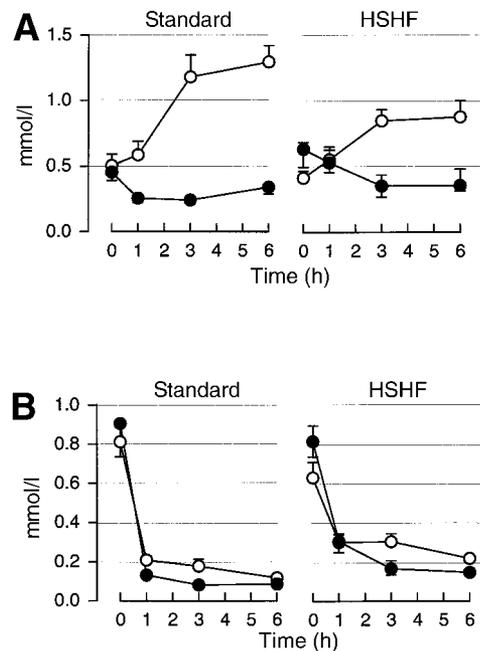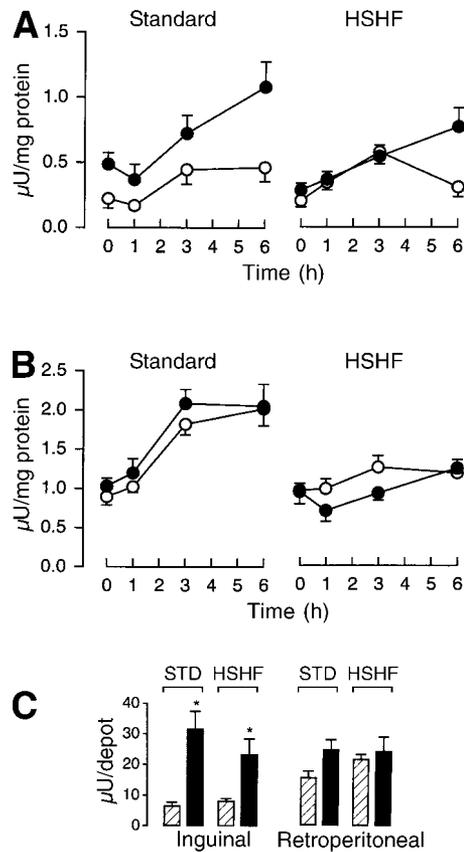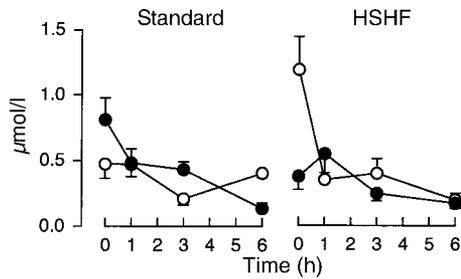
FIG. 5. Plasma corticosterone concentrations after a 24-h fast (0 h time point) and after 1, 3, or 6 h of refeeding with standard diet ad libitum in rats chronically fed standard or HSHF diet and treated (●) or not (○) with the PPAR-γ agonist COOH for 3 weeks. Each point represents the average ± SE of six animals. The 2 × 2 ANOVA results that follow represent level of significance (P value) of the main effects of diet (D) and time (T) and their interaction (D × T) in untreated and COOH-treated rats, and that of the main effects of agonist (A) and time and their interaction (A × T) in standard diet–fed and HSHF diet–fed rats. In untreated, D = NS, T < 0.0001, D × T = 0.001; in COOH-treated, D = NS, T = 0.002, D × T = NS; in standard diet–fed, A = NS, T = 0.001, A × T = 0.02; in HSHF diet–fed, A = 0.04, T = 0.0003, A × T = 0.003.

FIG. 6. mRNA levels of LPL (A), 11β-HSD-1 (B), and UCP-1 (C) in inguinal (*left*) and retroperitoneal (*right*) adipose tissue after a 24-h fast and after 6 h of refeeding with standard diet ad libitum in rats chronically fed standard diet and treated or not with the PPAR-γ agonist COOH for 3 weeks. Each column represents the average ± SE of six animals. Note different scales in *C* for retroperitoneal and inguinal depots. The 2 × 2 × 2 ANOVA results that follow represent level of significance (P value) of the main effects of depot (De), nutritional state (Nu), and agonist (A) and their interactions. Only P < 0.05 values are given; other effects and interactions were NS. LPL: De < 0.0001, A < 0.0001, De × A = 0.004, De × Nu × A = 0.03. 11β-HSD-1: A < 0.0001, De × A = 0.02. UCP-1: De = 0.003, Nu = 0.006, A = 0.005, De × Nu = 0.006, De × A = 0.005, Nu × A = 0.009, De × Nu × A = 0.01. ▨, control; ■, COOH.

but rather as a naive metabolic response to a first encounter with the high-carbohydrate diet. Insulin resistance in HSHF diet–fed rats was illustrated by their higher postprandial glucose excursion and weaker NEFA reduction in the face of identical insulinemia compared to standard diet–fed rats and by the lack of response of their visceral WAT LPL to refeeding (7). Likewise, the lack of effect of COOH on fasting levels of glucose, insulin, and lipids is undoubtedly attributable to the long duration of the fasting period. Remarkably, PPAR-γ activation greatly reduced the insulin response to refeeding in both standard diet–fed and HSHF diet–fed animals. This confirms the well-established insulin-sensitizing action of PPAR-γ agonists (9,13,14), which extends to the insulin-mediated inhibition of lipolysis, as demonstrated by decreased plasma NEFA concentrations.

COOH administration resulted in adipocyte remodeling (smaller, more numerous cells) typical of PPAR-γ agonists in general (9–11) in both inguinal and retroperitoneal depots. As has been determined in pilot studies, SF yields approximately fourfold more total RNA than VF, most likely because it is richer in nonadipocyte cells. Furthermore, 2 weeks of exposure to PPAR-γ agonists such as rosiglitazone doubles the amount of total RNA per unit tissue weight, which reflects the increase in cell number. In the present study, COOH exerted strong, depot-specific actions on LPL activity, which extended to LPL gene expression, as suggested by mRNA levels. In standard diet–fed rats, COOH upregulated LPL activity relative to total protein in inguinal WAT specifically, resulting in higher fasting LPL. Interestingly, the postprandial activation of LPL was relatively larger in COOH-treated rats than in controls, as enzyme activation appeared to be prolonged beyond 3 h, a time at which LPL had stabilized in untreated rats. This effect of COOH is all the more remarkable because it occurred in the face of a much reduced postprandial insulin increase. This raises the intriguing possibility that the PPAR-γ agonist could affect the posttranslational activation of LPL. Alternatively, the upregulation of LPL expression by PPAR-γ agonists seen here and previously (32–34) could simply make more enzyme available for activation. In HSHF rats, although

total depot LPL activity was greatly elevated by COOH, there was no increase in fasting LPL relative to total protein, as was observed in standard diet–fed rats. However, as in the latter, the agonist prolonged the late postprandial rise in enzyme activity.

In sharp contrast to its action on subcutaneous WAT LPL, COOH had no effect on retroperitoneal LPL regardless of diet. Whether the lack of effect of PPAR-γ activation on LPL expression in VF is the result of PPAR-γ's inability to directly affect LPL gene transcription through its previously identified peroxisome proliferator response element (33) or is secondary to PPAR-γ–induced changes in other signaling pathways that modulate LPL expression is unknown. As was shown in our previous study (7), retroperitoneal LPL in HSHF diet–fed animals was unresponsive to postprandial activation. The PPAR-γ agonist was unable to overcome the resistance of visceral LPL to feeding-induced activation, thus indicating that any PPAR-γ agonist–mediated insulin sensitization of adipocytes (35) did not extend to insulin-mediated postprandial activation of LPL in VF.

As was the case in visceral WAT, COOH did not affect LPL activity relative to total protein in BAT. However, in contrast to visceral fat, the large increase in BAT weight elicited by PPAR-γ agonist treatment was accompanied by

a parallel sixfold elevation in total tissue availability of the enzyme. It can be suggested that the latter contributed substantially to the seven- to eightfold increase in BAT weight through lipid uptake. COOH and other PPAR-γ agonists (36,37) therefore appear to direct BAT metabolism toward lipid storage.

The present findings extend those of previous studies that have reported a robust hypotriglyceridemic effect of PPAR-γ agonists, as assessed through single time point measurements (10,15), by demonstrating that chronic PPAR-γ activation abolished the postprandial increase in TG at least until the 6th h of refeeding. This action was all the more powerful because refeeding elicited almost a threefold increase in plasma TG levels. In fact, in rats chronically fed standard diet, COOH decreased triglyceridemia postprandially to half of that seen after a 24-h fast. In the absence of an effect of PPAR-γ activation on muscle LPL, it is reasonable to conclude that the COOH-induced increase in the availability of LPL in subcutaneous WAT and BAT contributed significantly to such a large reduction in circulating TGs. In addition to increasing LPL expression and activity in these tissues, PPAR-γ agonists may favor TG clearance through vasodilation (38), which could conceivably augment the delivery rate of TG-rich lipoproteins to LPL-rich tissues. By virtue of its potentiating effect on the postprandial lowering of NEFAs, COOH may also have lowered hepatic VLDL TG secretion. The respective contribution of these major determinants of triglyceridemia remains to be established.

Treatment with COOH was depot specific, not only at the level of LPL activity, but also at the level of lipid deposition. Indeed, the agonist nearly doubled inguinal depot mass (most of that being stored TGs), whereas it decreased retroperitoneal depot accretion by half despite overt adipogenesis. This effect is similar to that reported for other PPAR-γ agonists in humans (17–20). Although the mechanisms by which lipid deposition is blunted in VF by COOH remain to be understood, several possible explanations can be proposed. The lack of effect of the PPAR-γ agonist on LPL expression and activation in VF, as opposed to the large increase in SF, may in part limit lipid uptake by visceral relative to subcutaneous adipocytes, but at the same time argues against a contribution of decreased uptake of TG-derived fatty acids to the frank reduction in VF accretion. Enhanced lipolysis and fatty acid export from VF is also improbable because plasma NEFAs were not increased, and were even further decreased postprandially, by COOH. These considerations raise the as yet unproven possibility that COOH may have enhanced fatty acid oxidation specifically in VF. This hypothesis is supported by the observation that COOH upregulated UCP-1 expression by a much greater magnitude in the visceral than in the subcutaneous depot. As a result, locally increased thermogenesis may have favored lipid loss in the retroperitoneal versus the inguinal depot, without overtly affecting whole-body energy balance. It must be noted, however, that although PPAR-α agonists stimulate fatty acid oxidation in adipocytes (39,40), the ability of PPAR-γ agonists to do so is unestablished. Furthermore, the metabolic efficiency of UCP-1 in PPAR-γ agonist−treated rodents remains speculative because, as was seen here and in previous studies (36), increased

expression of UCP-1 in BAT by PPAR-γ agonists occurs in the face of a large expansion of the tissue, thereby suggesting that at least in BAT, newly synthesized UCP-1 is not actively uncoupling mitochondrial respiration.

A second PPAR-γ−modulated pathway that has been shown to be responsible for adipose tissue homeostasis involves the enzyme 11β-HSD-1 (23), which locally converts inactive corticosteroids into potent glucocorticoid receptor agonists (41,42). Here we found that PPAR-γ agonist treatment caused 11β-HSD-1 expression to be greatly diminished in VF, but not in SF. As a result, the local production of bioactive glucocorticoids in VF, to which it is particularly sensitive because of high glucocorticoid receptor density (43,44), may have been decreased, thereby reducing the local lipogenic drive and supporting the noted diminution in fat accretion in this depot. In this regard, it is worth noting that patients with hypercorticosteroidism display increased visceral obesity (45,46) and metabolic maladies that show a striking similarity to type 2 diabetes (47). Furthermore, 11β-HSD-1−null mice are resistant to diet-induced insulin resistance (48), whereas transgenic mice overexpressing 11β-HSD-1 exhibit increased corticosterone levels, visceral obesity, and insulin-resistant diabetes (49). Therefore, it is not unreasonable to suggest that a decrease in adipocyte and systemic glucocorticoid levels may contribute to the global adipose depot−specific effects of PPAR-γ activation and to global insulin sensitization as well.

In conclusion, this study demonstrated that the PPAR-γ agonist COOH exerted depot-specific effects on adipose LPL expression and activity that were accompanied by equally depot-specific differences in fat deposition. COOH increased LPL activity in BAT and subcutaneous WAT, but not in visceral WAT. It is likely that such changes in LPL contributed, at least in part, to the abrogation of postprandial hypertriglyceridemia through increased TG clearance. The depot specificity of COOH action extended to fat deposition, with SF being increased at the expense of VF. Our data demonstrated that such depot-specific actions may be mediated by the differential expression of genes involved in the modulation of various aspects of lipid metabolism, including LPL, UCP-1, and 11β-HSD-1. A more profound understanding of the role of PPAR-γ agonists in preventing VF accretion in humans and rodent models now awaits the assessment of the effects of such compounds on metabolic parameters, including fatty acid oxidation, thermogenesis, glucocorticoid metabolism, and lipogenesis, in individual fat tissues.

REFERENCES

1. Zilversmit DB: Atherogenic nature of triglycerides, postprandial lipidemia, and triglyceride-rich remnant lipoproteins. *Clin Chem* 41:153–158, 1995
2. Després J-P, Lemieux I, Prud'homme D: Treatment of obesity: need to focus on high risk abdominally obese patients. *BMJ* 322:716–720, 2001
3. Roche HM, Gibney MJ: The impact of postprandial lipemia in accelerating atherothrombosis. *J Cardiovasc Risk* 7:317–324, 2000
4. Goldberg IJ, Merkel M: Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front Biosci* 6:D388–D405, 2001
5. Cohen JC, Berger GM: Effects of glucose ingestion on postprandial lipemia and triglyceride clearance in humans. *J Lipid Res* 31:597–602, 1990
6. Picard F, Naïmi N, Richard D, Deshaies Y: Response of adipose tissue lipoprotein lipase to the cephalic phase of insulin secretion. *Diabetes* 48:452–459, 1999
7. Picard F, Boivin A, Lalonde J, Deshaies Y: Resistance of adipose tissue lipoprotein lipase to insulin action in rats fed an obesity-promoting diet. *Am J Physiol Endocrinol Metab* 282:E412–E418, 2002
8. Picard F, Richard D, Timofeeva E, Deshaies Y: Abnormal insulin and β-adrenergic modulation of lipoprotein lipase during refeeding after prolonged fasting in the Zucker rat. *Diabetologia* 43:866–874, 2000
9. Spiegelman BM: PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes* 47:507–514, 1998
10. Auwerx J, Schoonjans K, Fruchart JC, Staels B: Regulation of triglyceride metabolism by PPARs: fibrates and thiazolidinediones have distinct effects. *J Atheroscler Thromb* 3:81–89, 1996
11. de Souza CJ, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, Burkey BF: Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes* 50:1863–1871, 2001
12. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T: The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPAR-gamma) deficiency and PPAR-gamma agonist improve insulin resistance. *J Biol Chem* 276:41245–41254, 2001
13. Saltiel AR, Olefski JM: Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* 45:1661–1669, 1996
14. Picard F, Auwerx J: PPAR-γ and glucose homeostasis. *Annu Rev Nutr* 22:167–197, 2002
15. Lefebvre AM, Peinado Onsurbe J, Leitersdorf I, Briggs MR, Paterniti JR, Fruchart JC, Fievet C, Auwerx J, Staels B: Regulation of lipoprotein metabolism by thiazolidinediones occurs through a distinct but complementary mechanism relative to fibrates. *Arterioscler Thromb Vasc Biol* 17:1756–1764, 1997
16. Willson TM, Lambert MH, Kliewer SA: Peroxisome proliferator-activated receptor γ and metabolic disease. *Annu Rev Biochem* 70:341–367, 2001
17. Kawai T, Takei I, Oguma Y, Ohashi N, Tokui M, Oguchi S, Katsukawa F, Hirose H, Shimada A, Watanabe K, Saruta T: Effects of troglitazone on fat distribution in the treatment of male type 2 diabetes. *Metabolism* 48:1102–1107, 1999
18. Kelly IE, Han TS, Walsh K, Lean ME: Effects of a thiazolidinedione compound on body fat and fat distribution of patients with type 2 diabetes. *Diabetes Care* 22:288–293, 1999
19. Miyazaki Y, Mahankali A, Matsuda M, Glass L, Mahankali S, Ferrannini E, Cusi K, Mandarino LJ, DeFronzo RA: Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care* 24:710–719, 2001
20. Mori Y, Murakawa Y, Okada K, Horikoshi H, Yokoyama J, Tajima N, Ikeda Y: Effect of troglitazone on body fat distribution in type 2 diabetic patients. *Diabetes Care* 22:908–912, 1999
21. Way JM, Harrington WW, Brown KK, Gottschalk WK, Sundseth SS, Mansfield TA, Ramachandran RK, Willson TM, Kliewer SA: Comprehensive messenger ribonucleic acid profiling reveals that peroxisome proliferator-activated receptor gamma activation has coordinate effects on gene expression in multiple insulin-sensitive tissues. *Endocrinology* 142:1269–1277, 2001
22. Albrektsen T, Frederiksen KS, Holmes WE, Boel E, Taylor K, Fleckner J: Novel genes regulated by the insulin sensitizer rosiglitazone during adipocyte differentiation. *Diabetes* 51:1042–1051, 2002
23. Berger J, Tanen M, Elbrecht A, Hermanowski-Vosatka A, Moller DE, Wright SD, Thieringer R: Peroxisome proliferator-activated receptor-γ ligands inhibit adipocyte 11β-hydroxysteroid dehydrogenase type 1 expression and activity. *J Biol Chem* 276:12629–12635, 2001
24. Fajardo N, Deshaies Y: Long-term α₁-adrenergic blockade attenuates diet-induced dyslipidemia and hyperinsulinemia in the rat. *J Cardiovasc Pharmacol* 32:913–919, 1998
25. Reaven GM, Risser TR, Chen Y-DI, Reaven EP: Characterization of a model

26. Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW: Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *Am J Physiol Endocrinol Metab* 251:E576–E583, 1986
27. Kraegen EW, James DE, Storlien LH, Burleigh KM, Chisholm DJ: In vivo insulin resistance in individual peripheral tissues of the high fat fed rat: assessment by euglycaemic clamp plus deoxyglucose administration. *Diabetologia* 29:192–198, 1986
28. Hallfrisch J, Cohen L, Reiser S: Effects of feeding rats sucrose in a high fat diet. *J Nutr* 111:531–536, 1981
29. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275, 1951
30. Wilson MD, Blake WL, Salati LM, Clarke SD: Potency of polyunsaturated and saturated fats as short-term inhibitors of hepatic lipogenesis in rats. *J Nutr* 120:544–552, 1990
31. Chan CB, De Leo D, Joseph JW, McQuaid TS, Ha XF, Xu F, Tsushima RG, Pennefather PS, Salapatek AM, Wheeler MB: Increased uncoupling protein-2 levels in beta-cells are associated with impaired glucose-stimulated insulin secretion: mechanism of action. *Diabetes* 50:1302–1310, 2001
32. Staels B, Schoonjans K, Fruchart JC, Auwerx J: The effects of fibrates and thiazolidinediones on plasma triglyceride metabolism are mediated by distinct peroxisome proliferator activated receptors (PPARs). *Biochimie* 79:95–99, 1997
33. Schoonjans K, Peinado Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J: PPAR-α and PPAR-γ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J* 15:5336–5348, 1996
34. Kobayashi J, Nagashima I, Hikita M, Bujo H, Takahashi K, Otabe M, Morisaki N, Saito Y: Effect of troglitazone on plasma lipid metabolism and lipoprotein lipase. *Br J Clin Pharmacol* 47:433–439, 1999
35. Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, Umesono K, Akanuma Y, Fujiwara T, Horikoshi H, Yazaki Y, Kadowaki T: Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *J Clin Invest* 101:1354–1361, 1998
36. Kelly LJ, Vicario PP, Thompson GM, Candelore MR, Doebber TW, Ventre J, Wu MS, Meurer R, Forrest MJ, Conner MW, Cascieri MA, Moller DE: Peroxisome proliferator-activated receptors γ and α mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. *Endocrinology* 139:4920–4927, 1998
37. Fukui Y, Masui S, Osada S, Umesono K, Motojima K: A new thiazolidinedione, NC-2100, which is a weak PPAR-gamma activator, exhibits potent antidiabetic effects and induces uncoupling protein 1 in white adipose tissue of KKAy obese mice. *Diabetes* 49:759–767, 2000
38. Fujishima S, Ohya Y, Nakamura Y, Onaka U, Abe I, Fujishima M: Troglitazone, an insulin sensitizer, increases forearm blood flow in humans. *Am J Hypertens* 11:1134–1137, 1998
39. Vazquez M, Roglans N, Cabrero A, Rodriguez C, Adzet T, Alegret M, Sanchez RM, Laguna JC: Bezafibrate induces acyl-CoA oxidase mRNA levels and fatty acid peroxisomal beta-oxidation in rat white adipose tissue. *Mol Cell Biochem* 216:71–78, 2001
40. Cabrero A, Alegret M, Sanchez RM, Adzet T, Laguna JC, Vazquez M: Bezafibrate reduces mRNA levels of adipocyte markers and increases fatty acid oxidation in primary culture of adipocytes. *Diabetes* 50:1883–1890, 2001
41. Sandeep TC, Walker BR: Pathophysiology of modulation of local glucocorticoid levels by 11beta- hydroxysteroid dehydrogenases. *Trends Endocrinol Metab* 12:446–453, 2001
42. Quinkler M, Oelkers W, Diederich S: Clinical implications of glucocorticoid metabolism by 11β-hydroxysteroid dehydrogenases in target tissues. *Eur J Endocrinol* 144:87–97, 2001
43. Pedersen SB, Borglum JD, Moller-Pedersen T, Richelsen B: Characterization of nuclear corticosteroid receptors in rat adipocytes: regional variations and modulatory effects of hormones. *Biochim Biophys Acta* 1134:303–308, 1992
44. Pedersen SB, Jonler M, Richelsen B: Characterization of regional and gender differences in glucocorticoid receptors and lipoprotein lipase activity in human adipose tissue. *J Clin Endocrinol Metab* 78:1354–1359, 1994
45. Rebuffé-Scrive M, Krotkiewski M, Elfverson J, Bjorntörp P: Muscle and adipose tissue morphology and metabolism in Cushing's syndrome. *J Clin Endocrinol Metab* 67:1122–1128, 1988
46. Mayo-Smith W, Hayes CW, Biller BM, Klibanski A, Rosenthal H, Rosenthal DI: Body fat distribution measured with CT: correlations in healthy

of dietary-induced hypertriglyceridemia in young, nonobese rats. *J Lipid Res* 20:371–378, 1979

subjects, patients with anorexia nervosa, and patients with Cushing syndrome. *Radiology* 170:515–518, 1989

47. Peeke PM, Chrousos GP: Hypercortisolism and obesity. *Ann N Y Acad Sci* 771:665–676, 1995

48. Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ: 11β-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A* 94:14924–14929, 1997

49. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294:2166–2170, 2001