Original Article

Mechanisms of the Depot Specificity of Peroxisome Proliferator–Activated Receptor γ Action on Adipose Tissue Metabolism

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In this study, we aimed to establish the mechanisms whereby peroxisome proliferator–activated receptor γ (PPARγ) agonism brings about redistribution of fat toward subcutaneous depots and away from visceral fat. In rats treated with the full PPARγ agonist COOH (30 mg · kg^{-1} · day^{-1}) for 3 weeks, subcutaneous fat mass was doubled and that of visceral fat was reduced by 30% relative to untreated rats. Uptake of triglyceride-derived nonesterified fatty acids was greatly increased in subcutaneous fat (14-fold) and less so in visceral fat (4-fold), with a concomitant increase, restricted to subcutaneous fat only, in mRNA levels of the uptake-, retention-, and esterification-promoting enzymes lipoprotein lipase, aP2, and diacylglycerol acyltransferase 1. Basal lipolysis and fatty acid recycling were stimulated by COOH in both subcutaneous fat and visceral fat, with no frank quantitative depot specificity. The agonist increased mRNA levels of enzymes of fatty acid oxidation and thermogenesis much more strongly in visceral fat than in subcutaneous fat, concomitantly with a stronger elevation in O_2 consumption in the former than in the latter. Mitochondrial biogenesis was stimulated equally in both depots. These findings demonstrate that PPARγ agonism redistributes fat by stimulating the lipid uptake and esterification potential in subcutaneous fat, which more than compensates for increased O_2 consumption; conversely, lipid uptake is minimally altered and energy expenditure is greatly increased in visceral fat, with consequent reduction in fat accumulation. *Diabetes* 55:2771–2778, 2006

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Acadl, long-chain acyl-CoA dehydrogenase; ATGL, adipose triglyceride lipase; DGAT-1, diacylglycerol acyltransferase 1; GyK, glycerol kinase; KRB, Krebs-Ringer bicarbonate; LPL, lipoprotein lipase; mCPT-1, muscle-type carnitine palmitoyltransferase 1; NEFA, nonesterified fatty acid; PDK, pyruvate dehydrogenase kinase; PGC-1α, peroxisome proliferator–activated receptor γ coactivator 1α; PPAR, peroxisome proliferator–activated receptor; TZD, thiazolidinedione; UCP-1, uncoupling protein 1; WAT, white adipose tissue.

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Individuals with visceral fat deposition are at high risk of developing the metabolic syndrome, type 2 diabetes, and cardiovascular disease (1,2), in contrast with those with similar amounts of adipose tissue stored in subcutaneous depots (3). Visceral fat releases more nonesterified fatty acids (NEFAs) into circulation than subcutaneous fat (4,5), which is liable to expose the liver to high amounts of NEFAs and to increase hepatic glucose production and VLDL secretion (6,7). High plasma NEFAs lead to lipid accumulation in nonadipose tissues and interfere with insulin signaling (8,9). Also, enlarged visceral fat secretes a wide range of proinflammatory cytokines that reduce insulin signaling and promote endothelial dysfunction (10).

Peroxisome proliferator–activated receptor (PPAR)γ is a ligand-activated nuclear receptor that is highly expressed in mammalian white adipose tissue (WAT), in which it regulates the expression of a number of genes involved in lipid and glucose metabolism. PPARγ agonists of the thiazolidinedione (TZD) class are currently used for the treatment of insulin resistance and type 2 diabetes. The mechanisms involved in the insulin-sensitizing effect of PPARγ agonists are not completely understood but appear to involve changes in WAT metabolism to a large extent. In WAT, TZDs affect adipokine secretion and favor lipid uptake, retention, and fatty acid oxidation (11,12), which together lessen the proinflammatory and lipid burden on nonadipose tissues.

TZDs increase overall adiposity (13,14) by favoring lipid deposition in subcutaneous fat while reducing or maintaining visceral fat mass (14–16). Redistributing fat from lipolytic visceral fat to more anabolic subcutaneous fat is thought to play a role in the insulin-sensitizing activity of PPARγ agonists in humans. The mechanisms of such fat redistribution are not known. TZDs strongly induce differentiation of human preadipocytes isolated from subcutaneous fat but not those from visceral fat (17,18). However, the depot specificity of PPARγ agonism on overall adipose lipid metabolism has not been addressed in detail. Our previous study suggested that PPARγ agonism may lead to the redistribution of WAT by exerting depot-specific actions on several aspects of adipose lipid metabolism (19).

Fat storage represents the balance between accretion (uptake, synthesis, and esterification) and depletion (release of lipolytic products, oxidation, energy-consuming cycling, and thermogenesis). This study aimed to establish by which of these pathways PPARγ agonism leads to

TABLE 1
Primers used for quantitation of mRNA by real-time qPCR

Gene	Accession no.	5' primer (5'–3')	3' primer (5'–3')
aP2	NM_053365	ATGTGTGATGCCTTTGTGGG	CCCAGTTTGAAGGAAATCTC
Acadl	NM_012819	AGCTCCCACAGGAAAGGCTT	CTCGAGCATCCACGTAGGCT
ATGL	XM_341960	CACTTTAGCTCCAAGGATGA	TGGTTCAGTAGGCCATTCCT
DGAT-1	NM_053437	TATTACTTCATCTTTGCTCC	AAAGTAGGTGACAGACTCAG
GyK	NM_024381	CCTGTCCATTGAAATGTGTCATCC	GCCATGAAGCCATGACAATTAGTG
LPL	NM_012598	AACCTTTGTGGTGATCCATGGA	CGAAATCCGCATCATCAGGA
L27	NM_022514	CTGCTCGCTGTCGAAATG	CCTTGCGTTTCAGTGCTG
mCPT-1	NM_013200	CGGAAGCACACCAGGCAGTA	GCAGCTTCAGGGTTTGTCGGAATA
PDK-2	NM_030872	GGGGTGTCCCCTTGAGGAAGAT	TTCTTGGGCTCTGTGCTGGG
PDK-4	NM_053551	TTCTACTCGGATGCTCATGA	CACCTCGGTCAGAAATCTTG
PEPCK	NM_198780	TGGGTGATGACATTGCCTGG	ACCTTGCCCTTATGCTCTGCAG
PGC-1α	NM_031347	TCCTGTTACTATTATGAATCAAGCC	AAACCATAGCTGTCTCCATCATCC
PPARγ 2	AF156666	GGTGAAACTCTGGGAGATCC	TGAGGGAGTTTGAAGACTCTTC
UCP-1	NM_012682	TGGTGAGTTCGACAACTTCC	GTGGGCTGCCCAATGAATAC

depot-specific fat accretion. To this end, determinants of adipose lipid metabolism were assessed in subcutaneous fat and visceral fat, including triglyceride-derived fatty acid uptake and retention, lipolysis, fatty acid reesterification, and energy expenditure. Quantification of the level of expression of major genes of these pathways was combined with their assessment at the functional level.

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats (90–100 g, $n = 12$ per protocol, three protocols; Charles River Laboratories, St. Constant, QC, Canada) were housed individually in stainless-steel cages (23 ± 1°C, 12:12-h light/dark cycle, lights on at 0700). 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. Initially, rats had free access to tap water and a stock diet (Charles River Rodent Diet no. 5075; Ralston Products, Woodstock, ON, Canada). Rats were then fed a purified high-sucrose, high-fat diet (composition detailed in ref. 20) to maximize the effect of PPARγ agonism on lipid flux. During the 3-week feeding period, half of the animals were given the non-TZD PPARγ agonist COOH [2-(2-(4-phenoxy-2-propylphenoxy)ethyl)indole-5-acetic acid] as an adjunct to their diet at a dose of 30 mg · kg^{-1} · day^{-1}, previously shown to bring about frank redistribution of fat in the rat (19).

Serum and tissue sampling. Rats were killed by decapitation after a 10-h fast, trunk blood was centrifuged (1,500*g*, 15 min, 4°C), and serum was stored at −80°C. Tissue samples (inguinal depot as representative of subcutaneous fat and retroperitoneal depot as representative of visceral fat) were homogenized and processed exactly as described earlier (21,22) and stored at −80°C until measurements of enzyme activities. Fresh samples were used for fat cell isolation and explant incubation as described below.

Adipose tissue mRNA levels. Total RNA was isolated using QIAzol and the RNeasy Lipid Tissue kit (QIAGEN, Mississauga, ON, Canada). For cDNA synthesis, Expand Reverse Transcriptase (Invitrogen, Burlington, ON, Canada) was used following the manufacturer's instructions, and cDNA was diluted in DNase-free water (1:25) before quantification by real-time PCR (primers listed in Table 1). mRNA transcript levels were measured using a Rotor Gene 3000 system (Montreal Biotech, Montreal, QC, Canada). Chemical detection of the PCR products was achieved with SYBR Green I (Molecular Probes, Willamette Valley, OR). Data are expressed as the ratio between the expression of the target gene and the housekeeping gene L27.

Adipocyte isolation and cell-size distribution. Adipocytes were isolated from fat pads by a slight modification of the method of Rodbell (23). Briefly, fat pads were removed and placed in 2.5% Krebs-Ringer bicarbonate (KRB) buffer. The minced tissue was incubated in 2.5% KRB containing 0.25 mg/ml collagenase (Invitrogen) at 37°C for 18–21 min with shaking at 150 cycles/min. Cells were then filtered through a nylon mesh, and collagenase was removed by repeated (three times) washings with fresh KRB. Cell size distribution (in 10 μm increments, 20–260 μm) was determined microscopically in cell suspensions containing 0.4% trypan blue. Structures <20 μm were not considered. Cell diameter was measured in at least 300 cells/depot for each rat.

Adipose tissue uptake of triglyceride-derived fatty acids. Control and COOH-treated (18 days) rats were cannulated into the jugular vein under isoflurane anesthesia. After 3 days of recovery, 10-h fasted rats were injected through the jugular catheter with 0.75 ml/kg of 10% Intralipid containing ^{3}H-9,10–labeled trioleoylglycerol (570 dpm/nmol fatty acid; kindly provided by Drs. T. and G. Olivecrona, Umeå University, Umeå, Sweden) diluted 1:6 with 20% Intralipid (150 mg/kg of triglycerides were injected) and prepared as described previously (24). Twenty minutes after injection, rats were killed by ketamine-xylazine injection. Radioactivity content of tissues was quantified as described previously (24). Lipid uptake is expressed as percent injected dose.

Adipose tissue lipoprotein lipase activity. Tissue homogenates were incubated with a substrate mixture containing [carboxyl-^{14}C]triolein, and [^{14}C]NEFAs released by lipoprotein lipase (LPL) were separated and counted (21). LPL activity is expressed as microunits (1 μU = 1 μmol NEFAs/h at 28°C) per total depot to illustrate global tissue availability.

Adipose tissue PEPCK activity. Tissue homogenates were incubated with [^{14}C]bicarbonate and incorporation of radioactivity into acid-stable malate was measured after $^{14}CO_2$ evaporation (22). PEPCK activity was calculated as nanomoles of phosphoenolpyruvate converted to malate per minute at 37°C and is expressed per unit of total tissue protein (25).

[1-^{14}C]Pyruvate incorporation into lipids. As a measure of fatty acid reesterification into triglycerides (26), isolated adipocytes (~7,500/well) were incubated for 2 h in 2.5% KRB without glucose in a humidified atmosphere of 5% CO_2 and 95% O_2 at 37°C. KRB was supplemented with 5 mmol/l pyruvate and 1 μCi of [1-^{14}C]pyruvic acid (Na salt; Amersham, Piscataway, NJ). Total lipids were extracted (27), and [1-^{14}C]pyruvate incorporation was counted.

Lipolysis. Pieces of tissue (20–25 mg) obtained from 10-h fasted rats were incubated for 2 h in 1 ml of 2.5% KRB with or without insulin (100 pmol/l) in a humidified atmosphere of 5% CO_2 and 95% O_2 at 37°C. Explants were then removed, and incubation media were frozen until measurement of NEFAs (Wako, Richmond, VA) and glycerol (Sigma, Oakville, ON, Canada). NEFA and glycerol release are expressed per unit of DNA determined using the DNeasy Tissue kit (QIAGEN).

Oxygen consumption. Isolated adipocytes were washed in 2.5% KRB. Excess buffer was removed, and 200 μl of floating packed cells was divided into aliquots in a BD Oxygen Biosensor System (BD Biosciences, Mississauga, ON, Canada) in triplicate. Plates were read on a FluoStar Galaxy fluorometer (BMG Technologies, Durham, NC) at 1-min intervals for 120 min at an excitation wavelength of 485 nm and emission wavelength of 610 nm. Results are expressed as the slope of fluorescence intensity/time (seconds) × 1,000/μg DNA.

Mitochondrial mass. Isolated adipocytes were incubated at 37°C for 30 min in 2.5% KRB with 100 nmol/l MitoTracker Green FM (Molecular Probes and Invitrogen), a mitochondrion-specific dye, and images were acquired with an Evolution QEi camera (MediaCybernetics, Silver Spring, MD) mounted on an Olympus BX51 microscope (Olympus America, Melville, NY). Quantification was achieved using Image Pro Plus 5.0 (MediaCybernetics). The fluorescence-to-cell surface × 100 ratio was measured on 25 different cells for each depot, and averages were calculated for each rat.

Plasma analytes. Thawed plasma samples were used to measure levels of glucose (glucose oxidase method; YSI 2300 STAT Plus glucose analyzer),

TABLE 2
Morphometric and plasma variables in rats treated or not treated with COOH for 3 weeks

	Control	COOH
Food intake (MJ)	8.4 ± 0.4	8.8 ± 0.5
Final body weight (g)	326 ± 13	356 ± 20
Body weight gain (g)	172 ± 11	199 ± 15
Glucose (mmol/l)	7.8 ± 0.2	7.5 ± 0.2
Insulin (pmol/l)	252 ± 53	70 ± 11*
Triglycerides (mmol/l)	2.4 ± 0.3	1.4 ± 0.2*
NEFAs (mmol/l)	0.61 ± 0.02	0.44 ± 0.05*
Glycerol (mmol/l)	0.17 ± 0.01	0.21 ± 0.02*
NEFA-to-glycerol ratio	3.7 ± 0.2	2.1 ± 0.2*

Data are means ± SE of six rats. *Different from control, $P < 0.05$.

insulin (RIA; Linco Research, St. Charles, MO), triglycerides (enzymatic; Roche Diagnostics, Montreal, QC, Canada), NEFAs, and glycerol (as above).
Statistical analysis. Data are means ± SE. Morphometric and serum variables were analyzed by Student's unpaired *t* test. Adipose tissue variables were first analyzed by factorial ANOVA to establish the individual and interactive effects of fat localization, with two levels (subcutaneous and visceral fat), and PPARγ agonist treatment, with two levels (control and COOH). Individual pairwise between-group comparisons were then made using Fisher's protected least significant difference test. Significance was set at $P < 0.05$.

RESULTS

Morphometry, serum variables, and fat distribution. The 21-day COOH treatment tended to increase food intake, body weight, and body weight gain; however, the effect did not reach significance (Table 2). The agonist did not affect fasting glucose but significantly reduced circulating insulin (−72%). As also anticipated, circulating triglycerides and NEFAs were reduced by the agonist (−28 and −42%, respectively). Serum glycerol was slightly increased (24%) by COOH, resulting in a large decrease (−43%) in the serum NEFA-to-glycerol ratio. Confirming WAT remodeling by COOH, subcutaneous fat weight accretion from the beginning to the end of the 3-week treatment was more than twofold larger in treated (gain of 9.5 g) than in control rats (gain of 4.5 g), whereas that of visceral fat was reduced by 32% in treated (gain of 3.6 g) versus control rats (gain of 5.3 g), such that the visceral fat-to-subcutaneous fat weight ratio was reduced by more than half (1.0 vs. 0.4, control vs. COOH, respectively) (Fig. 1*A*). Total DNA content was markedly increased by COOH in subcutaneous fat (threefold) but remained unaffected in visceral fat (Fig. 1*B*). PPARγ$_2$ expression level was higher in visceral fat than in subcutaneous fat and was not affected by the agonist (Fig. 1*D* and *E*). COOH brought about the expected shift in adipocyte size distribution toward smaller cell diameters in subcutaneous fat and visceral fat, the shift being, however, much more marked in the latter than in the former (Fig. 1*C*). Indeed, COOH reduced peak diameter by <10 μm in subcutaneous fat and by ~25 μm in visceral fat. Of note was the large COOH-induced increase in the relative number of cells of the smallest diameters (>50 μm) in subcutaneous fat, probably representing newly differentiated adipocytes, such an increase being barely detectable in visceral fat.

Determinants of adipose fatty acid uptake and esterification. To gain insight into the mechanisms implicated in COOH-induced WAT remodeling, we first examined the expression of genes involved in fatty acid handling and measured functional uptake of fatty acid derived from lipoprotein-bound triglycerides. COOH increased both the mRNA level (Fig. 2*A*) and total tissue activity (Fig. 2*B*) of the triglyceride-hydrolyzing enzyme LPL twofold in subcutaneous fat but remained without effect in visceral fat. Levels of mRNA of the fatty acid binding protein aP2, a major adipose PPARγ target involved in adipogenesis, long-chain fatty acid uptake and retention (28,29) were increased twofold by COOH in subcutaneous fat, but remained unaffected in visceral fat (Fig. 2*C*). At the functional level, COOH increased the in vivo uptake of fatty acid derived from the hydrolysis of triglycerides in both depots, the effect being, however, much more marked in subcutaneous fat (14-fold) than in visceral fat (4-fold) (Fig. 2*D*). Diacylglycerol acyltransferase-1 (DGAT-1) catalyzes the terminal, rate-limiting step in triglyceride synthesis, and its overexpression favors fat gain (30). COOH increased DGAT-1 expression twofold in subcutaneous fat (Fig. 2*E*), suggesting an increase in fatty acid commitment to triglyceride synthesis, whereas DGAT-1 mRNA in visceral fat was unchanged by the agonist.

Lipolysis. Although PPARγ agonism reduces serum NEFA levels, there is evidence that it stimulates adipocyte lipolysis (31–33) concomitantly with stimulation of fatty acid reuptake and reesterification, which counter the release of lipolytic products. The possible contribution of COOH-mediated modulation of lipolysis to differential fat accretion was therefore assessed. In control rats, as anticipated, basal glycerol and NEFA release was higher in visceral fat than in subcutaneous fat (Fig. 3*A*, *top panel*). COOH increased basal glycerol release in both subcutane-

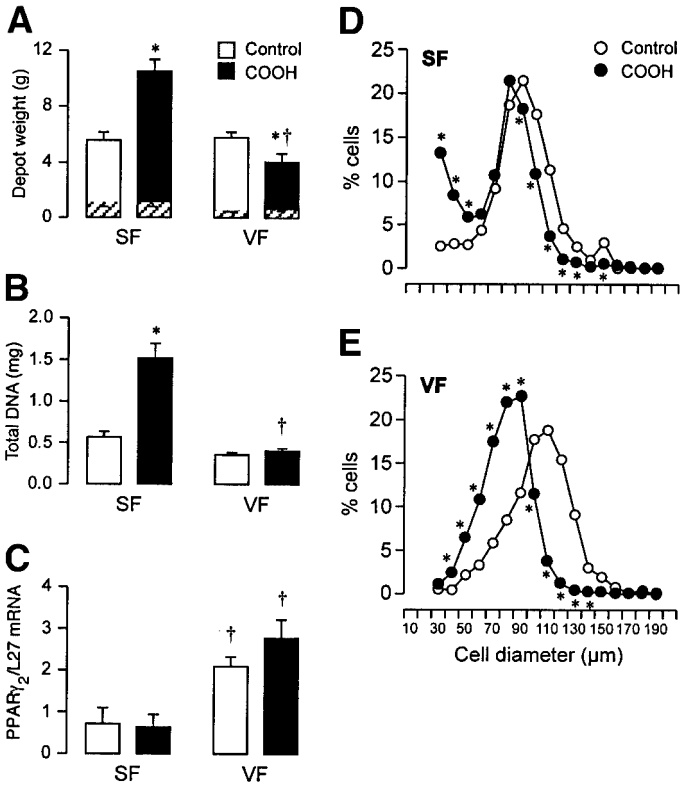

FIG. 1. Depot weight (*A*), total DNA (*B*), PPARγ2 mRNA level (*C*), and adipocyte size distribution of subcutaneous fat (SF) (*D*) and visceral fat (VF) (*E*) of rats treated or not treated with COOH for 3 weeks. In *A*, hatched areas indicate depot weights measured in a separate group of rats at the beginning of the 3-week treatment period. In *D* and *E*, numbers on the *x*-axis indicate the upper limit of a 10-μm range; SEs were omitted for clarity. Data are means ± SE of six rats. *$P < 0.05$ vs. the same depot in the control group; †$P < 0.05$ vs. subcutaneous fat in the same treatment group.

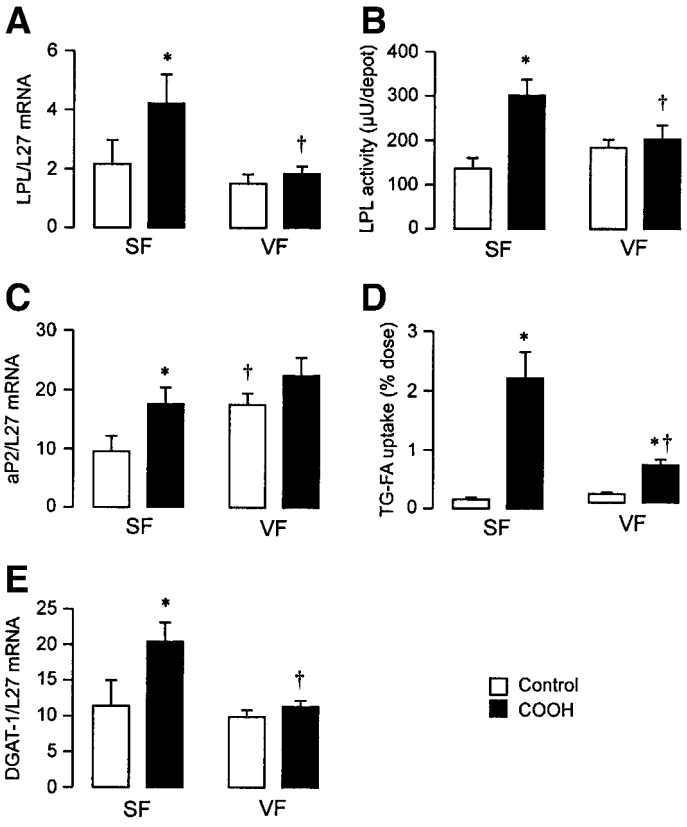

FIG. 2. LPL mRNA level (*A*) and hydrolytic activity (*B*), mRNA levels of aP2 (*C*), uptake of chylomicron triglyceride (TG)-derived labeled fatty acids (FA) (*D*), and mRNA level of DGAT-1 (*E*) in subcutaneous fat (SF) and visceral fat (VF) of rats treated or not treated with COOH for 3 weeks. Data are means ± SE of six rats. **P* < 0.05 vs. the same depot in the control group; †*P* < 0.05 vs. subcutaneous fat in the same treatment group.

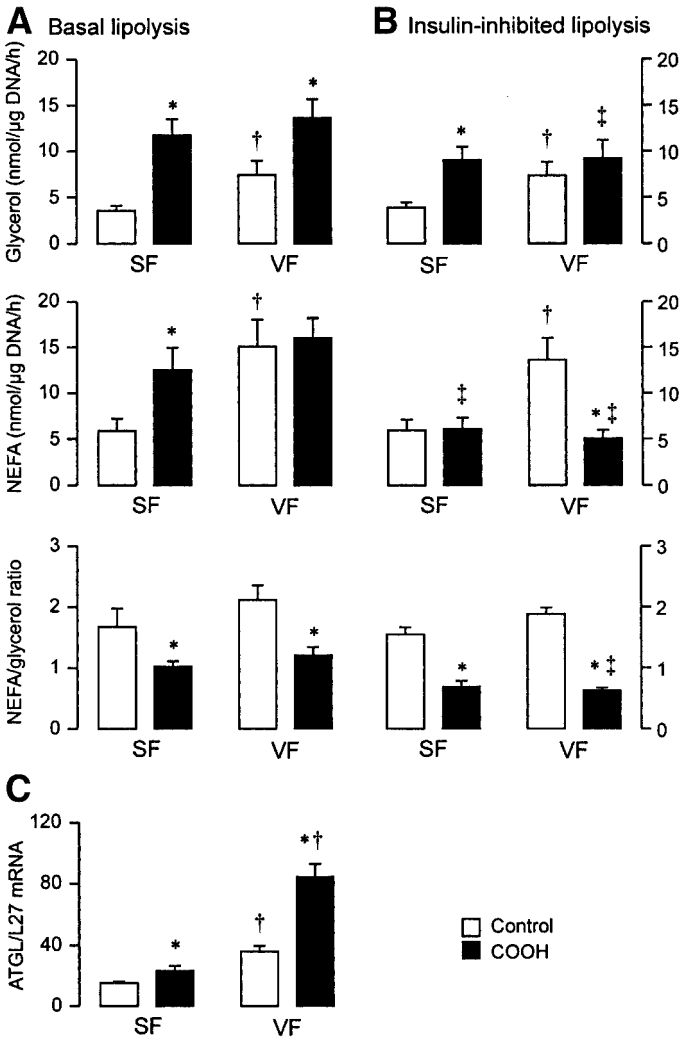

FIG. 3. Basal (*A*) and insulin-inhibited (*B*) release of glycerol (*top panels*) and NEFAs (*middle panels*), the NEFA-to-glycerol ratio (*A* and *B*, *bottom panels*), and the mRNA level of ATGL (*C*) in subcutaneous fat (SF) and visceral fat (VF) explants isolated from rats treated or not treated with COOH for 3 weeks. Data are means ± SE of six rats. **P* < 0.05 vs. same depot in the control group; †*P* < 0.05 vs. subcutaneous fat in the same treatment group; ‡*P* < 0.05 vs. the same depot and treatment incubated without insulin (*A*).

ous fat (threefold) and visceral fat (twofold). Because of increased fatty acid reesterification (see below), the effect of COOH on NEFA release was of lesser magnitude (nil in the case of visceral fat) (Fig. 3*A*, *middle panel*) than that predicted by the increase in glycerol release, resulting in a 40% reduction in the NEFA-to-glycerol ratio in both depots (Fig. 3*A*, *bottom panel*). The combined stimulatory effects of COOH on lipolysis and fatty acid reesterification observed in vitro were reflected in plasma levels of lipolytic products, that is, higher glycerol levels and lower NEFA levels and NEFA-to-glycerol ratio in COOH versus control rats (Table 2). A physiological concentration of insulin did not affect glycerol and NEFA release from tissues of untreated rats (Fig. 3*B*) but did tend to reduce glycerol and NEFA release in tissues of COOH-treated rats, suggesting sensitization of adipose lipolysis to insulin action. Of note is the fact that fatty acid cycling (reflected by the reduced NEFA-to-glycerol ratio) was amplified in the presence of insulin (subcutaneous fat −56% and visceral fat −67% compared with untreated vs. −40% without insulin), indicating additivity of the positive action of chronic COOH and acute insulin on fatty acid reesterification. Finally, expression levels of adipose triglyceride lipase (ATGL) (Fig. 3*C*), recently highlighted as an important determinant of basal lipolysis, were higher in visceral fat than in subcutaneous fat and were increased by COOH in both visceral fat (2.3-fold) and subcutaneous fat (1.5-fold), in general congruence with treatment effects on lipolytic rates.

Determinants of glycerol and fatty acid cycling. PPARγ agonism stimulates in WAT the reesterification into triglycerides of both glycerol and fatty acids released by intracellular lipolysis, thereby favoring lipid retention, through enhancing the expression of glycerol kinase (GyK) (glycerol phosphorylation) and PEPCK (glyceroneogenesis) (26). Their possible contribution to differential fat accretion was therefore assessed. COOH increased PEPCK activity (Fig. 4*A*) and mRNA levels (Fig. 4*B*) in both depots. Basal activity and mRNA of PEPCK were lower in subcutaneous fat and the relative increase induced by the PPARγ agonist was slightly larger in subcutaneous fat than in visceral fat. In parallel with PEPCK, [1-^{14}C]pyruvate incorporation into lipids (a functional measure of glyceroneogenesis) was more important in visceral fat than in subcutaneous fat and was increased by COOH in both depots (Fig. 4*C*). COOH also increased mRNA levels of GyK two- to threefold in both subcutaneous fat and visceral fat (Fig. 4*D*).

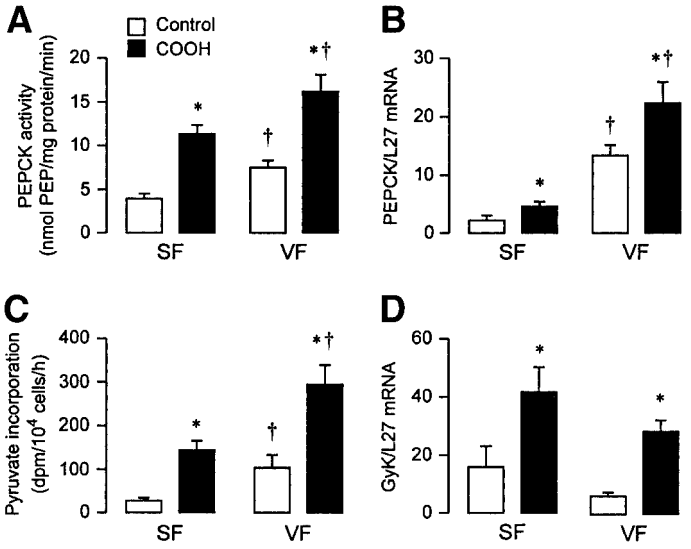

FIG. 4. PEPCK activity (*A*) and mRNA level (*B*), incorporation of [1-^{14}C]pyruvate into lipids (*C*), and GyK mRNA level (*D*) in subcutaneous fat (SF) and visceral fat (VF) of rats treated or not treated with COOH for 3 weeks. Data are means ± SE of six rats. **P* < 0.05 vs. the same depot in the control group; †*P* < 0.05 vs. subcutaneous fat in the same treatment group.

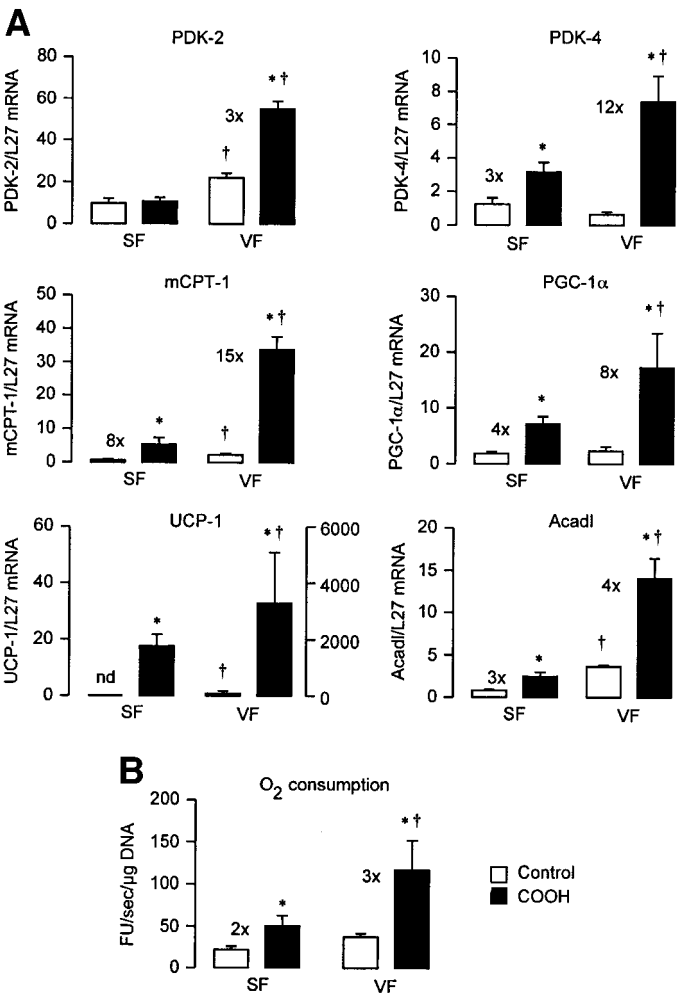

FIG. 5. mRNA levels of PDK-2, PDK-4, mCPT-1, PGC-1α, UCP-1, and Acadl (*A*) and adipocyte O_2 consumption (*B*) in subcutaneous fat (SF) and visceral fat (VF) of rats treated or not treated with COOH for 3 weeks. Data are means ± SE of six rats. **P* < 0.05 vs. the same depot in the control group; †*P* < 0.05 vs. subcutaneous fat in the same treatment group.

Determinants of fatty acid oxidation and energy expenditure. Despite favoring whole-body positive energy balance, PPARγ agonists locally increase WAT energy expenditure (11). We therefore sought to determine whether COOH favored the latter differentially in subcutaneous fat and visceral fat. Pyruvate dehydrogenase kinase (PDK)-2 and PDK-4 phosphorylate and inactivate the pyruvate dehydrogenase complex and thereby facilitate fatty acid oxidation. PDK-2 mRNA expression was augmented by COOH only in visceral fat, and PDK-4 mRNA was increased much more strongly in visceral fat than in subcutaneous fat (Fig. 5*A*). A similar pattern (stimulation by COOH in both depots but more marked in visceral fat than in subcutaneous fat) was observed for muscle-type carnitine palmitoyltransferase 1 (mCPT-1), the limiting enzyme in fatty acid transport to the mitochondrion, PPARγ coactivator 1α (PGC-1α), a major cofactor involved in thermogenesis, and long-chain acyl-CoA dehydrogenase (Acadl), which plays an important role in β-oxidation. Likewise, COOH increased the expression of uncoupling protein 1 (UCP-1), a key thermogenic gene, to a much greater extent in visceral fat than in subcutaneous fat (note scale change in Fig. 5*A*). At the functional level, in accordance with its effect on gene expression, COOH increased O_2 consumption in both adipose depots but significantly more so in visceral fat than in subcutaneous fat (Fig. 5*B*).

COOH increased mitochondrial density identically (2.5-fold) in adipocytes isolated from subcutaneous fat and visceral fat (Fig. 6*A*) and induced important changes in mitochondrial distribution, morphology, and interaction with lipid stores in both depots (Fig. 6*B*; only visceral fat cells are depicted). Unlike in control cells, mitochondria in adipocytes of COOH-treated rats displayed a reticular shape and appeared to surround clusters of small lipid droplets, indicating a major rearrangement of the lipid-metabolizing infrastructure of the cell.

DISCUSSION

In this study, we aimed to identify the mechanisms whereby PPARγ agonism results in the redistribution of adipose tissue to subcutaneous depots at the expense of visceral fat. It was found that COOH, a non-TZD agonist that mimics the fat remodeling seen in humans treated with TZDs, increased lipid uptake and esterification capacity to a greater extent in subcutaneous fat than in visceral fat. Conversely, COOH enhanced the expression of genes involved in fatty acid oxidation and thermogenesis, as well as O_2 consumption, to a greater degree in visceral fat than in subcutaneous fat. In this model, fat redistribution elicited by PPARγ agonism is therefore the consequence of concerted changes in multiple pathways of adipose lipid metabolism.

Remodeling of WAT by COOH included cell proliferation exclusively in subcutaneous fat, in which numerous very small adipocytes were present, as well as a reduction in peak adipocyte size indicative of reduced fat content, which was much more marked in visceral fat than in subcutaneous fat. The study is in line with the depot-specific action of PPARγ agonism on fat cell differentiation established in isolated human preadipocytes (17,18). In

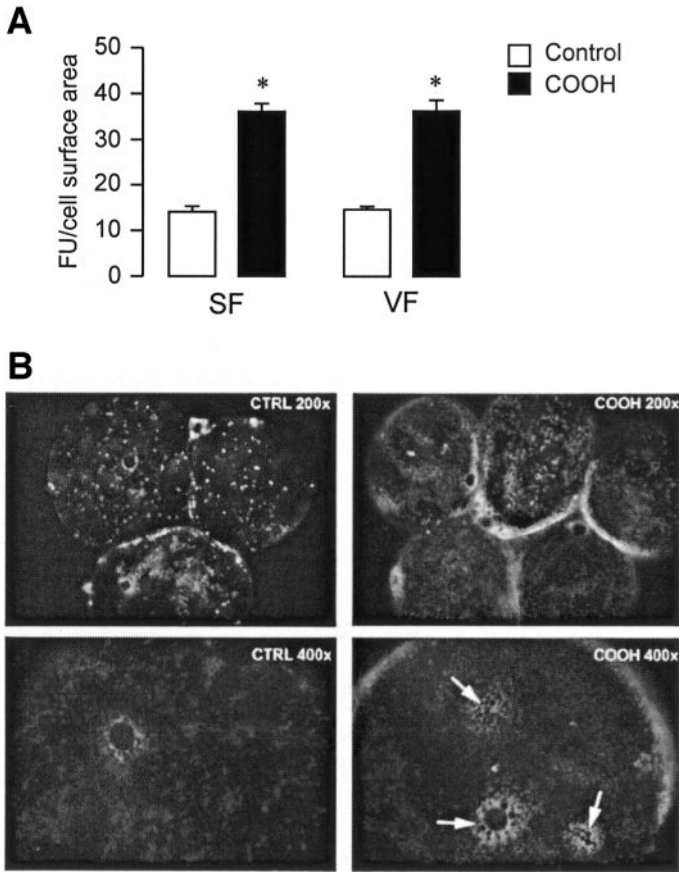

FIG. 6. Mitochondrial mass in live adipocytes isolated from subcutaneous fat (SF) and visceral fat (VF) of rats treated or not treated with COOH for 3 weeks (*A*) and representative micrographs of adipocytes isolated from the retroperitoneal depot of control and COOH-treated rats (*B*). In *A*, data are means ± SE of six rats in each of which 25 cells were analyzed. **P* < 0.05 vs. the same depot in the control group. In *B*, white arrows indicate clusters of small lipid droplets surrounded by mitochondria. FU, fluorescence units; CTRL, control.

addition to its effect on fat remodeling, COOH promoted changes in plasma variables that are hallmarks of PPARγ agonism, namely decreases in fasting insulinemia, triglyceridemia, and NEFAs (29).

The induction of "lipid trapping" by WAT is regarded as one important mechanism of the insulin-sensitizing action of PPARγ agonism, and the lipid trapping–insulin sensitivity relationship has been directly demonstrated in rodents (34). The present study extends these findings by demonstrating that PPARγ agonism increases the uptake by WAT not only of circulating NEFAs (34,35) but also of lipoprotein triglyceride-derived fatty acids. In congruence with this functional depot specificity, LPL expression and activity, as well as aP2 expression, were increased by COOH specifically in subcutaneous fat, extending our previous findings on the role of LPL in this important phenomenon (19). The increase in newly differentiating adipocytes avidly taking up NEFAs released from hydrolysis of circulating triglyceride probably contributed to this depot-specific effect of COOH. The study also revealed a currently unrecognized subcutaneous fat–specific increase in mRNA levels of DGAT-1, suggestive of a preferential commitment of fatty acids taken up by subcutaneous fat toward esterification (30). The agonist also increased triglyceride-derived fatty acid uptake in visceral fat, albeit to a much lesser extent than in subcutaneous fat, without altering LPL or aP2; however, these two proteins are not the only factors affecting triglyceride-derived lipid uptake (36). Finally, in a separate study with chow-fed rats treated with COOH for 3 weeks and then fasted for 24 h, quantification of mRNA levels of SREBP-1c, a PPARγ target and a master regulator of the expression of lipogenic genes, revealed a two- to threefold COOH-induced stimulation that was identical in subcutaneous fat and visceral fat (M.L., Y.D., unpublished observations). Taken together, the above findings establish that, specifically in subcutaneous fat, COOH increased the relative number of small adipocytes and the potential for hydrolysis of lipoprotein-bound triglycerides and subsequent fatty acid uptake, sequestering, and esterification, thus creating conditions favoring fat accretion.

A stimulatory effect of PPARγ agonists on adipose lipolysis was reported earlier in obese Zucker rats (31,32) and was confirmed here and in our recent study (33). Higher lipolysis is counteracted by increased fatty acid reesterification into triglycerides, as discussed below, as well as by increased fatty acid reuptake by adipocytes (34,35). In the present study, basal lipolysis in untreated rats was, as expected, higher in visceral fat than in subcutaneous fat and was robustly increased by COOH in both depots. Of note is the fact that lipolysis was reduced by a low, physiological concentration of insulin only in fat explants from COOH-treated animals, confirming higher sensitivity to the antilipolytic effect of insulin (31,32). Thus, increased fatty acid cycling together with potentiation of the sensitivity of lipolysis to insulin action counteract net fatty acid release from adipocytes. COOH stimulated lipolysis in both depots roughly equally, and the lipolytic rate did not appear to contribute to the depot-specific effect of COOH on fat accretion. The study also confirmed our previous study (33) showing that ATGL is a PPARγ target. Quantitative depot specificity of the COOH effect on ATGL mRNA appeared more pronounced than that on lipolysis; however, other lipolysis-related enzymes are altered by PPARγ agonism (33) and probably influence, together with depot differences in reesterification rates, the net release of lipolytic products.

The TZD-induced reduction in circulating NEFAs was recently shown to be associated with an increase in PEPCK-mediated glyceroneogenesis in WAT (26). However, the contribution of this pathway to PPARγ-induced fat redistribution has not been addressed. As shown previously (26), basal PEPCK expression and activity and [1-^{14}C]pyruvate incorporation into lipids were higher in visceral fat than in subcutaneous fat. The expression levels of GyK, which under control conditions does not contribute much to glycerol recycling, was similar in both depots. We show here that COOH robustly increased PEPCK, [1-^{14}C]pyruvate incorporation into lipids, and GyK expression in both depots. The relative increase (fold over control) tended to be larger in subcutaneous fat than in visceral fat; however, absolute values remained higher in the latter than in the former. Therefore, as in the case of lipolysis, the rate of reesterification of lipolytic products does not appear to contribute to a large extent to the depot-specific action of PPARγ agonism on fat accretion in this model.

TZDs increase the expression of genes involved in lipid oxidation and energy expenditure (28,29) and promote mitochondrial biogenesis in WAT (11,12,37,38). The resulting increase in energy expenditure probably comprises contributions from fatty acid oxidation, energy-consuming

fatty acid/triglyceride cycling, and uncoupling of oxidative phosphorylation. In concordance with the above actions, COOH was found here to increase the expression of several transcripts involved in fatty acid oxidation, thermogenesis, and mitochondrial proliferation. First, mRNA levels of PDK-2 and -4, which lower glucose utilization and facilitate fatty acid oxidation (39), were increased by COOH. PPARγ agonism was shown earlier to reduce PDK-4 expression in muscle but to increase its expression in WAT (40). The present study extends these findings by showing that stimulation of PDK-4 expression is more marked in visceral fat than in subcutaneous fat and further revealing a concomitant stimulation of PDK-2, which occurred exclusively in visceral fat. In addition, the expression of mCPT-1, Acadl, and UCP-1 followed the same pattern, with a particularly striking between-depot difference in the case of UCP-1, confirming and extending our previous study (19). Such differential gene expression had a functional impact because COOH stimulated O_2 consumption in both tissues, but more strongly so in visceral fat than in subcutaneous fat, such that energy expenditure, which was similar in both depots of control rats, became nearly threefold higher in visceral fat than in subcutaneous fat of COOH-treated rats. Stimulation of energy expenditure contributed in all likelihood to restrain fat accretion in visceral fat. In subcutaneous fat, the stimulation of lipid uptake clearly overwhelmed the increase in energy expenditure, leading to the establishment of a balance favoring fat deposition.

The higher activation of O_2 consumption in visceral fat than in subcutaneous fat led us to postulate that COOH might exert depot-specific effects on mitochondrial biogenesis. However, it was found that COOH strongly increased mitochondrial mass in both depots, indicating that the larger stimulation of energy expenditure in visceral fat relative to subcutaneous fat was instead associated with the increase in oxidative/uncoupling capacity resulting from the changes in enzyme expression described above. In addition to increasing the number of mitochondria, COOH induced important changes in mitochondrial distribution, morphology, and interaction with lipid stores, as reported earlier in rats and dogs treated with rosiglitazone (11,37). The augmentation of mitochondrial mass coupled with a higher contact surface between mitochondria and lipid droplets may have contributed to the overall stimulatory action of COOH on O_2 consumption.

The observed COOH-induced increase in the potential for fatty acid oxidation, energy dissipation, mitochondrial biogenesis, and O_2 consumption confirm earlier findings with TZDs (11,38) and indicate that white adipocytes, either newly differentiated or mature, have acquired features that normally characterize brown adipose cells. A further indication of this is the COOH-induced increase in the expression of PGC-1α, a molecular switch that turns on several key components of the adaptive thermogenic program in brown fat (41). Overexpression of PGC-1α in white adipocytes increases UCP-1 expression, proteins of the respiratory chain, and fatty acid oxidation exactly as occurs in brown adipose cells (42). In the present study, a stronger COOH-mediated induction of PGC-1α expression in visceral fat than in subcutaneous fat therefore constitutes a likely mechanism explaining the depot-specific magnitude of the activation of oxidative/thermogenic gene expression and O_2 consumption. Why such depot specificity of action of COOH on PGC-1α did not translate into differences in mitochondrial mass is unknown but may conceivably involve interaction with the many other factors modulating the complex mitochondrial biogenesis program (11). Finally, it is worth noting that the PPARγ-mediated increase in WAT energy expenditure does not appear to be of sufficient magnitude to have an impact on whole-body energy balance.

The reasons for various pathways of lipid metabolism being differentially recruited by PPARγ agonism in subcutaneous fat and visceral fat have yet to be established. There is no major difference in PPARγ expression between human subcutaneous fat and visceral fat (43–45), whereas the higher expression in visceral fat compared with subcutaneous fat observed here confirms an earlier study in high-fat–fed rats (46). PPARγ$_2$ expression was unchanged by COOH, ruling out the possibility that receptor expression levels might explain the depot-specific modulation of fat accretion by exogenous PPARγ agonism. PPARγ activity is, however, highly regulated by the recruitment of a vast array of nuclear coactivators and corepressors (47,48) with widely diverging metabolic consequences. Differential recruitment of such cofactors in subcutaneous fat and visceral fat by the PPARγ receptor-agonist complex constitutes an attractive possibility to explain, at the molecular level, the depot-specific actions of PPARγ agonism.

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