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Up-Regulation of Peroxisome Proliferator-Activated Receptors (PPAR-α) and PPAR-γ Messenger Ribonucleic Acid Expression in the Liver in Murine Obesity: Troglitazone Induces Expression of PPAR-γ-Responsive Adipose Tissue-Specific Genes in the Liver of Obese Diabetic Mice*

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ABSTRACT

Peroxisome proliferator-activated receptors (PPARs) are transcription factors that play an important role in the regulation of genes involved in lipid utilization and storage, lipoprotein metabolism, adipocyte differentiation, and insulin action. The three isoforms of the PPAR family, *i.e.* α, δ, and γ, have distinct tissue distribution patterns. PPAR-α is predominantly present in the liver, and PPAR-γ in adipose tissue, whereas PPAR-δ is ubiquitously expressed. A recent study reported increased PPAR-γ messenger RNA (mRNA) expression in the liver in *ob/ob* mice; however, it is not known whether increased PPAR-γ expression in the liver has any functional consequences. The expression of PPAR-α and -δ in the liver in obesity has not been determined. We have now examined the mRNA levels of PPAR-α, -δ, and -γ in three murine models of obesity, namely, *ob/ob* (leptin-deficient), *db/db* (leptin-receptor deficient), and serotonin 5-HT2c receptor (5-HT2cR) mutant mice. 5-HT2cR mutant mice develop a late-onset obesity that is associated with higher plasma leptin levels. Our results show that PPAR-α mRNA levels in the liver are increased by 2- to 3-fold in all three obese models, whereas hepatic PPAR-γ mRNA levels are increased by 7- to 9-fold in *ob/ob* and *db/db* mice and by 2-fold in obese 5-HT2cR mutant mice. PPAR-δ mRNA expression is not altered in *ob/ob* or *db/db* mice. To determine whether increased PPAR-γ expression in the liver has any functional consequences, we examined the effect of troglitazone treatment on the hepatic mRNA levels of several PPAR-γ-responsive adipose tissue-specific genes that have either no detectable or very low basal expression in the liver. The treatment of lean control mice with troglitazone significantly increased the expression of adipocyte fatty acid-binding protein (aP2) and fatty acid translocase (FAT/CD36) in the liver. This troglitazone-induced increase in the expression of aP2 and FAT/CD36 was markedly enhanced in the liver in *ob/ob* mice. Troglitazone also induced a pronounced increase in the expression of uncoupling protein-2 in the liver in *ob/ob* mice. In contrast to the liver, troglitazone did not increase the expression of aP2, FAT/CD36, and uncoupling protein-2 in adipose tissue in lean or *ob/ob* mice. Taken together, our results suggest that the effects of PPAR-γ activators on lipid metabolism and energy homeostasis in obesity and type 2 diabetes may be partly mediated through their effects on PPAR-γ in the liver. (*Endocrinology* **141:** 4021–4031, 2000)

PEROXISOME proliferator-activated receptors (PPARs) are well characterized transcription factors that are members of the nuclear hormone receptor superfamily (1, 2). There are three subtypes of PPARs, namely, α, δ, and γ, that have distinct tissue distribution patterns. PPAR-α is mainly present in liver, heart, and kidney (3). PPAR-δ is ubiquitously expressed, whereas PPAR-γ is predominantly expressed in adipose tissue and to a lesser extent in spleen, cells of the hemopoietic system, liver, and skeletal muscle (3–5). PPARs form heterodimers with the retinoid X receptor and bind to specific PPAR response elements in the promoter region of their target genes (1, 2). Several endogenous ligands and drugs have been identified that bind to PPARs and activate gene transcription. Fatty acids such as arachidonic acid and its analog, 5,8,11,14-eicosatetraynoic acid; hypolipidemic drugs such as fibrates and Wy-14,463; and leukotriene B4 preferentially bind to PPAR-α (1, 2). On the other hand, PPAR-γ is activated by polyunsaturated fatty acids, prostaglandin J2, and the thiazolidinedione group of drugs that includes troglitazone, rosiglitazone, and pioglitazone (1, 2). Thiazolidinediones are insulin-sensitizing agents (6) that have recently been approved for the treatment of type 2 diabetes.

PPARs play an important role in the regulation of genes involved in lipid utilization and storage, lipoprotein metabolism, adipocyte differentiation, and insulin action (7–10). Specifically, PPAR-α regulates the expression of genes that

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encode for enzymes involved in peroxisomal proliferation and fatty acid oxidation in peroxisomes and mitochondria (7). Some of the these PPAR-α-responsive genes include acyl-coenzyme A (acyl-CoA) synthetase, acyl-CoA oxidase, acyl-CoA dehydrogenase, and carnitine palmitoyltransferase I. Additionally, genes coding for apoliopoproteins AI, AII, and CIII are also altered by PPAR-α (8) suggesting a role for PPAR-α in the regulation of lipoprotein metabolism.

In contrast to PPAR-α, PPAR-γ plays an important role in the regulation of genes involved in adipocyte differentiation, lipid storage, and glucose metabolism. It has been shown that PPAR-γ regulates the transcription of adipocyte fatty acid-binding protein (aP2), lipoprotein lipase, and phosphoenolpyruvate carboxykinase in adipose tissue (9, 10). Additionally, PPAR-γ activators up-regulate the expression of acyl-CoA synthetase, fatty acid transporters, and uncoupling protein-2 (UCP2), and down-regulate the expression of leptin and tumor necrosis factor-α in adipocytes (9, 10).

Obesity and type 2 diabetes in both humans and animal models are associated with several abnormalities of lipid metabolism and insulin resistance (11). Despite the significant role of PPARs in the regulation of lipid metabolism and insulin action, limited data are available on the expression and regulation of PPAR-α and/or PPAR-γ in obesity and diabetes. PPAR-α messenger RNA (mRNA) levels are increased in streptozotocin-induced diabetic rat liver (12), whereas PPAR-γ expression is reduced in streptozotocin-induced diabetic mouse adipose tissue (13). The mRNA expression of PPAR-γ is not altered in adipose tissue in leptin-deficient *ob/ob* or gold thioglucose-treated obese mice (13). Similarly, studies in humans have found no difference in the mRNA levels of PPAR-α, -δ, or -γ in adipose tissue among lean controls or obese or type 2 diabetic subjects (14).

The liver plays a pivotal role in the regulation of fatty acid and lipoprotein metabolism. Although PPAR-α is abundantly expressed in liver, basal expression of PPAR-γ in the liver is very low (3–5). A recent study reported increased expression of PPAR-γ mRNA in the liver in *ob/ob* mice (15). However, it is not known whether other rodent models of obesity display similar changes or whether increased PPAR-γ expression in the liver in *ob/ob* mice has any functional consequences. The expression of PPAR-α and PPAR-δ in the liver in rodent models of obesity has not been reported. In the present study we examined the mRNA expression of PPAR-α, -δ, and -γ in the liver in three murine models of obesity, namely, *ob/ob* (leptin-deficient), *db/db* (leptin-receptor deficient), and serotonin 5-HT2c receptor (5-HT2cR) mutant mice. 5-HT2cR mutant mice develop a late-onset obesity that is associated with higher plasma leptin levels (16). To determine whether increased hepatic PPAR-γ mRNA expression has any functional consequences, we compared the effects of troglitazone (a PPAR-γ activator) treatment on the expression of several PPAR-γ-responsive genes in liver and adipose tissue of lean control and *ob/ob* mice.

Materials and Methods

Materials

[α-^{32}P]Deoxy-CTP (3000 Ci/mmol, 10 mCi/ml) was purchased from NEN Life Science Products (Boston, MA). The multiprime DNA labeling system was purchased from Amersham International (Aylesbury, UK); minispin G-50 columns were obtained from Worthington Biochemical Corp. (Freehold, NJ); Oligo(deoxythymidine) cellulose type 77F was purchased from Pharmacia LKB Biotechnology AB (Uppsala, Sweden); nitrocellulose and Nytran were obtained from Schleicher & Schuell, Inc. (Keene, OH). The complementary DNA (cDNA) clones for PPAR-α, -δ, and -γ were provided by Dr. Nathan M. Bass, University of California (San Francisco, CA). Kodak XAR5 film (Eastman Kodak Co., Rochester, NY) was used for autoradiography.

Animals

Male *ob/ob* (strain C57BL/6J *ob/ob*), *db/db* (strain C57BL/KsJ *db/db*), and their respective age-matched lean littermates were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were maintained in a normal light cycle room (light from 0600–1800 h; dark from 1800–0600 h) at controlled temperature conditions and were provided with standard rodent chow (Simonsen Laboratories, Gilroy, CA) and water *ad libitum*. Mice were allowed to acclimatize for at least 1 week before study. To examine the effect of PPAR-γ activation, control and *ob/ob* mice were fed powdered chow or powdered chow containing troglitazone (Parke-Davis, Ann Arbor, MI) at a concentration of 200 mg/100 g chow for 10 days. The food consumption of mice was monitored daily, and the chow was replaced. Food consumption of both groups of mice remained constant throughout the experiment. At the end of all experiments mice were anesthetized with halothane, then tissues were obtained, frozen in liquid nitrogen, and stored at −70 C until further analysis. All animal experimentations were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and were approved by an institutional review board.

Generation of obese mice lacking serotonin 5-HT2c receptors

5-HT2cR mutant mice were generated by introducing a nonsense mutation into exon 5 of the cognate gene (17), thereby placing a stop codon within the fifth putative *trans*-membrane segment of the receptor and eliminating the carboxyl-terminal half of the protein. To reduce genetic heterogeneity, the 5-HT2cR mutant mice were backcrossed for 13–14 generations to a C57BL/6 background. Genotyping was performed by PCR analysis of DNA from tail tissue. The growth curves of the resultant mutant mice demonstrated the development of obesity of late onset, with body weights of mutant mice diverging from wild-type mice after about 20 weeks of age despite the fact that both young and old mutant mice were hyperphagic (16). Both 3-month-old (hyperphagic but not obese) and 9-month-old (hyperphagic and obese) mutant mice were used for measuring PPAR-α and -γ mRNA levels in the liver.

Isolation of RNA and Northern blotting

Total RNA was isolated by a variation of the guanidinium thiocyanate method (18) as described previously (19). Total RNA from adipose tissue was used for Northern blotting, whereas polyadenylated [poly(A)$^+$] RNA from liver was isolated using oligo(deoxythymidine) cellulose. Total or poly(A)$^+$ RNA was quantified by measuring the absorption at 260 nm. Equal amounts of total (20 μg/lane) or poly(A)$^+$ RNA (10 μg/lane) were loaded on 1% agarose-formaldehyde gels and electrophoresed. The uniformity of sample application was checked by UV visualization of the acridine orange-stained gels before transfer to Nytran membranes. The cDNA probe hybridization was performed as described previously (19). The blots were exposed to x-ray films at −70 C for various durations (indicated in the figure legends) to ensure that measurements were made on the linear portion of curve, and the bands were quantified by densitometry. The blots were also probed for cyclophilin as a housekeeping gene, and the densitometric values were normalized relative to cyclophilin.

Serum chemistry and total hepatic lipids

Serum glucose, triglycerides, cholesterol, FFA, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels were measured using standard enzyme assays kits. The total lipid contents in the liver in control and *ob/ob* mice treated with troglitazone were measured by the Bligh-Dyer technique (20).

Statistics

The results are presented as the mean ± SEM. Statistical significance between two groups was determined using Student's *t* test. Comparisons among several groups were performed by ANOVA, and statistical significance was calculated by using Bonferroni's multiple comparison test.

Results

Expression of PPAR isoforms in the liver in murine models of obesity

We initially examined the mRNA expression of three PPAR isoforms in the liver in *ob/ob* and *db/db* mice (8 weeks

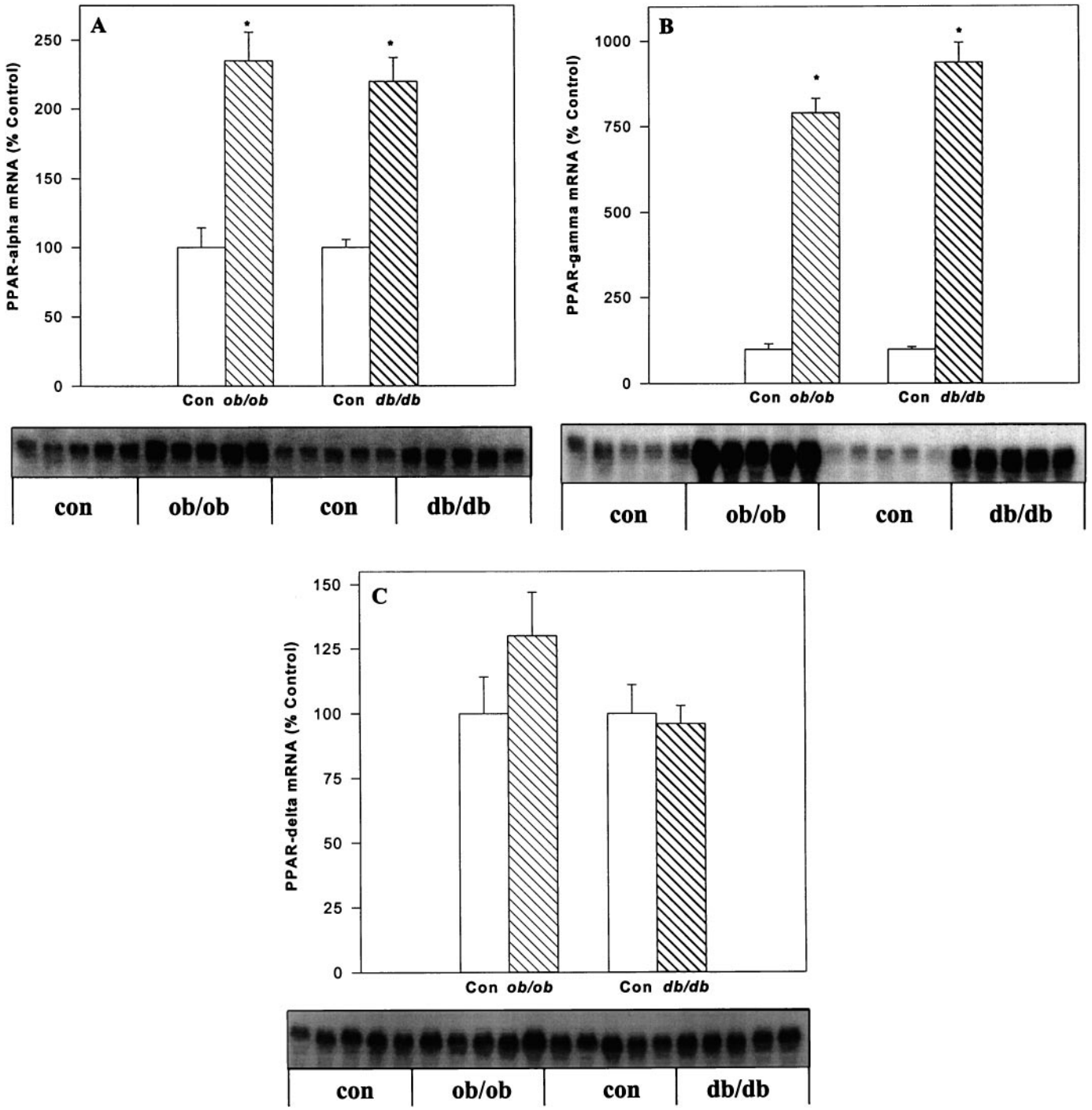

FIG. 1. PPAR mRNA levels in the liver of *ob/ob* and *db/db* mice. Age- and sex-matched control and obese mice were killed, and livers were obtained for poly(A)$^+$ RNA isolation. Northern blots were prepared and probed with PPAR-α (A), PPAR-γ (B), and PPAR-δ (C) cDNAs as described in *Materials and Methods*. The duration of exposure of the film was 16 h for PPAR-α and PPAR-γ, and 24 h for PPAR-δ. Data are presented as the mean ± SEM percentage of the control value, as quantified by densitometry and normalized to cyclophilin (n = 5 for each group). A: *, $P < 0.001$; B: *, $P < 0.001$.

age) and their age- and sex-matched lean littermates (Fig. 1). PPAR-α mRNA was abundantly expressed in the liver in control animals and was increased by 2.3-fold in *ob/ob* and by 2.2-fold in *db/db* mice (Fig. 1A). On the other hand, the basal expression of PPAR-γ was relatively low in control animals, but PPAR-γ mRNA levels were increased by 7.9-fold in *ob/ob* and by 9.4-fold in *db/db* mice compared with those in their lean littermates (Fig. 1B). PPAR-δ was also abundantly present in the liver in control animals; however, PPAR-δ mRNA levels in *ob/ob* or *db/db* mice were not significantly different from those in controls (Fig. 1C). As reported by others (13), PPAR-γ mRNA levels in adipose tissue were not significantly different between control and *ob/ob* or *db/db* mice (data not shown).

We next compared hepatic PPAR-α and PPAR-γ mRNA levels in young (3 months) and old (9 months) 5-HT2cR mutant mice to those in their wild-type littermates. 5-HT2cR mutant mice develop late-onset obesity (16). Although both young and old 5-HT2cR mutant mice are hyperphagic, only old (9 months) mice are modestly obese (40% overweight compared with genetically identical, wild-type littermates). The data presented in Fig. 2A demonstrate that PPAR-α mRNA levels in wild-type and young 5-HT2cR mutant mice are the same; however, there is a 2.1-fold increase in PPAR-α mRNA levels in the old obese 5-HT2cR mutant mice compared with those in the age-matched wild-type littermates. Similarly, PPAR-γ mRNA levels were comparable in young control and 5-HT2cR mutant mice, whereas there was a 2.2-fold increase in PPAR-γ mRNA expression in the liver in old obese 5-HT2cR mutant mice (Fig. 2B). These data suggest that the increase in both PPAR-α and PPAR-γ mRNA expression is related to the development of obesity in this model.

To determine the relationship between the onset of obesity and the increase in PPAR-α and -γ mRNA expression in the liver, we next examined PPAR-α and -γ mRNA levels in the liver of *ob/ob* mice at the age of 5 and 10 weeks. The *ob/ob* mice were only modestly obese (body weight 40% over that in age-matched controls) at the age of 5 weeks, whereas they weighed almost 150% over age-matched controls at the age of 10 weeks. The data presented in Fig. 3A demonstrate that PPAR-α mRNA levels in the liver were comparable between control and *ob/ob* mice at the age of 5 weeks and were increased by 2.8-fold in *ob/ob* mice at the age of 10 weeks. On the other hand, PPAR-γ mRNA levels in the liver were increased by 7.7- and 9.3-fold in 5- and 10-week-old *ob/ob* mice, respectively (Fig. 3B). These data suggest that the increase in PPAR-γ mRNA expression in the liver occurs earlier than that in PPAR-α during the course of obesity.

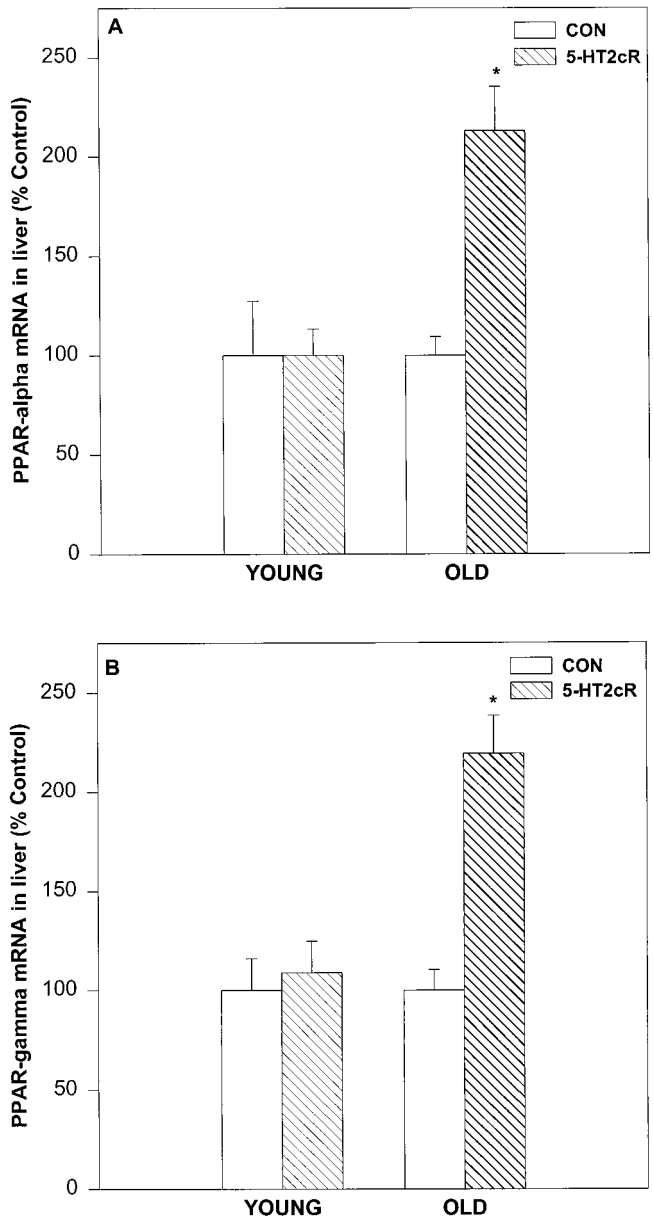

FIG. 2. PPAR-α and PPAR-γ mRNA levels in the liver of young and old 5-HT2cR mutant mice. Young (3 months) and old (9 months) 5-HT2cR mutant mice and their age- and sex-matched littermates were killed, and the livers were obtained for poly(A)$^+$ RNA isolation. Northern blots were prepared and probed with PPAR-α (A) and PPAR-γ (B) cDNAs as described in *Materials and Methods*. Data are presented as the mean ± SEM percentage of the control value, as quantified by densitometry and normalized to cyclophilin (n = 5 for each group). A: *, $P < 0.002$; B: *, $P < 0.001$.

Effect of troglitazone on PPAR-γ-responsive genes in liver and adipose tissue of ob/ob mice

Troglitazone is a member of the thiazolidinedione group of drugs that are synthetic ligands for PPAR-γ and are known to activate the transcription of genes that contain PPAR response elements (6). To determine whether increased expression of PPAR-γ in the liver in *ob/ob* mice has any functional consequences, we examined the effect of troglitazone treatment (200 mg/100 g chow for 10 days) on several PPAR-γ-responsive genes, including aP2, FAT/CD36, and UCP2 mRNA levels in the liver. Like PPAR-γ, all of these proteins are predominantly expressed in adipose tissue and have either no detectable or very low basal expression in the liver of control animals. We and others have recently shown that FAT/CD36 and UCP2 are overexpressed in the liver of *ob/ob* mice (19, 21, 22). We hypothesized that if PPAR-γ in the liver of *ob/ob* mice is functionally active, then troglitazone treatment should induce mRNA expression of aP2, FAT/CD36, and UCP2 in the liver of *ob/ob* mice. The data presented in

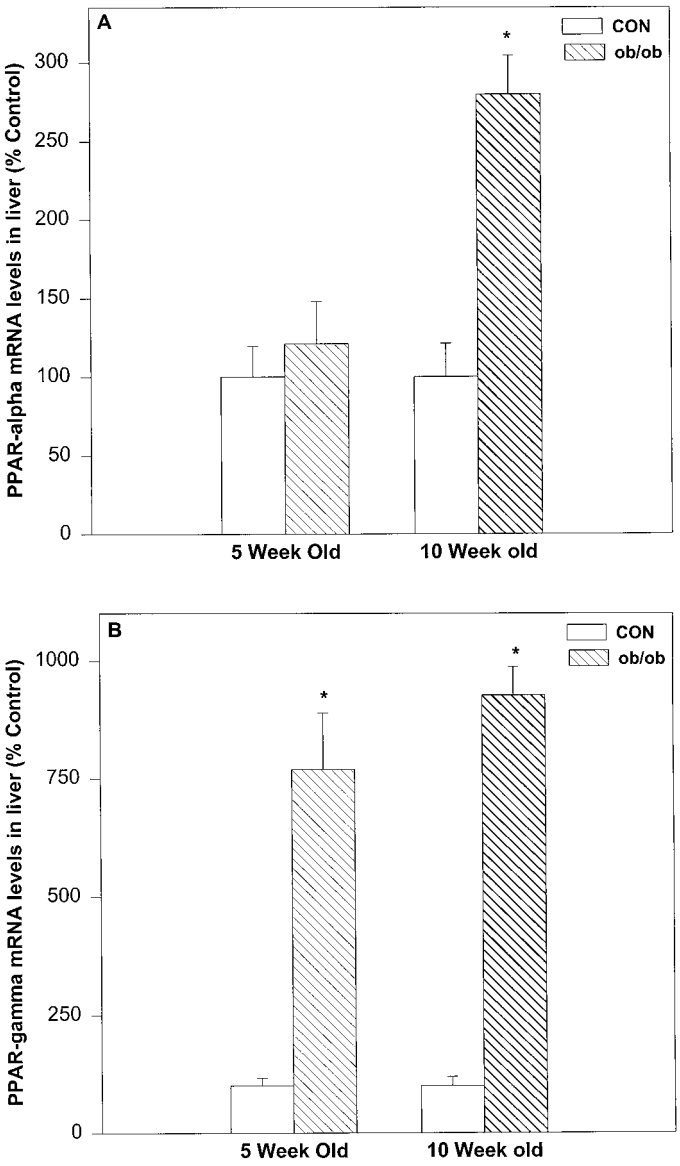

FIG. 3. Effect of age on PPAR-α and PPAR-γ mRNA levels in the liver of *ob/ob* mice. Five- and 10-week-old *ob/ob* mice and their age- and sex-matched control littermates were killed, and livers were obtained for poly(A)$^+$ RNA isolation. Northern blots were prepared and probed with PPAR-α (A) and PPAR-γ (B) cDNAs as described in *Materials and Methods*. Data are presented as the mean ± SEM percentage of the control value, as quantified by densitometry and normalized to cyclophilin (n = 5 for each group). A: *, $P < 0.001$; B: *, $P < 0.001$.

Table 1 demonstrate that, as expected, 10 days of troglitazone treatment significantly lowered serum glucose, triglyceride, and FFA levels in the *ob/ob* mice. As clinical studies have shown that troglitazone may produce liver damage in a small percentage of patients undergoing therapy (23), we also measured serum transaminase levels in these animals. Both serum ALT and AST levels were significantly higher in *ob/ob* mice (Table 1); however, troglitazone had no significant effect on serum ALT and AST levels (Table 1) in lean or obese mice.

Figure 4A presents Northern blots showing the expression of aP2, FAT/CD36, and UCP2 in the liver in control and *ob/ob* mice treated with troglitazone, and the densitometric analysis of the each Northern blot is presented in fig. 4, B–D. Because the basal expression of these genes is very low in liver, the fold increase observed here should only be considered a rough estimate of induction. Northern blots showing the expression of PPAR-γ (the target receptor for troglitazone) and cyclophilin (a housekeeping gene used as an internal control) are also presented in Fig. 4A and demonstrate that PPAR-γ and cyclophilin mRNA levels are not altered by troglitazone treatment in control and *ob/ob* mice. As shown previously (19, 21, 22), there was a marked increase in FAT/CD36 (32-fold) and UCP2 (3.5-fold) expression in the liver in *ob/ob* mice, whereas there was a slight increase in aP2 expression in the liver in *ob/ob* mice. In lean controls, troglitazone significantly increased aP2 (6-fold) and FAT/CD36 (8-fold) mRNA expression in the liver, but had no effect on hepatic UCP2 expression. Compared with lean mice, troglitazone produced a marked increase in aP2 (170-fold), FAT/CD36 (62-fold), and UCP2 (7-fold) mRNA levels in the liver of *ob/ob* mice. Compared with untreated *ob/ob* mice, the troglitazone-induced increase was 2-fold for FAT/CD36 and UCP2 and 57-fold for aP2 expression in the liver of *ob/ob* mice. These data suggest that activation of hepatic PPAR-γ by troglitazone induces the transcription of PPAR-γ-responsive genes in the liver.

To exclude the possibility that adipocyte infiltration in the liver of *ob/ob* mice may be responsible for the pronounced increase seen in hepatic aP2, FAT, or UCP2 mRNA with troglitazone treatment, the liver Northern blots were also hybridized with cDNA for the glucose transporter GLUT4 that is primarily expressed in adipose tissue and skeletal muscle. No GLUT4 signal was detectable in either control or *ob/ob* livers (both untreated and troglitazone treated) after up to 48 h of exposure (data not shown), suggesting that the increase in PPAR-γ-responsive genes in the liver is selective and is not due to adipocyte contamination of the *ob/ob* liver.

We also examined the effect of troglitazone treatment on PPAR-α-responsive genes, including acyl-CoA oxidase (ACO) and carnitine palmitoyltransferase (CPT I). These genes contain PPAR response elements, and it has been suggested that PPAR-γ agonists may also activate PPAR-α-responsive genes (15). The data presented in Fig. 5A show that the mRNA levels of ACO in the liver are slightly higher in untreated *ob/ob* mice, and are increased (5-fold) by troglitazone treatment in *ob/ob* mice. On the other hand, CPT I mRNA levels were 2-fold higher in the liver in untreated *ob/ob* mice and were not further increased by troglitazone in *ob/ob* mice (Fig. 5B). The mRNA levels of apolipoprotein CIII, another PPAR-α-responsive gene, were neither altered in *ob/ob* mice nor influenced by troglitazone treatment (data not shown), suggesting that only some of the PPAR-α-responsive genes are altered by treatment with troglitazone. The mRNA levels of PPAR-α itself were also not altered by troglitazone treatment in lean or obese mice (data not shown).

To examine the effect of troglitazone-induced up-regulation of PPAR-responsive genes in the liver on lipid accumulation, we measured total hepatic lipid content in both control and *ob/ob* mice after troglitazone treatment (Fig. 6). The livers from *ob/ob* mice contained 59% more total lipids on a per g basis ($P < 0.001$). Although troglitazone treatment had

TABLE 1. Effects of troglitazone (Tro) treatment on serum glucose, triglyceride, cholesterol, free fatty acids, and transaminases in control and *ob/ob* mice

Exp group	Serum glucose (mmol/liter)	Serum triglycerides (mmol/liter)	Serum cholesterol (mmol/liter)	Serum FFA (mmol/liter)	Serum ALT (U/liter)	Serum AST (U/liter)
Control mice	8.04 ± 0.28	1.19 ± 0.06	1.96 ± 0.07	1.24 ± 0.07	44.8 ± 3.1	36.3 ± 4.3
Control + Tro	7.77 ± 0.39	0.98 ± 0.02	1.99 ± 0.08	1.15 ± 0.05	36.4 ± 2.7	34.1 ± 3.8
P value *vs.* control	NS	<0.02	NS	NS	NS	NS
ob/ob mice	20.6 ± 1.40	1.51 ± 0.06	4.45 ± 0.20	1.69 ± 0.07	99.9 ± 8.0	88.0 ± 8.9
P value *vs.* control	<0.001	<0.005	<0.001	<0.002	<0.001	<0.001
ob/ob + Tro	11.0 ± 0.85	0.68 ± 0.03	4.12 ± 0.23	1.04 ± 0.05	101.4 ± 9.9	74.8 ± 9.4
P value *vs. ob/ob*	<0.001	<0.001	NS	<0.001	NS	NS

Control and *ob/ob* mice were fed powdered chow or powdered chow containing troglitazone (200 mg/100 g chow) for 10 days. The food consumption, and the body weights of mice were monitored daily. At the end of 10-day treatment, mice were killed, and blood and tissues were obtained. Serum concentrations of glucose, triglycerides, cholesterol, free fatty acids (FFA), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured by standard enzyme assay kits.

no significant effect on total hepatic lipid content in control mice, it produced a 19% decrease in total lipid content in the liver of *ob/ob* mice ($P < 0.05$), suggesting that troglitazone may enhance the utilization of lipids in the liver of obese mice.

We next examined the effect of troglitazone treatment on aP2, FAT/CD36, and UCP2 mRNA levels in adipose tissue in control and *ob/ob* mice. It is important to note that PPAR-γ expression is not altered in adipose tissue in obese mice. Figure 7A presents Northern blots showing the expression of aP2, FAT/CD36, and UCP2 in adipose tissue in control and *ob/ob* mice treated with troglitazone, and the densitometric analysis of the each Northern blot is presented in Fig. 7, B–D. As shown previously (19, 22), the mRNA levels of FAT/CD36 and UCP2 were increased in adipose tissue of untreated *ob/ob* mice, whereas aP2 mRNA expression was slightly decreased (Fig. 7A). In lean controls, troglitazone increased aP2 mRNA levels by 40%, and a similar trend was seen in *ob/ob* mice (Fig. 7B). Troglitazone significantly decreased FAT/CD36 mRNA levels in adipose tissue of both control and *ob/ob* mice (Fig. 7C). Troglitazone slightly decreased UCP2 mRNA levels in adipose tissue of control mice, and a similar trend was seen in *ob/ob* mice (Fig. 7D). Hence, consistent with no significant change in PPAR-γ expression in adipose tissue of obese mice, there was no significant difference in troglitazone action on adipose tissue between lean and obese mice.

Discussion

Despite the importance of PPARs in the regulation of lipid metabolism and energy homeostasis, very few studies have examined the expression and regulation of different PPAR isoforms in rodent models of obesity. In an early study Vidal-Puig *et al.* examined the association between PPAR-γ and obesity and reported that PPAR-γ expression was not altered in the adipose tissue in either genetic (*ob/ob* mice) or acquired (gold-thioglucose-induced) models of obesity (13). Similarly, no differences were found in PPAR-γ expression in adipose tissue between lean controls and Zucker fatty rats (24). However, a recent study reported that PPAR-γ expression is increased in the liver of *ob/ob* mice (15).

In the present study we have extended these observations and report that both PPAR-α and PPAR-γ mRNA levels are markedly increased in the liver of *ob/ob* (leptin-deficient) and *db/db* (leptin receptor deficient) mice, whereas hepatic PPAR-δ mRNA expression and adipose tissue PPAR-γ expression are not altered in these models. The increase in PPAR-γ mRNA levels in the liver in *ob/ob* and *db/db* mice is more pronounced compared with the increase in PPAR-α mRNA expression. Moreover, the up-regulation of PPAR-γ in the liver of *ob/ob* mice appears to be more sensitive to the development of obesity, as it is seen at an earlier age (5 weeks) when these animals are only 40% overweight compared with their lean littermates. The hepatic expression of PPAR-α is not elevated at this age in *ob/ob* mice.

Our results also demonstrate that both PPAR-α and PPAR-γ mRNA levels in the liver are increased in old (obese) 5-HT2cR mutant mice, but are not altered in young (lean) 5-HT2cR mutant mice. The lack of change in PPAR-α and PPAR-γ mRNA levels in young 5-HT2cR mutant mice and the increase in old 5-HT2cR mutant mice further support the concept that the increased expression of PPAR-α and PPAR-γ mRNA in the liver is related to the development of obesity. The obesity in 5-HT2cR mutant mice is different from that in *ob/ob* and *db/db* mice in several respects. First, 5-HT2cR mice develop a modest obesity (30–40% increase in body weight), whereas *ob/ob* and *db/db* mice are markedly obese (100–150% increase in body weight). Second, obesity develops late in 5-HT2cR mice (by 8–9 months of age), whereas *ob/ob* and *db/db* mice develop obesity as early as 4 weeks of age. Third, young 5-HT2cR mutant mice have normal plasma leptin levels and are sensitive to the anorectic effects of exogenous leptin, whereas old 5-HT2cR mutant mice have higher plasma leptin levels and are partially resistant to the anorectic effect of exogenous leptin (16). In contrast, *ob/ob* and *db/db* mice are leptin deficient and leptin receptor deficient, respectively (25, 26). Fourth, although obese 5-HT2cR mutant mice develop glucose intolerance and insulin resistance, they do not develop hyperlipidemia (16), which is commonly seen in both *ob/ob* and *db/db* mice. Despite these differences, a common denominator among these three murine models is that development of obesity is accompanied by increased expression of PPAR-α and PPAR-γ in the liver.

In the present study we also demonstrate that up-regulation of hepatic PPAR-γ allows the activation of several PPAR-γ-responsive genes (aP2, FAT/CD36, and UCP2) in the liver by troglitazone. Like PPAR-γ itself, these genes are predominantly expressed in adipose tissue and have either no detectable or very low basal expression in the liver of lean control animals. We and others have recently reported that

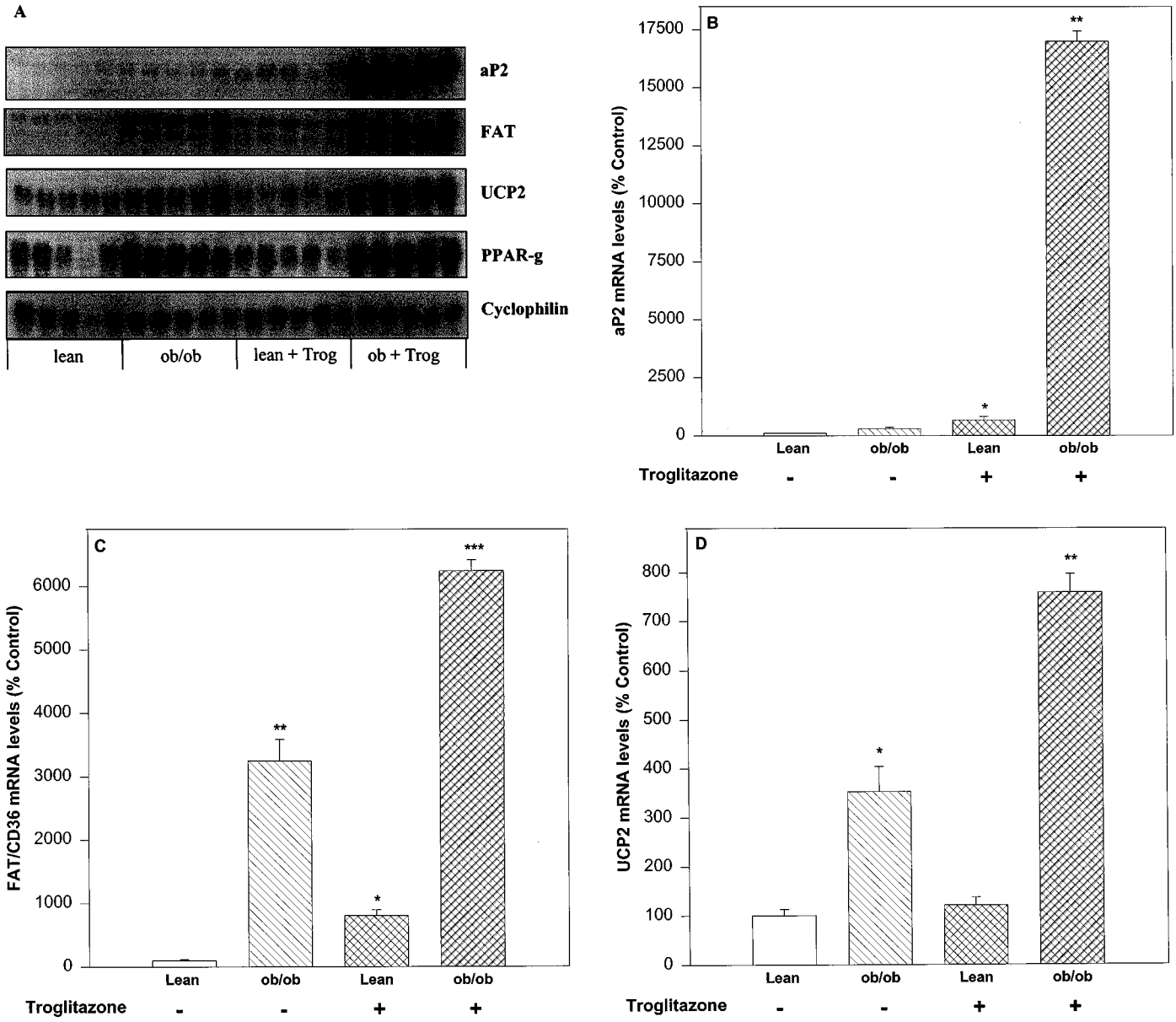

FIG. 4. Effect of troglitazone treatment on PPAR-γ-responsive genes in the liver of control and *ob/ob* mice. Control and *ob/ob* mice were fed a diet containing troglitazone (200 mg/100 g chow) for 10 days. At the end of treatment, mice were killed, and livers were obtained for poly(A)$^+$ RNA isolation. Northern blots were prepared and probed with aP2, FAT/CD36, and UCP2 cDNAs as described in *Materials and Methods*. A, Northern blot showing effects of troglitazone on aP2, FAT/CD36, and UCP2 mRNA in the liver. B—D, Data for aP2 (B), FAT/CD36 (C), and UCP2 (D) mRNAs in experimental groups presented as a percentage of the control value, as quantified by densitometry. Data are presented as the mean ± SEM (n = 5 for each group). B: *, $P < 0.05$ *vs.* untreated control mice; **, $P < 0.001$ *vs.* treated control and untreated *ob/ob* mice. C: *, $P < 0.05$ *vs.* untreated control mice; **, $P < 0.001$ *vs.* untreated control mice; ***, $P < 0.001$ *vs.* treated control and untreated *ob/ob* mice. D: *, $P < 0.001$ *vs.* untreated control mice; **, $P < 0.001$ *vs.* treated control and untreated *ob/ob* mice.

FAT/CD36 and UCP2 mRNA expression is significantly increased in the liver in *ob/ob* mice (19, 21, 22), whereas aP2 expression in the liver is not significantly altered (present study) in *ob/ob* mice. Our present results demonstrate that troglitazone, a synthetic ligand for PPAR-γ, significantly increases mRNA levels of aP2 and FAT/CD36 in the liver of lean control mice. Moreover, troglitazone markedly enhances the mRNA expression of aP2, FAT/CD36, and UCP2 in the liver of *ob/ob* mice. It is possible that the increase in FAT/CD36 and UCP2 expression in the liver of untreated *ob/ob* mice (19, 21, 22) is due to activation of elevated hepatic PPAR-γ by endogenous ligands. Similarly, the marked induction of aP2, FAT/CD36, and UCP2 in the *ob/ob* liver by troglitazone can be attributed to its ability to bind to hepatic PPAR-γ, resulting in the enhanced transcription of these genes in the liver. These results raise the possibility that the effects of PPAR-γ activators on lipid metabolism and energy balance may partly be mediated through their effects in the liver. Our data demonstrating that total hepatic lipid content is significantly decreased in the liver of obese mice after troglitazone treatment suggest that PPAR-γ activators may increase the utilization of lipids in the liver in obese diabetic

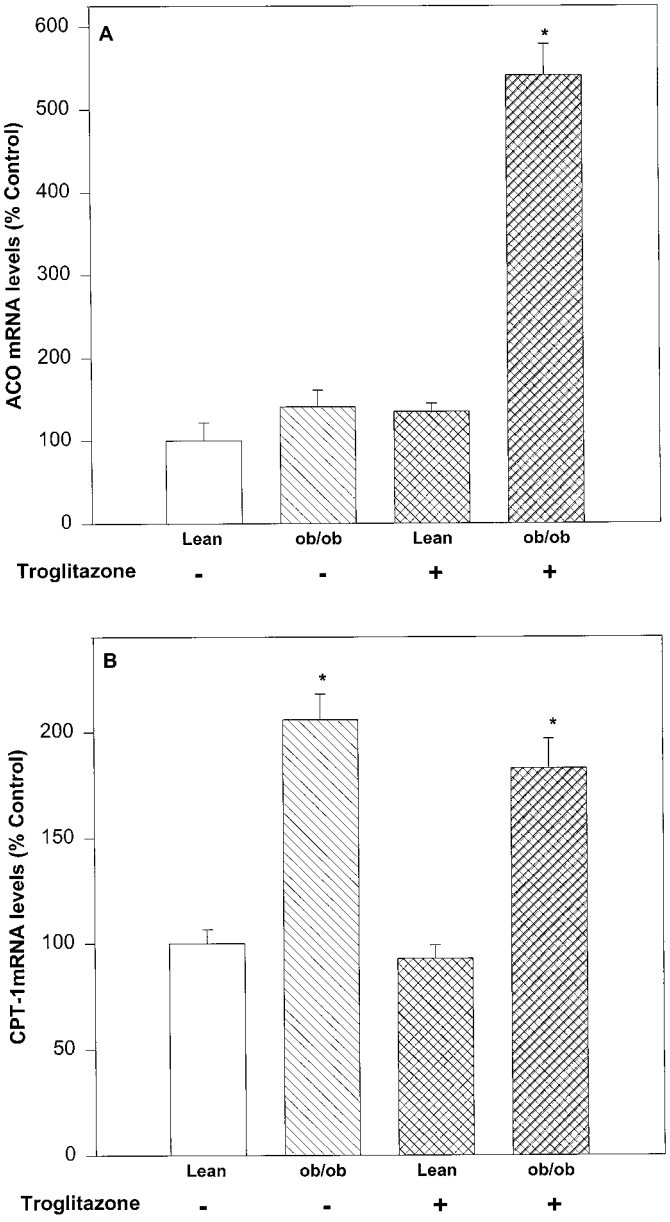

FIG. 5. Effect of troglitazone treatment on PPAR-α-responsive genes in the liver of control and *ob/ob* mice. Control and *ob/ob* mice were fed a diet containing troglitazone (200 mg/100 g chow) for 10 days. At the end of treatment mice were killed, and livers were obtained for poly(A)$^+$ RNA isolation. Northern blots were prepared and probed with ACO (A) and CPT I (B) cDNAs as described in *Materials and Methods*. Data are presented as the mean ± SEM percentage of the control value, as quantified by densitometry (n = 5 for each group). A: *, $P < 0.001$ *vs.* control and untreated *ob/ob* mice. B: *, $P < 0.001$ *vs.* control mice.

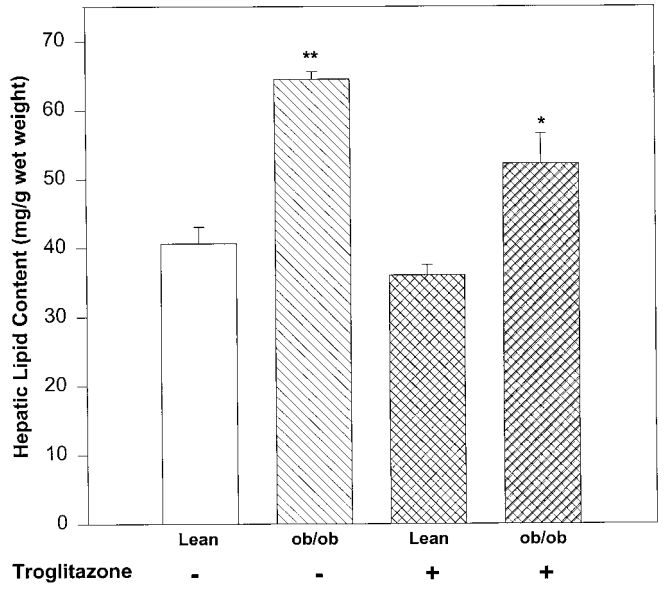

FIG. 6. Effect of troglitazone treatment on total lipid content in the liver in control and *ob/ob* mice. Control and *ob/ob* mice were fed a diet containing troglitazone (200 mg/100 g chow) for 10 days. At the end of treatment mice were killed, and livers were obtained. The lipids were extracted by the Bligh-Dyer technique as described in *Materials and Methods*. Data are presented as the mean ± SEM (n = 5 for each group). *, $P < 0.05$ *vs.* untreated *ob/ob* mice. B: **, $P < 0.001$ *vs.* control mice.

mice, and this may be an additional mechanism by which PPAR-γ activators decrease serum lipid levels. Our hypothesis is further supported by the studies by Burant *et al.* (27), who demonstrated that troglitazone improved insulin sensitivity and lowered serum lipid levels in a transgenic mouse model in which white and brown adipose tissues were ablated through targeted expression of diphtheria toxin A chain under the control of the adipocyte-specific aP2 promoter.

Because PPAR-α and PPAR-γ recognize similar DNA response elements, Edvardsson *et al.* proposed that thiazolidinediones may activate PPAR-α-responsive genes in the liver of obese mice (15). This hypothesis was based on semiquantitiative data obtained from analysis of two-dimensional gels of proteins from livers of *ob/ob* mice treated with agonists of PPAR-α (WY14,643) and PPAR-γ (BRL 49653). Their results showed that spots with the mobilities of ACO, peroxisomal bifunctional enzyme, and 3-ketoacyl thiolase were up-regulated by WY14,643 and BRL 49653 (15). Our results demonstrate that troglitazone, a well characterized PPAR-γ agonist, increases the mRNA expression of ACO, but has no effect on CPT I or apolipoprotein CIII mRNA levels. It is important to note that ACO is involved in peroxisomal oxidation of fatty acids, whereas CPT I is the major regulatory protein in mitochondrial fatty acid oxidation (7). On the other hand, apolipoprotein CIII is a potent inhibitor of lipoprotein lipase (8). All of these genes contain PPAR response elements and are activated by PPAR-α agonists (1, 2, 7, 8). The ability of troglitazone to induce some of the PPAR-α-responsive genes (*i.e.* ACO) raises the possibility that troglitazone may be an activator of both PPAR-γ and PPAR-α isoforms or that activation of PPAR-γ is able to interact with response elements in certain PPAR-α-responsive genes. The inability of troglitazone to induce other PPAR-α-responsive genes such as CPT I and apolipoprotein CIII suggests that these proteins may require the presence of other coactivators or may be under the dominant regulatory control of other transcription factors.

Although others have previously shown that various thiazolidinediones induce aP2 mRNA expression in differentiating cultured white adipocytes (28), the results obtained *in*

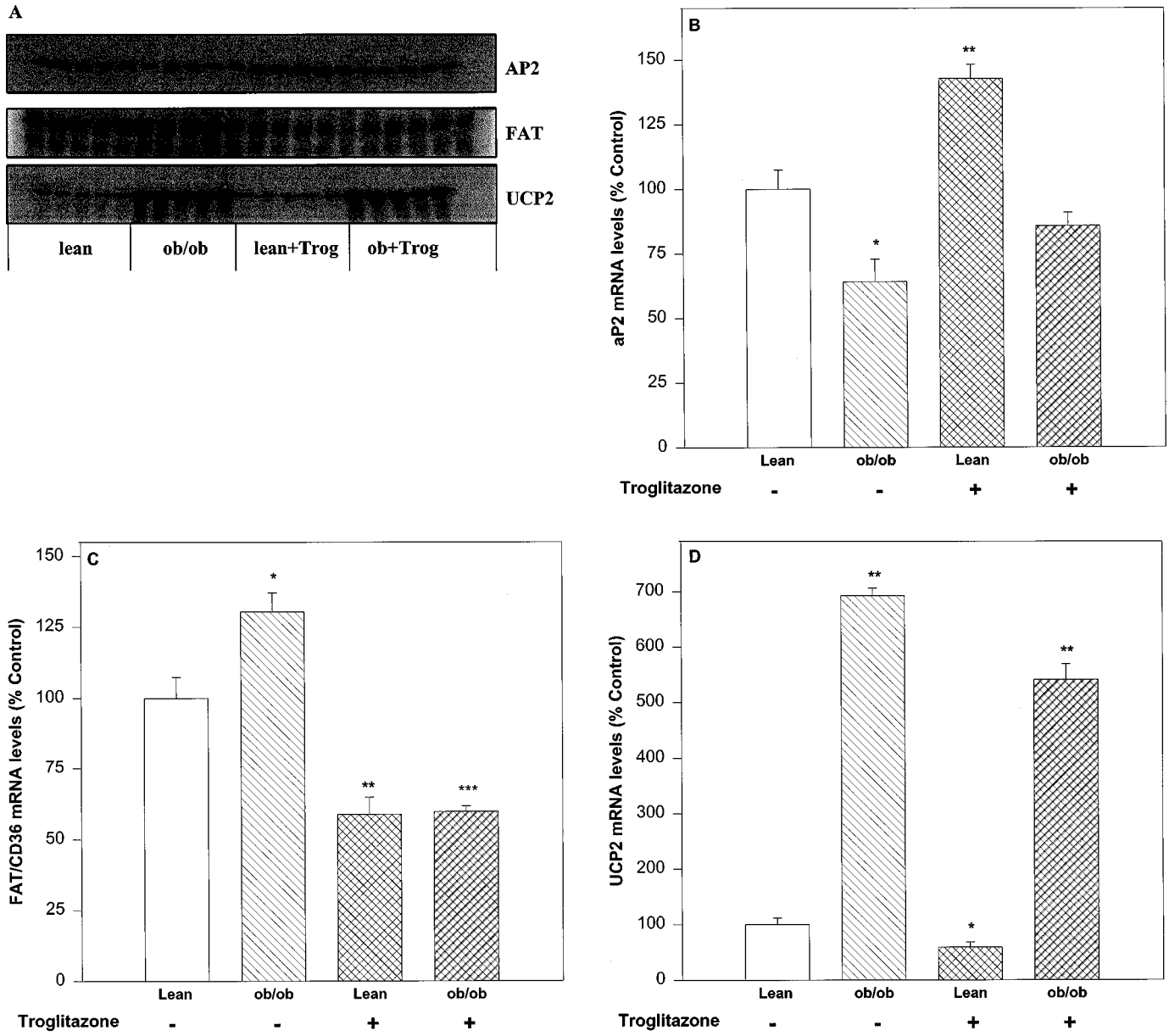

FIG. 7. Effect of troglitazone treatment on PPAR-γ-responsive genes in adipose tissue in control and *ob/ob* mice. Control and *ob/ob* mice were fed a diet containing troglitazone (200 mg/100 g chow) for 10 days. At the end of treatment, mice were killed, and adipose tissue was obtained for total RNA isolation. Northern blots were prepared and probed with aP2, FAT/CD36, and UCP2 cDNAs as described in *Materials and Methods*. A, Northern blot showing the effects of troglitazone on aP2, FAT/CD36, and UCP2 mRNA in the liver. B—E, Data for aP2 (B), FAT/CD36 (C), and UCP2 (D) mRNAs in experimental groups presented as a percentage of the control value, as quantified by densitometry. Data are presented as the mean ± SEM (n = 5 for each group). B: *, $P < 0.05$ *vs.* lean mice; **, $P < 0.05$ *vs.* untreated control mice. C: *, $P < 0.05$ *vs.* lean mice; **, $P < 0.01$ *vs.* untreated control mice; ***, $P < 0.001$ *vs.* untreated *ob/ob* mice. D: *, $P < 0.001$ *vs.* lean mice; **, $P < 0.05$ *vs.* untreated control mice.

vivo have been different. For example, BRL 49653 increased aP2 mRNA levels in adipose tissue in rats fed a high fat diet, but not in chow-fed rats (29). Similarly, although PPAR-γ activators increased UCP2 expression in cultured white and brown fat cells (30, 31), they had no effect on UCP2 mRNA levels in white adipose tissue in lean or Wistar fatty rats (32, 33). Our results demonstrate that despite producing a marked increase in aP2, FAT/CD36 and UCP2 mRNA expression in the liver, troglitazone had no significant stimulatory effect on the expression of these same genes in white adipose tissue from chow fed *ob/ob* mice. In fact, the expression of FAT/CD36 and UCP2 was significantly decreased, whereas mRNA levels of aP2 were not significantly different in adipose tissue of *ob/ob* mice after troglitazone treatment. The inability of troglitazone to induce the same set of PPAR-γ-responsive genes that are normally abundant in adipose tissue suggests that the stimulatory effect of troglitazone *in vivo* may depend upon several factors, such as the basal expression of specific genes as well as the local expression of PPAR-γ, and the presence of endogenous ligands. It is pos-

sible that because of higher basal expression of PPAR-γ in adipose tissue and abundant presence of endogenous ligands, *i.e.* fatty acids, these genes are already maximally induced, and hence, troglitazone is unable to produce any further increase in their transcription. A decrease in the expression of some of these genes by troglitazone in adipose tissue can be partly explained by the recently proposed hypothesis of Miles *et al.* (34) that PPAR-γ activators may act as partial agonist-antagonists *in vivo* and thus compete with putative endogenous agonists.

Because the exact physiological functions of aP2, UCP2, and FAT/CD36 are not known, we can only speculate on the consequences of their up-regulation in the *ob/ob* liver by troglitazone. aP2 is a member of family of intracellular fatty acid-binding proteins that binds long chain fatty acids with high affinity (35). It is possible that a troglitazone-induced increase in aP2 in the liver in *ob/ob* mice prevents the adverse effects of FFA on cells and membranes. UCPs have been proposed to be involved in the regulation of energy balance in part by increasing FA utilization (36). A troglitazone-induced increase in UCP2 expression could have both beneficial as well as harmful effects. On the one hand, UCP2 could dispose of excess energy by uncoupling mitochondrial respiration from oxidative phosphorylation, thereby increasing energy expenditure and using fatty acids. On the other hand, an increase in UCP2 could deplete hepatic ATP stores and make the liver more susceptible to injurious stimuli (21). Finally, FAT/CD36 is believed to facilitate fatty acid transport, promote phagocytosis of apoptotic cells, and function as a receptor for oxidized LDL (37). Recent studies by Aitman *et al.* have shown that a syndrome of spontaneous hypertension, hypertriglyceridemia, abdominal obesity, and insulin resistance in rats is due to a defective CD36 gene (38). Moreover, CD36 knockout mice have higher serum triglyceride and FFA levels (39). Conversely, overexpression of FAT/CD36 in transgenic mice reduces their serum triglyceride and FFA levels (40). It is possible that the troglitazone-induced increase in FAT/CD36 in the livers of obese mice may be an additional mechanism by which troglitazone lowers serum triglyceride levels and improves insulin sensitivity. As both PPAR-α and PPAR-γ are up-regulated in the liver in rodent models of obesity, it is possible that combination therapy with PPAR-α and PPAR-γ agonists may have a synergistic effect in lowering serum lipid levels and improving insulin sensitivity.

In summary, in this study we have shown that PPAR-α and PPAR-γ mRNA expression is up-regulated in the liver in three distinct murine models of obesity, whereas PPAR-δ expression is not altered. Moreover, treatment with troglitazone induces the expression of several PPAR-γ-responsive genes, including aP2, FAT/CD36, and UCP2, in the liver of *ob/ob* mice, raising the possibility that the effects of PPAR-γ agonists on lipid metabolism and energy balance may partly be mediated through their effects in the liver.

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