Liver Peroxisome Proliferator-activated Receptor γ Contributes to Hepatic Steatosis, Triglyceride Clearance, and Regulation of Body Fat Mass*

Oksana Gavrilova‡§, Martin Haluzik‡, Kimihiko Matsusue¶, Jaime J. Cutson‡, Lisa Johnson‡, Kelly R. Dietz‡, Christopher J. Nicoll‡, Charles Vinson¶, Frank J. Gonzalez¶, and Marc L. Reitman‡§

From the §Diabetes Branch, NIDDK and ¶Laboratory of Metabolism, NCI, National Institutes of Health, Bethesda, Maryland 20892

Peroxisome proliferator-activated receptor γ (PPARγ) is a nuclear receptor that mediates the antidiabetic effects of thiazolidinediones. PPARγ is present in adipose tissue and becomes elevated in fatty livers, but the roles of specific tissues in thiazolidinedione actions are unclear. We studied the function of liver PPARγ in both lipoatrophic A-ZIP/F-1 (AZIP) and wild type mice. In AZIP mice, ablation of liver PPARγ reduced the hepatic steatosis but worsened the hyperlipidemia, triglyceride clearance, and muscle insulin resistance. Inactivation of AZIP liver PPARγ also abolished the hypoglycemic and hypolipidemic effects of rosiglitazone, demonstrating that, in the absence of adipose tissue, the liver is a primary and major site of thiazolidinedione action. In contrast, rosiglitazone remained effective in non-lipoatrophic mice lacking liver PPARγ, suggesting that adipose tissue is the major site of thiazolidinedione action in typical mice with adipose tissue. Interestingly, mice without liver PPARγ, but with adipose tissue, developed relative fat intolerance, increased adiposity, hyperlipidemia, and insulin resistance. Thus, liver PPARγ regulates triglyceride homeostasis, contributing to hepatic steatosis, but protecting other tissues from peroxisome accumulation and insulin resistance.

*The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

†To whom correspondence should be addressed: Diabetes Branch, NIDDK, NIH, Bldg. 10, Rm. 8N-250, 10 Center Dr., Bethesda, MD 20892-1770. Tel.: 301-435-5370; Fax: 301-402-5788; E-mail: oksanag@bdg10.niddk.nih.gov.

‡Present address: Merck Research Laboratories, Rahway, New Jersey 07065.

§The abbreviations used are: PPARγ, peroxisome proliferator-activated receptor γ; TZD, thiazolidinediones; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase; SREBP-1, sterol response element-binding protein; LDLR, low density lipoprotein receptor; WAT, white adipose tissue; BAT, brown adipose tissue.

and, at higher doses, inhibit endogenous glucose production (largely a liver function) (7–9). However, muscle and liver have low PPARγ levels. Several lines of evidence suggest that TZDs act directly on adipose tissue, with secondary effects in skeletal muscle and liver. 1) Adipose tissue is the only insulin-responsive tissue expressing high levels of PPARγ (10–12). 2) TZDs stimulate insulin action in cultured adipose tissue (13) but not in isolated muscle (14) or primary hepatocytes (15). 3) PPARγ is essential for adipocyte differentiation (16, 17). 4) Overexpression of PPARγ and its activation by TZDs stimulate adipocyte differentiation (18), leading to accumulation of small adipocytes, which are more insulin-sensitive than large adipocytes (19). 5) Finally, in vivo, PPARγ agonists cause massive changes in gene expression in adipose tissue but affect a much smaller number of genes in liver and muscle (20). Thus, adipose tissue appears to be a direct target and the major site of TZD action.

Interestingly, in the near absence of adipose tissue, TZDs still have beneficial effects. Troglitazone improved diabetes and hyperlipidemia in lipoprotein patients with various degrees of fat loss (21) and in a mouse model of late onset lipatrophy (22). In the A-ZIP/F-1 (hereafter AZIP) mouse, a model of severe congenital lipoprotein diabetes (23), the effect of therapy depends on genetic background. In FVB/N AZIP mice, TZDs lower circulating triglyceride levels but do not affect the diabetes (24). The latter apparently results from the opposite effects on muscle and liver, with increased insulin sensitivity in skeletal muscle and decreased insulin sensitivity in liver (25). In contrast, in C57BL/6J AZIP mice, rosiglitazone improves both hyperlipidemia and diabetes.2 Taken together, these data suggest that PPARγ agonists may act directly on non-adipose tissues.

We focused on liver PPARγ as a potential direct target of TZDs, because PPARγ mRNA levels are markedly elevated in steatotic livers of the AZIP mice (24, 26, 27). Other mouse models of hepatic steatosis also exhibit increased liver PPARγ expression (12, 22, 28–32), indicating that increased PPARγ mRNA levels are a general property of steatotic liver. However, it is unclear whether the up-regulation of PPARγ causes the steatosis or whether the steatosis causes the elevated PPARγ levels.

The aim of this study is to understand the role of liver PPARγ in both wild type and adipose-deficient mice. We used the Cre-lox system to inactivate hepatocyte PPARγ and demonstrate that liver PPARγ is an important regulator of lipid homeostasis in wild type and lipoatrophic AZIP mice, contributes to development of hepatic steatosis in lipopatroy and in
diet-induced obesity, and mediates most of the effects of rosiglitazone in lipatrophic mice.

EXPERIMENTAL PROCEDURES

Animals—Studies were approved by the NIDDK animal care and use committee. Generation of the AZIP (23), PPARγ 

fl/fl (33), and Alb-Cre (34) mice have been reported. Hemizygous AZIP males (FVB/N background) were bred with PPARγ 

fl/fl Alb-Cre females (mixed FVB/N, 129, and C57BL/6J background). AZIP males from the PPARγ 

fl/fl LKO). All mice studied were homozygous for the PPARγ floxed allele, which had no affect on the phenotype of the wild type or AZIP mice. Sex-matched littermates were used as controls. Mice were typically reared three per cage on a 12-h light/dark cycle (lights on 0600–1800) and fed water and NIH-07 diet (Zeigler Brothers, Inc., Gardners, PA) ad libitum. When indicated, powder AIN-93G diet (35) (Dyets, Bethlehem, PA) or high fat diet (D12451; Research Diets, Inc., New Brunswick, NJ) was used. Rosiglitazone (3 mg/kg/day; Smith Kline Beecham, West Sussex, United Kingdom) was blended with food and administered for 5 weeks. Mice were euthanized in the non-fasted state between 0900 and 1200. Tissues were fixed in neutralized 10% formalin and processed by American Histolabs (Gaithersburg, MD).

Southern Blot Analysis—Genomic DNA was isolated from total liver of 11-week-old male mice, digested with BamHI, and analyzed as described (33). RNA Analysis—Total liver RNA was prepared and analyzed by Northern blot by phosphorimaging as described previously (24, 26). RNase protection assay was performed as described (36).

Western Blot Analysis—Nuclear extracts from total liver were isolated using an NE-PER nuclear and cytoplasmic extraction reagents kit (Fierce, Rockford, IL). Hepa-1 cells were transfected with an expression vector for Dr. Wahl, Universite de lausanne, lausanne, Switzerland), using LipofectAMINE reagent (Invitrogen). 10 μg of total protein from Hepa-1 cells and 10 μg of protein from nuclear extracts were subjected to electrophoresis on a 4 to 15% Tris-HCl gradient gel (Bio-Rad), transferred to Immobilon-P membranes (Millipore, Bedford, MA), and probed according to the manufacturer’s recommendations with anti-PPARγ antibodies. Immunoreactive proteins were detected using an enhanced chemiluminescence blot detection system (Amersham Biosciences).

Biochemical Assays—Serum was obtained from tail or retro-orbital veins in the non-fasted state. Serum glucose, triglyceride, free fatty acid, insulin, and leptin were assayed as described (37). Serum adiponectin and serum estradiol were measured by radioimmunoassay (MADP-60HK; Linco Research, Inc., St. Charles, MO and DSL-39100; Diagnostic System Laboratories, Inc., Webster, TX, respectively). Tissue triglyceride was measured as described (26).

Euglycemic-hyperinsulinemic Clamps—Mice were fasted for 12 h. Clamps were performed as described (38, 39) with a target plasma insulin of 4 μg/ml and plasma glucose of 110–120 mg/dl.

Indirect Calorimetry—Oxygen consumption and carbon dioxide production were measured by indirect calorimetry in 40-week-old female mice as described (40).

In Vivo Triglyceride Secretion—Triglyceride secretion was measured as the increase in circulating triglyceride after inhibiting triglyceride clearance with WR1339 (41, 42). Mice were fed a fat-free diet (Frosted Flakes; Kellogg Company, Battle Creek, MI) for 4 h and then anesthetized with avertin (43). WR1339 (T-8761; Sigma; 100 μl of a 1:10 dilution in phosphate-buffered saline) was injected via tail vein, and blood samples were withdrawn at 0, 30, 60, and 120 min. Plasma triglycerides were measured colorimetrically. Data are expressed as mg triglyceride/kg body weight/h, assuming plasma volume is 3.5% of body weight.

Triglyceride Clearance—Triglyceride clearance was measured in mice fasted for 4 h and then gavaged (at ~1200) with 400 μl of peanut oil. Blood was taken hourly for 6 h from the tail vein, and plasma triglycerides were measured colorimetrically.

Statistical Analysis—Values are reported as mean ± S.E. Statistical significance was determined using a two-tailed t test or analysis of variance, as appropriate.

RESULTS

Liver-specific Ablation of the PPARγ Gene—We generated liver-specific PPARγ knockout mouse by crossing PPARγ-flxed mice (33) with Alb-Cre mice (34). The wild type Alb-Cre PPARγ 

fl/fl (hereafter WT LKO) offspring were then crossed with lipoatrophic AZIP PPARγ 

fl/fl mice to produce AZIP mice lacking PPARγ in liver (AZIP LKO). Alb-Cre is expressed exclusively in hepatocytes of the postpartum liver (34), providing maximum recombination by six weeks of age (44). Therefore, we analyzed mice at 11 weeks of age and older. Southern blot analysis demonstrated that exon 2 of the PPARγ gene, encoding the DNA binding domain, was indeed deleted in both WT LKO and AZIP LKO livers (90 and 70%, respectively; see Fig. 1a). The remaining unrecombined floxed allele is likely to be from non-parenchymal cells, which do not express the Alb-Cre (45). To confirm the efficiency of targeting, we measured liver PPARγ mRNA levels. As reported (24, 26), the AZIP livers

![Fig. 1. Liver-specific inactivation of PPARγ gene. a, Southern blot analysis of recombination in liver. Genomic DNA was isolated from total liver of 11-week-old female mice, digested with BamH1, and analyzed as described in Ref. 33. b, Northern blot analysis of PPARγ mRNA. Total liver mRNA from 11-week-old female mice was probed for PPARγ and β-actin. c, RNase protection analysis of PPARγ mRNA. Total liver RNA was hybridized with riboprobes for PPARγ and β-actin and subjected to digestion with RNase H. d, Western blot analysis. 10 μg of protein from nuclear extracts of total liver were subjected to electrophoresis and probed with anti-PPARγ antibodies. Control lane contains 10 μg of total protein from Hepa-1 cells transfected with PPARγ1 expressing vector. *, nonspecific bands.](image-url)
Liver-specific PPARγ Knockout

expressed six times more PPARγ mRNA than the WT livers (Fig. 1b). Northern blot analysis revealed a 2- and 3-fold reduction of PPARγ mRNA levels in the WT LKO and AZIP LKO mice, respectively. RNase protection assay demonstrated that in the AZIP LKO liver most of the residual PPARγ mRNA is a truncated transcript from the recombinant null allele (Fig. 1c). Similarly, no detectible wild type PPARγ mRNA was found in the WT LKO liver (38). Truncated PPARγ transcripts carry a new stop codon, which is expected to produce 10- and 13-kDa translation products (PPARγ1 or PPARγ2, respectively), which appear to be unstable (35). Western blot analysis revealed that the AZIP liver expressed predominantly PPARγ2 isofrom (Fig. 1d), similarly to the steatotic liver of the ob/ob mouse (32). In the AZIP LKO mice, PPARγ2 protein levels were dramatically reduced; PPARγ1 levels were also affected; however, the amount of the residual PPARγ1 protein was comparable with that in the WT mice. In contrast to lipostrophic mice, WT liver contained predominantly PPARγ1 band, which was not affected by the knockout. Taken together, these data suggest that in the wild type liver most of the detectable PPARγ protein is produced by non-parenchymal cells, whereas in the steatotic liver of the AZIP mice hepatocytes is the major source of PPARγ protein.

**Loss of Liver PPARγ Improves Hepatic Steatosis in the AZIP Mice**—Inactivation of liver PPARγ did not cause a major phenotype in 11-week-old female non-lipoatrophic mice, with no effect on body weight, liver weight, liver triglyceride, in vivo triglyceride secretion, or serum glucose, insulin, triglyceride, or free fatty acid levels (Fig. 2 and not shown). In contrast, inactivation of liver PPARγ significantly altered the AZIP phenotype. The increased body and liver weights of the AZIP mice were reduced by liver PPARγ ablation (Fig. 2, a and b) without effect on other organs (not shown). The reduction in liver weight correlated with a decrease in liver triglyceride content (30%), in vivo triglyceride secretion rate (60%), and mRNA levels of several genes involved in fatty acid synthesis (up to 40%), suggesting a multifaceted role for liver PPARγ in triglyceride homeostasis (see Fig. 2, c and d and Table I). Male AZIP LKO mice also had smaller, less steatotic livers compared with the AZIP control mice, but this difference became significant only at the age of 20 weeks (not shown). The more profound phenotype in females was not because of the alteration of estradiol levels, because they were comparable in WT, WT LKO, AZIP, and AZIP LKO mice (24.6 ± 1.6, 22.7 ± 2.7, 22.9 ± 1.3, and 26.7 ± 3.4 pg/ml, respectively; seven-nine mice/group).

**Inactivation of Liver PPARγ Impairs Triglyceride Clearance**—The reduced triglyceride secretion in the AZIP LKO mice might be expected to lower circulating triglyceride levels. However, disruption of liver PPARγ actually increased the already elevated serum triglyceride levels in the AZIP mice (Fig. 2e), suggesting a defect in triglyceride clearance. To directly test this hypothesis, we measured serum triglyceride levels after an oral lipid load (Fig. 2f). In WT mice, serum triglycerides peaked at 140 mg/dl at 2 h and rapidly returned to baseline levels. In AZIP mice, serum triglycerides rose to 874 mg/dl at 3 h, demonstrating impairment of triglyceride clearance in the absence of adipose tissue. The AZIP LKO mice were even more fat intolerant, with serum triglycerides reaching 1099 mg/dl at 4 h and remaining elevated 6 h after the lipid dose. Thus, disruption of liver PPARγ exacerbates the hyperlipidemia of AZIP mice by impairment of triglyceride clearance.

Interestingly, whereas 11-week-old WT LKO mice did not have elevated serum triglyceride levels, they did show a small delay in triglyceride clearance (Fig. 2f). The area under the curve above the baseline was 256% higher in the WT LKO than in the WT mice (p = 0.008). These data suggest that liver PPARγ contributes to triglyceride clearance even in wild type, non-lipoatrophic mice.

**Loss of Liver PPARγ Exacerbates Muscle Insulin Resistance in the AZIP Mice**—Because high circulating triglyceride levels often correlate with increased muscle triglyceride content and insulin resistance, we measured triglyceride levels in skeletal muscle. Loss of liver PPARγ caused a 33% increase in muscle triglyceride levels in the AZIP mice (from 2.7 ± 0.3 to 3.6 ± 0.4 μmol/g; p = 0.09). We did not detect significant differences in non-fasting serum glucose or insulin levels in the AZIP and AZIP LKO mice; in both strains, serum glucose levels were four times higher, and insulin levels were 100 times higher than the WT controls (Fig. 2, g and h). However, fasting blood glucose was significantly higher in the AZIP LKO compared with the AZIP mice (257 ± 30 and 182 ± 28 mg/dl, respectively; p = 0.04), suggesting that loss of liver PPARγ makes lipoatrophic mice even more diabetic.

To evaluate glucose homeostasis, we performed euglycemic-hyperinsulinemic clamps (Fig. 2, i and j). In WT mice, loss of PPARγ in liver had no measurable effect on any of the clamp parameters. In AZIP mice, endogenous glucose production was less suppressed by insulin than in the WT controls (58 versus 93%), indicating hepatic insulin resistance. Loss of liver PPARγ did not cause significant changes in endogenous glucose production or its suppression by insulin, suggesting that under the conditions used liver insulin resistance was comparable in the AZIP and AZIP LKO mice (Fig. 2j). In contrast, inactivation of PPARγ in liver did affect muscle insulin sensitivity. In AZIP mice, muscle glucose uptake rate was 49% of the WT controls but only 24% in the AZIP LKO mice (p = 0.04), indicating that loss of liver PPARγ worsens muscle insulin resistance in lipoatrophic mice (Fig. 2j).

**Increased Body Fat Mass in Mice Lacking Liver PPARγ**—At the age of 11 weeks WT LKO did not have a major phenotype, showing only a small impairment of triglyceride clearance (Fig. 2). To study metabolic consequences of this defect, we analyzed 40-week-old WT and WT LKO mice (Fig. 3). Loss of liver PPARγ had no significant effect on body weight, liver weight, food intake, or metabolic rate (Fig. 3, a and b, and not shown). However, the WT LKO mice had more adipose tissue (Fig. 3e). The small delay in triglyceride clearance observed in 11-week-old WT LKO mice became more apparent with age, coinciding with persistent hyperlipidemia (Fig. 3, d and e). At the age of 40 weeks, the WT LKO mice also had elevated serum glucose and insulin levels, indicating insulin resistance (Fig. 3, f and g). Increased adiposity in the WT LKO mouse correlated with a 2.3-fold increase in serum leptin and a 1.9-fold decrease in serum adiponectin levels (not shown). Thus, in non-lipoatrophic mice, loss of liver PPARγ caused fat intolerance and with aging, hyperlipidemia, obesity, and insulin resistance.

To see whether an obesity phenotype can be also induced in younger mice, we compared the effects of the AIN-93G (lipogenic, simple carbohydrate diet) and NIH-07 (standard chow) diets on adipose tissue mass in WT and WT LKO mice (Fig. 3h). WT and WT LKO mice fed the NIH-07 diet and WT mice on the AIN-93G diet had a similar amount of WAT and BAT. In contrast, after only five weeks on the AIN-93G diet WT LKO mice had WAT depots two times larger than the controls. No difference in body weight was detected because of the range of body weights in each experimental group. However, within the same body mass range, the WT LKO mice on the AIN-93G diet had the largest amount of WAT (not shown). Serum leptin levels correlated with the WAT mass (not shown). Thus, the AIN-93G diet unmasked a predisposition to obesity in liver-specific PPARγ knockout mice. Taken together, these data demonstrate that hepatic PPARγ plays a role in regulation of
triglyceride partitioning between liver and other tissues, contributing to regulation of body fat mass and glucose homeostasis in non-lipoatrophic mice.

Liver PPARγ Regulates Genes Involved in Lipid Metabolism—To assess the direct transcriptional effects of liver PPARγ ablation, we measured target mRNA levels (Table I). On a chow diet AZIP mice have increased levels of lipogenic mRNA, including PPARγ, sterol response element-binding protein 1 (SREBP-1), fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC), and stearoyl-CoA desaturase (SCD1) (24).

Fig. 2. Liver-specific inactivation of PPARγ lessened hepatic steatosis but exacerbated hyperlipidemia in AZIP mice. Female mice were analyzed at the age of 11 weeks (n = 6–14) in non-fasting state (a–e, g, and h), after 5-h fasting (f), and during euglycemic-hyperinsulinemic clamps (i and j). a, body weight; b, liver weight; c, liver triglyceride; d, in vivo triglyceride secretion; e, serum triglyceride; f, plasma triglyceride clearance after oral triglyceride load; g, serum glucose; h, serum insulin; i, muscle glucose uptake; j, suppression of endogenous glucose production. WT; □, WT LKO; ▼, AZIP; ▼, AZIP LKO; *, significant effect of liver-specific PPARγ knockout (p < 0.05) within WT or AZIP genotype.
Liver-specific PPARγ Knockout

Female mice (from Fig. 2) were maintained on NIH-07 diet and euthanized at 900–1200 in the non-fasting state. Liver mRNA levels were analyzed by Northern blotting and quantitated by phosphorimaging. Levels of LPL and VLDLR mRNA were below the level of detection. Data are mean ± S.E., expressed as percent of WT (n = 4–5).

| Gene   | WT LKO | AZIP | AZIP LKO |
|--------|--------|------|----------|
| PPARγ  | 100 ± 23 | 52 ± 4 | 575 ± 116 | 171 ± 39 |
| PPARα  | 100 ± 10 | 71 ± 4 | 104 ± 11 | 113 ± 8 |
| SREBP-1| 100 ± 27 | 157 ± 45 | 306 ± 49 | 202 ± 40 |
| FAS    | 100 ± 24 | 65 ± 16 | 149 ± 22 | 111 ± 26 |
| ACC    | 100 ± 24 | 85 ± 19 | 197 ± 37 | 132 ± 32 |
| SCD1   | 100 ± 43 | 46 ± 7 | 359 ± 46 | 212 ± 43 |
| APOB   | 100 ± 14 | 75 ± 6 | 136 ± 12 | 137 ± 6 |
| CPT1   | 100 ± 13 | 90 ± 4 | 116 ± 15 | 89 ± 14 |
| CD36   | 100 ± 31 | 61 ± 11 | 1055 ± 73 | 886 ± 64 |
| LDLR   | 100 ± 14 | 126 ± 31 | 143 ± 17 | 79 ± 6 |
| LRP    | 100 ± 16 | 195 ± 19 | 112 ± 22 | 149 ± 5 |
| apoB   | 100 ± 9 | 87 ± 9 | 67 ± 12 | 73 ± 10 |
| MTP    | 100 ± 18 | 42 ± 6 | 71 ± 10 | 78 ± 5 |
| HL     | 100 ± 8 | 158 ± 11 | 113 ± 12 | 129 ± 18 |
| EL     | 100 ± 16 | 196 ± 29 | 177 ± 33 | 230 ± 16 |
| aP2    | 100 ± 21 | 111 ± 15 | 368 ± 90 | 253 ± 27 |
| FABP   | 100 ± 15 | 76 ± 1 | 79 ± 5 | 77 ± 1 |
| ADRP   | 100 ± 22 | 64 ± 11 | 239 ± 18 | 183 ± 6 |

* p < 0.05 for difference between WT and AZIP mice.

Liver PPARγ ablation reduced the FAS, ACC, and SCD1 mRNA levels (26, 33, and 41%, respectively). Expression of SREBP-1, a master regulator of lipogenesis, was not affected, suggesting that PPARγ acts either downstream of or parallel to SREBP-1.

AZIP mice have 10-fold elevated levels of liver CD36 mRNA, which encodes a lipoprotein receptor/fatty acid transporter. Ablation of liver PPARγ reduced CD36 mRNA levels by only 16 and 39% in the AZIP and WT mice, respectively. Low density lipoprotein receptor (LDLR) mRNA levels were reduced by 45%, but only in the AZIP mice. In contrast, LDLR-related protein, hepatic lipase, and endothelial lipase mRNA levels were increased in both WT LKO and the AZIP LKO mice. Lipoprotein lipase mRNA and very low density lipoprotein receptor mRNA were barely detectable in all strains and did not appear to be affected by either AZIP transgene or PPARγ null allele (not shown). Levels of mRNAs encoding other lipid metabolism genes (acyl-CoA oxidase, carnitine palmitoyl transferase, apolipoprotein B, microsomal triglyceride transfer protein, fatty acid-binding protein) were not significantly affected by ablation of liver PPARγ.

Steatotic AZIP livers also have elevated levels of adipose differentiation-related protein (ADRP) and adipose fatty acid-binding protein (aP2) mRNA (3.6 and 2.4, respectively). ADRP is a protein covering lipid droplets in non-adipose cells (47), whereas aP2 is a cytosolic fatty acid-binding protein, normally expressed in adipocytes (48). The levels of both ADRP and aP2 mRNA were reduced in the AZIP LKO mice, suggesting that liver PPARγ also contributes to regulation of lipid transport and storage within the hepatocytes.

Liver PPARγ Is Essential for Rosiglitazone Effects in the AZIP Mice—To assess the contribution of liver PPARγ to the antidiabetic effects of rosiglitazone, a PPARγ agonist, we first analyzed rosiglitazone effects in the lipoatrophic AZIP mice (Fig. 4). As noted previously (24), rosiglitazone increased liver weight and hepatic triglycerides and lowered serum triglycerides in the AZIP mice (Fig. 4, a–d). In addition, it elevated serum alanine aminotransferase and aspartate aminotransferase levels, indicating liver damage (not shown). All these effects were abolished in the AZIP LKO mice, demonstrating that they are mediated by liver PPARγ expression.

Previously, rosiglitazone treatment did not improve serum glucose and insulin levels in AZIP mice with a pure FVB/N genetic background (24). Here, using mice of mixed genetic background, we observed partial improvement of the diabetes, with a 2-fold decrease in serum glucose and 45% increase in serum insulin (Fig. 4, e and f; p = 0.001 and p = 0.09, respectively). Loss of liver PPARγ abolished the beneficial effect of rosiglitazone in AZIP mice.

Taken together, these data demonstrate that, in the absence of adipose tissue, liver PPARγ mediates triglyceride lowering and contributes to the hypoglycemic effects of rosiglitazone. Interestingly, rosiglitazone caused a significant decrease in serum-free fatty acid levels in both AZIP and AZIP LKO mice, suggesting that tissues other that liver and fat contribute to this effect (Fig. 4g).

Rosiglitazone treatment had no effect on liver mRNA levels in WT mice (not shown). In contrast, in the AZIP mice, rosiglitazone treatment increased mRNA levels of ACC, FAS, SCD1, acyl-CoA oxidase, CD36, and aP2 (Fig. 4h and not shown). All effects of rosiglitazone were abolished in the AZIP LKO mice, suggesting a role for liver PPARγ in regulation of genes involved in lipogenesis, fatty acid transport, storage, and oxidation.

Liver PPARγ Is Not Required for Rosiglitazone Effects in Non-lipoatrophic Mice—We next asked whether liver PPARγ is essential for the antidiabetic effects of rosiglitazone in a typical mouse, with adipose tissue. The WT and WT LKO mice were made insulin-resistant with a high fat diet (Fig. 5). After three months of high fat feeding, the WT and WT LKO mice had comparably elevated serum glucose and insulin levels, suggesting a similar degree of insulin resistance (Fig. 5, a and b). Serum triglycerides tended to be slightly higher in mice without liver PPARγ (p = 0.2), whereas free fatty acids were not different (Fig. 5, c and d). Both WT and WT LKO mice responded to rosiglitazone by lowering serum glucose, insulin, triglyceride, and free fatty acid levels and by improving glucose and fat clearance (Fig. 5, a–d, and not shown). Thus, in non-lipoatrophic mice liver PPARγ is not required for the antidiabetic and hypolipidemic effects of rosiglitazone.

In both WT and WT LKO mice, rosiglitazone increased adipose tissue mass and reduced liver size and steatosis (Fig. 5, e and f, and not shown). These observations are consistent with the hypothesis that the primary effect was increased triglyceride storage in adipose tissue, with a secondary reduction in liver triglyceride levels. Rosiglitazone treatment increased serum adiponectin levels in both WT and WT LKO mice (2.9- and 3.6-fold, respectively), whereas the increase in serum leptin was significant only in the WT LKO mice.

Thus, rosiglitazone had very similar effects in the WT and WT LKO mice, demonstrating that in non-lipoatrophic mice liver PPARγ contributes relatively little to the beneficial effects of rosiglitazone. These data suggest that adipose tissue is the major site of thiazolidinedione action in typical mice with adipose tissue.

**DISCUSSION**

Here we demonstrate that liver PPARγ contributes to triglyceride homeostasis, regulating both triglyceride clearance and the lipogenic program. A role for hepatic PPARγ had not been appreciated previously, possibly because of its low abundance in non-steatotic liver. Inactivation of liver PPARγ reduced hepatic steatosis in both lipoatrophic AZIP mice and mice with diet-induced obesity. In the AZIP mouse, this correlated with reduced triglyceride output from the liver and lower
expression of FAS, ACC, and SCD1, suggesting that in lipoatrophic mice liver PPARγ modulates hepatic steatosis by regulating lipogenic genes.

Interestingly, reduction of hepatic steatosis did not improve the metabolic phenotype of the AZIP mice, actually worsening the triglyceride clearance, hyperlipidemia, and muscle insulin resistance. Inactivation of PPARγ in liver of the ob/ob mice caused similar effects (36). These data suggest that hepatic steatosis develops not only as a result of increased triglyceride production but is also because of enhanced lipid uptake by liver. The degree of hepatic steatosis and efficiency of triglyceride clearance in the AZIP mouse depends on genetic background (27). FVB/N AZIP mice have high circulating triglyceride and fatty liver, whereas C57BL/6J AZIP mice have low circulating triglyceride but much more steatotic liver. Thus, it is possible that some the effects of liver PPARγ deficiency might be influenced by background genotype. Further understanding of the mechanisms contributing to hepatic steatosis will come from identifying the relevant modifier genes.

Improvement in hepatic steatosis has also been achieved in the ob/ob mouse by inactivation of SCD1 (49) or of SREBP-1 (50). SREBP-1 proteins are transcription factors that regulate multiple lipogenic genes, including FAS, ACC, and SCD1 (51). Similar to PPARγ, SREBP-1 mRNA levels are elevated in steatotic livers (24, 52). Transgenic mice overexpressing the constitutively active form of SREBP-1 proteins have fatty liver, supporting a role for SREBP-1 in development of the steatosis (53, 54). Because liver-specific disruption of PPARγ did not affect SREBP-1 levels, we conclude that PPARγ directly contributes to hepatic steatosis, acting either downstream of or in parallel with SREBP-1.

Interestingly, whereas liver-specific disruption of PPARγ and SREBP-1 both reduced lipogenesis and triglyceride content in liver (50), elevation of circulating triglyceride occurred only when PPARγ was missing. These data suggest that the role of hepatic PPARγ in regulation of lipid uptake by liver is not shared with SREBP-1. The target genes and molecular mechanisms by which liver PPARγ mediates lipid clearance remain largely unknown. Lack of liver PPARγ caused a 2-fold decreased in LDLR mRNA levels in the mice. This is in agreement with a recently published observation (55) that adenoviral overexpression of PPARγ increased LDLR mRNA levels in livers of the PPARγ null mice. Thus, LDL receptor might be one of the mediators of PPARγ-regulated lipid clearance by liver. CD36, a lipoprotein receptor/fatty acid transporter, is another likely candidate. PPARγ agonists increase CD36 mRNA levels in multiple tissues, including adipose, muscle, liver, and macrophages (30, 33, 56–59). Loss of function mutation of CD36 gene significantly blunted hypolipidemic and insulin sensitizing effects of pioglitazone in spontaneously hypertensive

![Image](https://example.com/image.png)
The observation that some patients with lipodystrophy have PPARγ mutations (63–65). Moreover, mice heterozygous for a PPARγ null allele are protected from high fat diet-induced obesity and have increased insulin sensitivity (17, 66). A mechanistic explanation for the obesity caused by liver-specific PPARγ deficiency follows from our observation that PPARγ ablation reduces the ability of the liver to take up triglyceride. This was the sole phenotype detected in young non-lipoatrophic mice, supporting the idea that it has an etiologic role in the later effects. Because of reduced hepatic uptake, more triglycerides are stored in other sites such as adipose tissue and possibly muscle, thereby causing insulin resistance. The obesity of the liver PPARγ ablated mice is dependent on dietary exposure and animal age, features that it shares with the common forms of human obesity.

It has been well established that PPARγ agonists, including TZDs, act as insulin sensitizers in human and animal models, but the identity and relative importance of target tissues are still debated (5). The prevailing hypothesis proposes that TZDs act directly on adipose tissue, with secondary effects in skeletal muscle and liver. Improvement of insulin sensitivity in muscle, liver, and adipose tissue (7–9) is accompanied by increase of peripheral adiposity (21, 67, 68). Although insulin signaling in muscle and adipose tissue could be stimulated by PPARγ agonists within a day, improvement of insulin action in liver requires longer treatment (15) and higher doses (8), consistent with an indirect effect. When adipose tissue was present, ablation of liver PPARγ did not influence the effectiveness of rosiglitazone treatment. Similarly, muscle-specific PPARγ knockout mice also showed intact response to rosiglitazone treatment (69). Taken together, these data demonstrate that in a typical mouse neither liver PPARγ nor muscle PPARγ are essential for the beneficial effects of rosiglitazone, suggesting that TZDs normally act via adipose tissue.

In contrast, in the absence of adipose tissue, liver becomes the major site of TZD action. Loss of liver PPARγ abolished most of the effects of rosiglitazone in the AZIP mouse, including a reduction in circulating glucose and triglyceride levels, and an increase in hepatic triglyceride content. Thus, possible mechanisms by which TZDs improve metabolic abnormalities in lipoatrophic mice may include redistribution of triglyceride away from the circulating compartment into the liver and increased fat oxidation. TZDs decrease respiratory quotient in lipoatrophic mice (24) and humans (21), but which tissue is responsible for lipid oxidation is unclear. Liver is likely to contribute, because rosiglitazone induced hepatic acyl-CoA oxidase mRNA in a PPARγ-dependent way. Our observation that liver PPARγ is not required for reduction of free fatty acids by rosiglitazone suggests that tissues other that liver and adipose tissue may also contribute to fat oxidation.

It is important to note that efficacy of TZD therapy in lipoatrophic mice depends on background genotype. In FVB/N AZIP mice, rosiglitazone lowered circulating triglyceride but had no effect on diabetes (24), presumably because of the opposite effects of muscle and liver (25). In contrast, C57BL/6J AZIP mice improved both hyperlipidemia and diabetes, similar to a mouse model of late onset lipoatrophy (22). In this study, in the AZIP mice of mixed genetic background, rosiglitazone lowered hyperglycemia but increased insulin levels, probably because of improvement of β-cell function. Which modifier genes determine the way lipoatrophic mice respond to TZD therapy is not known. Given the range of responses even within the same transgenic mouse model, it might be hard to predict the outcome of TZD therapy in human lipoatrophy, a heterogeneous group of syndromes (70, 71).

An important unanswered question concerns the similarity between mouse and human hepatic steatosis. We are unaware of any published studies quantitating PPARγ levels in steatotic
liver from humans. In typical type 2 diabetic patients rosiglitazone reduced hepatic triglyceride (9). Troglitazone treatment was also efficacious in reducing liver size in a heterogeneous group of lipodystrophic patients, correlating with an increase in adipose mass (21). It is possible that worsening of hepatic steatosis with TZD treatment is a species-specific manifestation, similar to the hepatomegaly caused by PPARα agonists in rodents (46, 72). However, more studies are needed to evaluate the role of TZD therapy in patients with complete lipoatrophy.

Acknowledgments—We thank Dr. P. Gordon for stimulating discussion, Dr. R. Walczak for probes, and Drs. P. Gorden, D. LeRoith, D. Moller, and A. Mir for helpful comments on the manuscript.

REFERENCES
1. Xu, H. E., Lambert, M. H., Montana, V. G., Parks, D. J., Blanchard, S. G., Brown, P. J., Sternbach, D. D., Lehmann, J. M., Wisely, G. B., Willson, T. M., Kiewer, S. A., and Milburn, M. V. (1999) Mol. Cell 3, 397–403
2. Forman, B. M., Tontonoz, P., Chen, J., Brun, R. P., Spiegelman, B. M., and Evans, R. M. (1995) Cell 83, 803–812
3. Berger, J., and Moller, D. E. (2002) Annu. Rev. Med. 53, 301–327
4. Foyt, H. L., Ghazzi, M. N., Hanley, R. M., Saltiel, A. R., and Whitcomb, R. W. (2000) in Diabetes mellitus: A Fundamental and Clinical Text (LeRoith, D., Taylor, S. I., and Olefsky, J. M., eds) 2nd Ed., pp. 788–800, Williams & Wilkins, Philadelphia
5. Olefsky, J. M. (2000) J. Clin. Invest. 106, 467–472
6. Kersten, S., Desvergne, B., and Wahli, W. (2000) Nature 405, 421–424
7. Inzucchi, S. E., Maggs, D. G., Spollet, G. R., Page, S. L., Rife, P. S., Walton, V., and Shulman, G. I. (1998) N. Engl. J. Med. 338, 667–672
8. Maggs, D. G., Buchanan, T. A., Burant, C. F., Cline, G., Gumbiner, B., Hsueh, W. A., Inzucchi, S., Kelley, D., Nolan, J., Olefsky, J. M., Polonsky, K. S., Silver, D., Valiquett, T. R., and Shulman, G. I. (1998) Ann. Intern. Med. 128, 176–185
9. Mayerson, A. B., Hundul, R. S., Dufour, S., Lebon, V., Befroy, D., Cline, G., Enechson, S., Inzucchi, S., Shulman, G. I., and Petersen, K. F. (2002) Diabetes 51, 807–814
10. Tontonoz, P., Hu, E., Graves, R. A., Budavari, A. I., and Spiegelman, B. M. (1994) Genes Dev. 8, 1234–1238
11. Chawla, A., Schwartz, E. J., Decaen, D. D., and Lazar, M. A. (1994) Endocrinology 135, 788–800
12. Vidal-Puig, A., Jimenez-Linan, M., Lowell, B. B., Hamann, A., Hu, E., Spiegelman, B., Flier, J. S., and Moller, D. E. (1996) J. Clin. Invest. 97, 2533–2541
13. Berger, J., Biswas, C., Hayes, N., Ventre, J., Wu, M., and Doebber, T. W. (1996) Endocrinology 137, 1984–1990
14. Zierath, J. R., Ryder, J. W., Doebber, T., Woods, J., Wu, M., Ventre, J., Li, Z., McCrary, C., Berger, J., Zhang, B., and Moller, D. E. (1998) Endocrinology 139, 5054–5064
15. Jiang, G., Dallas-Yang, Q., Li, Z., Szalkowski, D., Liu, F., Shen, X., Wu, M., Zhou, G., Doebber, T., Berger, J., Moller, J., and Zhang, B. H. (2002) Diabetes 51, 2412–2419
16. Rosen, E. D., Sarraf, P., Troy, A. E., Bradwin, G., Moore, K., Milstone, D. S., Spiegelman, B. M., and Mottonen, R. M. (1999) Mol. Cell 4, 611–617
17. Kobata, N., Terauchi, Y., Miki, H., Tamemoto, H., Yamauchi, T., Komeda, K., Sato, S., Nakano, R., Ishii, C., Sugiyama, T., Eto, K., Tsukamoto, Y., Okuno, A., Murakami, K., Sekihara, H., Hasegawa, G., Naito, M., Toyoshiba, Y., Tanaka, S., Shiota, K., Kitamura, T., Fujita, T., Eikoh, O., Azawa, S., Nagai, R., Tobe, K., Kimura, S., and Kadowaki, T. (1999) Mol. Cell 4, 597–609
18. Tontonoz, P., Hu, E., and Spiegelman, B. M. (1994) Cell 79, 1147–1156
19. Okuno, A., Tamemoto, H., Tobe, K., Ueki, K., Mori, Y., Iwamoto, K., Umemoto, K., Akamatsu, Y., Fujisawa, T., Horikoshi, H., Yasaki, Y., and Kadowaki, T. (1998) J. Clin. Invest. 101, 1354–1361
20. Way, J. M., Harrington, W. W., Brown, K. K., Gottschalk, W. K., Sundseth, S. M., Manfield, T. A., Baxter-Chandran, R. K., Willson, T. M., and Klevay, S. A. (2001) Endocrinology 142, 1269–1277
21. Arisugu, E., Duncan-Morin, J., Sebring, N., Rother, K. I., Gottlieb, N., Lieberman, J., Herzon, D., Kleiner, D. E., Reynolds, J., Premkumar, A., Summer, A. E., Hofnagle, J., Reitman, M. L., and Taylor, S. I. (2000) Ann. Intern. Med. 133, 263–274
22. Burant, C. F., Seenan, S., Hirano, K., Tai, T. A., Lohmiller, J., Lukenes, J., Davidson, N. O., Ross, S., and Graves, R. A. (1997) J. Clin. Invest. 100, 2900–2908
23. Moitra, J., Mason, M. M., Olive, M., Krylov, D., Gavrilova, O., Marcussamuels, B., Peigenbaum, L., Lee, E., Aoyama, T., Eckhaus, M., Reitman, M. L., and Vinson, C. (1998) Genes Dev. 12, 3168–3181
24. Chao, L., Marcussamuels, B., Mason, M. M., Moitra, J., Vinson, C., Aisugu, E., and Gavrilova, O. (1998) J. Clin. Invest. 106, 1221–1228
25. Kim, J. K., Fillmore, J. J., Chao, L., Gavrilova, O., Higashimoru, T., Chen, S., Chou, C., Chen, Y., Haluzik, M., Reitman, M. L., and Vinson, C. (2000) J. Clin. Invest. 106, 1211–1218
26. Aisugu, E., Gavrilova, O., and Reitman, M. L. (2002) J. Clin. Invest. 109, 1777–1784
27. Colombo, C., Haluzik, M., and Reitman, M. L. (2002) J. Biol. Chem. 277, 24484–24489
28. Chou, C. J., Haluzik, M., Gregory, C., Dietz, K. R., Vinson, C. Gavrilova, O., and Reitman, M. L. (2002) J. Biol. Chem. 277, 24484–24489
29. Costet, P., Legrande, C., More, J., Edgar, A., Galisier, P., and Pineau, T. (1998) J. Biol. Chem. 273, 29577–29585
30. Jennas, N. R., Tecott, L. H., Nonogaki, K., Beigneux, A., Moser, A. H., Grunfeld, C., and Feingold, K. R. (2000) Endocrinology 141, 4021–4031
31. Bedoucha, M., Atzpodien, E., and Boelsterli, U. A. (2001) J. Hepatol. 35, 17–23
32. Rahman, R., Masih-Khan, E., Lo, M., van Bremen, C., McManus, B. M., and
Akiyama, T. E., Sakai, S., Lambert, G., Nicol, C. J., Matsuura, K., Pimprale, S., Lee, Y. H., Ricote, M., Glass, C. K., Brewer, H. B., Jr., and Gonzalez, F. J. (2002) *Mol. Cell. Biol.* 22, 2607–2619

Yakar, S., Liu, J. L., Stannard, B., Butler, A., Accili, D., Sauer, B., and LeRoith, D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 7324–7329

Reeves, P. G., Nielsen, F. H., and Fahey, G. C., Jr. (1983) *J. Nutr.* 113, 1939–1953

Matsusue, K., Haluzik, M., Lambert, G., Yim, S. H., Gavrilova, O., Ward, J. M., Brewer, B. Jr., Reitman, M. L., and Gonzalez, F. J. (2003) *J. Clin. Investig.* 111, 737–747

Gavrilova, O., Marcus-Samuels, B., Graham, D., Kim, J. K., Shulman, G. I., Castle, A. L., Vinson, C., Eckhaus, M., and Reitman, M. L. (2000) *J. Clin. Investig.* 105, 271–278

Kim, J. K., Michael, M. D., Previs, S. F., Peroni, O. D., Mauvais-Jarvis, F., Neschen, S., Kahn, B. B., Kahn, C. R., and Gi, S. (2000) *J. Clin. Investig.* 105, 1791–1797

Haluzik, M., Dietz, K. R., Kim, J. K., Marcus-Samuels, B., Shulman, G. I., Gavrilova, O., and Reitman, M. L. (2002) *Diabetes* 51, 2113–2118

Gavrilova, O., Marcus-Samuels, B., and Reitman, M. L. (2000) *Diabetes* 49, 1919–1925

Gorensztaijn, J., Rone, M. S., and Kotlar, T. J. (1976) *Biochem. J.* 156, 539–543

Tietge, U. J., Bakillah, A., Maugaes, C., Tsukamoto, K., Hussain, M., and Rader, D. J. (1999) *J. Lipid Res.* 40, 2134–2139

Hagan, B., Constantini, F., and Lacy, E. (1986) *Manipulating with Mouse Embryo, Laboratory Manual*, p. 271, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Hayhurst, G. P., Lee, Y. H., Lambert, G., Ward, J. M., and Gonzalez, F. J. (2001) *Mol. Cell. Biol.* 21, 1393–1403

Pustic, C., Shiota, M., Nuswender, K. D., Jetton, T. L., Chen, Y., Moates, J. M., Shelton, K. D., Lindner, J., Cherrington, A. D., and Magunson, M. A. (1999) *J. Biol. Chem.* 274, 305–315

Palmer, C. N., Hsu, M. H., Griffin, K. J., Raucy, J. L., and Johnson, E. F. (1998) *Mol. Pharmacol.* 53, 14–22

Brassemael, D. L., Barber, T., Wolins, N. E., Blanche-Mackie, E. J., and Londo, C. (1997) *J. Lipid Res.* 38, 2249–2263

Bernlohr, D. A., Doering, T. L., Kelly, T. J., Jr., and Lane, M. D. (1985) *Biochem. Biophy. Res. Commun.* 132, 850–855

Cohen, P., Miyazaki, M., Secchi, N. D., Hagg-Greenberg, A., Lieb, W., Soukas, A. A., Sharma, R., Hudgins, L. C., Ntambi, J. M., and Friedman, J. M. (2002) *Science* 297, 240–243

Yahagi, N., Shimano, H., Hasty, A. H., Matsuzaka, T., Ide, T., Yoshikawa, T., Amemiya-Kudo, M., Tomita, S., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K., Ouga, J., Harada, K., Gotoda, T., Nagai, R., Ishibashi, S., and Yamada, N. (2002) *J. Biol. Chem.* 277, 19353–19357

Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002) *J. Clin. Investig.* 109, 1125–1131

Shimomura, I., Bashmakov, Y., and Horton, J. D. (1999) *J. Biol. Chem.* 274, 30028–30032

Shimano, H., Horton, J. D., Hammer, R. E., Shimomura, I., Brown, M. S., and Goldstein, J. L. (1996) *J. Clin. Investig.* 98, 1575–1584

Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L. (1997) *J. Clin. Investig.* 99, 846–854

Yu, S., Matsuura, K., Kasharsidery, P., Cao, W. Q., Yeldandi, V., Yeldandi, A. V., Rao, M. S., Gonzalez, F. J., and Reddy, J. K. (2003) *J. Biol. Chem.* 278, 498–505

Gerhold, D. L., Liu, F., Jiang, G., Li, Z., Xu, J., Lu, M., Sachs, J. R., Bagchi, A., Fridman, A., Holder, D. J., Doebber, T. W., Berger, J., Elbrecht, A., Molter, D. E., and Zhang, B. B. (2002) *Endocrinology* 143, 2106–2118

Singh Abhuja, H., Liu, S., Crombie, D. L., Boehm, M., Leibowitz, M. D., Heyman, R. A., Deppe, C., Nagy, L., Tontonoz, P., and Davies, F. J. (2001) *Mol. Pharmacol.* 59, 765–773

Chawla, A., Barak, Y., Nagy, L., Liao, D., Tontonoz, P., and Evans, R. M. (2001) *Nat. Med.* 7, 48–52

Moore, K. J., Rosen, E. D., Fitzgerald, M. L., Randow, F., Andersson, L. P., Altshuler, D., Milstone, D. S., Mortensen, R. M., Spiegelman, B. M., and Freeman, M. W. (2001) *Nat. Med.* 7, 41–47

Qi, N., Kadowo, I., Zielek, V., Landa, V., Kren, V., Peshodsingha, H. A., Lexin, E. S., Abumrad, N. A., Pravenec, M., and Kurtz, T. W. (2002) *J. Biol. Chem.* 277, 48501–48507

Coburn, C. T., Knapp, F. F., Jr., Febbraio, M., Beets, A. L., Silverstein, R. L., and Abumrad, N. A. (2000) *J. Biol. Chem.* 275, 32523–32529

Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lopez, P., Chien, K. R., Koder, A., and Evans, R. M. (1999) *Mol. Cell*, 5, 595–599

Barroso, I., Gurnell, M., Crowley, V. E., Agostini, M., Schwabe, J. W., Soos, M. A., Maslen, G. L., Williams, T. D., Lewis, H., Schafer, A. J., Chatterjee, V. K., and O'Rahilly, S. (1999) *Nature* 402, 880–883

Agarwal, A. K., and Garg, A. (2002) *J. Clin. Endocrinol. Metab.* 87, 488–491

Hegele, R. A., Cao, H., Frankowski, C., Mathews, S. T., and Leff, T. (2002) *Diabetes* 51, 3596–3599

Miles, P. D., Barak, Y., He, W., Evans, R. M., and Olefsky, J. M. (2000) *J. Clin. Investig.* 105, 287–292

Kelly, I. E., Han, T. S., Walsh, K., and Lean, M. E. (1999) *Diabetes Care* 22, 288–293

Mori, Y., Murakawa, Y., Okada, K., Horikoshi, H., Yokoyama, J., Tajima, N., and Beda, Y. (1999) *Diabetes Care* 22, 908–912

Chen, L., Szanto, I., Ristow, M., Gonzalez, F., and Kahn, C. R. (2001) *Diabetes* 50, A57 (abstr. 230-OR)

Reitman, M. L., Arigito, E., Gavrilova, O., and Taylor, S. I. (2000) *Trends Endocrinol. Metab.* 11, 410–416

Garg, A. (2000) *Am. J. Med.* 108, 143–152

Issemann, I., and Green, S. (1990) *Nature* 347, 645–650