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Article

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There is uncertainty about the site(s) of action of the antidiabetic thiazolidinediones (TZDs). These drugs are agonist ligands of the transcription factor PPARγ, which is abundant in adipose tissue but is normally present at very low levels in liver and muscle. We have studied the effects of TZDs in A-ZIP/F-1 mice, which lack white adipose tissue. The A-ZIP/F-1 phenotype strikingly resembles that of humans with severe lipoatrophic diabetes, including the lack of fat, marked insulin resistance and hyperglycemia, hyperlipidemia, and fatty liver. Rosiglitazone or troglitazone treatment did not reduce glucose or insulin levels, suggesting that white adipose tissue is required for the antidiabetic effects of TZDs. However, TZD treatment was effective in lowering circulating triglycerides and increasing whole body fatty acid oxidation in the A-ZIP/F-1 mice, indicating that this effect occurs via targets other than white adipose tissue. A-ZIP/F-1 mice have markedly increased liver PPARγ mRNA levels, which may be a general property of fatty livers. Rosiglitazone treatment increased the triglyceride content of the steatotic livers of A-ZIP/F-1 and *ob/ob* mice, but not the "lean" livers of fat-transplanted A-ZIP/F-1 mice. In light of this evidence that rosiglitazone acts differently in steatotic livers, the effects of rosiglitazone, particularly on hepatic triglyceride levels, should be examined in humans with hepatic steatosis.

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Introduction

Diabetes mellitus is a disease defined by elevated blood glucose levels. The causes of type 2 diabetes are not well understood. It is thought that both resistance of target tissues to the action of insulin and decreased insulin secretion ("β-cell failure") occur. Major insulin-responsive tissues for glucose homeostasis are liver, in which insulin stimulates glycogen synthesis and inhibits gluconeogenesis; muscle, in which insulin stimulates glucose uptake and glycogen synthesis; and white adipose tissue (WAT), in which insulin stimulates glucose uptake and inhibits lipolysis. The relative importance of each tissue is not known; nor are the details of how they interact.

To understand the physiological roles of adipose tissue, we have generated a transgenic line of mice, named A-ZIP/F-1, which has virtually no white adipose tissue (1). These mice express a dominant negative protein in adipose tissue that heterodimerizes with and inactivates members of the C/EBP and JUN families of B-ZIP transcription factors. The A-ZIP/F-1 phenotype strikingly resembles that of humans with severe lipoatrophic diabetes, including the lack of fat, marked insulin resistance and hyperglycemia, hyperlipidemia, fatty liver, and organomegaly. The detailed mechanisms by which the lack of WAT causes insulin resistance are not established. Contributing components probably include deficiency of leptin (and other hormones) and the lack of a place to store triglycerides (2–5). It has been postulated that increased intracellular triglycerides (or metabolites such as fatty acyl-CoA or malonyl-CoA) inside nonadipose tissues could cause insulin resistance (6, 7).

The TZDs are a class of antidiabetic agents that act by increasing insulin sensitivity (8, 9). Currently, two drugs in this class are approved for use in the United States, rosiglitazone (formerly BRL49653; ref. 10) and pioglitazone. TZDs are agonist ligands for the transcription factor peroxisome proliferator-activated receptor γ (PPARγ), and their antidiabetic effects are thought to be mediated through PPARγ (11). PPARγ is expressed at high levels in adipose tissue, colon, and activated macrophages, and at lower levels in other tissues (12–17). The tissues at which TZDs act to increase insulin sensitivity are debated (8, 9, 18). TZDs increase glucose utilization (largely a muscle function) and, at higher doses, inhibit endogenous glucose production (largely a liver function) (19). However, liver and muscle have low PPARγ levels. Thus, WAT has been a leading candidate for the target of TZD actions, as it is the only insulin-responsive tissue with a high level of PPARγ. Additionally, PPARγ and its agonist ligands stimulate

adipose differentiation (20), leading to increased numbers of small adipocytes, which are thought to be more insulin sensitive than are large, lipid-laden adipocytes (21). However, Burant et al., studying a partially lipoatrophic mouse, concluded that troglitazone effectively treated diabetes, independent of adipose tissue (22). Here, we have reexamined this question using the more severely lipoatrophic A-ZIP/F-1 mice and do not see an effect of TZDs on insulin and glucose levels. However, rosiglitazone greatly increases the hepatic steatosis of these mice, which may be due to their increased hepatic expression of PPARγ.

Methods

Mice. Generation and characterization of the "fatless" A-ZIP/F-1 mice have been reported (1, 3–5, 23). Studies were approved by the NIDDK animal care and use committee. All A-ZIP/F-1 mice used in this study were hemizygous females on the FVB/N background, produced by breeding hemizygous males with wild-type (WT) females. Controls were age- and sex-matched FVB/N mice. C57BL/6J-*lep$^{ob/ob}$* female mice were purchased from The Jackson Laboratory (Bar Harbor, Maine, USA). Mice were typically reared three to a cage on a 12-hour light/dark cycle (lights on at 0600–1800 hours), fed water and pellet (NIH-07) or powder (AIN 93G; Dyets, Bethlehem, Pennsylvania, USA) diet ad libitum. Food was blended daily with rosiglitazone (0.012 mg/g diet, for a dose of ~3 mg/kg/d; Smith Kline Beecham, West Sussex, United Kingdom) in a coffee grinder. Troglitazone (Parke-Davis, Ann Arbor, Michigan, USA) treatment was given by oral gavage at dose of 10 mg/100 μl H$_2$O daily for 2 weeks. All mice were sacrificed at between 0900 and 1200 hours. Tissues were fixed in neutralized 10% formalin and then processed by American Histolabs (Gaithersburg, Maryland, USA).

RNA analysis. cDNA probes for mouse acyl-CoA oxidase (AOX) and PPARα (courtesy of J. Peters, National Cancer Institute; ref. 24) and phosphoenolpyruvate carboxykinase (PEPCK) (25) were excised from plasmids. cDNA probes for sterol regulatory element binding protein-1 (SREBP1), stearoyl-CoA desaturase-1 (SCD1), and fatty acid synthase (FAS) were prepared by PCR (26) with mouse liver cDNA. PCR, using mouse liver cDNA as template with the following primers, was used to make probes for carnitine palmitoyl transferase I (CPT1: 5′-primer, 5′GAGAGCTGGCAACACCATCCACGC3′; 3′-primer, 5′GGGATTTGGCGTAGCTGTCGATGG 3′), PPARγ (5′-primer, 5′ACAAGACTACCCTTTACTGAAATTACCATG3′; 3′-primer, 5′GGCACTTCTGAAACCGACAGTACTGACATTTATTT3′), and insulin receptor substrate-2 (IRS2: 5′-primer, 5′GCGGCCTCATCTTCTTCACTGC3′; 3′-primer, 5′GGTTCCACGGCCTAGCCTGTGG3′). Total RNA was extracted using TRIzol (Life Technology Inc., Rockville, Maryland, USA). Northern blots (on Maximum Strength Nytran Plus; Schleicher & Schuell, Keene, New Hampshire, USA) were hybridized using Rapid-hyb (Amersham Pharmacia, Piscataway, New Jersey, USA) according to the manufacturer's instructions and exposed to film or quantitated with a BioImaging Analyzer with BAS 1500 MacBus software (Fuji Medical Systems, Standish, Maine, USA). Background was corrected by subtracting the average of an adjacent area on the blot.

Biochemical assays. FFAs, triglycerides, and insulin were assayed as described elsewhere (4). Glycated hemoglobin was assayed using a kit (442-B; Sigma Chemical Co., St. Louis, Missouri, USA). Glucose was measured using a Glucometer Elite (Bayer Corp., Elkhart, Indiana, USA), and serum chemistries were measured by the Clinical Pathology Department of the NIH Clinical Center. Liver triglyceride was measured by ethanolic KOH hydrolysis followed by a radiometric assay for glycerol (23).

Indirect calorimetry. Oxygen consumption and carbon dioxide production were measured as described previously (4). Measurements were made simultaneously with mice from different groups. In fasting animals, food was removed at onset of recording; data points from the 4th to 7th hours inside the metabolic chamber were used for analysis. Resting oxygen consumption was calculated as the average of the points with less than 21 ambulating beam breaks per minute.

Fat transplantation. Five-week-old A-ZIP/F-1 females (~17 g) were anesthetized with pentobarbital (0.01 ml/g body weight of 5 mg/ml in 5% ethanol/PBS, intraperitoneally). Donor fat pads (parametrial) from age-matched WT female mice sacrificed by cervical dislocation were placed into sterile 0.9% saline. Four fat pads were implanted subcutaneously through small incisions in the shaved skin of the back, with one piece per incision (4). Silk sutures (4-0) were used to close incisions. Mice were housed singly for 3 days after surgery and then at three to a cage. Tail venous blood (~150 μl) was withdrawn at noon 1 day before surgery, and weekly thereafter. Upon sacrifice, the grafts were examined visually, weighed, and fixed for histological analysis.

Statistical analysis. Values are reported as means ± SEM. Statistical significance was determined using two-tailed *t* test or ANOVA, as appropriate.

Results

Rosiglitazone does not improve the diabetes of A-ZIP mice. Treatment of A-ZIP/F-1 mice for 5 weeks with rosiglitazone had no effect on their threefold elevated glucose levels or the 200-fold elevated insulin levels (Figure 1). Similarly, treatment for 2 weeks with troglitazone failed to reduce the glucose and insulin levels (Table 1). As a positive control for TZD effectiveness, we treated leptin-deficient *ob/ob* mice with rosiglitazone (Figure 1). As expected (27), the *ob/ob* mice showed vastly improved insulin levels and normalization of serum glucose.

A glucose tolerance test was performed to characterize the insulin resistance further. In the A-ZIP/F-1 mice, the baseline 6-hour–fasted blood glucose values were elevated, and the glucose levels rose higher and remained elevated for longer than in WT controls (Figure 2). Rosiglitazone treatment of the A-ZIP/F-1 mice did not alter the abnormal glucose tolerance test. Thus,

in the lipoatrophic and diabetic A-ZIP/F-1 mice, rosiglitazone did not ameliorate the insulin resistance, confirming that adipose tissue is required for the glucose- and insulin-lowering effects of TZDs.

Rosiglitazone improves the lipid profile of A-ZIP/F-1 mice. In addition to their insulin-sensitizing actions, TZDs lower serum triglycerides and FFAs (22, 28–30). In marked contrast to its lack of effect on glucose and insulin levels, rosiglitazone treatment lowered serum triglyceride and FFA levels to normal in the A-ZIP/F-1 mice (Figure 1). Troglitazone was also effective at lowering the triglycerides and FFA levels (Table 1), suggesting that this is a general property of TZDs. Taken together, these data demonstrate that TZDs can reduce serum triglyceride and FFA levels in mice lacking adipose tissue.

Rosiglitazone exacerbates hepatic steatosis of A-ZIP/F-1 mice. We next examined the mechanism by which TZDs lower triglycerides and FFAs in lipoatrophic mice. Improvement of serum triglycerides and FFAs could be caused by redistribution from the circulation to tissue sites. Because A-ZIP/F-1 mice lack WAT, increased lipid deposition in this tissue cannot occur. However, the liver can store prodigious amounts of triglyceride. At baseline, A-ZIP/F-1 livers are already very steatotic (1, 5, 23). However, the livers from rosiglitazone-treated mice were even larger (1.87-fold) and lighter in color than the untreated A-ZIP/F-1 livers (Figure 3; Table 2). Histologically, the rosiglitazone-treated livers showed increased vacuolation, indicative of lipid deposition. Rosiglitazone treatment had no effect on the gross appearance or histology of WT livers.

Next, hepatic triglyceride content was measured directly. Rosiglitazone caused a 3.5-fold increase in liver triglyceride concentration in the A-ZIP/F-1 mice and a 1.4-fold increase in the *ob/ob* mice, compared with a 35% reduction in WT mice (Figure 3i). When triglyceride content was expressed as a total per liver, the effect of rosiglitazone was even more remarkable (A-ZIP/F-1, 432 ± 125 vs. 2,781 ± 414 μmol, $P < 0.001$; *ob/ob*, 1,497 ± 172 vs. 2,516 ± 744 μmol, $P = 0.01$; WT, 54 ± 7 vs. 31 ± 4 μmol, $P = 0.02$; in each pair, values are for control and rosiglitazone-treated, respectively). In contrast to rosiglitazone, troglitazone treatment did
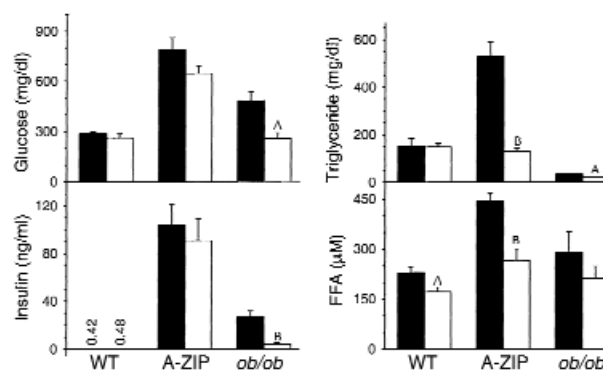

Figure 1

Serum chemistries of nonfasted female mice. WT or A-ZIP/F-1 mice, 4–5 weeks old, were treated with a control (filled bars) or rosiglitazone (open bars) diet for 5 weeks. Female *ob/ob* mice, 6 weeks old, were treated for 2 weeks. Data are mean ± SEM ($n = 6$). [A]$P < 0.05$ for differences within each genotype between control and rosiglitazone-treated mice. [B]$P < 0.005$ for differences within each genotype between control and rosiglitazone-treated mice.

not increase liver triglyceride in A-ZIP/F-1 mice (Table 1). Similar to the effect of rosiglitazone, troglitazone treatment of control mice reduced liver triglycerides.

During rosiglitazone treatment, the A-ZIP/F-1 mice gained weight, which was quantitatively accounted for by the increase in liver size (Table 2). Rosiglitazone also caused a significant, but less dramatic increase in liver weight in the *ob/ob* mice. By comparison, rosiglitazone treatment of the WT mice caused no change in body weight and a small reduction in liver weight.

Given the marked steatosis and hepatomegaly (to 18% of body weight), we looked for evidence of altered liver function or hepatitis. Serum biochemistries in rosiglitazone-treated A-ZIP/F-1 mice showed unchanged albumin and slight increases in transaminases that were not statistically significant (Table 2). Serum total bilirubin, lipase, and cholesterol, on the other hand, decreased significantly. In addition, there was no histological evidence of hepatitis. These data suggest that the liver is functioning normally, with little evidence for liver injury, despite the massive steatosis.

Table 1

Treatment of A-ZIP/F-1 mice with troglitazone

	WT, Control	WT, Troglitazone	A-ZIP/F-1, Control	A-ZIP/F-1, Troglitazone
Body weight (g)	22.8 ± 0.8	22.3 ± 0.8	23.9 ± 1.3	23.7 ± 0.9
Food intake (g/d)	3.92 ± 0.18	3.83 ± 0.16	5.33 ± 0.18	4.91 ± 0.28
Liver weight (g)	1.07 ± 0.02	1.06 ± 0.03	2.60 ± 0.26	2.68 ± 0.16
BAT weight (mg)	120 ± 13	144 ± 3	67 ± 5	53 ± 6
Glucose (mg/dl)	294 ± 18	271 ± 23	929 ± 90	859 ± 58
Insulin (ng/ml)	0.93 ± 0.20	0.82 ± 0.08	113 ± 24	99 ± 31
FFA (μM)	312 ± 43	264 ± 54	928 ± 150	617 ± 92
Triglyceride (mg/dl)	300 ± 43	259 ± 34	919 ± 83	595 ± 58[A]
Liver triglyceride (μmol/g)	50 ± 6	24 ± 5[A]	279 ± 34	281 ± 21
Liver triglyceride (μmol/liver)	53 ± 7	25 ± 5[A]	732 ± 116	751 ± 63

Female WT or A-ZIP/F-1 mice, 6–8 weeks old, were treated with troglitazone for 2 weeks. Animals were sacrificed in the fed state. Data are mean ± SEM ($n = 6$). [A]$P < 0.05$ for difference within genotype between control and troglitazone-treated mice.

Figure 2

Glucose tolerance testing. Intraperitoneal glucose tolerance tests using 2 mg glucose/kg were performed at approximately 8 hours after lights on, after a 6-hour fast (4). Female WT (circles) and A-ZIP/F-1 (triangles) mice were treated with rosiglitazone (open symbols) or control diets (filled symbols) for 2–4 weeks at the time of testing. Tail vein blood was collected immediately before and at 15, 30, 60, and 120 minutes after glucose injection. Data are the average of two tests per mouse, performed 1 week apart, with six mice per group. A-ZIP/F-1 mice are different from WT at $P < 0.05$ for all points, with no effect of rosiglitazone treatment.
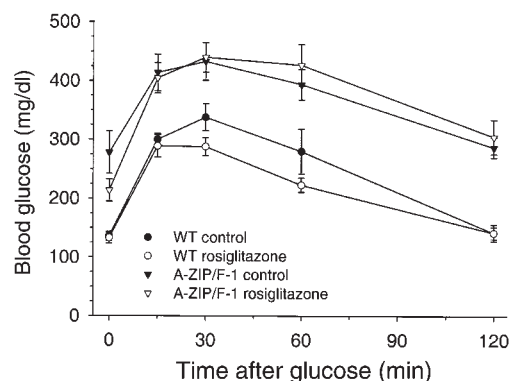
Rosiglitazone increases respiratory exchange ratio. The respiratory exchange ratio (RER; the ratio of CO_2 produced to O_2 consumed) is a measure of metabolic fuel source. Pure carbohydrate oxidation gives an RER of 1.00, whereas pure fat oxidation gives a ratio of approximately 0.71 (31). Fasting lipoatrophic humans (32) and A-ZIP/F-1 mice (33) have an elevated RER, indicating increased net fractional oxidation of carbohydrate. In WT mice, 4–7 hours of fasting was sufficient to reduce the RER maximally, irrespective of treatment with rosiglitazone. Under identical conditions, the A-ZIP/F-1 mice did not show a maximally reduced RER unless treated with rosiglitazone (Table 2). Thus rosiglitazone increases net fractional lipid oxidation, suggesting that increased fat oxidation (or decreased lipogenesis) contributes to rosiglitazone's ability to reduce serum FFA and triglyceride levels.

Diabetes of fat-transplanted mice does not respond to rosiglitazone. We next studied whether the antidiabetic effect of rosiglitazone could be restored in A-ZIP/F-1 mice by transplantation of WT adipose tissue. A submaximal transplant amount (400 mg) (4) was chosen, enabling the potential for detection of a rosiglitazone effect. Similar to previous observations (4, 5), fat transplantation improved the glucose and insulin levels beginning

2 weeks after surgery, whereas sham-operated mice had persistent hyperglycemia and hyperinsulinemia (Figure 4, a and b). However, we did not detect any effect of rosiglitazone treatment on the final glycated hemoglobin (sham control, 11.6 ± 1.1%; sham rosiglitazone, 12.3 ± 0.8%; transplanted control, 7.2 ± 0.6%; transplanted rosiglitazone, 7.8 ± 1.2%; WT 6.6 ± 0.5%), glucose, or insulin levels in the fat-transplanted mice (data not shown). The rosiglitazone treatment was effective, as the weight of the brown adipose tissue in both the sham- and fat-transplanted groups was increased by rosiglitazone treatment (sham, from 60 ± 10 to 177 ± 21 mg; transplanted, from 27 ± 3 to 66 ± 6 mg).

We next examined the effect of fat transplantation on the increased hepatic size and steatosis caused by rosiglitazone treatment (Figure 4, c and d). As expected (4, 5), fat transplantation greatly decreased liver weight (by 42%) and triglyceride content (by 82%), but did not reduce either to WT levels. However, rosiglitazone treatment did not increase hepatic triglyceride content; the steatotic effect of rosiglitazone was abolished by WAT transplantation.

Hepatic PPARγ expression correlates with rosiglitazone-induced increase in liver triglyceride content. Given that hepatic levels of PPARγ are a possible determinant of

Table 2

Characteristics of rosiglitazone-treated mice

	WT, Control	WT, Rosiglitazone	A-ZIP/F-1, Control	A-ZIP/F-1, Rosiglitazone	ob/ob, Control	ob/ob, Rosiglitazone
Body weight (g)	21.9 ± 0.5	22.1 ± 0.4	22.4 ± 0.4	24.2 ± 0.4[A]	39.3 ± 0.6	41.3 ± 0.6[A]
Liver weight (g)	0.95 ± 0.02	0.83 ± 0.03[A]	2.28 ± 0.13	4.27 ± 0.31[B]	3.69 ± 0.11	4.51 ± 0.19[B]
BAT weight (mg)	125 ± 6	271 ± 14[B]	65 ± 12	150 ± 17[B]	340 ± 12	531 ± 28[A]
Aspartate aminotransferase (U/l)	158 ± 18	126 ± 18	211 ± 57	323 ± 71		
Alanine aminotransferase (U/l)	51 ± 9	44 ± 7	101 ± 25	189 ± 47		
Lipase (U/l)	46 ± 3	47 ± 8	53 ± 7	35 ± 4[A]		
Cholesterol (mg/dl)	118 ± 5	119 ± 4	171 ± 11	134 ± 12[A]		
Albumin (g/dl)	3.3 ± 0.1	3.2 ± 0.1	3.7 ± 0.1	3.9 ± 0.3		
Total bilirubin (mg/dl)	0.31 ± 0.05	0.25 ± 0.03	0.55 ± 0.10	0.24 ± 0.03[A]		
Lactate dehydrogenase (U/l)	721 ± 108	564 ± 97	1,220 ± 390	1830 ± 320		
Oxygen consumption (ml/h/g$^{0.75}$)	10.33 ± 0.36	10.86 ± 0.24	8.25 ± 0.24	8.23 ± 0.20		
RER	0.699 ± 0.006	0.692 ± 0.005	0.730 ± 0.007	0.698 ± 0.007[A]		

Female WT or A-ZIP/F-1 mice, 4–5 weeks old, were treated with rosiglitazone for 5 weeks. Female *ob/ob* mice, 6 weeks old, were treated for 2 weeks. Animals were sacrificed in the fed state. Data are mean ± SEM (n = 6). [A]$P < 0.05$ for differences within each genotype between control and rosiglitazone-treated mice. [B]$P < 0.005$ for differences within each genotype between control and rosiglitazone-treated mice.

the response to rosiglitazone treatment, we measured hepatic PPARγ mRNA levels. WT mice had levels of PPARγ mRNA near the limit of detection by Northern analysis. In contrast, in A-ZIP/F-1 mice, the PPARγ mRNA levels were increased (3- to 17-fold; quantitation of the increase is difficult owing to the low levels in the WT mice; Figure 4, e and f, and Figure 5). Rosiglitazone treatment had little effect on PPARγ mRNA levels in WT mice, but increased them 1.4-fold in A-ZIP/F-1 mice. Fat transplantation reversed the increase in the A-ZIP/F-1 mice. In skeletal muscle, Northern blots demonstrated barely detectable levels of PPARγ mRNA in A-ZIP/F-1 mice, FVB/N controls, and *ob/ob* mice, which did not change upon rosiglitazone treatment (data not shown). Thus, there is a striking correlation between the hepatic PPARγ mRNA levels, the hepatic triglyceride content, and the response to rosiglitazone treatment. These data suggest that PPARγ levels are an important determinant of hepatic triglyceride content, presumably increasing lipogenesis and possibly fatty acid uptake.

The aP2 gene is transcribed predominantly in adipose and other PPARγ-responsive tissues (34, 35). Hepatic aP2 mRNA levels were similar in A-ZIP/F-1 and control mice but were induced by rosiglitazone treatment only in the A-ZIP/F-1 mice (FVB/N controls, 100 and 59, vs. A-ZIP/F-1, 101 and 565; first value is untreated, second is rosiglitazone-treated; data are given in arbitrary units and are averages of two different pooled samples). These data indicate that hepatic PPARγ drives liver expression of aP2, a gene usually thought of as an adipose marker.

We also quantitated the liver mRNAs encoding transcription factors and metabolic enzymes involved in lipogenesis, β-oxidation, and glucose homeostasis. In A-ZIP/F-1 mice, the lipogenic genes, fatty acid synthase (FAS) and stearoyl-CoA desaturase-1 (SCD1), were both higher than in control mice (Figure 5). After 5 weeks of rosiglitazone treatment, SCD1 levels increased approximately 1.7-fold in both WT and A-ZIP/F-1 mice, whereas FAS levels did not change. Liver mRNA levels for SREBP1 were slightly elevated in A-ZIP/F-1 mice, but were not affected by rosiglitazone treatment.

To study the effect of rosiglitazone on β-oxidation genes, we measured PPARα, carnitine palmitoyl transferase-1 (CPT1), and acyl-CoA oxidase (AOX) mRNA levels (Figure 5). These mRNAs were all expressed at similar levels in control WT and A-ZIP/F-1 mice. Rosiglitazone treatment significantly increased mRNA levels only for AOX, which catalyzes the first step of β-oxidation. The AOX increase was less than that seen with treatment with PPARα agonist treatment (24).

Phosphoenolpyruvate carboxykinase (PEPCK) mRNA levels indicate the level of gluconeogenesis. In A-ZIP/F-1 mice, the PEPCK mRNA levels were comparable to those of control mice. IRS-2 mRNA levels, which correlate with insulin signaling (36), were reduced by 80% in A-ZIP/F-1 mice and were slightly less reduced after rosiglitazone treatment.

In summary, the mRNA levels in untreated A-ZIP/F-1 mice demonstrate increased expression of lipogenic enzymes, with no change in enzymes involved in β-oxidation. Rosiglitazone treatment caused a modest increase in AOX mRNA levels in A-ZIP/F-1, but not WT mice, consistent with an increase in β-oxidation.
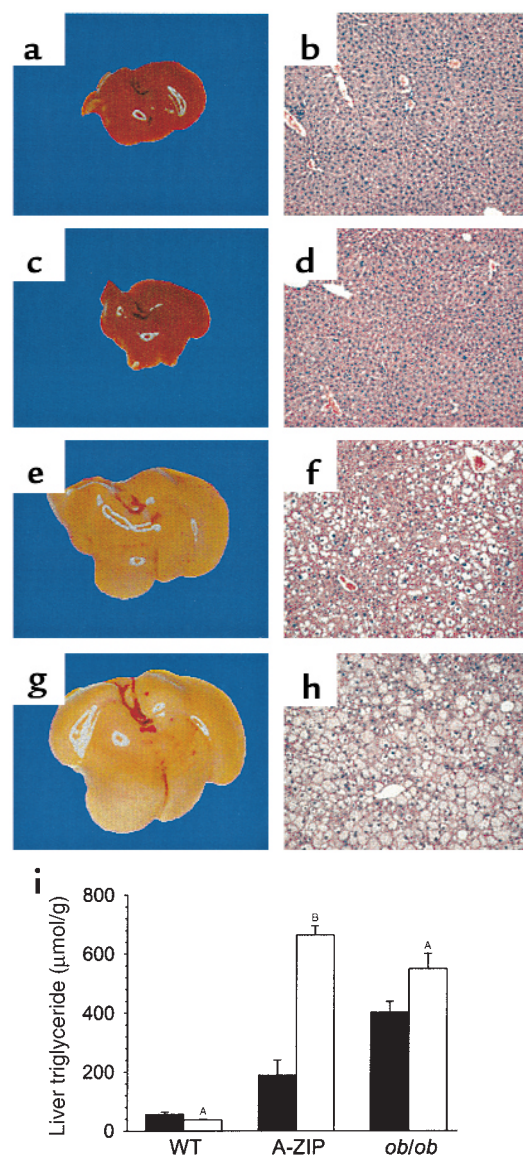

Figure 3

Rosiglitazone exacerbates hepatic steatosis. Livers from WT (**a–d**) and A-ZIP/F-1 (**e–h**) mice, either treated with rosiglitazone (**c**, **d**, **g**, **h**) or not (**a**, **b**, **e**, **f**) for 5 weeks, are shown at the same magnification. Hematoxylin and eosin–stained sections of the same livers (original magnification, ×100) show steatosis of the control A-ZIP/F-1 mice, which worsens with rosiglitazone treatment. Liver triglyceride content (**i**) is shown for control (filled bars) and rosiglitazone-treated (open bars) mice (after 5 weeks of treatment for the A-ZIP/F-1 mice and 2 weeks for the *ob/ob* mice). Data are mean ± SEM (*n* = 5–6). [A]$P < 0.05$ for differences within each genotype between control and rosiglitazone-treated mice. [B]$P < 0.005$ for differences within each genotype between control and rosiglitazone-treated mice.

Discussion

White adipose tissue is required for the insulin-sensitizing actions of TZDs. We show that rosiglitazone (or troglitazone) treatment of lipoatrophic A-ZIP/F-1 mice does not improve their hyperglycemia or hyperinsulinemia. This result is most consistent with the hypothesis that WAT is required for the antidiabetic effects of TZDs. The adipose site for TZD action is concordant with the observation that PPARγ is normally expressed at high levels in adipose tissue and not the other major insulin target tissues, muscle and liver. The effect of the TZDs in diabetics without lipoatrophy may be through the stimulation of adipocyte differentiation, generating more small adipocytes, which are more insulin sensitive. The TZDs may also increase insulin sensitivity by stimulating adipocyte glucose, fatty acid, and triglyceride uptake; reducing systemic levels; or altering release of adipose signaling molecules that affect insulin sensitivity in other tissues.

Fat transplantation did not confer rosiglitazone-responsiveness to the A-ZIP/F-1 mice. There are a number of possible explanations for this failure. First, the transplanted fat may have lowered the serum glucose and insulin too efficiently, such that a rosiglitazone effect was masked. Second, although fat implants vascularize and contain nerves, their function and regulation are not entirely normal (4), so the transplanted fat may not have been able to respond to TZDs. Third, the transplanted adipocytes may have enlarged to the point at which they were poorly responsive to TZDs. Finally, the transplanted WAT may have exhausted its proliferative potential. Thus the lack of restoration of the antidiabetic effects of rosiglitazone by fat transplantation does not detract from our belief in the importance of adipose tissue in mediating the antidiabetic effects of TZDs.

Mice lacking PPARγ have been produced (37–39). Homozygous *PPARγ* disruption is embryonic lethal, but *PPARγ*^{+/−} mice are viable. Surprisingly, when challenged with a high fat diet, *PPARγ*^{+/−} mice show increased insulin sensitivity relative to *PPARγ*^{+/+} controls (38, 40). A proposed explanation is that the *PPARγ*^{+/−} adipose tissue is not as lipid laden and is thus more insulin-sensitive, possibly with a contribution from the paradoxically higher leptin levels (41).

The conclusion that WAT is required for the antidiabetic effects of TZDs contrasts with that reached by Burant et al. (22). Using the aP2/DTA mouse model of lipoatrophy, they concluded that troglitazone action is independent of adipose tissue. The aP2/DTA mouse has a normal amount of WAT at weaning, which begins to diminish by approximately 5 months of age, and by the time of troglitazone treatment (8–9 months), these mice showed an approximately 90% reduction in gonadal fat. The effectiveness of thiazolidinediones in aP2-nSREBP-1c lipoatrophic mice with intermediate levels of adipose tissue (42) has not been reported.

There are multiple reasons for the difference between our conclusions and those of Burant et al. The mice express different transgenes and have different genetic backgrounds. Also, the greater residual adipose tissue of the aP2/DTA mouse may enable improvement in the diabetes via direct adipose effects, just as troglitazone effectively treats the diabetes of patients with partial lipodystrophy (32).

Figure 4
Effect of rosiglitazone treatment on fat-transplanted A-ZIP/F-1 mice. WAT (400 mg) was transplanted into A-ZIP/F-1 mice at 5 weeks of age. Serum glucose (**a**) and insulin (**b**) were measured weekly (note that the insulin scale is logarithmic). Rosiglitazone (or control) treatment was begun 2 weeks after transplantation. Symbols are filled circles, sham-operated A-ZIP/F-1; open circles, sham-operated, rosiglitazone-treated A-ZIP/F-1; filled triangles, transplanted A-ZIP/F-1; open triangles, transplanted, rosiglitazone-treated A-ZIP/F-1; and filled squares, WT FVB/N. Hepatic weight (**c**), triglyceride content (**d**), and PPARγ mRNA (**e**, **f**) levels in control (filled bars) or rosiglitazone-treated (open bars) mice at 8 weeks after transplant. WAT transplants increased to 329 ± 59% and 398 ± 87% of initial weight in the control and rosiglitazone-treated groups, respectively. mRNA levels are expressed as a percent of WT levels. Each lane of the Northern blot is from a different mouse. Data are mean ± SEM (*n* = 5–6).
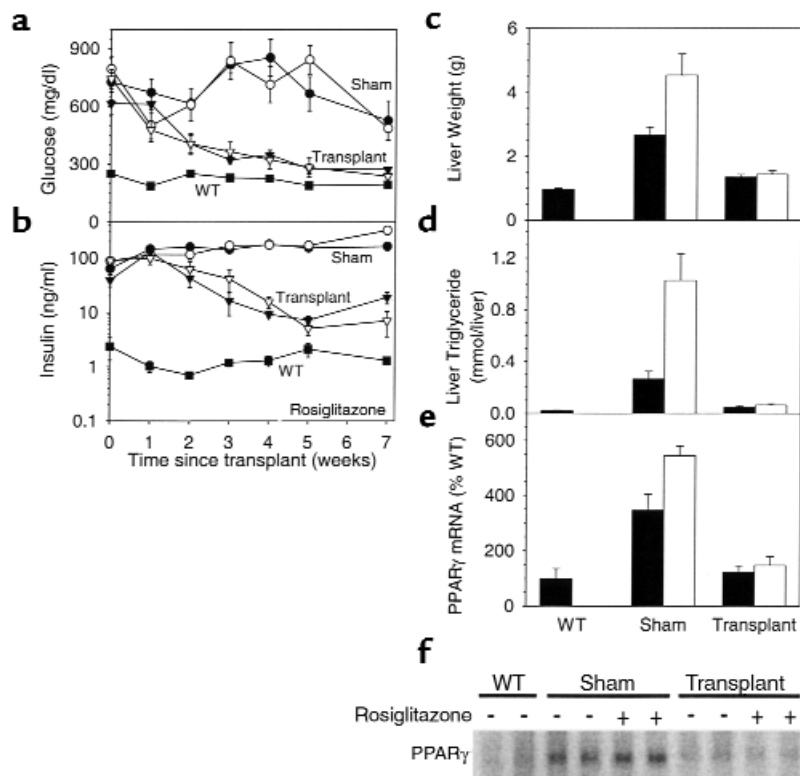
Rosiglitazone improves hypertriglyceridemia in A-ZIP/F-1 mice, independently of white adipose tissue. In contrast to its lack of antidiabetic effects, rosiglitazone lowered serum triglycerides and FFAs in A-ZIP/F-1 mice. Possible mechanisms are decreased dietary intake, decreased fatty acid production, increased β-oxidation, and redistribution away from the circulating compartment. Food intake was not decreased, indicating that triglyceride availability did not change. Although we have not measured fatty acid production directly, neither FAS nor SCD1 mRNA was decreased (and in fact these mRNAs were increased), indicating that decreased hepatic fatty acid synthesis is unlikely. In contrast, increased fat oxidation probably does contribute to the improvement in lipid levels, as hepatic AOX mRNA levels are increased and the elevated respiratory quotient was reversed by rosiglitazone treatment. We do not know which tissues contribute to the increased lipid oxidation in the A-ZIP/F-1 mice; muscle and liver are candidates. It is also possible that redistribution of circulating triglycerides into tissue occurs. For example, uptake of circulating lipids may contribute to the fatty liver.

TZD actions and PPARγ physiology in mice with normal compared with steatotic livers. PPARγ is normally expressed prominently in white adipose tissue, with much lower levels in liver and skeletal muscle (12–14, 17). The A-ZIP/F-1 mice have increased hepatic PPARγ mRNA levels. This increase in PPARγ may be a general property of steatotic livers, as it is present in *ob/ob* mice (43), UCP-DTA mice fed a high-fat diet (14), PPARα knockout mice (44), and slightly in aP2/DTA mice (22). The mechanistic relationship between the steatosis and increased PPARγ mRNA levels is unclear. Do increased PPARγ levels cause increased lipogenesis and/or lipid uptake, leading to steatosis? Does hepatic steatosis per se lead to increased PPARγ levels? Or are both true? One possibility is that during increased energy availability (such as obesity or a high-fat diet), a circulating factor stimulates hepatic PPARγ transcription, turning on the lipogenic program and producing a fatty liver.

The very different responses to rosiglitazone treatment of A-ZIP/F-1 and *ob/ob* mice (with PPARγ-rich, fatty livers) compared with WT and fat-transplanted A-ZIP/F-1 mice (with low PPARγ and normal or near-normal liver lipid) highlight a fundamental difference between normal and lipoatrophic mice. In normal mice, there is little hepatic PPARγ and thus little direct hepatic effect of rosiglitazone treatment. The major rosiglitazone action on adipocytes provides more storage capacity for triglyceride, resulting in slightly lower hepatic (and other tissue) triglyceride levels. In contrast, in lipoatrophic mice, the steatotic liver is a major direct target for rosiglitazone action and becomes more lipogenic upon rosiglitazone treatment, further increasing liver lipid content.

Although both rosiglitazone and troglitazone failed to lower glucose and insulin, and both did reduce triglycerides, there were significant differences between these two TZDs. Notably, only rosiglitazone increased hepatic triglyceride content. It is possible that one or
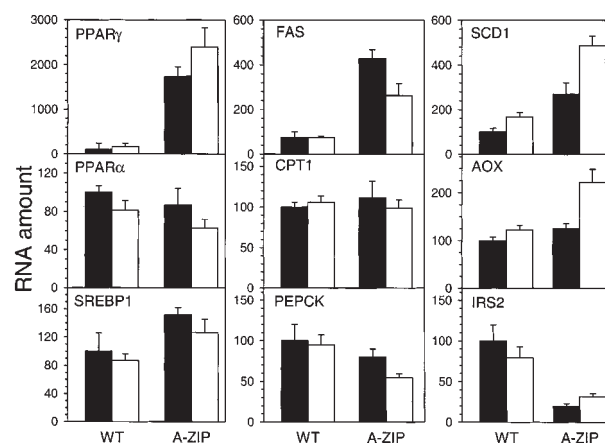

Figure 5

Liver mRNA levels. Results are for control (filled bars) and rosiglitazone-treated (open bars) mice after 5 weeks of treatment. Data are expressed as a percentage of WT control and are mean ± SEM (n = 4–5). By two-way ANOVA, the genotype effect is significant (P < 0.05) for all except PPARα and CPT1; the treatment effect is significant for SCD1 and AOX, and there is a significant genotype x treatment interaction only for AOX.

both of these drugs have differential effects via PPARγ-independent mechanisms (45). Another likely explanation is that troglitazone is a partial PPARγ agonist in certain assays, whereas rosiglitazone is a full agonist, giving the drugs overlapping but distinct effects on gene expression (46). Recent reports of PPARγ antagonist (47) and weak agonist (48) ligands hint at the future pharmacological diversity. In theory, an ideal PPARγ ligand would target adipose tissue, but not liver, and increase insulin sensitivity, but not lipogenesis. Generation of A-ZIP/F-1 mice with liver-specific disruption of PPARγ will test the contribution of hepatic PPARγ to generation of the fatty liver.

Obesity is a major risk factor for both diabetes and hepatic steatosis, so it is likely that both conditions will be present in a significant number of people. Given that the physiology of PPAR action (particularly PPARα) can be different between mice and humans, our results raise clinical questions that can only be answered with data from humans. Do people with steatotic livers have increased hepatic PPARγ levels? What about patients with type 2 diabetes? Do humans with hepatic steatosis respond differently to (certain) TZDs than do people with lean livers? We are not aware of studies measuring PPARγ levels in steatotic human livers. Nor do we know of reports specifically comparing the effects of TZDs in patients with fatty livers compared with controls. However, when a group of lipoatrophy patients was treated with troglitazone, both improved diabetes and a reduced liver volume (presumably due to reduced triglyceride content) were observed (32). The diabetes and liver volume improved even in the four patients with the most severe generalized lipoatrophy (E. Arioglu and S.I. Taylor, personal communication). One interpretation is that there are

differences in physiology between humans and mice.

Our results suggest that the antidiabetic actions of TZDs require adipose tissue. However, the absence of adipose tissue causes severe hepatic steatosis with high PPARγ levels. In this situation, rosiglitazone treatment worsened the steatosis, while lowering circulating triglycerides and increasing net fractional fatty acid oxidation. We postulate that the state of the liver determines the mouse's response to rosiglitazone treatment, with PPARγ-rich fatty livers becoming even more lipid-laden, whereas normal livers actually lose triglycerides.

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