Pharmacogenetic Evidence That \( Cd36 \) Is a Key Determinant of the Metabolic Effects of Pioglitazone*

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Pioglitazone, like other thiazolidinediones, is an insulin-sensitizing agent that activates the peroxisome proliferator-activated receptor \( \gamma \) and influences the expression of multiple genes involved in carbohydrate and lipid metabolism. However, it is unknown which of these many target genes play primary roles in determining the antidiabetic and hypolipidemic effects of thiazolidinediones. To specifically investigate the role of the \( Cd36 \) fatty acid transporter gene in the insulin-sensitizing actions of thiazolidinediones, we studied the metabolic effects of pioglitazone in spontaneously hypertensive rats (SHR) that harbor a deletion mutation in \( Cd36 \) in comparison to congenic and transgenic strains of SHR that express wild-type \( Cd36 \). In congenic and transgenic SHR with wild-type \( Cd36 \), administration of pioglitazone was associated with significantly lower circulating levels of fatty acids, triglycerides, and insulin as well as lower hepatic triglyceride levels and epididymal fat pad weights than in SHR harboring mutant \( Cd36 \). Additionally, insulin-stimulated glucose oxidation in isolated soleus muscle was significantly augmented in pioglitazone-fed rats with wild-type \( Cd36 \) versus those with mutant \( Cd36 \). The \( Cd36 \) genotype had no effect on pioglitazone-induced changes in blood pressure. These findings provide direct pharmacogenetic evidence that in the SHR model, \( Cd36 \) is a key determinant of the insulin-sensitizing actions of a thiazolidinedione ligand of peroxisome proliferator-activated receptor \( \gamma \).

Thiazolidinediones such as pioglitazone and rosiglitazone have attracted considerable attention for the treatment of type II diabetes. The insulin-sensitizing effects of these compounds are believed to be related at least in part to their ability to bind to the peroxisome proliferator activated receptor \( \gamma \) (PPAR\( \gamma \)), a nuclear hormone receptor that regulates the expression of multiple genes involved in the control of carbohydrate and lipid metabolism (1–4).

Although many genes and metabolic pathways are likely to be involved in the insulin-sensitizing action of PPAR\( \gamma \) ligands, it has been proposed that the effect of thiazolidinediones on genes regulating free fatty acid (FFA) transport and metabolism may be one of the key mechanisms responsible for the antidiabetic effects of these drugs (2, 3, 5–10). Chronic elevations of FFA levels are a well known determinant of insulin resistance (11–14). Accordingly, a number of investigators have suggested that thiazolidinediones may attenuate insulin resistance by increasing the metabolic clearance of FFA and decreasing FFA levels in the circulation.

The fatty acid transporter \( Cd36 \) is one of a number of molecules that mediate the uptake of FFA by adipocytes and muscle cells (15–16) and is a well known target of PPAR\( \gamma \) ligands (17). Mutations in \( Cd36 \) have been found to be associated with impaired carbohydrate and lipid metabolism in both humans and laboratory animals (18–22). Recently, Hevener et al. (6) reported that infusion of FFA induces reductions in systemic glucose disposal rate that are paralleled by decreases in muscle expression of \( Cd36 \). These investigators also found that oral administration of the thiazolidinedione troglitazone can attenuate the decreases in \( Cd36 \) expression and glucose disposal rate otherwise induced by infusion of FFA (6). Based on these correlational observations, Hevener and colleagues have proposed that the insulin-sensitizing effects of thiazolidinediones may at least be partly mediated by their effects on \( Cd36 \) expression and FFA metabolism (6).

The availability of animal models lacking \( Cd36 \) provides an opportunity to use a pharmacogenetic approach to specifically test the proposed role of \( Cd36 \) in the insulin-sensitizing actions of thiazolidinediones. Based on the hypothesis of Hevener and colleagues, it might be predicted that the insulin-sensitizing

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effects of thiazolidinediones would be attenuated in animals lacking Cd36 (6). We now report a positive test of this hypothesis by showing that the insulin-sensitizing actions of pioglitazone are impaired in spontaneously hypertensive rats harboring mutant Cd36 compared with congenic and transgenic strains of spontaneously hypertensive rats expressing wild-type Cd36. Whereas previous studies have demonstrated that PPARy ligands alter the expression of many genes related to carbohydrate and lipid metabolism (3, 4), the current findings provide direct pharmacogenetic evidence that Cd36 is a key determinant of the insulin-sensitizing actions of pioglitazone, a PPARy ligand widely used for the treatment of type II diabetes.

EXPERIMENTAL PROCEDURES

Animals—We compared the SHR progenitor strain carrying a spontaneous mutation in Cd36 that abolishes membrane expression of Cd36 protein to a congenic strain and transgenic strain of SHR that express wild-type Cd36 (19–21). The progenitor strain of SHR with defective Cd36 descends from inbred SHR originally obtained from National Institutes of Health and was beyond the F90 generation when derivation of the congenic and transgenic strains was initiated. The defect in Cd36 in the SHR progenitor strain has been previously described in detail. The SHR-BN/Shr/Ny (inbred as SHR) strain was derived by backcrossing an inbred Brown Norway strain (Charles River Laboratories) with the SHR progenitor strain for 8 generations (19, 21). The SHR-4 congenic strain is 99% genetically identical to the SHR progenitor strain, which differs only with respect to the region of chromosome 4 that includes Cd36. Details regarding derivation and characterization of the SHR-4 congenic strain have been previously described (19, 21). SHR-4 congenic rats of the N8F8 generation were used in the current study. The SHR/Ola-TgNCd36 (abbreviated as SHR-TG19) transgenic rats were derived by microinjecting SHR progenitor zygotes with wild-type Cd36 cDNA under control of the elastase factor 1a promoter (20). Details regarding derivation of the transgenic strain and the expression pattern of the Cd36 transgene have been previously described (20). In the current studies, we used SHR-TG19 transgenic rats of the F7 generation that were homozygous for the Cd36 transgene as confirmed by progeny testing. For in vivo studies, a minimum of 8 animals was studied in each group and for in vitro studies, a minimum of 5 animals was studied in each group. All experiments were performed in agreement with the Animal Protection Law of the Czech Republic (311/1997) which corresponds fully to the European Community Council recommendations for the use of laboratory animals 86/609/ECC. The experiments were approved by the Ethics Committee of the Institute of Physiology, Czech Academy of Sciences, Prague, and by the Animal Protection Committee of the Charles University, Faculty of Medicine in Prague, Brno, Czech Republic. Serum insulin concentrations were determined using random primers and the cDNA was amplified by real-time PCR using QuantiTect SYBR Green reagents (Qiagen) on an Opticon continuous fluorescence detector (MJ Research) running 35 cycles of the protocol: 1 min denaturation at 95 °C, 30 s annealing at 58.9 °C, and 1 min extension at 72 °C. The upstream primers were CAAATGCTGGACCCAACACA (cyclophilin A) and TCAAGGTGTGTCACAAAGGCC (Cd36); the downstream primers were TGGCATCTAAACACCATCTACG (cyclophilin A) and AGGATAAACAACCACTGCATG (Cd36). The RNA was reverse transcribed using random primers and the cDNA was amplified by real-time PCR using primers specific for Cd36 and the cDNA was measured using a continuous fluorescence detector (MJ Research) running 35 cycles of the following protocol: 1 min denaturation at 95 °C, 30 s annealing at 51.2 °C for Cd36 or 58.9 °C for cyclophilin, followed by 30 s extension at 72 °C. Post-PCR melting curves confirmed the specificity of single-target amplification and -fold expression of Cd36 relative to cyclophilin was determined in triplicate using the pref ed methods of Mathur et al. (25).

Statistical Analysis—All data are expressed as means ± S.E. Two-way analysis of variance was used to test for effects of strain, pioglitazone treatment, and strain × treatment interactions on the dependent variables.
variables of interest. Differences between control versus treatment groups within a strain were evaluated by Student’s t test, and differences among strains within groups were evaluated by one-way analysis of variance with Dunnett’s method to adjust for multiple comparisons using the SHR strain as the control. Statistical significance was defined as p < 0.05.

RESULTS

Before starting the high fructose diet, mean body weights of the three strains were similar with the SHR-4 congenic strain (193 ± 3 g) weighing slightly less (p < 0.05) than the SHR progenitor strain (209 ± 3 g) and SHR-TG19 transgenic strain (214 ± 3 g) (Fig. 1A). Within each strain, rats treated with pioglitazone gained the same amount of weight as untreated rats (Fig. 1B), so no excess weight gain was observed during the pioglitazone treatment. The SHR-4 congenic rats gained −10 grams more than the SHR progenitor rats and the SHR-TG19 transgenic rats (both p < 0.05), perhaps because of their slightly lower body weights at the beginning of the study (Fig. 1B).

Compared with control treatment (no pioglitazone), administration of pioglitazone was associated with significant reductions in NEFA levels in both the SHR-4 congenic (p < 0.005) and SHR-TG19 transgenic (p < 0.001) strains expressing wild-type Cd36, but this effect did not achieve statistical significance in the SHR progenitor strain harboring defective Cd36 (p = 0.06) (Fig. 2A). Without pioglitazone treatment, mean serum NEFA levels were 0.72 ± 0.06, 0.77 ± 0.05, and 0.70 ± 0.06 mmol/L in the SHR-4, SHR-TG19, and SHR progenitor strains, respectively. With pioglitazone treatment, serum NEFA levels were 0.37 ± 0.03, 0.47 ± 0.02, and 0.56 ± 0.04 mmol/liter, respectively. With pioglitazone treatment, analysis of variance showed a significant strain effect on NEFA levels (p < 0.005) with significantly lower values in the SHR-4 congenic and SHR-TG19 transgenic strains compared with the SHR progenitor strain.

Compared with control treatment (no pioglitazone), administration of pioglitazone was also associated with significant reductions in triglyceride levels in the SHR-4 congenic (p < 0.005) and SHR-TG19 transgenic (p < 0.005) strains expressing wild-type Cd36 but not in the SHR progenitor strain harboring defective Cd36 (p = 0.38) (Fig. 2B). Without pioglitazone treatment, mean serum triglyceride levels were 1.7 ± 0.2, 1.7 ± 0.1, and 1.8 ± 0.2 mmol/liter in the SHR-4, SHR-TG19, and SHR progenitor strains, respectively. With pioglitazone treatment, serum triglyceride levels were 1.0 ± 0.1, 1.3 ± 0.1, and 1.5 ± 0.2 mmol/liter, respectively. Analysis of variance showed a significant strain effect on NEFA levels (p < 0.005) with significantly lower values observed in the pioglitazone-treated SHR-4 congenic and SHR-TG19 transgenic strains compared with the pioglitazone-treated SHR progenitor strain (p < 0.05 by Dunnett’s method).

The effects of pioglitazone on serum insulin levels are shown in Fig. 3. Analysis of variance showed a significant effect of strain (p < 0.002) and treatment (p < 0.001) on serum insulin levels. Pioglitazone treatment was associated with significantly lower serum insulin levels in the SHR congenic and transgenic strains but not in the SHR progenitor strain harboring mutant Cd36. Serum insulin levels in pioglitazone-treated SHR congenic rats, 0.48 ± 0.03 nmol/liter, and in pioglitazone-treated SHR-TG19 transgenic rats, 0.58 ± 0.05 nmol/liter, were significantly lower than in pioglitazone-treated SHR, 0.75 ± 0.05 nmol/liter (both p < 0.05). There was no significant effect of pioglitazone on serum glucose levels in the three strains (SHR, 5.7 ± 0.2 mmol/ liter with pioglitazone versus 5.3 ± 0.1 mmol/liter without pioglitazone; SHR-TG19 transgenic, 5.7 ± 0.1 mmol/liter with pioglitazone versus 5.6 ± 0.1 mmol/liter without pioglitazone; SHR-4 congenic, 6.2 ± 0.2 mmol/liter with pioglitazone versus 6.1 ± 0.2 mmol/liter without pioglitazone). In the absence of pioglitazone, oral glucose tolerance was not significantly different among the three strains by analysis of variance, although there was a tendency for better glucose tolerance in the SHR-TG19 strain compared with the SHR-4 congenic strain and SHR progenitor strain (AUC = 155 ± 23 mmol/liter−1·2 h−1, 189 ± 18 mmol/liter−1·2 h−1, and 181 ± 2 mmol/liter−1·2 h−1, respectively) (Fig. 4). With pioglitazone, oral glucose tolerance was significantly better in both the SHR-TG19 transgenic strain (AUC = 163 ± 10 mmol/liter−1·2 h−1) and the SHR-4 congenic strain (AUC = 154 ± 7 mmol/liter−1·2 h−1), compared with the SHR progenitor strain (AUC = 193 ± 16 mmol/liter−1·2 h−1, both p < 0.05) (Fig. 4). Glucose tolerance in the SHR-4 congenic rats fed pioglitazone was also significantly better than in the SHR-4 congenic rats not given pioglitazone (p < 0.05) (Fig. 4). Glucose tolerance was similar in SHR with or without pioglitazone and in SHR-TG19 rats with and without pioglitazone. In the SHR-TG19 strain, the lack of effect of pioglitazone on the oral glucose tolerance AUC may have been related to its modest level of Cd36 expression and the more modest effects of pioglitazone on NEFA levels in this strain.

To investigate mechanisms that might mediate the influence of Cd36 on the insulin-sensitizing actions of pioglitazone, we studied pioglitazone effects on insulin-stimulated glucose oxi-
Cd36 Mediates the Metabolic Effects of Pioglitazone

In the absence of insulin, pioglitazone treatment had no effect on glucose oxidation in soleus muscles from any of the strains (data not shown). However, in the presence of insulin, pioglitazone treatment induced increases in glucose oxidation in both the SHR transgenic and congenic strains, although statistical significance was achieved only in the TG19 strain \((p < 0.05)\) (Fig. 5). No effect on insulin-stimulated glucose oxidation was observed in the SHR progenitor strain with mutant \(Cd36\) (Fig. 5). As a consequence, insulin-stimulated glucose oxidation in SHR treated with pioglitazone, \(141 \pm 21\) nmol glucose/g tissue \(2\ h\^{-1}\), was significantly lower \((p < 0.05)\) than in the pioglitazone-treated SHR-4 congenic strain, \(276 \pm 51\) glucose/g tissue \(2\ h\^{-1}\), and in the pioglitazone-treated SHR transgenic strain, \(316 \pm 44\) glucose/g tissue \(2\ h\^{-1}\) (Fig. 4). No differential effects of pioglitazone treatment were observed on incorporation of glucose into diaphragm muscle glycogen or into adipose tissue lipids between any of the strains (data not shown).

Epididymal fat pad weights, which were similar among the three strains, were significantly increased by pioglitazone treatment (Fig. 6). The magnitude of the increase appeared greatest in the SHR progenitors, where epididymal fat pad weight at the end of the treatment, \(1.4 \pm 0.4\ g/100\ g\ body\ weight\), was significantly greater \((p < 0.05)\) than in both the SHR-4 congenic strain, \(1.1 \pm 0.3\ g/100\ g\ body\ weight\), and the SHR-TG19 strain, \(1.29 \pm 0.3\ g/100\ g\ body\ weight\) (Fig. 6). In the absence of pioglitazone, hepatic triglyceride levels (Fig. 7) were similar in the SHR progenitors with mutant \(CD36\) and in the SHR-TG-19 strain, which expresses \(CD36\) in the liver, \(15.6 \pm 1.5\ \mu\text{mol/g}\) and \(14.4 \pm 0.6\ \mu\text{mol/g}\), respectively (20). In both strains, hepatic triglycerides were significantly higher than in the SHR-4 congenic strain, \(8.1 \pm 0.9\ \mu\text{mol/g}, p < .05\). Administration of pioglitazone appeared to reduce hepatic triglyceride levels in all three strains, however, this effect was greatest in the transgenic rats (Fig. 7). Thus, in rats fed pioglitazone, hepatic triglyceride levels were significantly reduced in both the SHR-4 congenic and SHR-TG19 transgenic strains as compared with the SHR progenitor strain \((6.1 \pm 0.3\ \text{and} 8.1 \pm 0.3\ \mu\text{mol/g} \text{versus} 12.2 \pm 0.6\ \mu\text{mol/g} \text{respectively}, p < 0.05)\). No strain or treatment effects were detected on triglyceride levels in isolated soleus muscle.

Northern blot analysis of fat tissue showed abundant expression of wild-type \(Cd36\) message in the SHR-4 congenic strain and low expression of the \(Cd36\) transgene in the SHR-TG19 strain (Fig. 8). Endogenous mutant \(Cd36\) transcripts were also present in the SHR-TG19 strain and the SHR progenitor (Fig. 8). Administration of pioglitazone was associated with increased expression of wild-type \(Cd36\) transcript in the SHR-4 congenic strain and of the mutant \(Cd36\) transcripts in the SHR progenitor and SHR-TG19 strains consistent with previous reports of a functional binding site for PPAR\(\gamma\)-RXR het-
erodimers in the Cd36 promoter (17). Real-time PCR studies were used to estimate the effect of pioglitazone on expression of wild-type Cd36 using primers designed to specifically amplify the wild-type gene and not the mutant variant. In the SHR-4 congenic strain, administration of pioglitazone was associated with a significant increase in expression of wild-type Cd36 compared with SHR-4 congenic rats not given pioglitazone (Fig. 9). Real-time PCR confirmed low level expression of the wild-type Cd36 transgene in the SHR-TG19 strain (Fig. 9). However, in contrast to the SHR-4 congenic strain, in which pioglitazone increased the expression of wild-type Cd36, no effect of pioglitazone was detected on expression of the Cd36 transgene in the SHR-TG19 strain (Fig. 9). As expected, using either Northern blot or real-time PCR analysis, expression of wild-type Cd36 could not be detected in the SHR progenitor strain that harbors only mutant Cd36 (Figs. 8 and 9).

Consistent with the results of previous studies (19), mean arterial pressures were significantly lower in the SHR-4 congenic strain than in the SHR progenitor strain and administration of pioglitazone caused significant decreases in mean arterial pressure of ~10 mmHg in both the SHR progenitor and the SHR-4 congenic strains (data not shown). The pioglitazone-induced changes in blood pressure were associated with reciprocal changes in heart rate that were similar between the two strains (data not shown).

**DISCUSSION**

Multiple gene targets and metabolic pathways are thought to be involved in the ability of PPARγ ligands to enhance carbohydrate and lipid metabolism (3, 4). Large scale gene expression profiling studies have documented effects on expression of numerous genes in multiple insulin-sensitive tissues (3). However, the relative roles of specific target genes in the insulin-sensitizing effect of a PPARγ ligand remain ill defined because such information is difficult to discern from studies that simply correlate changes in gene expression with changes in metabolic phenotypes. A number of studies have proposed that the insulin-sensitizing effects of PPARγ ligands may be mediated in part by promoting uptake and oxidation of fatty acids by adipose tissue and by shunting lipids away from liver and skeletal muscle (2, 3, 5–10). However, other findings suggest that improving fatty acid utilization by muscle tissues may also play an important role. Burant and colleagues have reported that the PPARγ ligand troglitazone can still improve glucose tolerance in aP2/ΔTA lipodystrophic mice that lack subcutaneous or intra-abdominal fat (26). Hevener et al. (6) showed that the thiazolidinedione troglitazone could attenuate the decreases in insulin sensitivity and in skeletal muscle expression of CD36 that are induced by systemic infusion of free fatty acids (6). CD36 is a membrane protein that facilitates a major fraction of fatty acid uptake/utilization by fat and muscle tissues (16, 27). The Cd36 gene is one of the many whose expression can be strongly influenced by PPARγ ligands (3, 4).

In the current study, we directly tested the role of Cd36 in the insulin-sensitizing actions of the thiazolidinedione pioglitazone. We examined the ability of pioglitazone to improve blood lipid levels and insulin sensitivity of glucose oxidation in the SHR strain, which expresses mutant Cd36 (19–21) and exhibits loss of CD36 function in fatty acid uptake (22). Con-
genic and transgenic SHR strains that we derived from the SHR progenitor and which differed by expression of wild-type CD36 and by restoration of optimal fatty acid uptake (19–22) were used to test the specificity of the effects observed in the SHR. The availability of these strains allows for a direct pharmacogenetic test of the role of Cd36 in the insulin-sensitizing effects of thiazolidinediones.

We used the SHR strain, which carries a functionally defective Cd36 and an SHR-4 congenic strain, which is 99% genetically identical to the SHR progenitor strain and in which the expression of wild-type, functional Cd36 is under control of its native promoter (19). A transgenic line, SHR-TG19, which is genetically identical to the SHR progenitor strain except for Cd36 was also used. In the transgenic line, the wild-type, functional Cd36 is under control of the elongation factor 1α promoter. It is expressed at modest levels in tissues that ordinarily express relatively large amounts of Cd36 (e.g. fat, heart, skeletal muscle) (20) and exhibits significant expression in liver and kidney (20) where levels are ordinarily low. Phenotypic differences between the SHR progenitor and the SHR transgenic strains with respect to the effect of pioglitazone can be solely attributed to Cd36 expression. A comparison of findings in the transgenic to those in the congenic strain would control for the possibility that some of the phenotypic differences observed may be related to the slightly atypical Cd36 expression pattern in the SHR-TG19. Taken together, the results in the transgenic and congenic strains versus the SHR progenitor strain provide a compelling pharmacogenetic test of the role of Cd36 in the metabolic actions of pioglitazone.

A considerable body of evidence suggests that the insulin-sensitizing actions of thiazolidinediones may be secondary to their effects on fatty acid levels and fatty acid metabolism (2, 3, 5–10). In addition, chronic elevations in fatty acid levels are a well-known cause of insulin resistance (11–14). Our findings confirm the important role of changes in fatty acid utilization in the insulin-sensitizing effect of thiazolidinediones. More significantly, they directly implicate Cd36 as a key determinant of these metabolic changes. In the current study, the ability of pioglitazone to reduce circulating levels of fatty acids was significantly impaired in the SHR as compared with the congenic and transgenic lines. These observations indicate that administration of pioglitazone decreases fatty acid levels in a Cd36-dependent fashion. The decrease in circulating fatty acids then results in an improvement of insulin sensitivity because failure to achieve this decrease in the SHR significantly diminishes the ability of pioglitazone to induce reductions in circulating insulin and to enhance insulin-stimulated glucose oxidation in soleus muscle. Rescue of Cd36 function in the congenic and transgenic strains restores the effects of pioglitazone on both fatty acid levels and insulin sensitivity. Thus, at least under the dietary and genetic conditions of the current study, Cd36 is an important determinant of key metabolic actions of pioglitazone. Despite the current findings implicating Cd36, however, it should be noted that in the SHR harboring mutant Cd36, a tendency for a modest effect of pioglitazone on some metabolic parameters including serum NEFA and hepatic triglyceride levels was also observed. Thus, although the current studies establish a role for Cd36 in the metabolic actions of pioglitazone, they should not be interpreted as excluding a role for other targets in the metabolic effects of thiazolidinediones.

Pioglitazone had significant hypolipidemic and insulin-sensitizing effects in both the SHR transgenic line and SHR congenic line although oral glucose tolerance testing was significantly improved only in the SHR congenic line, perhaps because of the much greater level of expression of wild-type Cd36 in this strain. In addition, real-time PCR experiments showed no response of the Cd36 transgene to pioglitazone, whereas expression of endogenous wild-type Cd36 in the SHR congenic line was substantially up-regulated by pioglitazone. Thus, whereas up-regulation of Cd36 expression may contribute to the metabolic effects of pioglitazone in the SHR congenic line, up-regulation of Cd36 would not appear to account for the metabolic effects of pioglitazone in the SHR transgenic strain. It is possible that the anti-diabetic effects of pioglitazone in the SHR transgenic strain and SHR congenic strain are partly mediated by the formation of new fat cells expressing functional Cd36 as opposed to defective Cd36. It is also possible that the increased adipose tissue mass in pioglitazone-treated animals results in increased release of adipocyte mediators that can regulate fatty acid utilization in other tissues, notably muscle, provided those tissues have functional Cd36. Examples of such mediators are leptin, resistin, and adiponectin (28, 29). This interpretation could also explain the interaction of pioglitazone treatment and Cd36 on the increase in fat pad weight observed in treated animals. The increase in fat pad weight was significantly greater in the SHR progenitors, which would reflect the ineffectiveness of the adipocyte mediators to enhance fatty acid utilization and clearance by peripheral tissues of these animals because they carry defective Cd36.

The current findings provide direct pharmacogenetic evidence that Cd36 is an important determinant of the metabolic effects of pioglitazone. However, they do not allow conclusions to be made with respect to the relative importance of Cd36 in specific tissues. Adipose tissue is generally thought to play an important role in mediating the metabolic effects of thiazolidinediones, but as shown by Burant and colleagues (26) and Chao et al. (10), it is not required for all of these effects. Cd36 facilitates a major fraction (>60%) of fatty acid uptake by adipose tissue and muscle (16, 27, 30), and it is possible that Cd36 expression in both tissues plays an important role in mediating the anti-diabetic effects of thiazolidinediones. The role of Cd36 in liver may also be worth exploring, given the potential effects of hepatic triglyceride levels on glucose production. Future studies could dissect the relative contribution of specific tissues by comparing the insulin-sensitizing effects of thiazolidinediones in strains where Cd36 expression is rescued in a tissue-specific fashion. This could be accomplished not only by deriving new transgenic strains of SHR that express wild-type Cd36 in fat, muscle, or liver, but also by capitalizing on tissue-transplantation experiments between histocompatible congenic, transgenic, and progenitor strains of SHR.

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