

# Unraveling the mechanism of action of thiazolidinediones

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Commentary

See related article,  
Volume 106, Number 10,  
pages 1221–1228.

Thiazolidinediones (TZDs) are a new class of antidiabetic agents and include three compounds that have come to clinical use – troglitazone (Rezulin®), rosiglitazone (Avandia®), and pioglitazone (Actos®) – as well as several others that have been limited to pre-clinical study. TZDs were initially discovered by screening compounds for a hypoglycemic action in the *ob/ob* mouse (1), and subsequently they were shown to improve insulin action in a variety of obese and diabetic animal models with insulin resistance (2). In these model systems, TZDs reduce plasma glucose and insulin levels and improve some of the abnormalities of lipid metabolism. Consistent with animal studies, clinical studies have shown that treatment of type 2 diabetic patients with TZDs can lower serum glucose and insulin levels, increase peripheral glucose uptake, and decrease triglyceride levels (3). In euglycemic clamp studies, this is associated with an increase in insulin sensitivity in peripheral tissues (mainly represented by muscle in clamp studies), with relatively little effect on hepatic glucose output (4).

Insights into the molecular mechanism of action of TZDs came from studies that demonstrated that these agents increase transcription of certain genes in adipose tissue (5, 6). The recognition that the promoter region of one of the genes, the fat-specific gene *aP2*, binds a transcription factor identified as PPAR $\gamma$  (7) led Spiegelman, Kletzien, and Kliewer (6–9) to propose that TZDs exert their effects by activating this nuclear receptor. Due to alternative splicing and alternative promoter usage, there are two isoforms of PPAR $\gamma$  (1 and 2), and both are predominantly expressed in fat cells (10). Subsequent studies have shown an excellent correlation between the hypoglycemic action of the TZDs and their affinity for PPAR $\gamma$ . Regulation of gene expression via PPAR $\gamma$  is tissue-specific (10), and

although its role in adipocyte differentiation is well documented (10), the normal functions of PPAR $\gamma$  remain unclear. Complete elimination of a functional *PPAR $\gamma$*  gene results in embryonic lethality, whereas *PPAR $\gamma$*  heterozygote knockout mice with a 50% reduction in PPAR $\gamma$  expression exhibit increased basal insulin sensitivity and resistance to high-fat diet-induced insulin resistance (11).

Despite the evidence that TZDs act by binding to PPAR $\gamma$  and can improve different insulin-resistant states in both human and animal studies, the exact mechanism and site of action of the TZDs are still unclear. Type 2 diabetes is a complex metabolic syndrome with alterations in glucose, lipid, and protein metabolism (12). Hyperglycemia in type 2 diabetes is due to a decrease in insulin action to stimulate glucose uptake in peripheral tissues, such as muscle and fat, as well as unsuppressed hepatic glucose output and relative hypoinsulinemia. Traditional thinking suggests that the defect in muscle glucose uptake accounts for most of the postprandial hyperglycemia, the increase in fasting glucose is due to the increased hepatic glucose output, and the lipid abnormalities are due to a combination of defects in fat and liver (12). However, recent studies in mice in which insulin resistance has been created in individual tissues by conditional gene knockout suggest that these classical views may have been oversimplified (13–15). Thus, the muscle-specific insulin receptor knockout mouse exhibits hypertriglyceridemia and increased FFAs (14), the liver-specific knockout exhibits only mild hyperglycemia and lower triglycerides (15), the fat-specific knockout has normal glucose tolerance (M.D. Michael and C.R. Kahn, unpublished observations), and the  $\beta$  cell-specific knockout of the insulin receptor produces a defect in glucose-stimulated insulin release (13).

The adipocyte has a special role in the overall pathogenesis of insulin resistance, since most type 2 diabetes is associated with obesity, and adipocytes from obese animals have been shown to exhibit a number of properties that may be associated with insulin resistance (Figure 1, left panel). These include the fact that obesity is associated with hypertrophy of the adipocyte itself, leading to insulin resistance as well as overproduction of FFAs, TNF- $\alpha$ , and leptin, all of which have been shown to have effects that induce insulin resistance in other peripheral tissues (11, 16). Therefore, the key question is, are the effects of TZDs mediated exclusively through direct action on the adipocyte, with indirect effects to improve insulin sensitivity in other tissues, or are other tissues also important targets of the TZD agents? Since PPAR $\gamma$  is most highly expressed in adipose tissue, and since TZDs have been shown to regulate gene expression in fat, most speculation has focused on the adipocyte as the site of primary response. However, low levels of PPAR $\gamma$  receptor mRNA can be observed in many tissues, leaving this issue unresolved.

In normal humans, up to 80% of insulin-stimulated glucose disposal occurs in skeletal muscle, and this is a major site of insulin resistance in type 2 diabetes (2). Studies using euglycemic, hyperinsulinemic clamps have demonstrated that TZD treatment is associated with improved insulin sensitivity and increased insulin-stimulated glucose disposal in muscle, with little effect on hepatic glucose output (4). TZDs have also been shown to increase insulin signaling in vitro using muscle culture cells (17). On the other hand, TZDs have been shown to lower levels of circulating FFAs that could contribute to insulin resistance (18). TZDs also stimulate adipocyte differentiation, generating small adipocytes that are more insulin-sensitive than large adipocytes (19) (Figure 1, right panel).

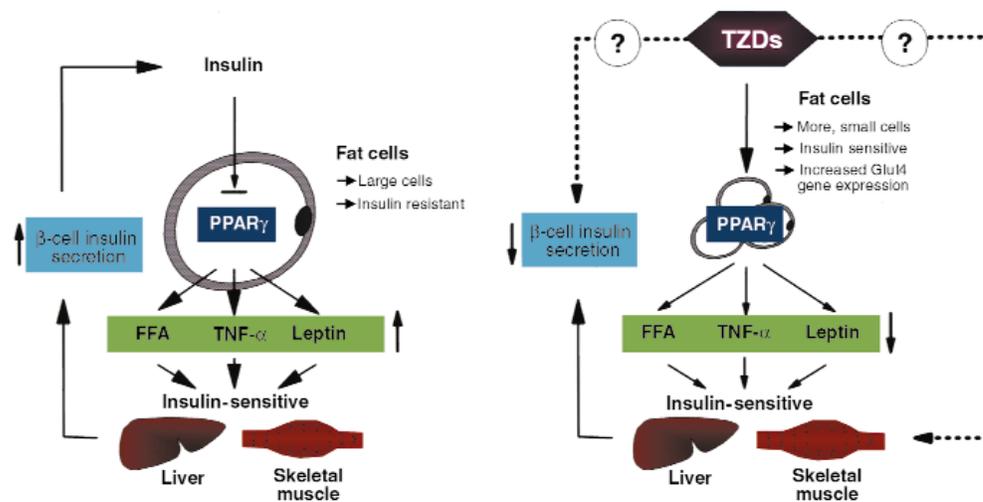
Recently, two groups of investigators have generated transgenic models of lipoatrophy that provide an opportunity to study the role of adipose tissue in the antidiabetic action of TZDs (20, 21). One of the lipoatrophic models was based on use of an attenuated form of the diphtheria toxin A chain (DTA) under the control of the adipocyte-specific *aP2* promoter to create a cell-specific toxigen (20). By 8–10 months of age, these mice have no visible subcutaneous or intra-abdominal adipose tissue and have severe diabetes with hyperinsulinemia, hyperglycemia, hyperlipidemia, and fatty liver (20, 22). When these animals were treated with troglitazone for 5 weeks, their insulin sensitivity was improved, glucose tolerance normalized, and serum insulin, cholesterol, triglyceride, and FFA levels were dramatically reduced, despite the absence of fat (22). These data suggest that TZDs do not require significant levels of adipose tissue to improve insulin sensitivity and that there is probably important and direct major action of troglitazone on other tissues, most likely skeletal muscle.

In a recent issue of the *JCI*, Chao et al. further explored the role of adipose tissue in the mechanism of action of TZDs, using an even more severe transgenic lipoatrophic mouse model, the A-ZIP/F-1 mouse (23). These mice express a dominant-negative protein, A-ZIP/F, under the control of the adipocyte-specific *aP2*

enhancer/promoter (21). This protein inhibits DNA binding and function of the B-ZIP transcription factors of both the C/EBP and JUN families, producing a severe disruption of adipocyte growth and differentiation. A-ZIP/F-1 mice have no visible white adipose tissue (WAT) and significantly reduced brown adipose tissue throughout life, and adults develop hyperglycemia with polyphagia, polyuria, and polydipsia, severe insulin resistance, and hyperlipidemia (21, 23). Also, as with *aP2*/DTA animals (22), treatment with rosiglitazone or troglitazone lowers serum triglyceride and FFA levels to normal in A-ZIP/F-1 mice, further supporting the finding that TZDs are able to correct hyperlipidemia in mice lacking adipose tissue. However, treatment of these mice with TZDs at concentrations that were effective in lowering glucose in *aP2*/DTA and *ob/ob* mice failed to reduce the serum glucose and insulin levels and to improve glucose tolerance in the A-ZIP/F-1 mice (23). This leads the authors to conclude that while the effects of TZDs on lipid metabolism do not depend on the action of these drugs on fat, their actions on glucose metabolism require the presence of adipose tissue.

Why the effect of TZDs is different in these two lipoatrophic diabetic animal models is unclear. The models are of different mechanism, severity, and duration of lipoatrophy and in mice of different genetic background. In some

humans with lipoatrophic diabetes, treatment with troglitazone results in remarkable improvement in hyperglycemia, as well as a significant decrease in triglyceride and FFA levels (24). This improvement is accompanied by the redistribution of, and a small but significant increase in, adipose tissue (24). However, it is unlikely that the increase in glucose disposal in *aP2*/DTA mice could be accounted for by the small amount of residual fat in this model. It is possible that TZDs stimulate this residual adipose tissue, which, in turn, conveys some signals (perhaps through leptin or TNF- $\alpha$ ) to skeletal muscle and improves insulin sensitivity (Figure 1, right panel). It is also possible that the direct effect of TZDs on skeletal muscle requires the presence of signals from other tissues, including WAT. These signals could be missing in the A-ZIP/F-1 mice, which have a complete absence of WAT. This hypothesis is also supported by the study of surgical implantation of adipose tissue in A-ZIP/F-1 mice (23, 25, 26). Both in vivo and in vitro experiments demonstrated that skeletal muscle from A-ZIP/F-1 mice has impaired response to insulin stimulation, and fat transplantation normalized glucose uptake and reversed insulin resistance in skeletal muscle from these animals. Interestingly, TZDs did not affect fat-transplanted A-ZIP/F-1 mice (23), perhaps because these mice already enjoy improved insulin sensitiv-



**Figure 1**

Left panel: Fat cells in the insulin resistance of type 2 diabetes are larger than in the normal subjects, with increased production of FFA, TNF- $\alpha$ , and leptin. This leads to insulin resistance in the liver and skeletal muscle, resulting in a need for increased peripheral insulin, which is generated by the  $\beta$  cells in the pancreas. Right panel: TZDs stimulate adipocyte differentiation, preferentially generating more numerous, smaller adipocytes. These small adipocytes are more insulin-sensitive, producing less FFA, TNF- $\alpha$ , and leptin. Therefore, insulin is more effective and there is less need for the  $\beta$  cells to increase their secretion of insulin.

ity as a result of the restoration of some WAT. Future studies using genetic techniques to create tissue-specific knockout of PPAR $\gamma$  in individual insulin-sensitive tissues, such as skeletal muscle, fat, and liver, as well as in pancreatic  $\beta$  cells, are needed; these animal models should answer not only the question of the primary site of the antidiabetic effect of TZDs, but also perhaps of the role of this receptor in normal function of each of these tissues.

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