PPARγ-mediated insulin sensitization: the importance of fat versus muscle

Ulrich Kintscher¹ and Ronald E. Law²

¹Center for Cardiovascular Research, Institut für Pharmakologie und Toxikologie, Campus Charité-Mitte, Charité-Universitätsmedizin Berlin, Berlin, Germany; and ²Takeda Pharmaceuticals North America, Inc., Lincolnshire, Illinois

PPARγ (peroxisome proliferator-activated receptor-γ) belongs to the family of PPARs, which also includes the isoforms PPARα and PPARβ/δ (16). The PPARγ gene gives rise to at least three mRNAs, PPARγ1, PPARγ2, and PPARγ3, that differ at their 5’ end as a consequence of alternate promoter usage and splicing (2). Proteins produced from PPARγ1 and PPARγ3 mRNAs are identical, whereas the PPARγ2 protein contains an additional NH₂-terminal region composed of 30 amino acids (2). PPARγ1 is expressed at substantial levels in cells of the monocyte/macrophage lineage, where it plays a pivotal role in regulating gene expression involved in lipid metabolism and inflammation (31). PPARγ2 expression is abundant in, and primarily restricted to, adipose tissue. However, low levels of PPARγ2 expression have been described in PMA- and TGF-β-stimulated monocytes (11, 12).

PPARγ, after heterodimerizing with the retinoid X receptor, is activated by binding certain synthetic ligands, known as insulin sensitizers, glitazones, or thiazolidinediones (TZDs), clinically used in oral antidiabetic therapy (3). In addition, multiple endogenous ligands have been identified, including fatty acids and fatty acid derivatives such as 15-deoxy-D¹₂,¹₄-prostaglandin J₂ (3). After ligand activation, PPARγ changes its conformational structure, which facilitates the release of corepressors and subsequent binding of a distinct set of nuclear coactivators, resulting in the regulation of gene transcription (2, 6).

PPARγ activation through binding of the synthetic TZDs results in a marked improvement in type 2 diabetic patients of insulin and glucose parameters resulting from an improvement of whole body insulin sensitivity (4, 21, 24, 28). The mechanism of PPARγ-mediated insulin sensitization by TZDs is still unclear. Despite the importance of skeletal muscle in insulin-induced glucose disposal, adipose tissue seems to be the major mediator of PPARγ action on insulin sensitivity. PPARγ has the highest expression levels in adipose tissue compared with other metabolic organs, such as skeletal muscle, liver, and pancreas (36). PPARγ is the master regulator of adipogenesis, thereby stimulating the production of small insulin-sensitive adipocytes (15, 35). The induction of adipogenesis associated with the capability for fatty acid trapping has been shown to be an important contributor to the maintenance of systemic insulin sensitivity (33). In addition, PPARγ activation in mature adipocytes induces a number of genes involved in the insulin-signaling cascade, thereby improving insulin sensitivity (20, 30, 34). However, there is still debate about the role of other...
metabolic tissues in mediating PPARγ-function in glucose and insulin homeostasis. Recently, the function of PPARγ in adipose tissue and skeletal muscle has been intensively characterized by using targeted deletion of PPARγ in those tissues.

**PPARγ Function: Lessons from Tissue-Specific Knockout Mice**

**PPARγ Deficiency in Fat**

A study by He et al. (8) utilized the Cre-recombinase-loxP system to generate mice with PPARγ deficiency only in fat. As a result of the LoxP site location on either side of exons 1 and 2 of the PPARγ gene, a complete loss of the PPARγ1 isotype and translation of a nonfunctional PPARγ2 isoform were achieved after Cre-mediated deletion. Placement of Cre cDNA under the control of the adipose-specific fatty acid-binding protein (aP2) promoter resulted in fat-specific deletion of PPARγ. aP2 is a downstream target of PPARγ during adipocyte differentiation (13). Usage of the aP2 promoter, therefore, deletes PPARγ after normal differentiation in adipocytes. This strategy allows one to study the role of PPARγ-mediated functions in mature adipocytes, but not during the early processes of adipogenesis.

Mice carrying the deletion of PPARγ in mature adipocytes showed a marked decrease of brown and white adipose mass, with >80% loss of white adipocytes (8). Adipocyte loss was compensated for by a hypertrophic response of the remaining adipocytes and an inflammatory reaction in adipose tissue (8). Metabolic measurements in adipose PPARγ-deficient mice demonstrated marked hyperlipidemia with elevated free fatty acid (FFA) and triglyceride levels, associated with a significant decrease in plasma adipokines (leptin, adiponectin). Despite these metabolic changes, systemic insulin sensitivity was maintained with no changes in fasting glucose and insulin levels, as well as no changes in glucose and insulin tolerance tests. However, adipocyte PPARγ was required to maintain insulin sensitivity in mice fed a high-fat diet. Insulin responsiveness of different metabolic organs, including adipose tissue, skeletal muscle, and liver, was tested in clamp studies under normal chow diet. These experiments revealed the presence of adipose tissue and hepatic insulin resistance, whereas muscle insulin sensitivity was maintained. Liver insulin resistance was a result of hepatic lipid accumulation (8).

TZD treatment in patients results in prominent improvement of whole body insulin sensitivity; however, the targeted metabolic tissue responsible for these effects is still unknown (4, 21, 28). To further clarify the insulin-sensitizing mechanism of TZDs, adipose PPARγ-deficient and control mice were treated with rosiglitazone (8). TZD treatment lowered FFA levels in control mice, which was not observed in adipocyte PPARγ-deficient mice, indicating that TZD-mediated regulation of plasma FFAs is dependent on PPARγ function in intact adipose tissue. Hepatic insulin resistance was not affected by TZD treatment.

Previous studies in fatless and lipodystrophic animal models revealed the development of systemic insulin resistance and diabetes. Surgical reimplantation of adipose tissue reversed the diabetic state in those animals, underscoring the importance of adipose tissue in maintaining insulin and glucose homeostasis (5, 22). Lack of PPARγ in mature adipocytes did impair insulin sensitivity under high-fat diet conditions, a nutritional environment prevalent in industrialized countries and rising in developing nations (8). Against the background of this epidemic in overnutrition, the importance of adipose PPARγ in maintaining intact systemic insulin sensitivity is evident from the study by He et al. (8). Impaired PPARγ function in fat resulted in hepatic insulin resistance. Unfortunately, muscle insulin sensitivity has not yet been evaluated in adipose PPARγ-deficient mice fed a high-fat diet. A further increase in FFA accumulation in skeletal muscle in response to a high-fat diet should also contribute to muscular insulin resistance. PPARγ deficiency in adipocytes also demonstrated that adipose tissue is a major mediator of TZD effects to decrease circulating FFAs by inhibiting lipolysis in fat. This mechanism is likely a major contributor to TZD insulin-sensitizing activity (17, 26, 27). Although the elegant work of He et al. provided important insight concerning the role of PPARγ function in mature adipocytes, it did not examine the role of this nuclear receptor in producing new insulin-sensitive, small adipocytes.

What is the role of PPARγ during the initiation of adipocyte differentiation? Koutnikova et al. (14) utilized a complex approach by introducing a genomic modification near exon B (introduction of a neomycin cassette 500 bp downstream) of the PPARγ2 isoform. The sole presence of the selection marker in proximity to exon B resulted in an almost complete loss of PPARγ2 in brown and white adipose tissue, as well as in liver and muscle. PPARγ1 is a downstream target of PPARγ2 during differentiation of white adipocytes, so it was not surprising that the PPARγ1 isoform was also undetectable in white adipose tissue (29, 32). In contrast, in brown adipose tissue the loss of PPARγ2 was compensated for by an increase in PPARγ1 expression. PPARγ1 mRNA expression level in muscle and liver remained unchanged. Because PPARγ2 is expressed predominantly in white adipose tissue, this model serves as an adipose-specific PPARγ knockdown.

One-week-old animals homozygous for the mutation had no white adipose tissue associated with a massive hepatomegaly due to steatosis and accumulation of lipids in heart, skeletal muscle, and kidneys (14). Serum FFA and triglyceride levels were dramatically elevated in young lypodystrophic animals. Adult mice showed significantly higher glucose levels during a glucose tolerance test, as well as increased glucose and insulin levels during regular feeding, indicating a state of insulin resistance in these animals. Serum FFA and triglyceride levels were decreased during adulthood in PPARγ adipose tissue knockdown mice despite their remaining lypodystrophic. Decreased plasma triglyceride and FFA levels likely resulted from a compensatory increase in FFA catabolism in skeletal muscle, since multiple genes involved in FFA oxidation were induced in muscle tissue of mutant animals. β-Oxidation genes in the liver, however, remained unchanged, and genes for hepatic gluconeogenesis were induced. These together led the authors to the conclusion that, in the absence of white adipose tissue, PPARγ in the liver contributes to impaired glucose tolerance, whereas the muscle might be a major compensatory organ for lipid metabolism (14).

Zhang et al. (38) selectively disrupted the PPARγ2 isoform in mice by replacing the initiation codon and the partial exon B with a red fluorescence protein-coding sequence and the neomycin gene cassette. PPARγ2 deletion resulted in a marked reduction of white adipose tissue mass, whereas brown adipose tissue mass was normal (38). PPARγ2-deficient mice had
impaired insulin sensitivity, as measured in an insulin tolerance test. However, insulin sensitivity was impaired only in male mice, not in female animals, suggesting a sex-specific regulation of PPARγ2-dependent insulin and glucose metabolism (38). Surprisingly, in the study by Zhang et al., lipodystrophy in PPARγ2-deficient mice did not result in lipid accumulation in nonadipose tissue (e.g., liver), and circulating triglyceride and cholesterol levels were equal among the groups. In addition, food intake and body weight were similar between wild-type and PPARγ2-deficient mice, and one has to ask where incorporated substrates (carbohydrates, fatty acids, etc.) are stored in lipodystrophic PPARγ2-deficient mice. Treatment of PPARγ2-deficient mice with the PPARγ ligand rosiglitazone still led to a normalization of systemic insulin resistance, suggesting that PPARγ2 is not essential for TZD-mediated insulin sensitization in this model (38).

What Have We Learned from PPARγ Deficiency in Fat?

The three studies discussed demonstrated that PPARγ is essential for the development and normal function of white adipose tissue (8, 14, 38). They have elegantly shown that PPARγ is required to keep up intact insulin sensitivity during caloric intake, even gaining importance during intake of high-fat diets. Certain mechanisms of the maintenance of insulin sensitivity through adipose PPARγ have been identified. By maintaining the intact function of white adipose tissue, PPARγ protects the liver against lipid overload, thereby ensuring intact hepatic insulin sensitivity. Adipose PPARγ guarantees a balanced and adequate regulation of secretion from adipose tissue of adipocytokines such as adiponectin and leptin, which are major contributors to a regular insulin response (7, 10, 19, 37). The role of skeletal muscle during these processes still remains unclear. Previous studies have shown that lipodystrophic or fatless animals develop severe insulin resistance and diabetes, mainly as a result of impaired muscular glucose disposal due to lipid accumulation and subsequent lipotoxicity (22, 27). However, He et al. (8) observed an intact insulin sensitivity in skeletal muscle of adipose PPARγ-deficient mice despite a marked accumulation of lipids. They hypothesized that a certain threshold of lipid accumulation must be reached in skeletal muscle to induce lipotoxic insulin resistance in skeletal muscle. This threshold is likely to be attained during intake of high-fat diets. Unfortunately, the authors did not perform these experiments in their study. In addition, Koutnikova et al. (14) demonstrated that the loss of PPARγ induced the expression of genes involved in muscular β-oxidation, thereby providing a compensatory enhanced FFA catabolism in skeletal muscle, contributing to the clearance of FFAs (23). Compensatory FFA catabolism, however, did not affect the insulin-resistant state in mutant animals (14). In addition, despite a decrease in serum FFA levels in adult animals, FFA serum levels were still elevated during caloric intake (14). Finally, increased muscular FFA oxidation could not prevent lipid accumulation in skeletal muscle of mutant animals, assuming the continued presence of lipotoxic impairment of muscular glucose metabolism (14). Further experiments are required to characterize the role of compensatory muscular FFA oxidation in the regulation of whole body lipid and glucose metabolism after adipocytic PPARγ loss.

In summary, two major mechanisms of adipose PPARγ-mediated preservation of systemic insulin sensitivity have been identified: 1) protection of nonadipose tissue against excessive lipid overload and maintenance of regular organ function (liver, skeletal muscle), and 2) balanced and adequate regulation of adipocytokine secretion (adiponectin, leptin).

Although PPARγ expressed in liver and skeletal muscle contributes importantly to glucose and lipid metabolism, these early and elegant studies using tissue-specific PPARγ knock-out mouse models pinpoint adipose tissue as the major target of TZD-mediated improvement of hyperlipidemia and insulin sensitivity (8, 14).

**PPARγ Deficiency in Skeletal Muscle**

To further study the role of skeletal muscle in PPARγ-mediated regulation of insulin and glucose metabolism, Hevener et al. (9), as well as Norris et al. (25), produced mice with a skeletal muscle-specific deletion of PPARγ. LoxP sites flanked exon 1 and/or 2 of the PPARγ, and Cre mice were carrying the Cre recombinase under the control of the muscle-specific muscle creatine kinase promoter (9, 25).

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**Table 1. Tissue-specific PPARγ knockout mouse models in adipose tissue and skeletal muscle**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Adipose Tissue</th>
<th>Muscle</th>
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<tbody>
<tr>
<td>He et al. (8)</td>
<td>PPARγloxPap2-Cre Mice</td>
<td>Mutant PPARγ2 (neocassette 500 bp downstream exon B)</td>
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<tr>
<td>Koutnikova et al. (14)</td>
<td>Mutant PPARγ2 (initiation codon/partial exon B replaced by RFP and neocassette)</td>
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<tr>
<td>Zhang et al. (38)</td>
<td>PPARγloxPMCK-Cre Mice</td>
<td>PPARγloxPMCK-Cre Mice</td>
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<tr>
<td>Norris et al. (25)</td>
<td>FFA↑, TG↑ (chow)</td>
<td>TG↑</td>
</tr>
<tr>
<td>Hevener et al. (9)</td>
<td>FFA↑, TG↑ (1 wk)</td>
<td>FFA↑, TG↑ (5 mo)*</td>
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<tr>
<td>Whole body IR</td>
<td>FFA↑, TG↑ (5 mo)*</td>
<td>Leptin↓</td>
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<tr>
<td>Lipid profile</td>
<td>FFA↑, TG↑ (1 wk)</td>
<td>Leptin↓</td>
</tr>
<tr>
<td>Adipocytokines</td>
<td>Adiponectin↓</td>
<td>Adiponectin↓</td>
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<td>Steatosis</td>
<td>Nonsteatosis</td>
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<tr>
<td>Muscle</td>
<td>Nonsteatosis</td>
<td>Nonsteatosis</td>
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PPARγ, peroxisome proliferator-activated receptor-γ; ap2, adipose-specific fatty acid-binding protein; IR, insulin resistance; HFD, high-fat diet; FFA, free fatty acids; TG, triglycerides; RFP, red fluorescence protein gene; m, male; f, female. Times (wk, mo) refer to age. *Serum levels in fasted mice. In the absence of specific indications, data are for the adult phenotype on a chow diet.
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PPARγ IN FAT AND MUSCLE

Mice deficient for PPARγ in skeletal muscle had significant whole body insulin resistance demonstrated either by insulin/glucose tolerance tests or by hyperinsulinemic euglycemic clamp studies (9, 25). However, the etiology of impaired insulin action in these mice remains to be defined. Hevener et al. (9) postulated that loss of PPARγ resulted in skeletal muscle insulin resistance followed by impaired insulin action in adipose tissue and liver. In contrast, Norris et al. (25) did not observe a change in muscular glucose disposal, whereas hepatic insulin sensitivity was impaired. Finally, the authors of both studies investigated the effects of TZDs in their model. TZD treatment did markedly improve systemic parameters of insulin sensitization independently of muscular PPARγ deficiency, once again underscoring the importance for adipose tissue and liver for TZD actions (9, 25).

WHAT HAVE WE LEARNED FROM PPARγ DEFICIENCY IN FAT AND SKELETAL MUSCLE?

In contrast to the studies in adipose-specific PPARγ-deficient mice, the data in muscle-specific PPARγ−/− mice demonstrate that whole body insulin sensitivity is, at least in part, dependent on an intact PPARγ system in skeletal muscle (Table 1). Despite the fact that PPARγ expression in muscle is only 5–10% of its expression in adipose tissue, PPARγ seems to play a central role in maintaining regular insulin-mediated signaling in muscle, resulting in normal glucose disposal (1, 18, 36). In addition, the studies by Hevener et al. (9) and Norris et al. (25) demonstrated that PPARγ deficiency in muscle results in secondary insulin resistance in adipose tissue and liver. These data suggest the existence of an important endocrine cross talk among muscle, fat, and liver, whose communicating molecules remain to be identified.

With respect to the pharmacological treatment of insulin resistance and type 2 diabetes mellitus, all studies in tissue-specific PPARγ knockout mice, except the study by Zhang et al. (38), in which only PPARγ2 was knocked out, have demonstrated that adipose tissue is the major primary target of TZD-induced insulin sensitization (8, 9, 14, 25).

In conclusion, recent studies in tissue-specific PPARγ knockout mice have greatly increased our understanding of the role of different metabolic tissues in PPARγ-mediated regulation of systemic insulin-stimulated glucose metabolism. The importance of adipose PPARγ to maintain systemic insulin sensitivity has convincingly been demonstrated. PPARγ-mediated adipogenesis plays an important role in the protection against nonadipose tissue insulin resistance. Surprisingly, a small amount of muscular PPARγ contributes to intact systemic insulin sensitivity by maintaining intact insulin-mediated glucose utilization in muscle. Future studies are required to characterize the molecular network involved in the endocrine cross talk among fat, muscle, and liver.

GRANTS

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REFERENCES


