

PPARadigms and PPARadoxes: expanding roles for PPAR γ in the control of lipid metabolism

Robert Walczak and Peter Tontonoz¹

Howard Hughes Medical Institute, Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA 90095-1662

Abstract The nuclear receptor PPAR γ is a central regulator of adipose tissue development and an important modulator of gene expression in a number of specialized cell types including adipocytes, epithelial cells, and macrophages. PPAR γ signaling pathways impact both cellular and systemic lipid metabolism and have links to obesity, diabetes, and cardiovascular disease. The ability to activate this receptor with small molecule ligands has made PPAR γ an attractive target for intervention in human metabolic disease. As our understanding of PPAR γ biology has expanded, so has the therapeutic potential of PPAR γ ligands. Recent studies have provided insight into the paradoxical relationship between PPAR γ and metabolic disease and established new paradigms for the control of lipid metabolism. This review focuses on recent advances in PPAR γ biology in the areas of adipocyte differentiation, insulin resistance, and atherosclerosis.—Walczak, R., and P. Tontonoz. PPARadigms and PPARadoxes: expanding roles for PPAR γ in the control of lipid metabolism. *J. Lipid. Res.* 2002. 43: 177–186.

Supplementary key words peroxisome proliferator-activated receptors • adipocyte • adipose tissue • diabetes • obesity

The past 10 years have seen a new paradigm emerge for the transcriptional regulation of metabolic pathways with the discovery of the peroxisome proliferator-activated receptors (PPAR α , PPAR γ , and PPAR δ), a trio of nuclear receptors activated by lipids. Characterization of PPARs led to the realization that certain nuclear receptors have evolved to recognize specific metabolic intermediates in the pathways that they control. Unlike the steroid receptors, which bind a single high affinity ligand, PPARs have the ability to recognize a range of structurally diverse molecules. The importance of this property became clear when it was discovered that the physiologic activators of these receptors are likely to be native and modified polyunsaturated fatty acids. Thus, the PPARs are transcription factors that regulate expression of genes involved in lipid metabolism, and the physiologic ligands for these receptors are lipids themselves.

PPAR γ was originally identified as a central regulator of gene expression and differentiation in adipose cells (1). It has subsequently been shown to regulate differentiation-

linked gene expression in several other tissues as well, including breast, colon, prostate, and macrophages. PPAR γ signaling pathways are now recognized to be involved in the control of lipid uptake, transport, storage, and disposal. PPAR γ binds to DNA as an obligate heterodimer with the retinoid X receptor (RXR). Naturally occurring ligands for PPAR γ include native and oxidized polyunsaturated fatty acids and prostanoids such as 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (2–4). The thiazolidinedione (TZD) anti-diabetic drugs have also been shown to be potent and selective ligands for PPAR γ , linking PPAR γ transcriptional activity to insulin sensitivity and glucose homeostasis (2, 5). Two glitazones, rosiglitazone (Avandia) and pioglitazone (Actos), are widely used for the treatment of type 2 diabetes.

PPAR γ AND ADIPOCYTE DIFFERENTIATION

A dominant regulator of adipocyte development

Advances in our understanding of lipid metabolism have identified the adipocyte as a central player in the control of energy balance and whole body lipid homeostasis. Storage and release of fatty acids and glycerol from the adipocyte impact overall lipid homeostasis as well as hepatic and peripheral glucose metabolism. Adipocytes also perform an important endocrine function by secreting numerous cytokines, bioactive peptides, and complement factors. Upon secretion into the bloodstream, adipocyte-derived signaling molecules act at distant sites to regulate energy homeostasis. PPAR γ functions as a key modulator of lipid homeostasis, both through its ability to promote

Abbreviations: BAT, brown adipose tissue; FATP, fatty acid transport proteins; FIAF, fasting induced adipose factor; LXR, liver X receptor; NIDDM, non-insulin-dependent diabetes mellitus; oxLDL, oxidized LDL; PDK4, pyruvate dehydrogenase kinase 4; PGAR, PPAR γ angiopoietin related; TZD, thiazolidinedione; RXR, retinoid X receptor; SHR, spontaneously hypertensive rat; WAT, white adipose tissue.

¹To whom correspondence should be addressed at Howard Hughes Medical Institute, UCLA School of Medicine, Box 951662, Los Angeles, CA 90095-1662.

e-mail: ptontonoz@mednet.ucla.edu

adipocyte differentiation, and its ability to influence the production of secreted mediators.

Studies over the past 7 years have identified PPAR γ as a dominant regulator of adipogenesis (1, 6). PPAR γ cooperates with other transcription factor families including the C/EBPs and SREBPs, to regulate adipocyte differentiation. Together, these transcription factors effect the complex changes in gene expression necessary for the development of the functional adipocyte. The interplay of transcription factor families in the regulation of adipose tissue differentiation has been the subject of recent comprehensive reviews (1, 7–9).

Much of our understanding of the transcriptional regulation of adipose tissue development has evolved from experiments with cultured cell lines. PPAR γ is induced during the conversion of preadipocytes to adipocytes, and is highly expressed in both white and brown adipose tissue (10). Ectopic expression of the nuclear receptor PPAR γ in fibroblasts results in expression of adipose specific genes and intracellular accumulation of lipid droplets, features that are specific for mature white adipocytes (11). These early observations have been supported by recent studies showing that PPAR γ mutants with dominant negative activity inhibit adipogenesis in cultured preadipocytes (12, 13).

Since homozygous inactivation of the PPAR γ gene in mice results in placental insufficiency and embryonic lethality (14), genetic proof of the importance of PPAR γ function for adipose tissue development has required some creative approaches. Barak and colleagues utilized a tetraploid-rescue approach to generate a single PPAR γ null pup that survived to term. This PPAR γ null pup completely lacked both white and brown adipose tissue and showed profound hepatic steatosis, consistent with other models of lipoatrophy (14). In addition, several laboratories have shown that embryonic fibroblasts derived from PPAR $\gamma^{-/-}$ fetuses fail to differentiate into adipocytes *in vitro* (14, 15). Rosen et al. demonstrated that mice chimeric for wild-type and PPAR γ null cells show little or no contribution of null cells to adipose tissue, despite the ability of these cells to contribute to the formation of other tissues (16). Finally, results obtained from murine models have also been supported by limited human studies. Several groups have identified gain of function mutations in PPAR γ that appear to correlate with severe obesity and/or hyperlipidaemia in different populations (17–19).

PPAR γ , PGC-1, and brown adipose tissue function

Although PPAR γ has been most extensively studied in models of white adipose tissue (WAT) differentiation, this same nuclear receptor is also important for brown adipose tissue (BAT) development and function. While the function of white adipocytes is to store energy in the form of triglycerides, brown adipocytes are specialized to dissipate energy in the form of heat. The thermogenic properties of brown adipocytes are conferred by the tissue-specific protein UCP-1, a mitochondrial proton transporter that uncouples respiration from oxidative phosphorylation. PPAR γ is abundantly expressed in both embryonic and

adult BAT (10, 14). Treatment of CD-1 rats with the PPAR γ selective ligand rosiglitazone results in marked increases in the mass of interscapular BAT (20). *In vitro*, the same ligand induces terminal differentiation of the brown preadipocyte cell line HIB-1B as well as expression of UCP-1 and other adipocyte-specific genes. Sears and colleagues have identified a PPAR γ response element in the enhancer region of UCP-1 promoter that appears to be important to its tissue-specific regulation (21).

An interesting feature of UCP-1 regulation by PPAR γ is a requirement for a specific coactivator protein, called PGC-1 (22). PGC-1 mRNA expression is dramatically elevated upon cold exposure of mice in brown fat and skeletal muscle, key thermogenic tissues. Ectopic expression of PGC-1 in white adipose cells stimulates expression of UCP-1 and mitochondrial enzymes of the respiratory chain, increases oxygen consumption, and increases the cellular content of mitochondrial DNA (23). Treatment of cultured human primary preadipocytes with rosiglitazone has also been reported to stimulate UCP-1 expression, presumably because there are brown adipocytes in these cultures (24). Together these data demonstrate that PPAR γ and its transcriptional cofactors are important not only for the storage of excess energy in WAT but also for energy dissipation in BAT.

PPAR γ as an adipocyte transcription factor

To date, only a limited number of genes are known to be direct targets of PPAR γ in adipose tissue. The majority of these encode proteins with direct links to lipid metabolism, including the adipocyte specific fatty acid binding protein aP2, lipoprotein lipase, the fatty acid transport proteins (FATP) and CD36, adipophilin, long chain acyl-CoA synthase, phosphoenolpyruvate carboxykinase, and liver X receptor α (LXR α) (1, 25). However, target gene searches have also identified a number of novel molecules, including the secreted molecules fasting induced adipose factor (FIAF)/PPAR γ angiopoietin related (PGAR) (26, 27), and resistin (28–30). Recently, IRS-2 gene expression was also reported to be induced in 3T3-L1 adipocytes and human primary adipocytes in response to TZDs (31). Whether or not these newly identified PPAR γ -regulated genes are direct targets is still under investigation.

PPAR γ AND INSULIN RESISTANCE

PPAR γ ligands regulate systemic lipid and glucose homeostasis

Insulin resistance is a common feature of obesity, non-insulin-dependent diabetes mellitus (NIDDM), and lipodystrophy. Although the role of PPAR γ in adipocyte development suggested a possible connection between PPAR γ activity and insulin sensitivity, the discovery that PPAR γ is the biologic target for the thiazolidinedione class of anti-diabetic drugs provided the first definitive link (2, 5, 32, 33). The discovery that TZDs are PPAR γ ligands presented a paradox because of the correlation of obesity and insulin resistance. These drugs stimulate adipocyte differentiation *in vitro* and *in vivo*, yet lower glucose and improve

lipid profiles in type 2 diabetics. Further evidence for a connection between PPAR γ and insulin sensitivity has come from studies of human populations. PPAR γ polymorphisms and dominant negative mutations have been associated with metabolic syndrome features such as hyperlipidemia, obesity, severe insulin resistance, diabetes mellitus, and hypertension (18, 19, 34).

Many labs have been working to decipher the mechanism by which PPAR γ ligands modulate systemic lipid metabolism and insulin sensitivity. One plausible model for the role of adipose tissue in systemic insulin resistance postulates a critical role for FFA. According to this model, insulin resistance is caused, at least in part, by alterations in partitioning of FFA between the adipose tissue, skeletal muscle, and liver (35). This imbalance results in intracellular accumulation of triglycerides and fatty acid metabolites in muscle and liver. A number of studies support the concept that TZDs reduce circulating FFA levels and improve insulin sensitivity in muscle and liver due to promotion of adipocyte differentiation and FFA uptake. Identification of the fatty acid transporters FATP and CD36 as PPAR γ targets provided a straightforward mechanism by which PPAR γ ligands may promote selective FFA uptake into adipose tissue (36–38). In fact, the CD36 gene was independently identified as a determinant of insulin resistance in the spontaneously hypertensive rat (SHR) model (39).

Comprehensive gene profiling studies have provided additional insight into the mechanism of action of PPAR γ agonists. Administration of a highly potent and selective PPAR γ agonist (GW1929) to rats revealed increased expression of genes involved in lipogenesis, fatty acid transport, lipid storage, and oxidation in both WAT and BAT (40). By contrast, a marked decrease was observed in the expression of a number of genes involved in fatty acid transport and oxidation in skeletal muscle, even though some of these genes are established PPAR targets in other tissues. These findings are consistent with the hypothesis that PPAR γ can improve insulin action by stimulating adipogenesis as well as the uptake and oxidation of fatty acids by adipose tissue. Interestingly, Way and colleagues also observed a marked decrease in expression of pyruvate dehydrogenase kinase 4 (PDK4) in GW1929 treated animals (40). PDK4 is a potent repressor of oxidative glucose metabolism in muscle, and its activity leads to TG accumulation. These authors speculate that the effects of GW1929 on the expression of PDK4 and other genes in muscle are likely to be secondary to changes in circulating FFA concentrations. The fact that no genes were induced in muscle in response to GW1929 in these studies, the fact that PPAR γ is not a transcriptional repressor, and the low level of PPAR γ expression in skeletal muscle collectively suggest that changes in muscle gene expression are secondary to direct effects in other tissues. A definitive test of this hypothesis is likely to require a tissue-specific PPAR γ knockout.

PPAR γ ligands regulate secretion of adipocyte-derived signaling molecules

Another potential mechanism by which PPAR γ agonists may affect lipid and glucose metabolism is through alter-

ations in the endocrine functions of adipose tissue. Adipocytes secrete a number of signaling molecules that have been termed adipocytokines. These endocrine factors have the potential to modulate gene expression and metabolism in other organs such as skeletal muscle, liver, and brain. TNF α is a potent inhibitor of adipocyte differentiation and has been suggested to act as an autocrine or paracrine regulator that limits adipose tissue expansion. Elevated TNF α levels are observed in several rodent models of obesity and NIDDM (1). Recent studies utilizing gene disruptions in mice knockouts confirmed that absence of TNF α or its receptor results in significantly improved insulin sensitivity (41, 42). PPAR γ ligands appear to antagonize TNF α -mediated inhibition on insulin signaling at several different levels. First, TZD treatment has been shown to decrease circulating TNF α levels in rodents (43, 44). Second, TZDs antagonize the inhibitory effect of TNF α on the insulin receptor and its substrate IRS-1 (45, 46). Third, TZD increase expression of IRS-2 and the p85 subunit of the PI3-kinase in adipocytes (31, 47). The paradigm of TNF α as a mediator of insulin resistance is based primarily on animal data and has not yet been thoroughly explored in humans. For example, Peiffer et al. have reported increased plasma levels of TNF α in male but not in female patients with NIDDM (48).

Leptin, the protein product of the *ob* gene is an adipocyte-derived hormone that controls body weight by modulating food intake and energy expenditure (49). Leptin's principal site of action is the hypothalamus where it binds to a tissue-specific variant of the leptin receptor (Ob-Rb) (50). Leptin expression is regulated by both hormonal and nutritional signals; it is decreased by fasting and catecholamines and increased by overfeeding, high fat diet, insulin, and glucocorticoids. A majority of obese humans exhibit elevated circulating leptin levels and have been postulated to be leptin resistant. *Ob* gene expression is dramatically repressed in 3T3-L1 adipocytes within a few hours after rosiglitazone treatment (51, 52). In rats, administration of pioglitazone increases food intake and adipose tissue weight while reducing *ob* mRNA (53).

Adiponectin was independently identified by several laboratories as a protein specifically and highly expressed in human adipose tissue (54, 55). A mouse homolog was designated AdipoQ (56) and ACRP30 (57). Adiponectin mRNA and protein are significantly reduced in obese mice, primates, and humans (56, 58, 59). By contrast, expression of adiponectin in mice is markedly enhanced by PPAR γ ligands, although it does not seem to be a direct target gene. Maeda and colleagues found that adiponectin levels were significantly reduced by TNF α , and this effect was antagonized by TZDs (60). Very recently, Yamauchi and colleagues reported that decreased expression levels of adiponectin coincide with insulin resistance in murine models and that physiological doses of adiponectin improve insulin resistance (61). Consistent with these results, Fruebis et al. have shown that a proteolytic product of the ACRP30 protein increases fatty acid oxidation in muscle and causes significant weight loss in mice (62).

Resistin gene expression is induced during adipocyte differentiation in culture and is restricted to WAT and to BAT in vivo (29). Its expression in adipose tissue and plasma levels is low during fasting and highly increased when animals are refed (29, 30). Steppan et al. found that administration of resistin impairs glucose tolerance and insulin action in mice. This same group also reported that circulating resistin was markedly increased in both genetic and diet-induced obesity. However, another group has reported that adipose tissue mRNA for resistin is significantly decreased in several models of obesity and diabetes (28). At present, there is no agreement as to whether resistin is positively or negatively regulated by TZDs (28, 29, 63).

Lessons from mouse models

Studies using mouse genetic models have also greatly advanced our understanding of the role of PPAR γ signaling in insulin resistance (Fig. 1). Several groups have analyzed the actions of TZDs in lipoatrophic mice. Total lipoatrophy in the A-ZIP/F-1 mouse is associated with insulin resistance, hyperglycemia, and an increase in muscle and liver triglyceride content (64). Transplantation of wild-type adipose tissue reversed the hyperglycemia, low-

ered insulin levels, and improved insulin sensitivity in these mice, demonstrating that diabetes in A-ZIP/F-1 mice is caused by the lack of adipose tissue. Chao et al. found that TZDs failed to reduce glucose and insulin levels in this model system, suggesting that white adipose tissue is required for the antidiabetic effects of these ligands (65). However, these authors also found that TZDs were effective in lowering circulating TG and in increasing whole body FFA oxidation in A-ZIP/F-1 mice, indicating that target tissues other than fat must be involved in these effects (65). In contrast, Graves and colleagues found troglitazone effective in lowering glucose and insulin in a different lipoatrophic model, the ap2/DTA transgenic mouse (66). The basis for the difference between these models is not yet clear.

Important advances have also come from analysis of the influence of PPAR γ gene dosage on lipid metabolism and insulin resistance in mice. Some of these studies are summarized in Fig. 2. In wild type mice, a high fat diet promotes adipocyte hypertrophy, obesity, triglyceride accumulation in skeletal muscle and liver, and insulin resistance (15). Kubota et al. have hypothesized that TZDs ameliorate insulin resistance in this model by stimulating de novo

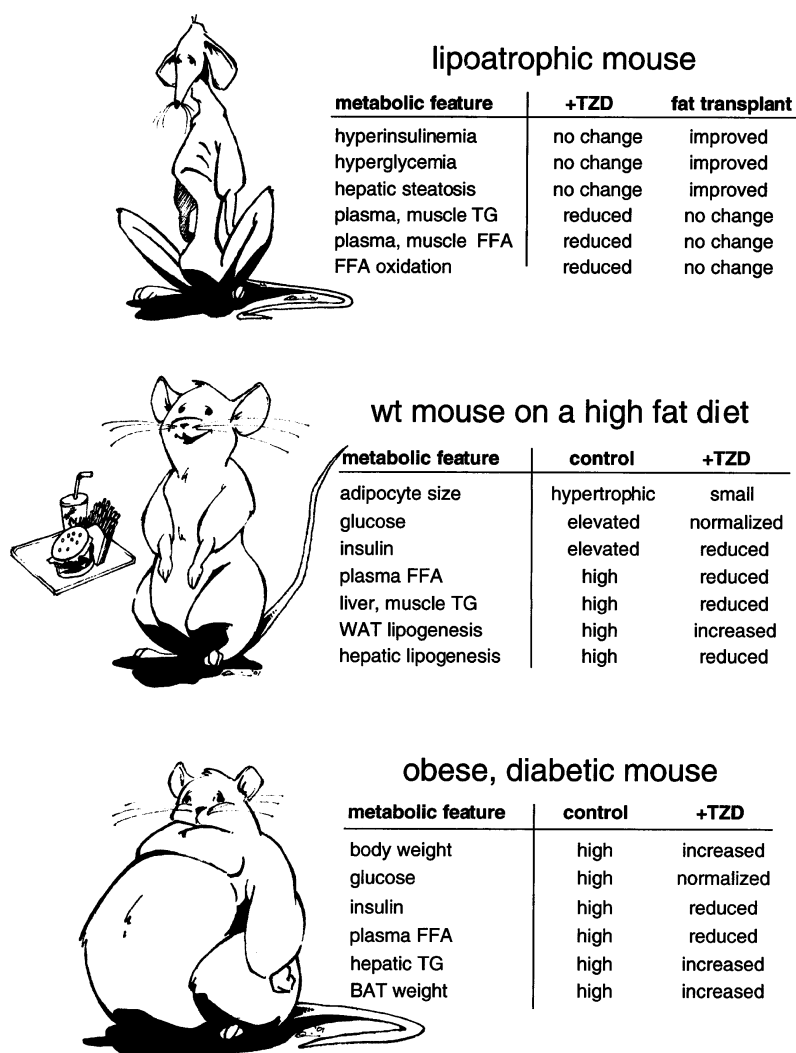
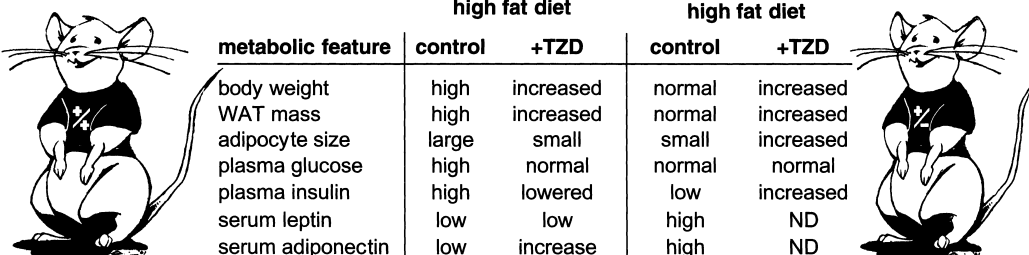


Fig. 1. PPAR γ ligands modulate lipid metabolism and insulin sensitivity: lessons from animal models. In mouse models of lipoatrophic diabetes such as the A-ZIP/F1 mouse, complete absence of adipose tissue is associated with severe insulin resistance and hyperglycemia (62, 63). Transplantation of wild type fat lowers basal glucose, prevents hyperinsulinemia, and significantly improves insulin sensitivity. However, the improvement in serum FFA and TG levels is very modest. On the other hand, thiazolidinedione (TZD) treatment does not reduce glucose or insulin levels, suggesting that white adipose tissue (WAT) is required for the antidiabetic effects of PPAR γ ligands. TZDs are effective in lowering circulating TGs and increasing whole body fatty acid oxidation in this model, indicating that these effects occur via tissues other than WAT. In wild-type (wt) mice, a high fat diet induces adipocyte hypertrophy, obesity, and insulin resistance (15, 66). TZD administration is hypothesized to reverse these metabolic disturbances by stimulating de novo adipogenesis. This action of PPAR γ ligands results in a redistribution of triglycerides from skeletal muscle and liver into the WAT. Blood glucose and insulin levels were normalized, and circulating FFA were decreased upon TZD treatment. In the ob/ob obese diabetic model, genetic absence of leptin results in increased food intake, obesity, and insulin resistance (1, 63). These animals are hyperinsulinemic, hyperglycemic, and have elevated FFA in the plasma, and increased TG levels in liver and skeletal muscle. TZD administration reduces basal insulin levels with a modest decrease in serum glucose and FFA.



metabolic feature	wild-type high fat diet		PPAR γ +/- high fat diet	
	control	+TZD	control	+TZD
body weight	high	increased	normal	increased
WAT mass	high	increased	normal	increased
adipocyte size	large	small	small	increased
plasma glucose	high	normal	normal	normal
plasma insulin	high	lowered	low	increased
serum leptin	low	low	high	ND
serum adiponectin	low	increase	high	ND

Fig. 2. PPAR γ agonist treatment and heterozygous PPAR γ deficiency both improve insulin resistance, although by different mechanisms (15, 66). Administration of TZDs to wild-type mice fed a high fat diet results in a potent stimulation of adipogenesis, thereby increasing fatty acid flux from liver and skeletal muscle into WAT. TZD treatment also induces apoptosis of hypertrophic adipocytes, thereby increasing the number of small, highly insulin sensitive adipocytes. This is hypothesized to ameliorate insulin resistance by decreasing circulating FFA and TNF α , and increasing levels of the insulin sensitizing hormone adiponectin. By contrast, PPAR γ heterozygosity prevents high fat diet induced adipocyte hypertrophy, and improves insulin sensitivity. This phenotype is associated with increased circulating leptin and adiponectin levels, and a significant increase in fatty acid β -oxidation rate in brown adipose tissue (BAT), liver and skeletal muscle. Paradoxically, the favorable PPAR γ ^{+/-} phenotype is largely reversed by TZD treatment.

adipogenesis, thus increasing the number of small insulin sensitive adipocytes and promoting TG redistribution from liver and muscle into the WAT. Unexpectedly, PPAR γ ^{+/-} mice were found to have smaller adipocytes and to be more insulin sensitive than wild type controls (15, 67). Moreover, adipocyte hypertrophy in response to high fat diet was partially prevented in PPAR γ ^{+/-} mice (15, 68). These observations suggest that reduced expression of PPAR γ may prevent adipocyte hypertrophy and thereby improve insulin sensitivity. Consistent with this hypothesis, PPAR γ ^{+/-} mice show reduced levels of factors associated with insulin resistance including FFA and TNF α , increased levels of leptin and adiponectin, and a significantly increased rate of fatty acid beta-oxidation. Paradoxically, this favorable insulin-sensitive phenotype of the PPAR γ ^{+/-} mice is reversed by TZD treatment (**Fig. 2**).

In conclusion, there appears to be an optimal amount of PPAR γ activity in adipose tissue required for optimal whole body lipid metabolism. Too little PPAR γ can lead to lipoatrophy and too much can lead to adipocyte hypertrophy, both of which can cause insulin resistance. PPAR γ agonists have opposite effects on adiposity and energy expenditure in PPAR γ ^{+/+} versus PPAR γ ^{+/-} mice. Heterozygous PPAR γ deficiency and TZD administration each prevent adipocyte hypertrophy and insulin resistance, although via different mechanisms. Heterozygous PPAR γ ^{+/-} deficiency increases fatty acid combustion and energy dissipation, whereas TZD stimulate de novo adipogenesis, thereby increasing fatty acid flux from muscle and liver into WAT.

PPAR γ AND MACROPHAGE LIPID METABOLISM

Oxidized lipids and atherosclerosis

The earliest events in atherogenesis include the recruitment of circulating monocytes to the artery wall, their differentiation into tissue macrophages, and their transformation

into foam cells through the accumulation of LDL-derived cholesterol (69). Macrophage lipid uptake is mediated primarily by scavenger receptors including scavenger receptor A (SR-A), CD36, and CD68. Considerable evidence has emerged to suggest that oxidized lipids play a critical role in these early events (70, 71). LDL acquires a number of novel biological activities as a result of oxidative modification, including the ability to bind scavenger receptors, the ability to attract circulating monocytes to the sites of endothelial injury, and the ability to regulate macrophage gene expression.

Recent work suggests that nuclear receptors including PPAR γ and the LXRs may mediate important effects of oxidized lipids on macrophages and other vascular cells. PPAR γ is expressed at a low level in circulating monocytes, and its expression is significantly increased upon differentiation to macrophages. Moreover, PPAR γ protein is present at high levels in the macrophage-derived foam cells of atherosclerotic lesions (38, 72). Treatment of macrophages with oxidized LDL (oxLDL) in vitro induces expression of both PPAR γ and LXR α mRNAs (25, 38). Internalization of oxLDL by macrophages provides the cell with activators of PPAR γ , such as oxidized fatty acids, as well as with activators of LXRs such as 27- and 25-hydroxycholesterol (3, 73, 74). PPAR γ ligands can also be produced locally in atherosclerotic lesions through the oxidation of fatty acids by 12/15 lipoxygenase (75).

PPAR γ regulates lipid uptake and efflux

Alterations in the rates of lipid uptake or efflux from the artery wall each have the potential to affect the development of atherosclerotic lesion. Work from a number of laboratories over the past 4 years have provided evidence supporting a role for PPAR γ in pathways for both lipid uptake and lipid efflux in macrophages (**Fig. 3**). PPAR γ was implicated in lipid uptake by the observation that the gene encoding the scavenger receptor CD36 is a direct target of PPAR γ /RXR heterodimer

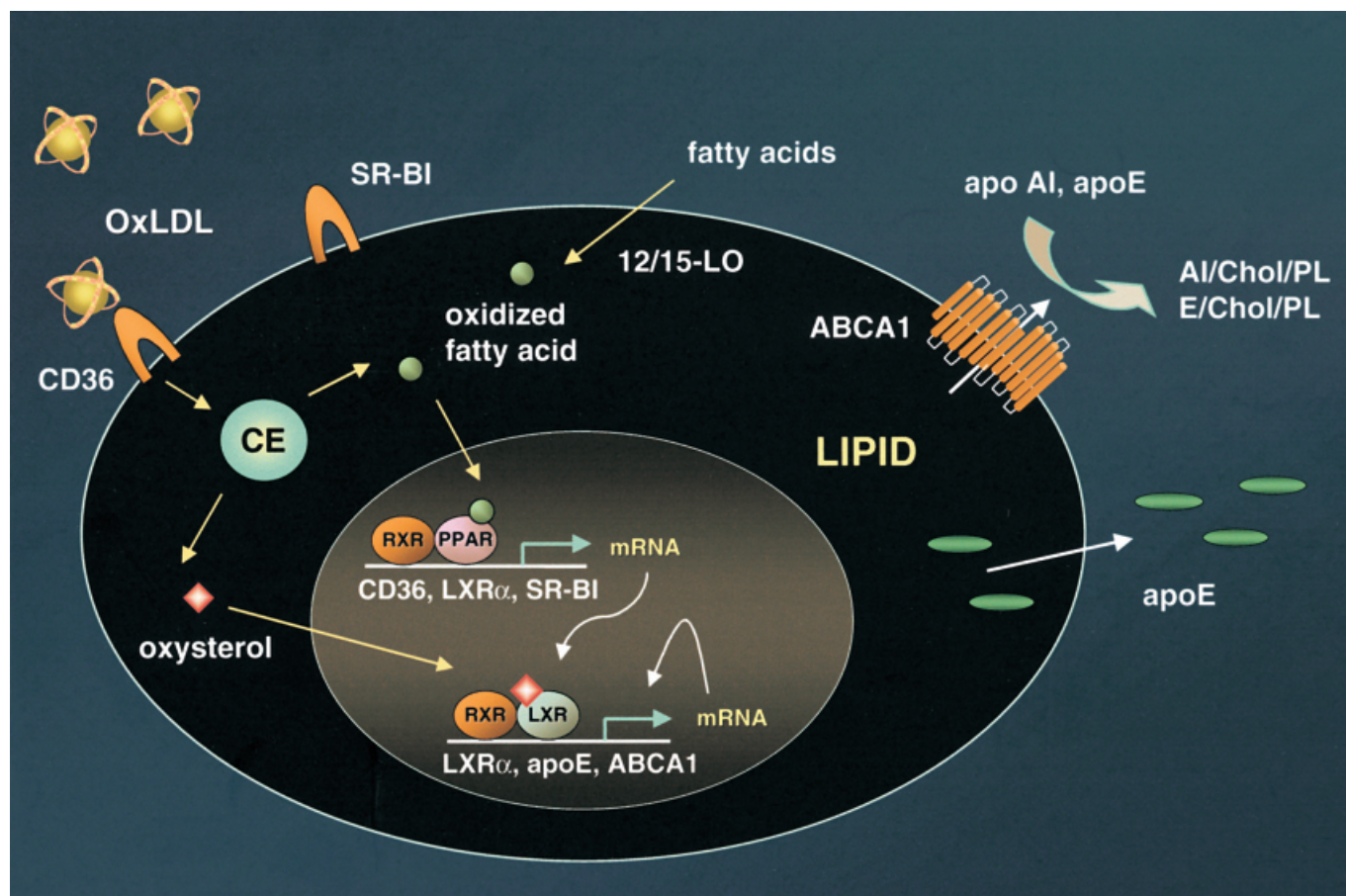


Fig. 3. Model for the role of PPAR γ in the regulation of macrophage lipid metabolism.

(38). A critical role for PPAR γ in the regulation of this gene is supported by both gain of function and loss of function studies. Retroviral expression and activation of PPAR γ in macrophages or fibroblasts induces expression of the CD36 gene. In addition, the ability of synthetic PPAR γ ligands to induce CD36 expression is abolished in PPAR γ null cells (76, 77). In NIH-3T3 fibroblasts (which lack scavenger receptors), expression of PPAR γ endows these cells with the ability to take up oxLDL. However, in differentiated macrophages in vitro, loss of PPAR γ expression does not significantly compromise oxLDL uptake, presumably due to compensation by other scavenger receptors (76, 77). In contrast to CD36, expression of SR-A is down regulated in response to PPAR γ activation (77, 78). Although the contribution of PPAR γ to lipid uptake in vivo is not yet clear, selective loss of CD36 in mice has been shown to decrease both macrophage lipid uptake and atherosclerosis (79, 80). It is important to note that CD36 expression is also regulated by PPAR γ in adipose tissue (36). Its regulation in this context is likely to contribute to uptake of free fatty acids for esterification into triglycerides (79). Interestingly, the related protein SR-BI has also been identified as a PPAR-responsive gene in human macrophages (81).

PPAR γ and atherosclerosis

The initial observation that PPAR γ activation promotes CD36 expression raised concerns about the effects of the thiazolidinediones on cardiovascular disease risk in humans. Patients with NIDDM are already at increased risk of developing atherosclerosis. Fortunately, available data to this point suggest that on balance the effect of systemic administration of synthetic PPAR γ ligands is antiatherogenic in both mice and humans. Although the mechanisms involved in this effect are not completely worked out, they are likely to include favorable alterations in plasma lipid profiles, amelioration of insulin resistance, and direct effects on cells of the artery wall.

Several studies have examined the effects of PPAR γ ligands in murine models of atherosclerosis. Li et al. demonstrated that two unrelated agonists (rosiglitazone and GW7845) reduced atherosclerosis in LDLR $^{-/-}$ mouse fed a Western diet (82). Markedly fewer and smaller lesions were found in male mice despite a significant increase in the expression of CD36 in the vessel wall. Subsequent studies showed a similar beneficial effect of TZDs in the apolipoprotein E (apoE) $^{-/-}$ and LDLR $^{-/-}$ models (83, 84). Limited human studies also support an antiatherogenic effect of PPAR γ ligands. For example, Minamikawa

et al. found a significant decrease in carotid artery thickness in Type 2 diabetics treated with troglitazone (85).

The observation that the net effect of systemic PPAR γ activation is antiatherogenic suggests that the potential of PPAR ligands to promote CD36 expression and lipid uptake is balanced by other systemic and/or local actions. One plausible explanation for this paradox involves the function of the nuclear receptor LXR α in macrophages. Recent data indicate that in differentiated macrophages, the PPAR γ and LXR signaling pathways converge to mediate a key response to lipid loading: a pathway for cholesterol efflux. PPAR γ and the LXRs cooperate to induce expression of the putative cholesterol/phospholipid transporter ABCA1 and apoE in macrophages (86, 87). The molecular basis for this cooperation is that the LXR α gene is itself a direct transcriptional target of PPAR γ . Ligand activation of PPAR γ leads to primary induction of LXR α expression and to coupled induction of the ABCA1 and apoE genes, which are both direct LXR targets (88–90). ABCA1 and apoE have both been implicated in the efflux of cholesterol and phospholipid to extracellular acceptors. As a consequence of this regulatory loop, PPAR γ and LXR ligands additively promote cholesterol efflux from macrophages (86, 87). In PPAR γ null macrophages, the ability of PPAR γ ligands to increase expression of ABCA1 and stimulate efflux is abolished (86). Thus, PPAR γ may be an important modifier of the reverse cholesterol transport pathway. This modulation may be particularly important in tissues where PPAR γ is highly expressed, such as macrophages, adipocytes, and certain epithelial cells.

The realization that PPAR γ could influence both lipid uptake and efflux raised the question of whether the net effect of PPAR γ ligands on macrophages within an atherosclerotic lesion would be to promote or impede foam cell formation. Studies in which PPAR γ ligands are administered to mice do not permit one to differentiate between systemic and local effects. Chawla et al. addressed this issue by examining atherosclerosis in LDLR $^{-/-}$ mice transplanted with wild type or PPAR γ null bone marrow (86). Loss of bone marrow PPAR γ expression led to a significant increase in atherosclerosis, suggesting that the PPAR γ -LXR-ABCA1 efflux pathway dominates in vivo. Moreover, these observations support the hypothesis that direct effects of PPAR γ ligands on cells of the artery wall are important for the antiatherogenic properties of these compounds.

Other local effects of PPAR γ ligands are also likely to contribute to their antiatherogenic effects. PPAR γ ligands inhibit macrophage production of inflammatory cytokines (78, 91), although some of this activity may be not be mediated by PPAR γ (76, 77). Oxidized lipid activators of PPAR γ have also been found to antagonize expression of CCR2 in monocytes, and thus may decrease monocyte recruitment into the vessel wall (92). Local effects on smooth muscle cells may also play a role. For example, TZDs have been shown to inhibit migration of smooth muscle cells in response to chemoattractive signals and thus may limit their recruitment into lesions (93). Thus, systemic treatment with PPAR γ ligands results in the si-

multaneous activation of many PPAR γ regulatory pathways. As outlined above, PPAR γ agonists have multiple effects on systemic lipid and glucose metabolism in addition to their effects in vascular cells. The net effect is likely to reflect a balance between local effects in the artery wall and systemic effects on lipid metabolism. ■

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