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Peroxisome proliferator-activated receptor gamma (PPAR γ) and its ligands: A review

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Abstract

Peroxisome proliferator-activated receptor gamma (PPAR γ) is a member of a class of nuclear hormone receptors intimately involved in the regulation of expression of myriad genes that regulate energy metabolism, cell differentiation, apoptosis and inflammation. Although originally discovered as a pivotal regulator of adipocyte differentiation, the roles that this transcription factor play in physiology and pathophysiology continue to grow as researchers discover its influence in the function of many cell types. This review highlights the roles that PPAR γ play in the regulation of gene expression associated with normal cell physiology as well as the pathophysiology of multiple diseases including obesity, diabetes and cancer. Additionally, naturally occurring and pharmaceutical ligands for the receptor as well as the potential role of PPAR γ as the receptor responsible for fatty acid-induced effects on gene expression will be described. © 2002 Elsevier Science Inc. All rights reserved.

1. Introduction

Nuclear receptors are integral regulators of gene transcription and intracellular function (see reviews [1,2]). The nuclear receptor superfamily includes members such as the estrogen; thyroid and glucocorticoid receptors as well as the subfamily of peroxisome proliferator-activated receptors (PPARs; Fig. 1A). Nuclear receptors are ligand-activated transcription factors that regulate diverse biologic events ranging from cell differentiation and development to lipid metabolism and energy homeostasis. Steroids, fatty acids, hormones, and vitamins A and D are among the natural lipophilic ligands that bind to this superfamily of receptors.

The PPAR subfamily of nuclear hormone receptors include distinct genes that code for several PPAR isoforms denoted: PPAR α , β/δ and γ [3]. The *PPAR γ* gene contains three

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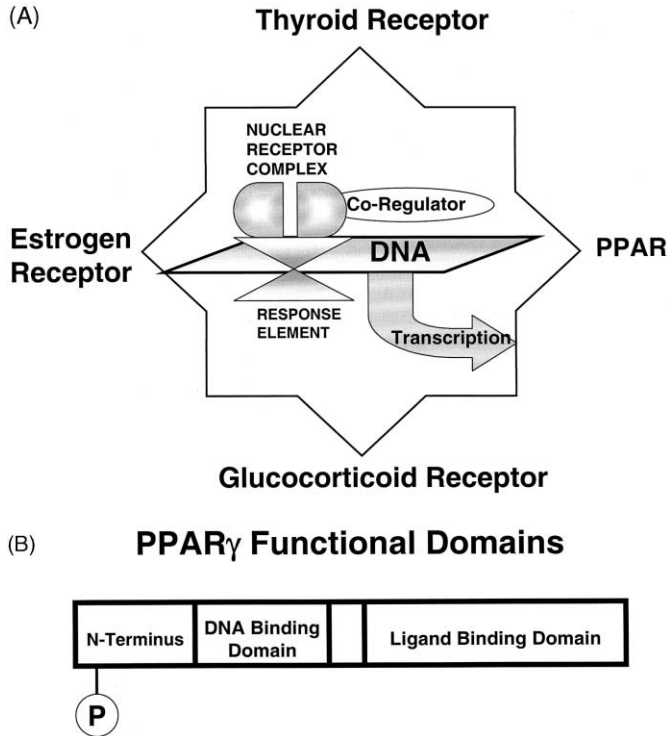


Fig. 1. Peroxisome proliferator-activated receptors (PPARs) are nuclear transcription factors. *Panel A:* This schematic represents the nuclear receptor superfamily that includes (but is not limited to) thyroid receptors, steroid receptors, and PPARs. Ligand-bound nuclear receptors bind response elements on DNA and regulate the transcription of genes involved in diverse physiological processes. Additionally, various co-regulators (including co-activators and co-repressors) bind the ligand-bound nuclear receptor complex and facilitate or inhibit, respectively, transcriptional activation. *Panel B:* The PPAR γ protein consists of three general functional domains including the N-terminus which regulated by phosphorylation (P), the DNA binding domain and the ligand binding domain. This general structure is common for all members of the PPAR family of receptors.

promoters that yield three RNA isoforms, γ_1 , γ_2 , and γ_3 by alternative promoter usage and splicing [4–7]. The PPAR γ_1 and γ_3 RNA transcripts both translate into PPAR γ_1 protein. Expression of PPARs is tissue dependent [8]. PPAR α is highly expressed in liver, cardiac myocytes, enterocytes and proximal tubule of the kidney. PPAR β/δ is ubiquitously expressed, whereas PPAR γ is highly expressed in adipose tissue and the immune system. PPAR γ_1 is expressed (in relatively low abundance) in many tissues, whereas PPAR γ_2 is predominantly expressed in adipocytes.

2. PPAR: key regulators of gene transcription

PPAR γ , like other members of the nuclear receptor superfamily, is characterized by three general functional domains: the N-terminal domain (a site for functional regulation by phosphorylation; [9–13]), the DNA binding domain and the ligand binding domain (see Fig. 1B).

The processes by which PPAR bind DNA and regulate transcription have been extensively reviewed (see [14,15]). Briefly, ligand binds to the PPAR molecule, causing a conformational change in the AF2 (activation function 2) domain found in the ligand-binding domain. Ligand-bound PPAR then forms a heterodimeric complex with other transcription factors (e.g., 9-*cis*-retinoic acid receptor; RXR). This heterodimeric complex comprises the functional transcription factor that then binds to peroxisome proliferator response elements (PPRE) on DNA. The PPRE are DR-1 elements (sequence AGGTCA and AGGTCA) found in the promoters of PPAR responsive genes such as aP2 [14]. This process, in most cases, activates transcription of various genes involved in diverse physiological and pathophysiological processes (Fig. 2, [16]). In addition to the heterodimer complex, it has been reported that a host of accessory proteins, denoted ‘co-activators’ or ‘co-repressors’ can bind to the nuclear receptors in a ligand-dependant manner and impact the transcriptional process by either remodeling chromatin structure and/or acting as adapter molecules that link the nuclear receptor complex to key transcriptional machinery [17,18].

Regulation of PPAR γ can occur at the level of gene expression, ligand availability (both endogenous and pharmacological ligands) and at the level of PPAR γ activity (phosphorylation status, [9]). The fact that genetic deletion of the *PPAR γ* gene in mice results in embryonic lethality at approximately Day 10 of gestation due to placental insufficiency [19,20] supports

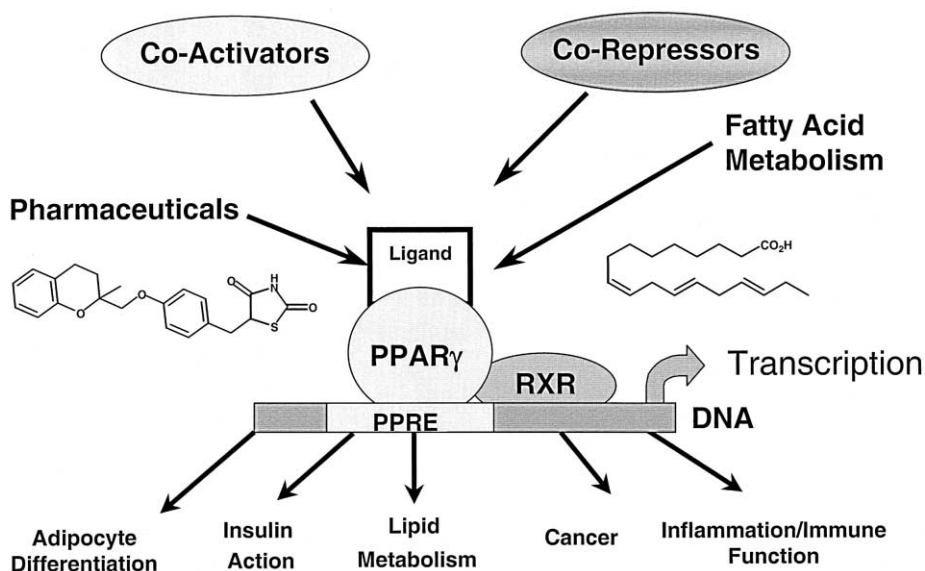


Fig. 2. Activation of peroxisome proliferator-activated receptor gamma (PPAR γ) regulates expression of genes involved myriad physiological and pathophysiological states. Upon ligand binding (either pharmaceutical or endogenous ligand such as fatty acids) PPAR γ undergoes a conformational change in the ligand binding domain and forms a heterodimeric complex with retinoic acid receptor (RXR). Various co-activators and co-repressors fine-tune the ability of the-activated PPAR γ :RXR complex bind to peroxisome proliferator response elements (PPRE) to regulate transcription. PPAR γ activation appears to play a role in diverse physiological and pathophysiological events including stimulation of adipocyte differentiation, stimulation of insulin action, regulation of lipid metabolism, inhibition of tumor cell proliferation and diverse effects on inflammatory processes.

the notion that PPAR γ (expression and presumably function) plays a crucial role in the regulation of developmental genes in multiple cell types including adipose, placenta and cardiac tissues [19].

3. PPAR γ ligands

Although there are a number of naturally occurring agents that activate PPAR γ (Fig. 3, [21]), the identity of the true natural ligand of this receptor is still a mystery. Various polyunsaturated acids activate PPAR γ in micromolar concentrations [22,23]. Since free fatty acids circulate within human plasma in micromolar levels [24], it is not unrealistic to consider the possibility of their functioning as the receptor's natural ligand. However, concentration of unsaturated fatty acids within the cell and the site of receptor activation are still unknown.

Some J-series prostaglandins have been shown to bind to PPAR γ in low micromolar range [25,26]. The PGD $_2$ derivative, 15-deoxy- $\Delta^{12,14}$ -PGJ $_2$ (15d-PGJ $_2$) is a high affinity ligand ($K_d = 300$ nM) that demonstrates anti-inflammatory [27,28] and anti-neoplastic activity [29]. Although it is still unknown whether 15d-PGJ $_2$ is present in adipose tissue, it has been shown to stimulate adipocyte differentiation *in vitro* [30,31].

More recently, researchers have found that human monocyte stimulation with the C-terminal fragment (C-36) of α 1-antitrypsin (AAT) activates PPAR α and PPAR γ with an observed increase of intracellular lipid accumulation within the culture [32]. Activation was specific to the C-36 fibrils and not found for the native protein or other fragments. AAT is a major proteinase inhibitor found in human plasma and most tissues. Interestingly, C-36 is also found in atherosclerotic plaques at primary sites of degradation. The physiological role of C-36 is under current investigation.

A number of synthetic PPAR γ ligands have been identified over the past 6 years (Fig. 4), of which the most well known are the thiazolidinediones (TZDs) [33,34]. Although the anti-diabetic activity of this class of compounds has been known for sometime, it wasn't until 1995 that TZDs were shown to be PPAR γ agonists [35]. TZDs, such as troglitazone, improve insulin resistance and lower plasma glucose levels in man [36]. In general, *in vitro*

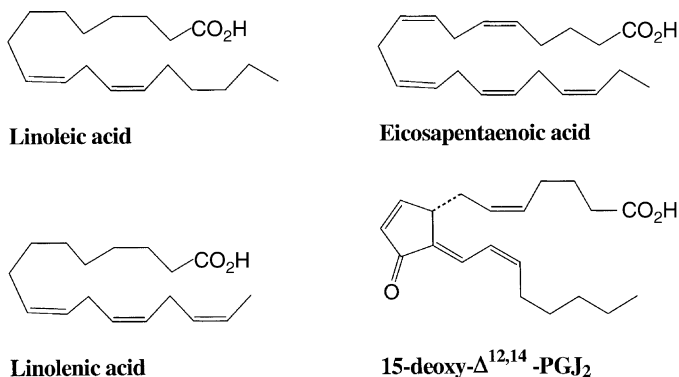


Fig. 3. Chemical structures of naturally occurring peroxisome proliferator-activated receptor gamma ligands.

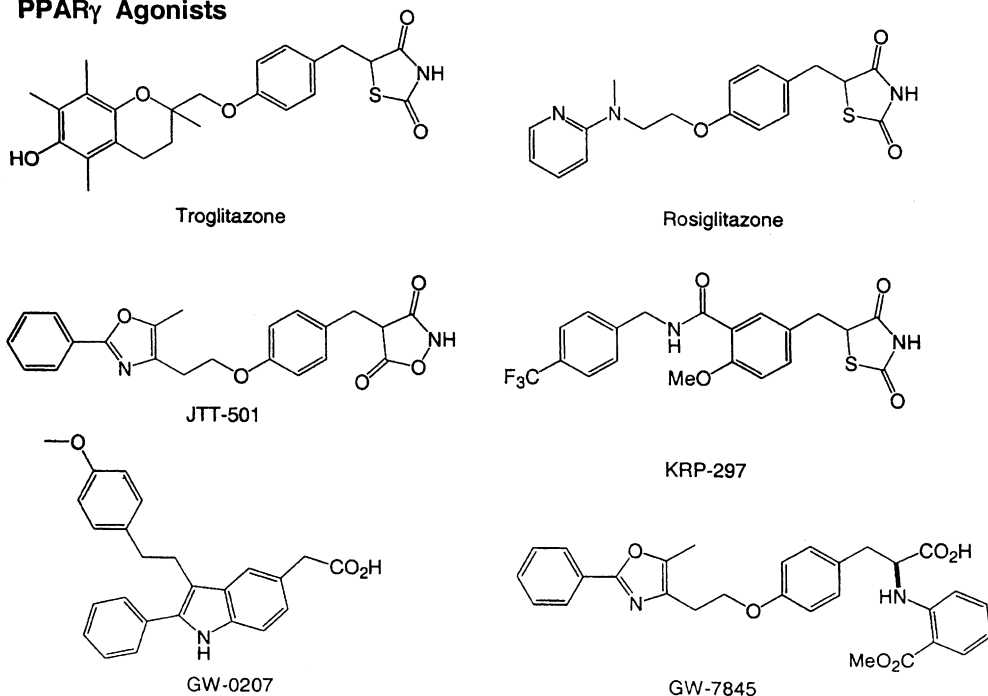
PPAR γ Agonists

Fig. 4. Chemical structures of pharmaceutical peroxisome proliferator-activated receptor gamma agonists.

potencies of TZDs correlate well with glucose lowering activity to provide additional evidence of this being a PPAR-mediated process [34,37]. Most TZDs are selective for PPAR γ over the PPAR α and PPAR β/δ subtypes [34,35], but there are some exceptions. Thiazolidinedione KRP-297 is a potent PPAR γ agonist and a weak PPAR α 127 agonist [38]. Rosiglitazone is the most potent and selective agent in this class.

The mechanism by which TZDs bind and activate PPAR γ has been investigated [39]. The PPAR γ LBD consists of 13 α -helices and a 4-strand β -sheet and provides a large binding pocket (1300 Å) that allows access to a number of structurally diverse ligands. The ternary complex of the PPAR γ LBD, rosiglitazone and the 88-amino acid fragment of human SRC-1 has been determined by X-ray crystallography and indicates that the TZD head group forms specific H-bonding interactions with His⁴⁴⁹, Tyr⁴⁷³, His³²³, Ser²⁸⁹ and Gln²⁸⁶. It is believed that interaction with Tyr⁴⁷³ triggers transcriptional activation since this residue lies in the C-terminal AF-2 helix.

Although TZDs have good anti-diabetic activity, most demonstrate only modest affinity for PPAR γ . Therefore, in the search for more potent (<50 nM) ligands, a number of non-TZD ligands have been reported. Isoxazolidinedione JTT-501 is a nanomolar PPAR γ agonist and micromolar PPAR α agonist that has been shown to improve insulin sensitivity in Zucker diabetic fatty (ZDF) rats [40]. More structurally diverse analogs have also been developed. Tyrosine-based agonist GW-7845 shows sub-nanomolar affinity for PPAR γ and excellent selectivity. This compound displays potent anti-diabetic, anti-carcinogenic and

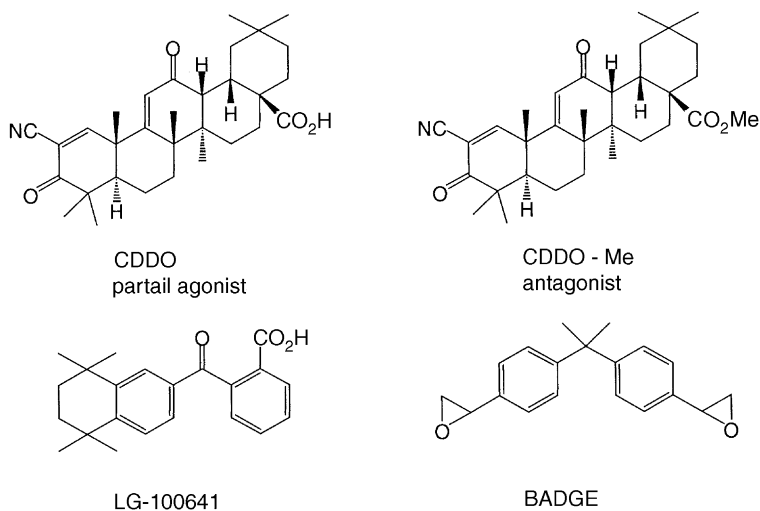


Fig. 5. Chemical structures of novel peroxisome proliferator-activated receptor gamma ligands.

anti-atherosclerotic activity in rodents [41–43]. Indole GW-0207 is a PPAR γ agonist that is equipotent to rosiglitazone with superior selectivity over the other receptor subtypes. It has excellent pharmacokinetics in rats and normalizes glucose levels in ZDF rats at 1/100 the dose of troglitazone [44].

A number of novel partial agonists and antagonists have been identified over the past few years (see Fig. 5). Triterpenoid 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), is a nanomolar affinity partial agonist [45] that has been reported to demonstrate anti-inflammatory properties and inhibit proliferation of many human tumor cell lines [42]. Interestingly, the corresponding methyl ester, CDDO-Me is a nanomolar PPAR γ antagonist that inhibits 3T3-L1 cell differentiation by rosiglitazone [45].

Bisphenol A diglycidyl ether (BADGE) is a recently identified micromolar antagonist that inhibits TZD induced differentiation of preadipocytes. BADGE has also been shown to inhibit hormone-mediated differentiation of 3T3-L1 and 3T3-F442A cells [46]. Acid LG-100641 is a potent PPAR γ antagonist that blocks TZD activation of the receptor, but not differentiation induced by retinoic X receptor ligands. Amazingly, LG-100641 promotes glucose uptake in adipocytes [47]. This compound should prove to be a useful tool for defining the role of PPAR γ in insulin resistance.

4. PPAR γ function in cell differentiation

4.1. PPAR γ : a pivotal regulator of adipocyte differentiation

Adipogenesis refers to the process of differentiation of preadipocyte precursor cells into adipocytes that are capable of lipid filling as well as the expression and secretion of myriad hormones and cytokines. Adipogenesis occurs prenatally and postnatally, both in response to

normal cell turnover and to support excess energy storage [48]. The program of adipocyte differentiation and associated induction of adipocyte gene expression has been extensively studied (see review [49]). PPAR γ (coupled to RXR) and C/EBP α are pivotal transcription factors involved early in the process of cell growth arrest followed by progression into the fully differentiated adipocyte phenotype [50]. The sufficiency of PPAR γ to drive adipocyte differentiation was illustrated in studies reporting that expression and activation of PPAR γ in fibroblasts and muscle cells triggered the adipocyte differentiation program [51,52]. The pivotal role that PPAR γ plays in the development of adipose tissue was confirmed with the finding that mouse embryos which lack the *PPAR γ* gene ($-/-$) were devoid of adipose tissue [19].

Detailed studies examining the mechanism(s) underlying the process of adipocyte differentiation have described complex interactions among PPAR γ /RXR, C/EBP proteins and ADD-1/SREBP-1. These transcription factors act synergistically to result in full induction of adipocyte-specific genes including aP2, PEPCK, leptin, ACS and lipoprotein lipase (see reviews [49,53]). Additionally, the transcription factor, ADD-1/SREBP-1, plays a pivotal role in cholesterol homeostasis, as it regulates the expression of multiple genes involved in fatty acid metabolism [54]. Thus, ADD-1/SREBP-1 may regulate the generation of endogenous PPAR γ ligands, thus enhancing PPAR γ activation of adipocyte gene expression and terminal differentiation [55].

In addition to stimulating adipocyte differentiation, data now suggest that PPAR γ activation stimulates apoptosis in mature, lipid-filled adipocytes [56]. This ligand-induced apoptosis in mature cells is coincident with stimulation of adipogenesis from preadipocyte precursors, resulting in a net increase in the number of small, relatively insulin-sensitive adipocytes [56,57].

In livestock species, data are very limited which describe the role of PPAR γ and its ligands in the regulation of adipogenesis. Bovine stromal-vascular cells were stimulated to differentiate into adipocytes following thiazolidinedione exposure [58]. Similarly, Wu *et al.* [59] reported that lipids, as well as the weak PPAR γ agonist, indomethacin, stimulated adipocyte differentiation in primary cultures of bovine stromal-vascular cells. Studies examining differentiation of porcine adipocytes in response to the thiazolidinedione, troglitazone, indicate that similar to findings in other species, porcine adipocyte differentiation is stimulated following PPAR γ activation, and this differentiation does not necessarily correlate with changes in PPAR γ protein expression [60].

5. Regulation of *PPAR γ* gene expression

Although considerable data exist which quantify effects of PPAR γ activation (primarily via treatment with thiazolidinediones), there is a relative paucity of data that describe the regulation of expression of PPAR γ , especially the differential regulation of expression of the various PPAR γ isoforms.

In livestock, the *PPAR γ* gene has been cloned in pigs [61,62] and cattle [63]. The porcine *PPAR γ* gene has been mapped to porcine chromosome 13 [64], and the nucleic acid sequence of porcine PPAR γ is highly conserved with the bovine, human and murine sequences; the amino acid identities ranged from 96 to 98% for all species [62]. Both PPAR γ 1 and PPAR γ 2 RNA

isoforms are highly expressed in porcine adipose tissue, although the $\gamma 1$ isoform predominates [62]. To date, the $\gamma 3$ RNA isoform has not been identified in livestock species.

The bovine *PPAR γ* gene has been assigned to bovine chromosome 22, and differential expression of *PPAR $\gamma 1$* and *PPAR $\gamma 2$* mRNA was quantified among various bovine tissues [63]. Specifically, *PPAR $\gamma 1$* expression was higher and more widely distributed than *PPAR $\gamma 2$* [63]. Interestingly, *PPAR γ* expression has been identified in bovine lutein cells [65] and limited data suggest that *PPAR γ* ligands may play a role in the secretion of progesterone by these cells. These data have led the authors to hypothesize that *PPAR γ* and its ligands may play a role in linking nutritional status to reproductive function in the bovine [65,66]. Additional data are needed to prove/refute this intriguing hypothesis.

A limited body of evidence suggests that *PPAR γ* expression is regulated nutritionally in rodents, humans, and livestock. In rodents, adipose tissue *PPAR γ* expression is regulated by insulin [67] and dietary manipulation (high fat diets or fasting; [67,68]), but not by obesity [67].

Porcine *PPAR γ* abundance in adipose tissue is regulated by nutritional status [62,69]. Expression of *PPAR $\gamma 2$* but not *PPAR $\gamma 1$* mRNA is significantly reduced in pigs that were fasted or allowed restricted food intake compared to *ad libitum* fed controls [62]. The pig data are consistent with the findings of Vidal-Puig *et al.* [67] who reported that fasting reduced *PPAR $\gamma 2$* mRNA abundance more significantly than *PPAR $\gamma 1$* in the mouse. *PPAR $\gamma 2$* expression is regulated by dietary fatty acids in swine. Specifically, *PPAR $\gamma 2$* expression in porcine subcutaneous (sc) adipose tissue is up regulated with dietary supplementation of 18:2 but not 16:0, 18:0 or $\omega 3$ fatty acids [70].

The mechanisms underlying differential regulation of *PPAR $\gamma 2$* versus *PPAR $\gamma 1$* protein expression in mammals are not well understood. It is tempting to speculate that nutritional, metabolic and/or endocrine factors that are modified with food restriction, fasting and other nutritional/physiological/pathologic states differentially modulate $\gamma 1$, $\gamma 2$ and $\gamma 3$ RNA expression and/or *PPAR γ* protein activity. Further research is needed to determine the functional importance of the splice variants and to delineate mechanisms that regulate their expression and activation in mammals.

6. Nutritional regulation of gene expression: *PPAR γ* as a fatty acid receptor

Dietary fat (fatty acid profile and total fat consumption) regulates gene expression in metabolic tissues; often effects are observed in a tissue-specific manner. For example, consumption of high fat diets leads to whole-body insulin resistance in rodents, and the mechanisms underlying this insulin resistance involve the insulin responsive glucose transporter, GLUT4. Increased dietary fat (but not caloric) consumption down-regulates GLUT4 protein expression in adipose tissue but not skeletal muscle [71] and expression is linked to arachidonic acid metabolites, presumably via activation of *PPAR γ* [72]. In contrast, high fat diets alter GLUT4 translocation to the plasma membrane and insulin signaling but not gene expression in skeletal muscle; culminating in insulin resistance [73].

In addition to total dietary fat intake, data are accumulating that *specific* dietary fatty acids may differentially regulate gene expression. Raclot *et al.* [74] reported that consumption of

dietary n-3 polyunsaturated fatty acids (n-3 PUFA) down-regulated (40–75% compared to control) expression of genes involved in lipid metabolism, adipogenesis and food intake regulation in retroperitoneal but not subcutaneous adipose tissue. Genes regulated by n-3 PUFA in retroperitoneal adipose tissue included leptin, CEBP/α, fatty acid synthase, lipoprotein lipase, hormone sensitive lipase and phosphoenolpyruvate carboxykinase. Regulation of expression of the aP2 fatty acid binding protein gene by PUFA may also be important as it has been implicated in the targeting of fatty acids to regulatory elements in the nucleus [75].

Clearly, dietary fatty acids can have profound effects on gene expression. However, the mechanism(s) underlying these effects have not been fully elucidated. As discussed earlier in this review, expression of genes important in adipocyte differentiation and lipid metabolism (e.g., aP2, lipoprotein lipase, and fatty acid synthase) is induced during TZD as well as PUFA treatment.

Thus, data are accumulating which indicate that at least one mechanism by which fatty acids can regulate gene transcription is by acting as ligands for nuclear receptors such as PPARγ which bind DNA and directly and regulate (positively or negatively) gene transcription.

7. Role of PPARγ in nutritional regulation of gene expression

In addition to regulating the expression of PPARγ RNA and protein, dietary fatty acids are also able to *activate* PPARs and promote diet-induced changes in gene expression in metabolically important tissues. Many fatty acids activate the various PPAR isoforms (see review [76]), and PPAR isoforms are implicated in mediating the anti-cancer effects of diverse fatty acids such as conjugated linoleic acids (CLAs).

CLA is a group of geometric and positional isomers of linoleic acid found in ruminant products [77]. Over the past few years, CLA has been credited with anti-cancer, anti-obesity, anti-atherogenic, and anti-diabetic properties [78]. CLA is a potent activator of PPARα [79]; CLA-activation of PPARα has been proposed as the mechanism underlying the anti-cancer effects reported in mammary, colon, skin and stomach tumors [76]. Evidence for the anti-diabetic effects of dietary CLA and the link to PPARγ was first provided by Houseknecht *et al.* [80]. A mixture of CLA isomers fed to prediabetic ZDF rats for 14-day normalized impaired glucose tolerance, and reduced serum triglyceride and insulin concentrations, similar to effects seen with troglitazone treatment [80]. Furthermore, CLA treatment of ZDF rats *in vivo* induced expression of the PPARγ-responsive *aP2* gene in adipose tissue [80], and the same mixture of CLA isomers was able to transactivate PPARγ response elements *in vitro* [80].

More recently, we have expanded this work to examine the effects of specific isoforms of CLA to regulate whole-body insulin action and prevent the development of hyperglycemia in the ZDF rat [81]. Dietary consumption of the t10c12 but not the c9t11 isomer of CLA prevented the development of hyperglycemia, nearly normalized impaired glucose tolerance, and significantly increased insulin-stimulated glucose uptake in isolated soleus muscles in ZDF rats [81]. Additionally, consumption of either CLA isomer rescued *UCP2* gene expression in skeletal muscle and adipose tissue of ZDF rats compared to controls [81]. All of these CLA effects on gene transcription are consistent with PPARγ activation. The c9t11 isomer has

been shown to preferentially bind PPAR α over PPAR γ [82] perhaps explaining the lack of efficacy of this isomer to prevent the onset of hyperglycemia. The relative potency of the t10c12 isomer for PPAR γ versus PPAR α has yet to be determined. Furthermore, additional studies are needed to determine the ability of specific CLA isomers to activate PPARs and to regulate insulin secretion in models of NIDDM.

As in rodents, dietary CLA has been shown to have anti-obesity and nutrient re-partitioning effects in swine. Dugan *et al.* [83] reported that supplementation of swine diets with 2% CLA caused a reduction in feed intake and adipose tissue mass with a corresponding increase in lean growth and feed efficiency. In agreement, Ostrowska *et al.* [84] reported that feeding swine CLA isomers resulted in increased lean body mass and reduced adiposity. In contrast, O'Quinn *et al.* [85] found no effect of modified tall oil (contains a mixture of CLA isomers) consumption on adiposity or growth performance in pigs. However, they found that modified tall oil increased saturation of fatty acids in adipose tissue and increased belly firmness resulting in improved carcass quality [85]. Differences among studies could be due, in part, to the different mixtures of CLA isomers fed. Presumably the effects of consumption of CLA isomers in swine are mediated via PPARs; further research is needed, testing specific isoforms and mixtures of CLA, to confirm this hypothesis.

8. Role of PPAR γ in the pathophysiology of diabetes, cancer and inflammation

PPAR γ and its ligands have now been implicated in the pathology and/or treatment of numerous diseases in man including obesity, diabetes, atherosclerosis and cancer. As several of these pathologies are also prevalent in companion animals, a review of the clinical and pharmaceutical literature follows.

9. PPAR γ ligands are insulin sensitizers

Non-insulin dependent diabetes mellitus (NIDDM), otherwise known as Type II diabetes, is characterized by defects in peripheral glucose uptake and utilization, hepatic glucose production and pancreatic β -cell dysfunction (see Fig. 6, [86]). Peripheral insulin resistance (skeletal muscle and adipose tissue) and hyperinsulinemia often precede the development of hyperglycemia and frank diabetes.

Pharmaceutical ligands for PPAR γ , including the TZD class of drugs, are potent insulin sensitizers that impact whole-body glucose utilization (see reviews [14,15,87]). Data from many laboratories have shown that various TZDs ameliorate insulin resistance in humans and animal models of NIDDM by increasing insulin action, including insulin-stimulated glucose uptake in skeletal muscle and adipose tissue [14,15,87]. Additionally, TZD treatment enhances insulin-stimulated inhibition of hepatic glucose output, reduces hypertriglyceridemia and plasma free fatty acids, and improves pancreatic β -cell function. There is strong correlation between the potency of TZD binding to PPAR γ and *in vivo* glucose lowering, and at least a portion of TZD effects is due to PPAR γ regulated induction of genes important in glucose and lipid metabolism [14,87].

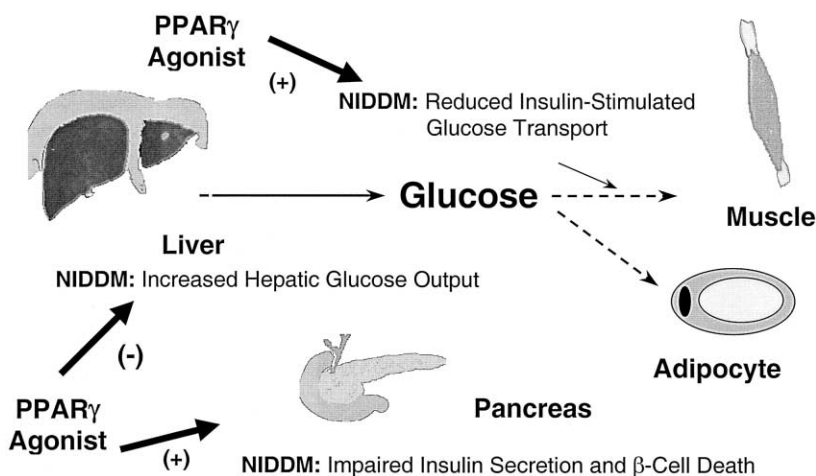


Fig. 6. Peroxisome proliferator-activated receptor gamma (PPAR γ) ligands improve insulin action defects in peripheral tissues of non-insulin dependent diabetes mellitus (NIDDM) patients. NIDDM patients and animal models of NIDDM are characterized by impaired insulin-stimulated glucose uptake into adipose tissue and skeletal muscle, increased hepatic glucose output in the presence of insulin, as well as impaired insulin secretion and loss of pancreatic β -cell population due to increased apoptosis. Treatment with PPAR γ agonists improved or normalized defects in each of these processes.

In addition to effects on gene expression related to glucose metabolism, the potent lipid-lowering effects of TZD could significantly impact insulin action as regulation of whole-body lipid metabolism can have profound effects on insulin-stimulated glucose transport and utilization by skeletal muscle [88]. Recently, Oakes *et al.* [89] reported that TZDs effect systemic fatty acid balance by lowering hepatic triglyceride production, elevating hepatic triglyceride clearance, enhancing insulin-stimulated suppression of fatty acid mobilization and increasing free fatty acid trafficking into adipocytes. Thus, TZDs, presumably via PPAR γ activation, have profound effects on lipid metabolism with potentially significant consequences on whole-body insulin action.

The precise mechanism(s) underlying all of the insulin-sensitizing effects of TZDs are not fully understood as PPAR γ expression is extremely low or absent in skeletal muscle, the primary site of whole-body glucose uptake and utilization. In contrast, PPAR γ expression is high in adipose tissue, a concept that has led to speculation that an adipocyte factor could mediate at least a portion of the TZD effects on whole-body insulin action. Recently Steppan *et al.* [90,91] reported the identification of an adipocyte secreted protein, resistin. Resistin expression is induced during adipocyte differentiation, circulates in high concentrations in obese mice; and impairs glucose tolerance and insulin action [90]. As adipocyte resistin expression is down regulated by several TZDs (rosiglitazone, pioglitazone and troglitazone; [90]), it is tempting to speculate that this is at least one mechanism by which PPAR γ activation improves whole-body insulin action.

10. PPAR gamma and beta cell function

An important component in the development of hyperglycemia and glucose intolerance in diabetic patients and animal models of NIDDM is reduced pancreatic beta cell function and/or mass. PPAR γ ligands may have therapeutic efficacy in preventing the loss of β -cell function as it has recently been reported that PPAR γ is expressed in normal human islet cells [92] and several studies conducted with ZDF rats report that treatment of prediabetic animals with thiazolidinediones prevented beta cell loss and development of hyperglycemia [93–96]. It has been hypothesized that at least one underlying mechanism is the TZD-induced prevention of lipid toxicity or β -cell apoptosis induced by pancreatic islet lipid accumulation [93–95].

11. Mutations in *hPPAR* γ gene impact insulin action

A commonly occurring mutation in the human *PPAR* γ 2 gene is the proline to alanine exchange in codon 12 (Pro12Ala). This mutation is associated with reduced transcriptional activity when assayed *in vitro* [97]. Furthermore, the Pro12Ala polymorphism in humans is associated with lower fasting insulin concentrations [97] improved insulin sensitivity [98,99], increased antilipolytic insulin sensitivity [100] and reduced risk of Type II diabetes [101,102]. Recently, Stefan *et al.* [103] provided evidence that Pro12Ala is associated with differential regulation of insulin secretion in response to free fatty acids in healthy human carriers.

In terms of PPAR γ regulation of adiposity, humans carrying the Pro12Ala polymorphism present significantly higher body mass index (BMI; [104,105]). Additionally the normal relationship between adiposity (BMI) and leptin gene expression is altered in Pro12Ala patients such that leptin levels are higher for a given BMI compared to non-carriers [106]. Interpretation of data from patients with this polymorphism is complicated by the recent finding of a gene–nutrient interaction at the PPAR γ locus [107]. Specifically, when the dietary ratio of polyunsaturated: saturated fatty acids is low, the BMI is higher in Pro12Ala patients than in the non-Ala carriers. When the ratio is high, the opposite result was observed with the Pro12Ala patients being leaner [107]. These data highlight the complexity of the biologic effects of PPAR γ activation and inactivation in the face of variation in dietary fat consumption (both level and composition of fatty acids).

The pivotal role that PPAR γ plays in the regulation of glucose homeostasis in humans was reinforced recently with the discovery of dominant negative mutations in the human *PPAR* γ gene that were associated with severe insulin resistance [108]. Interestingly, in spite of the profound insulin resistance, diabetes and hypertension, these patients displayed no abnormality in adiposity, suggesting the insulin sensitizing effects of PPAR γ are distinct from the regulation of adipogenesis [108].

12. PPAR γ in inflammation

PPAR γ has been implicated in the regulation of multiple inflammatory processes (see reviews [14,15,109,110]). Furthermore, PPAR γ ligands have been proposed as possible

therapeutics for inflammatory disease processes including inflammatory bowel disease and arthritis.

PPAR γ is expressed throughout the immune system in rodents, humans and pigs [3,62, 111,112] and PPAR γ expression is induced during monocyte to macrophage differentiation *in vitro* [110,113]. Multiple studies have now reported that PPAR γ ligands (at high μ M concentrations) including 15d-PGJ₂, TZDs and NSAIDs are able to stimulate differentiation of monocytes to macrophages and inhibit the induction of inducible nitric oxide synthase (iNOS), metalloproteinase-9 (MMP-9) and scavenger receptor A transcription [27]. Additionally, several studies have reported that PPAR γ ligands, especially 15d-PGJ₂, inhibit cytokine (TNF- α , IL-1 β and IL6) production *in vitro* (see Fig. 7, [15,109,110]).

The clinical relevance of PPAR γ regulation of inflammatory response has been highlighted by the findings that PPAR γ expression is induced during monocyte/macrophage differentiation involved in atherosclerotic foam cell formation and PPAR γ expression is high in murine and human atherosclerotic plaques [15,110]. In the case of foam cells, oxidized LDL (OxLDL) promotes foam cell formation and macrophage gene expression via activation of PPAR γ with subsequent induction of the OxLDL receptor, CD36, forming a positive feedback loop for foam cell formation (see Fig. 7, [113]). Thus, the pro-foam cell function of PPAR γ ligands must be viewed as *pro-atherogenic*.

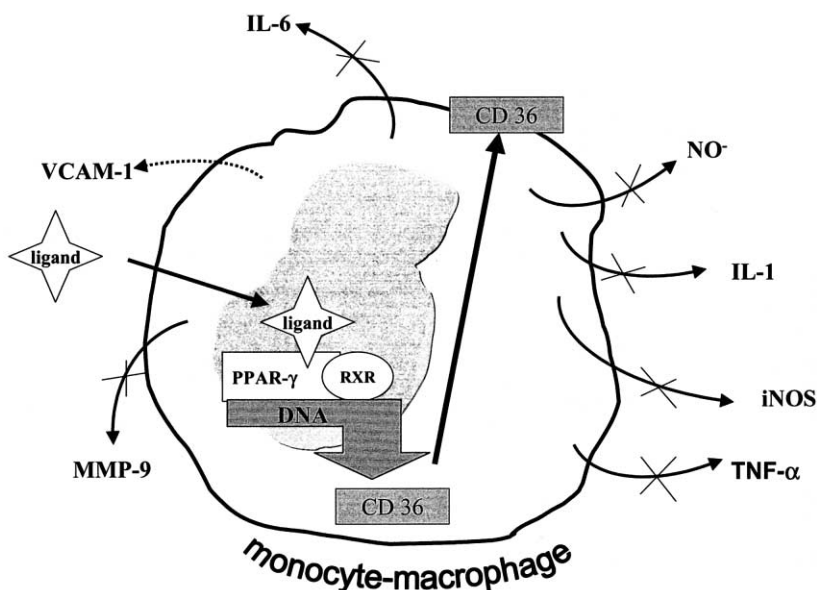


Fig. 7. Peroxisome proliferator-activated receptor gamma (PPAR γ) regulates immune cell function. This cartoon represents a monocyte-derived macrophage response to activation by a PPAR γ ligand. Ligand activation of PPAR γ triggers transcriptional induction of cell surface receptor CD 36 and inhibits production of various cytokines (Interleukin 1, IL-1; Interleukin 6, IL-6; tumor necrosis factor-alpha, TNF- α) adhesion molecules (vascular cell adhesion molecule, VCAM-1), metalloproteinase-9 (MMP-9), inducible nitric oxide synthase (iNOS) and nitric oxide (NO⁻).

In contrast to the negative, pro-atherogenic effects on foam cell formation, PPAR γ ligands appear to have positive effects on the vascular cell wall. There, TZD function to interfere with chemo-attraction and cell adhesion of various inflammatory cell types including monocytes and T-lymphocytes, primarily by inhibiting the expression of monocyte-chemoattractant protein-1 (MCP-1), VCAM-1 (vascular cell adhesion molecule-1) and intracellular adhesion molecule (ICAM; see reviews [15,110]). Obviously, more research is needed to determine the net impact of PPAR γ ligands on the pathology of atherogenesis, although encouraging clinical data showing troglitazone inhibits coronary arterial wall thickness [114] tends to support a therapeutic role for PPAR γ ligands in the prevention/treatment of atherosclerosis.

In addition to inflammation associated with atherosclerotic plaque formation, recent data support a potential therapeutic role for PPAR γ ligands in the treatment/prevention of inflammatory bowel disease. Suh *et al.* [115] reported that PPAR γ ligands inhibited epithelial inflammatory response in animal models of colitis. At least one of the proposed mechanisms includes inhibition of IL-1 β -induced activation of IL-8, presumably via inhibition of NF- κ B pathways [15].

Recently, the anti-inflammatory properties of PPAR γ ligands were tested for efficacy in ameliorating the cytokine-induced damage to cartilage associated with arthritis. Specifically, Bordji *et al.* [116] reported that PPAR γ and PPAR α RNA and protein are expressed in rat cartilage and that the PPAR γ ligands, 15d-PGJ₂ and troglitazone, were able to significantly ameliorate the IL-1 β induced reduction in proteoglycan synthesis and nitric oxide

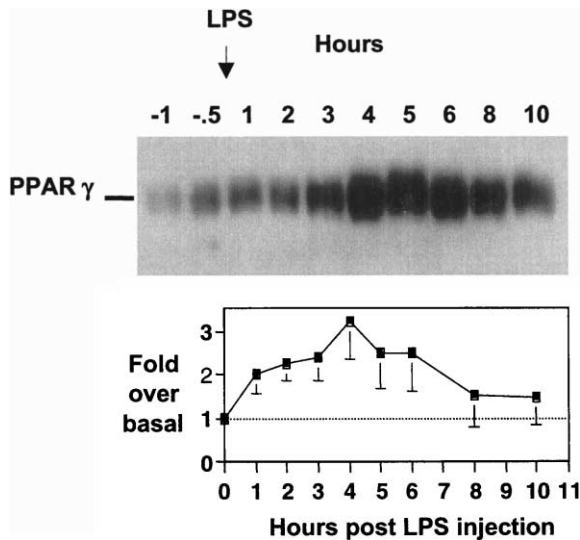


Fig. 8. Peroxisome proliferator-activated receptor gamma 1 (PPAR γ 1) expression in porcine peripheral white blood cells (WBC) is temporally regulated following *in vivo* LPS challenge. *Panel A* depicts a representative Western blot analysis of PPAR γ protein expression in porcine WBC following *in vivo* lipopolysaccharide challenge, while *Panel B* quantifies the dynamic increase in PPAR γ expression in WBC from castrate male pigs (n = 4). The symbol (*) indicates means for each time point are different (P < 0.05) from pre-LPS treatment concentrations. Reprinted with permission from Leininger *et al.* [112].

production [116]. In contrast, PPAR α agonists had no effect [116]. These authors speculate that PPAR γ ligands could have chondroprotective effects in patients suffering from rheumatoid or osteoarthritis.

To date, most of the data describing anti-inflammatory effects of PPAR γ ligands have been generated from *in vitro* studies and have focused on inflammatory responses related to atherogenesis. We published the first evidence that suggested a role for PPAR γ in the regulation of inflammatory responses associated with acute, systemic endotoxemia *in vivo* using the pig as a model (see Fig. 8, [112]). We found that PPAR γ protein is expressed in peripheral white blood cells of pigs, with the PPAR γ 1 RNA isoform predominating compared to PPAR γ 2 [112]. Furthermore, following acute *in vivo* LPS challenge, PPAR γ 1 expression significantly and transiently increased in porcine peripheral white blood cells, coinciding (or closely following) LPS-induced changes in plasma TNF- α , cortisol, insulin, glucose and free fatty acids [112]. These data suggest that PPAR γ and its ligands may play a role in the whole-body response to acute endotoxemia in the pig.

13. PPAR γ and cancer

The ability of PPAR γ to regulate cell differentiation and proliferation has inspired a number of researchers to explore the use of PPAR γ agonists as chemotherapeutic agents [117–119]. PPAR γ is highly expressed in human lipocarcinomas [120] and various other human tumors including breast [121,122], lung [123,124], colon [125,126], prostate [127], bladder [128] and gastric [129]. Furthermore, prostaglandin 15d-PGJ₂ and/or troglitazone induce apoptosis and growth inhibition of human breast [121,122], lung [123,124], colon [125,126], prostate [127], bladder [128], gastric [129] and thyroid [29] carcinoma cells *in vitro*.

In support of the *in vitro* data, there are now many reported examples of tumor growth suppression/arrest in tumor-bearing rodent models treated with PPAR γ agonist therapies (see reviews [117–119]). For example, troglitazone (500 mg/kg per day) treatment of nude mice implanted with papillary thyroid tumors reduced tumor growth and prevented distant metastasis [29]. Both estrogen receptor positive (MCF-7) and negative (MDA-MB-231) breast cancer cell lines undergo cell cycle arrest when treated with 15d-PGJ₂ or troglitazone [121,122] and similar effects are observed in rodent breast cancer *in vivo* models. Clay *et al.* [121] reported that 15d-PGJ₂ induces an irreversible apoptosis in an MDA-MB-231 nude mouse model and troglitazone inhibits tumor growth in BNX trile-immunodeficient mice [122].

Conflicting reports on the efficacy of PPAR γ activators for the treatment of colon cancer have been reported. Two independent groups have reported that treatment of APCmin⁺/mice with PPAR γ activators for 5–8 weeks results in an increase of tumors or polyps in the colon [130,131]. However, Sarraf *et al.* [125,126] have published experiments demonstrating that PPAR γ agonists induce apoptosis and inhibit growth of colon tumors *in vitro* and in nude mouse models. One possibility for the discrepancy observed between models is that APCmin⁺/mice are genetically predisposed to colon cancer.

Probably one of the most exciting experiments run to date is the Phase 2 clinical trial examining the efficacy of troglitazone treatment of advanced liposarcoma [132]. Patients were

administered troglitazone once daily (800 mg) for 6 weeks and biopsies showed a significant accumulation of lipid by the tumor cells compared to pretreatment suggesting drug-induced differentiation into adipocyte-like cells. In addition, levels of the biologic marker for cell proliferation, Ki-67, were decreased. These data suggest that troglitazone induced terminal adipocyte differentiation in the tumors.

These early experiments show potential for the treatment of various cancers with PPAR γ ligands. Additional research is needed to determine if *in vitro* and rodent data translate to efficacy in human and veterinary cancer patients; nevertheless, PPAR γ activators show promise for future cancer therapeutics.

14. Summary

PPAR γ is a nuclear receptor that plays a pivotal role in the regulation of gene transcription and cellular differentiation. Furthermore, it appears to link lipid and glucose metabolism and is being pursued as a therapeutic target for diverse pathophysiological states in humans including diabetes, cancer and inflammation. Although a considerable amount is known about the regulation of PPAR γ action in rodents and humans, there is a paucity of data examining PPAR γ expression and function in domestic species, including livestock and companion animals. Additional research is warranted to determine the role(s) the PPAR γ nuclear receptor plays in the biology of domestic species.

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