REVIEW

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Structure and physiological functions of the human peroxisome proliferator-activated receptor γ

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Abstract

The peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptor superfamily. To date, three different PPAR isotypes, namely PPAR-α, -δ, and -γ, have been identified in vertebrates and have distinct patterns of tissue distribution. Like all nuclear receptors, the human PPAR-γ (hPPAR-γ) is characterized by a modular structure composed of an N-terminal A/B domain, a DNA-binding domain with two zinc fingers (C domain), a D domain, and a C-terminal ligand-binding domain (E/F domain). Human PPAR-γ exists in two protein isoforms, hPPAR-γ1 and -γ2, with different lengths of the N-terminal. The hPPAR-γ2 isoform is predominantly expressed in adipose tissue, whereas hPPAR-γ1 is relatively widely expressed. Human PPAR-γ plays a critical physiological role as a central transcriptional regulator of both adipogenic and lipogenic programs. Its transcriptional activity is induced by the binding of endogenous and synthetic lipophilic ligands, which has led to the determination of many roles for PPAR-γ in pathological states such as type 2 diabetes, atherosclerosis, inflammation, and cancer. Of the synthetic ligands, the thiazolidinedione class of insulin-sensitizing drugs (ciglitazone, pioglitazone, troglitazone, rosiglitazone) is employed clinically in patients with type 2 diabetes.

Key words: PPAR-γ, adipogenesis, insulin resistance, thiazolidinediones.

Abbreviations: ADD-1/SREBP-1 – adipocyte determination and differentiation factor 1/sterol response element binding protein 1, aP2 – adipocyte fatty acid binding protein, BMI – body mass index, CAP – c-Cbl associating protein, C/EBP – CCAAT/enhancer binding protein, CBP/p300 – CREB (cAMP response element binding protein) binding protein, 15d-PGJ2 – 15-deoxy-$\Delta^{12,14}$-prostoglandin J2, DR-1 – direct repeat 1, GLUT4 – insulin-responsive glucose transporter, HAT – histone acetyl transferase, 15-HETE – 15-hydroxyeicosatetraenoic acid, 9- and 13-HODE – 9- and 13-hydroxyoctadecadienoic acid, IL-6 – interleukin 6, LBD – ligand-binding domain, MAPK – mitogen-activated protein kinase, NR – nuclear receptor, PEPCK – phosphoenolpyruvate carboxykinase, PPAR – peroxisome proliferator-activated receptor, PGC-1α – PPAR-γ coactivator-1α, PPRE – peroxisome proliferator response element, RXRα – retinoid X receptor-α, SRC1 – steroid receptor coactivator, TNF-α – tumor necrosis factor-α, TG – triglyceride, TZD – thiazolidinedione, WAT – white adipose tissue.

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INTRODUCTION

In recent years it has become evident that the societies of the developed countries are at immense risk of metabolic diseases, the so-called civilization diseases or X syndrome. In fact, the rise in the prevalence of specific endocrine-related diseases such as obesity and diabetes clearly suggests an importance of either environmental or genetic factors. The therapy of metabolic diseases assumes the recognition and detailed understanding of the molecular events that control these disorders and the development of therapeutics targeting the factors responsible for them. Recently, several different transcriptional factors have been identified as regulators of the expression of a set of genes involved in glucose and lipid metabolism. Among them, peroxisome proliferator-activated receptors (PPARs), belonging to the superfamily of nuclear receptors (NRs), have been shown to play a central role in the transcriptional control of genes encoding proteins involved in the above processes. Three different human PPAR subtypes have been identified so far, designated as PPAR-α, PPAR-β (also known as PPAR-δ, or NUCI in humans, or FAAR in rodents), and PPAR-γ [29, 56]. Each of them displays

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a distinct pattern of tissue distribution and a specific role. PPAR-α is predominantly expressed in the liver and skeletal muscles, participating in fatty-acids catabolism. PPAR-α also activates fatty-acid oxidation in the kidney, skeletal muscles, and heart [9]. It has been established that PPAR-β is present at moderate levels in all human tissues, with a higher expression in the placenta and the large intestine [6, 87]. Very little is known about the functions of PPAR-β. However, recent findings have implicated PPAR-β as an important regulator of energy expenditure as well as glucose and lipid metabolism [28, 72, 118, 132]. Of the three members of PPARs, PPAR-γ is the most frequently studied NR involved in the control of energy balance and both lipid and glucose homeostasis [59, 74, 112]. This transcriptional factor is believed to be a pivotal regulator of adipogenesis since it is expressed primarily in adipose tissue [22, 121]. PPAR-γ forms a heterodimer complex with retinoid X receptor-α (RXRα), which then binds to peroxisome proliferator response elements (PPREs) within the promoters of PPAR-γ-targeted genes [54, 65, 66]. The PPAR-γ/RXRα heterodimer may be activated by various ligands. Compared with classical NRs such as estrogen receptors, PPAR-γ interacts with a wide spectrum of natural [35, 36, 64] and synthetic [46, 71] lipophilic ligands. The natural ligands include polyunsaturated fatty acids [35, 64], prostaglandin (PG)J2 derivatives [36, 63], and oxidized fatty acids [89]. Of the synthetic ligands, thiazolidinediones (TZDs) are compounds that exhibit high affinity to PPAR-γ and are utilized to increase insulin sensitivity in diabetic patients [71, 96, 112].

This review summarizes current knowledge on the structure and physiological functions of hPPAR-γ and its contribution to the pathogenesis of type 2 diabetes.

GENOMIC ORGANIZATION OF THE *PPARG* GENE

The *PPARG* gene was cloned from vertebrates, including *Xenopus sp.* [29], mouse [148], and humans [40]. Phylogenetic analysis revealed that its structure is well conserved between humans and mice (99% similarity and 95% identity) [30, 149]. In humans, the *PPARG* gene is located on chromosome 3 at position 3p25 [40], whereas in the mouse it is found on chromosome 6 at position E3-F1 [58]. The *PPARG* gene exceeds 100 kb of genomic DNA and it includes 9 exons: A1, A2, B, and six exons designated 1–6 (Fig. 1A). In humans, four different subtypes of PPAR-γ mRNA (-γ1, -γ2, -γ3, and -γ4) transcribed from four different promoters have been identified (Fig. 1A) [31, 30, 116]. In contrast to the findings in humans, only two subtypes of PPAR-γ mRNA in mice (-γ1 and -γ2) have been detected [149]. hPPAR-γ1 mRNA is encoded by eight exons (A1, A2, 1-6), whereas hPPAR-γ2 and -γ3 mRNA are encoded by seven (hPPAR-γ2 and -γ3 mRNA contain B, 1-6 and A2, 1-6, respectively). hPPAR-γ4 mRNA is encoded by the six exons 1-6 that are common to all four hPPAR-γ mRNAs (Fig. 1A). mRNA analysis by RT-competitive PCR assay revealed that the hPPAR-γ2 mRNA level is significantly lower than that of the hPPAR-γ1 subtype in all tissues analyzed [30]. Thus in human tissues with two coexisting subtypes of mRNA, PPAR-γ1 always predominates quantitatively [30, 31, 87]. It has been established that adipose tissue and the large intestine exhibit the highest levels of hPPAR-γ1 mRNA, whereas it is barely detectable in skeletal muscle [30]. In contrast to hPPAR-γ1 mRNA, which is present in a broad range of tissues, hPPAR-γ2 and -γ4 mRNA are restricted to adipose tissue [116]. hPPAR-γ3 mRNA is expressed in white adipose tissue (WAT), the large intestine, and macrophages [16, 31, 101].

It is noteworthy that the nucleotide sequences of the mPPAR-γ1 and -γ2 promoter regions contain consensus binding sequences of transcription factors, such as CCAAT/enhancer binding protein (C/EBP), hepatocyte nuclear factors 5 and 3 (HNF5 and HNF3), and adipocyte determination and differentiation factor 1/sterol response element binding protein 1 (ADD-1/SREBP-1) [31, 149]. Moreover, in contrast to the mPPAR-γ2 promoter, the mPPAR-γ1 promoter contains a large block (of approximately 550 bp) of a (C+G)-rich sequence. It has been suggested that these CpG islands within the mPPAR-γ1 promoter may be methylated, resulting in the inhibition of expression of the associated genes [149].

So far, very little is known about the regulation of PPAR-γ gene expression. There are several reports describing the involvement of ADD-1/SREBP-1, members of the C/EBP family of transcription factors, and a retinoic acid receptor-related orphan receptor (RORα, also termed RZR) in this process [61, 104, 116, 138].

STRUCTURE OF THE PPAR-γ PROTEIN

Despite four distinct mRNA subtypes, the PPAR-γ exists in two protein isoforms (designated hPPAR-γ1 and -γ2) resulting from alternative promoter usage and alternative splicing at the 5' end of the gene. hPPAR-γ1, -γ3, and -γ4 mRNA give rise to an identical protein product, hPPAR-γ1 (477 aa), whereas hPPAR-γ2 mRNA encodes the hPPAR-γ2 protein with 30 extra N-terminal amino acids (505 aa) encoded by the B exon (Fig. 1A).

Like all NRs, PPARs share a similar modular structure with functionally distinct domains called A/B (ligand-independent activation domain), C (DNA binding domain), D (hinge domain), and E/F (ligand-binding domain – LBD) [93] (Fig. 1B). The N-terminal domain A/B has been relatively well conserved through evolution. The chief fragment of this domain is an α-helix fragment possessing a ligand-independent activating function (AF-1) [134]. It has been shown that the hPPAR-γ2 isoform exhibits tenfold higher activity than

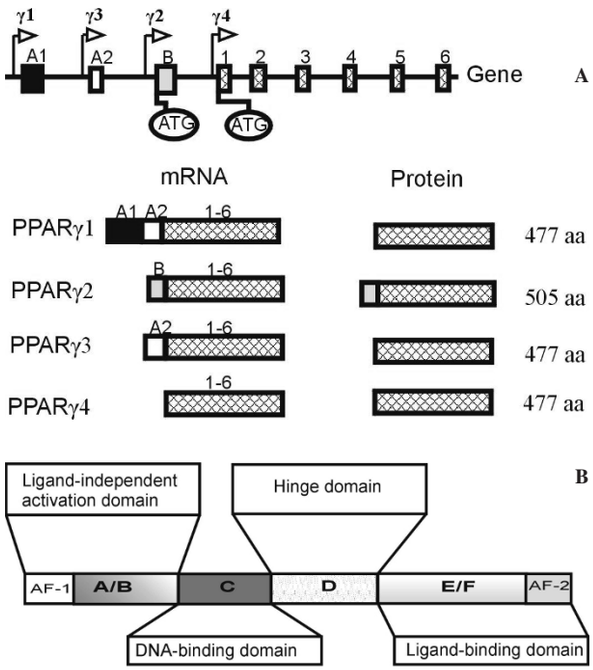

Fig. 1. Scheme of the genomic structure of the human *PPARG* gene and the structures of the two PPAR-γ protein isoforms (**A**). The *PPARG* gene is shown in 5' to 3' orientation. The location of the ATG start-codon is indicated. γ1, γ2, γ3, and γ4 represent the promoters of hPPAR-γ1, -γ2 -γ3, and -γ4 mRNA, respectively. The four subtypes of mRNA give rise to two different PPAR-γ proteins. Transcription on the promoters γ1, γ3, and γ4 results in the same protein of 477 amino acids. The PPAR-γ2 protein of 505 amino acids is produced by transcription from the promoter γ2. (**B**) Domain structure of PPARs. PPARs contain the following functional regions: an N-terminal A/B domain with AF-1 (ligand--independent activation domain), a C domain with two zinc fingers (DNA-binding domain), a D domain (hinge domain), and a C-terminal E/F domain containing AF-2 (ligand-binding domain).

hPPAR-γ1 in the presence of insulin in ligand-independent transcriptional activation [134]. This data may imply different functions of the two isoforms that differ only in their N-termini. So far, the role of the additional 30 amino-acid residues at the N-terminus of the hPPAR-γ2 protein has not been explained. However, there are some hypotheses about this fragment concerning its effect on an increase in receptor activity [7]. Furthermore, in the A/B domain is Ser-112 (also referred to as Ser-114 in the literature), which is subject to phosphorylation [108]. Since intramolecular communication between the A/B and the LBD domains is required for ligand binding, the phosphorylation at Ser--112 by mitogen-activated protein kinase (MAPK) disrupts interdomain communication, resulting in a reduction in ligand-binding affinity [108]. Moreover, the MAPK-dependent phosphorylation of this residue in the hPPAR-γ2 protein inhibits its ability to promote specific gene expression and the process of adipogenesis [52].

The C domain is the most conserved of all the functional domains [93]. It contains two zinc finger-like motifs that are responsible for binding of the receptor to the DNA promoter of target genes. Each of the zinc fingers is encoded by a separate exon (2 and 3). Besides the zinc fingers there are amino-acid motives determining the recognition of an appropriate DNA sequence which binds the receptor. Additionally, a large part of the domain takes part in dimerization with another NR, RXR. The less conserved domain D is treated rather as a flexible hinge between the C and E/F domains [93]. This site is also a docking domain for cofactors. In addition, it contains the nuclear localization signal, a sequence recognized by transporting proteins, which determines relocation of the synthesized protein from the cytoplasm to the cell nucleus. Moreover, some of the amino acids are involved in the activities of both nearby domains, leading to the dimerization and recognition of the target DNA sequence. The largest domain is the LBD (E/F domain) located at the C-terminus [90], and responsible for the binding of a specific ligand and activation of PPAR binding to PPREs in the target gene promoter. The LBD contains a fragment called ligand--dependent activation function 2 (AF-2), engaged in the recruitment of PPAR cofactors to assist the gene transcription processes [15].

The crystallographic structure of the LBD of PPAR-γ revealed numerous similarities to other NR structures [90, 126]. It has been determined that the unliganded LBD possesses a small β-sheet of four strands and 12 highly conserved α-helices that are folded into three layers to create a central hydrophobic ligand-binding pocket, almost entirely buried within the core of these α-helices [90, 126]. In addition to the structure of a typical NR, the PPAR-γ LBD contains one additional α-helix between the β-strand and helix 3. The conformation of helix 12 is critical for transcriptional activation since significant differences have been observed in the location of this helix for agonist and antagonist-bound forms of the estrogen receptor [18]. A "mouse trap" model of receptor activation has therefore been proposed. According to this model, helix 12, containing the conserved AF-2 core, closes the ligand binding site in response to a ligand, resulting in a transcriptionally active form of the receptor [99].

The recently solved X-ray structure of the PPAR--γ/RXRα LBD heterodimer as a complex with GW409544 and two LXXLL (L, leucine; X, any amino acid) peptides from steroid receptor coactivator (SRC1; Fig. 2A) revealed that the acidic head group of the ligand GW409544 forms hydrogen bonds with either His--323 (helix 5) or Tyr-473 (the AF2 helix) within PPAR-γ (Figs. 2B and C) [142]. In addition, comparison of the structures of GW409544 bound to PPAR-α and PPAR-γ, along with mutational analysis of their LBDs, resulted in the identification of the single amino acids which determine ligand selectivity. These are Tyr-314 in PPAR-α and His-323 in PPAR-γ, which constitute part of the network of hydrogen-binding residues involved in the activation of PPAR through its acidic ligands [142]. It is noteworthy that the identification of a major determinant of selectivity between PPAR-α and PPAR-γ has provided the opportunity to design new diabetes drugs.

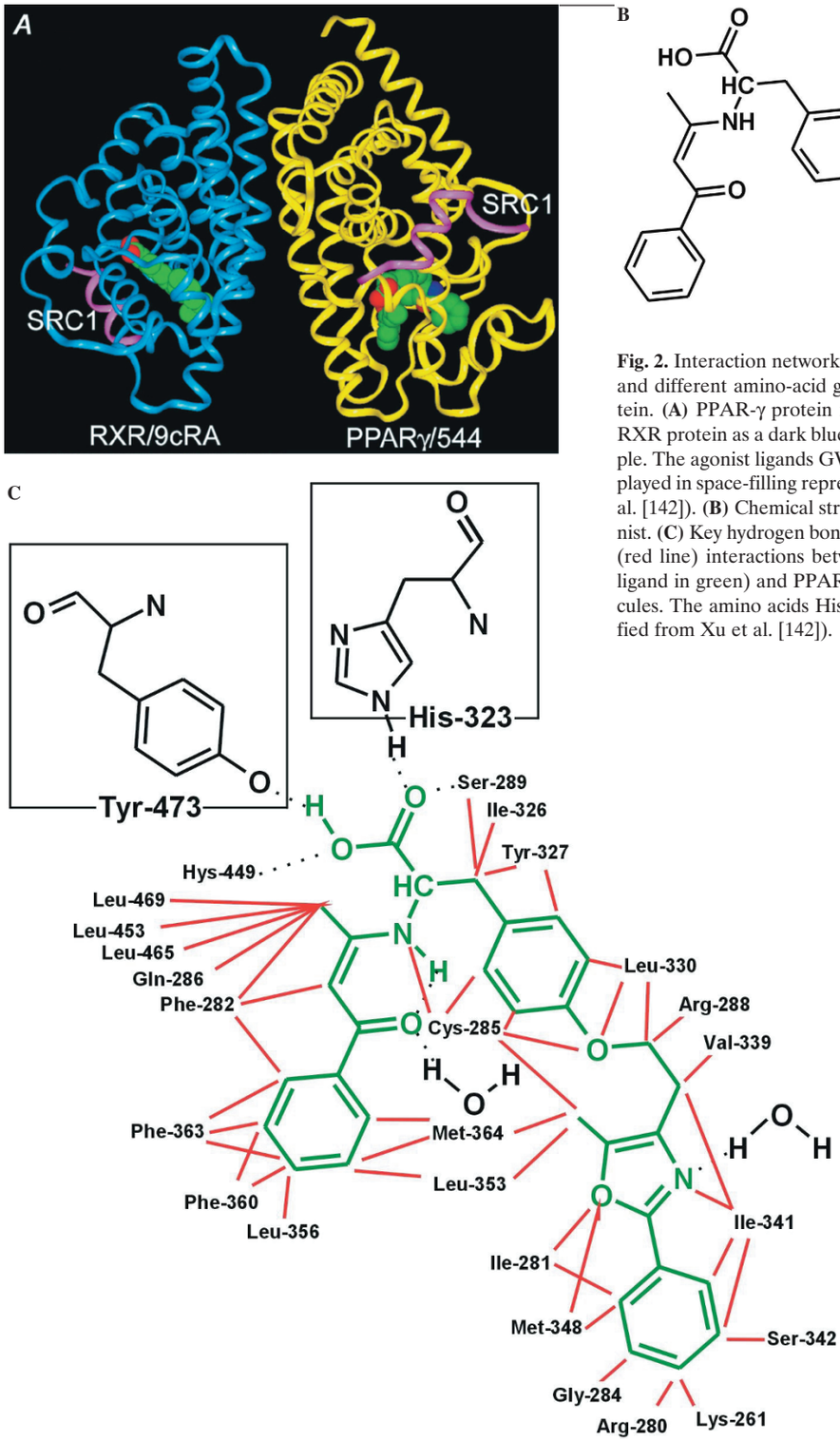

Fig. 2. Interaction network between the agonist ligand GW409544 and different amino-acid groups in the LBD of the PPAR-γ protein. **(A)** PPAR-γ protein is displayed as a yellow worm and the RXR protein as a dark blue worm with the LxxLL peptides in purple. The agonist ligands GW409544 and 9-*cis*-retinoic acid are displayed in space-filling representations colored by atom type (Xu et al. [142]). **(B)** Chemical structure of GW409544, the PPAR-γ agonist. **(C)** Key hydrogen bonds (black broken line) and hydrophobic (red line) interactions between GW409544 (the structure of the ligand in green) and PPAR-γ protein and two bound water molecules. The amino acids His-323 and Tyr-473 are indicated (modified from Xu et al. [142]).

Over the past several years, various natural and synthetic PPAR-γ ligands, including PPAR-γ agonists [71], PPAR-γ partial agonists [75], and PPAR-α/γ dual agonists [142], have been investigated. Among the known endogenous PPAR-γ agonists are polyunsaturated fatty acids (linoleic and arachidonic acids) [35, 64], PG-related compounds derived from nutrition or the metabolic pathway (15d-PGJ2 and delta12-PGJ2) [36, 63], and oxidized fatty acids (9- and 13-HODE, 15-HETE) [89] (Fig. 3). Since most of these natural compounds bind and activate PPAR-γ at micromolar concentrations [36, 63], it is not clear whether their relevant concentrations

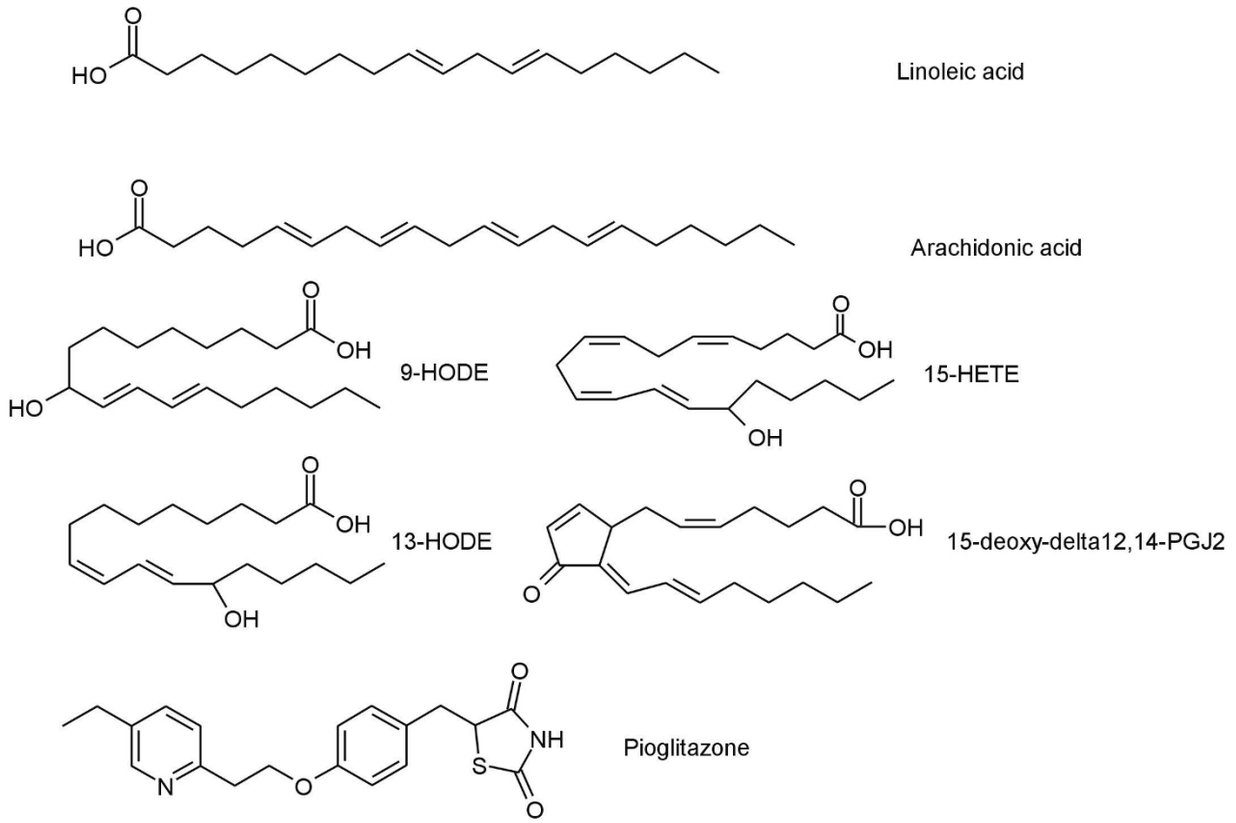

Fig. 3. Chemical structures of selected representatives of natural (linoleic, arachidonic acids and the PG-related compounds) and synthetic (pioglitazone – the TZD antidiabetic drug) ligands for PPAR-γ protein.

in the nuclei of target cells are sufficient for receptor activation. Recent studies on biologically relevant endogenous adipogenic PPAR-γ ligands have shown the existence of novel hydrophobic ligands specific to PPAR-γ, transiently generated in 3T3-L1 cells in response to increased cAMP during an early phase of adipogenesis [125]. These new data imply that the production of such ligand (not yet identified) is an important process affecting the adipogenic program.

The synthetic PPAR-γ agonists are thought to be factors determining adipocyte differentiation as well as potential antidiabetic drugs [70, 71, 122]. Compounds such as TZDs are used clinically as insulin sensitizers [62]. They activate PPAR-γ and decrease insulin resistance and glucose level in the serum of patients with type 2 diabetes [62]. Many drugs belonging to the TZD class exhibit high selectivity for PPAR-γ and minimal or no activity toward other subtypes-α and -β [136]. For example, troglitazone (Rezulin) is a very specific high--affinity PPAR-γ ligand with a K_d of 30–700 nM [71]. However, despite significant antidiabetic activities, TZDs cause several side effects, such as increased adiposity, oedema, and cardiac hypertrophy. From the therapeutic point of view, improvement of the pharmacological profiles of PPAR-γ ligands is highly required. Therefore, an alternative approach, relying on the identification of a partial agonist, was developed. It was recently reported that a PPAR-γ partial agonist similar to LSN862, i.e. (S)-2 methoxy-3-{4-[5-(4-phenoxy)pent--1-ynyl]phenyl}-propionic acid, has better antidiabetic activity and weaker side effects than the TZDs [98]. More recently, a novel family of PPAR-γ partial agonists (pyrazol-5-yl benzenesulfonamide derivatives) with either high potency or specificity *in vitro* or glucose-lowering efficacy *in vivo* has been identified [75]. Interestingly, the X-ray structures of the PPAR-γ-ligand complexes revealed a lack of hydrogen bonds between them. This is in sharp contrast to PPAR-γ agonists sharing a common binding mode in which the acidic head groups form a network of hydrogen interactions with His-323, His-449, and Tyr-473 within the ligand binding pocket [142]. Further molecular studies are required to understand how PPAR-γ partial agonists modulate transcriptional activity through the recruitment of coactivator and corepressor proteins.

THE GENE TRANSCRIPTION MECHANISM

The gene transcription mechanism is similar to that of all NRs, as shown in Fig. 4 [93]. In general, the C--terminal A/F domain of the receptor heterodimerizes with RXRα and the PPAR/RXRα complex binds to the PPRE sequence of the target gene promoters [54, 65,

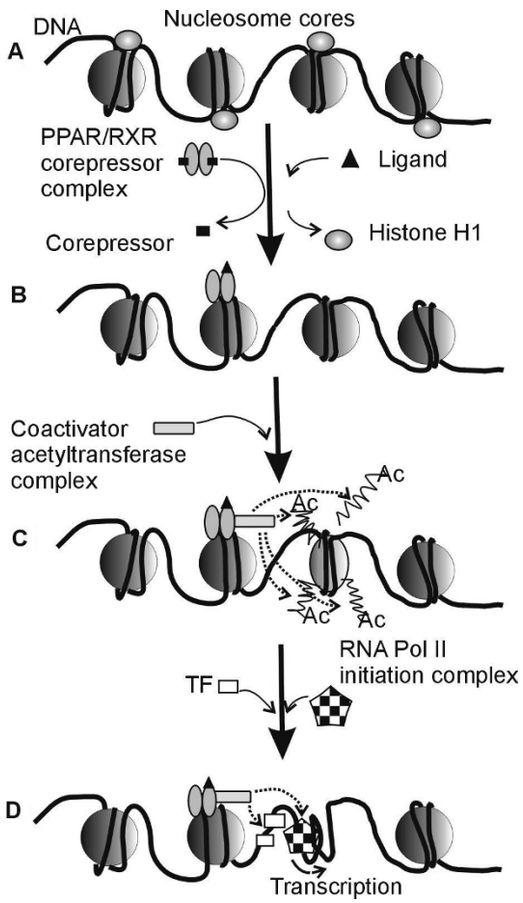

Fig. 4. Transcriptional activity of PPARs. **(A)** The promoter region with a PPRE, the TATA box, and the transcription start site is located in a repressive chromatin structure. The binding of ligand to the PPAR/RXR/corepressor complex causes the release of the corepressor from the ligand-activated PPAR/RXR complex. **(B)** The activated PPAR/RXR complex binds to the PPRE, inducing structural change in chromatin, with histone H1 released. The PPRE-bound PPAR/RXR targets a coactivator-acetyltransferase complex to the promoter. **(C)** The coactivator-acetyltransferase complex acetylates the histone tails (Ac), thereby generating a transcriptionally active structure. **(D)** Additional transcription factors (TF) and the RNA Pol II initiation complex are recruited to the accessible promoter and transcription is initiated.

66]. The PPRE motif contains a characteristic 13--nucleotide sequence, AGGTCA N AGGTCA (N – any nucleotide) that is composed of two hexanucleotides separated by one nucleotide (this type of sequences is called DR-1). The direct repeat 1 (DR-1) sites are specifically and almost exclusively recognized by the PPAR-γ/RXRα heterodimer. Each component of the heterodimer complex binds to one hexamer of the DR-1 motif. The unactivated PPAR-γ is associated with a corepressor that silences its transcriptional activity by the recruitment of histone deacetylases, such as the NR corepressor, the silencing mediator of retinoid and thyroid receptors (repression step). The binding of exo- or endogenous ligand to the receptor stimulates the release of the corepressor and the recruitment of a coactivator, which contains a protein with histone acetyl transferase (HAT) activity such as CREB (cAMP response element binding protein) binding protein (CBP/p300) or SRC1 (derepression step). The action of acetyltransferases leads to chromatin decondensation. In the following step, the HAT complex dissociates and either transcription factors or the RNA polymerase II initiation complex are recruited to the accessible promoter, resulting in transcription activation of the target gene (transcription activation step). Interactions of PPAR-γ with a coactivator or corepressor thus regulate its transcriptional activity by affecting the chromatin structure through the acetylation or deacetylation of histones, respectively. It should be mentioned that both coactivators and corepressors are highly versatile and are not selective for particular PPAR subtypes. It has been observed, for example, that all three members of the PPAR family are subjected to transcriptional coactivation by the transcription coactivator PPAR-γ coactivator-1α (PGC-1α), which is highly expressed in tissues with high mitochondrial metabolism, such as brown adipose tissue, heart, and skeletal muscle [41, 94; 128, 131]. Studies on the functional determination of the role of the coactivator PGC-1α in PPAR-γ activation in brown fat during cold exposure have shown that PGC-1α coactivates the PPAR-γ/RXRα heterodimer to induce mitochondrial genes that contribute to the process of adaptive thermogenesis [94]. It is noteworthy that PGC-1α binds to the hinge domain of PPAR-γ in a ligand-independent manner [94], whereas most coactivators utilize the LXXLL helical motifs to interact with the LBD of NRs [44, 79]. In addition, PGC-1α does not possess intrinsic HAT activity and it therefore recruits proteins with HAT activity, such as SRC1 and CBP/p300.

Besides the two distinct pathways for PPAR-γ activation (ligand-dependent and -independent), the activity of this transcription factor may also be modulated by posttranscriptional modification, such as phosphorylation. As mentioned previously, phosphorylation at Ser--112 (the N-terminal domain A/B) through the activated MAPK leads to the inhibition of PPAR-γ activity and adipocyte differentiation [1, 20, 52]. On the other hand, it is known that extracellular signals, for example growth factor/insulin, activate MAPK in cells. Taken together, MAPK activation through growth factor/insulin results in phosphorylation at Ser-112, causing inhibition of PPAR-γ transcriptional activity and, consequently, a decrease in body mass index (BMI), as shown in Fig. 5. The molecular mechanism of this process remains to be determined; however, these results create a link between cell membrane signaling and nuclear effectors.

THE ROLE OF PPAR-γ IN ADIPOGENESIS

Adipogenesis refers to the differentiation process of pre-adipocyte precursor cells into mature adipocytes

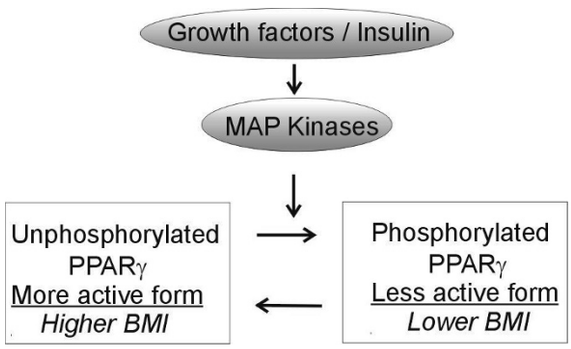

Fig. 5. Alternative pathway for PPAR-γ regulation. MAPK activation through extracellular signals (growth factors/insulin) phosphorylates at Ser-112 in the terminal domain A/B, resulting in a decrease in either PPAR-γ activity or adipocyte differentiation (lower BMI).

during which gene expression, cell morphology, and hormone sensitivity change. Pre-adipocytes can be differentiated into white (energy storage) and brown (energy dissipation) adipocytes. In the differentiation of white adipocytes, the expressions of numerous genes encoding proteins participating in fatty-acid metabolism is induced. It is known that the transcription factor PPAR-γ is an important regulator of the formation of adipose tissue [22, 121] since it induces several specific adipose markers, such as adipocyte fatty acid binding protein (aP2) [120], phosphoenolpyruvate carboxykinase (PEPCK) [119], and lipoprotein lipase (LPL) [107], and, moreover, the ectopic expression of PPAR-γ promotes adipogenesis in nonadipogenic fibroblastic cells such as NIH-3T3 cells [121]. In addition, PPAR-γ--deficient adipocytes of adult mice die within a few days [55] and PPAR-γ knockout mice are unable to develop adipose tissue [8, 103]. Consistent with the above, several PPAR-γ missense mutations (C190S, V290M, F388L, R425C, P467L) in humans are associated with partial lipodystrophy [2, 10, 45, 76, 106]. Although all these studies indicate a pivotal role of PPAR-γ in adipogenesis, it is likely one of several proteins involved in the regulation of this multifactoral process. Indeed, besides PPAR-γ, C/EBP transcription factors (C/EBP-α, -β, and -δ) expressed in distinct phases of adipogenesis have been shown to play important roles. C/EBP-β and -δ are activated in response to insulin or glucocorticoids in the initial stages of adipogenesis [137, 1139] and they, in turn, induce the transcription of PPAR-γ (Fig. 6). In addition, during the early stages of differentiation, another transcriptional factor, namely ADD1/SREBP1, has been found to affect the transcriptional activity of PPAR-γ [61] (Fig. 6). It has been suggested that this factor can modulate PPAR-γ activity through the production of endogenous ligands for PPAR-γ since it participates in the regulation of cholesterol homeostasis and in the expressions of several genes encoding proteins involved in lipid metabolism [32]. In the terminal stages of adipogenesis, PPAR-γ activates the expression of C/EBP-α; however, C/EBP-α, in response, also induces PPAR-γ gene expression through binding to the same DNA sites in the PPAR-γ promoter that are induced by C/EBP-β, and -δ [139]. Thus there is a positive feedback loop between PPAR-γ and C/EBP-α [138] (Fig. 6). The positive cross-regulation between these factors has been observed in C/EBP-α-deficient adipocytes, which accumulate fewer lipids and do not induce endogenous PPAR-γ [138]. In addition to the above transcription factors activating adipogenesis, there are secreted several factors involved in the control of this process, such as tumor necrosis factor (TNF)-α and leptin. TNF-α is a polypeptide hormone with pleiotropic effects on cellular proliferation and differentiation and is a potent inhibitor of adipogenesis. The exposure of 3T3-L1 adipocytes to TNF-α results in lipid depletion and a complete reversal of adipocyte differentiation [124]. In addition, suppression of several adipocyte genes, such as those encoding aP2, adipsin, and insulin-responsive glucose transporter (GLUT4), has been found [113, 117, 147]. This antiadipogenic effect of TNF-α most likely results from the downregulation of C/EBP-α and PPAR-γ expression [102, 141]. In the case of leptin, which induces lipolysis and glucose utilization in adipocytes, it has been shown that TZD-activated PPAR-γ inhibits leptin production [60]. This inhibition can be explained in terms of a functional antagonism between C/EBP-α and PPAR-γ on leptin promoter activity [49].

Apart from adipocyte differentiation, PPAR-γ activation promotes the apoptosis of mature adipocytes [92]. It has been reported that troglitazone, a PPAR-γ agonist of the TZD class, increases the population of small adipocytes in WAT and concomitantly decreases the population of large adipocytes. In addition, the percentage of apoptotic nuclei is increased by 2.5-fold in troglitazone-treated tissues, implying that large adipocytes lost by apoptosis may be counterbalanced by small adipocytes newly differentiated by troglitazone treatment. PPAR-γ activation by TZD thus leads to the accumulation of small adipocytes, which are more insulin sensitive than large adipocytes [92]. PPAR-γ has also been implicated in the differentiation of other cells and tissues, such as macrophages, breast, and colon [17, 86, 105, 123].

PPAR-γ AND INSULIN SENSITIVITY

Type 2 diabetes is characterized by insulin resistance, which is defined as the inability of insulin to stimulate glucose disposal in muscles and adipose tissue and to repress gluconeogenesis in the liver. Moreover, an increased level of insulin, produced by pancreatic beta cells, is not capable of compensating for this resistance. The metabolic consequence of insulin resistance is thus the disorder of lipids and glucose homeostasis in liver, muscles, and adipose tissue. Results of genetic studies indicate the involvement of PPAR-γ in glucose homeostasis. Consistent with this, several dominant-negative

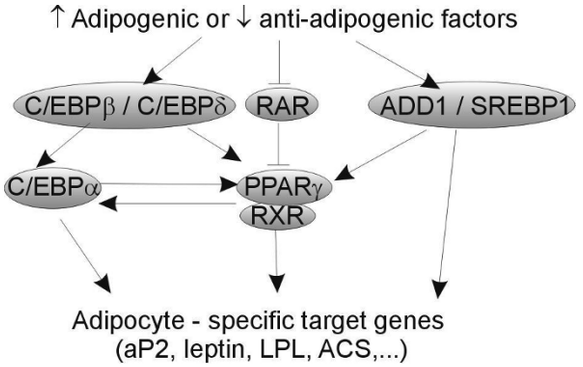

Fig. 6. The functional network between PPAR-γ and other transcription factors in gene activity modulation in adipocyte differentiation. C/EBP-β and -δ activate PPAR-γ expression during the early stages of adipogenesis. ADD1/SREBP1 promotes the transcriptional activity of PPAR-γ through the generation of endogenous ligands for PPAR-γ. Upon ligand stimulation, PPAR-γ induces the expression of C/EBP-α during the terminal stages of adipose differentiation. Through a positive feedback loop, C/EBP-α maintains the expression of PPAR-γ, resulting in adipocyte gene expression. Additionally, expression of the target genes can be modulated by an alteration of RXR activity from the non-permissive state (RXR bound to the peroxisome proliferators-activated receptor, RAR) to the permissive state (RXR bound to PPAR-γ). These changes may induce a recruitment of various cofactors affecting the target genes.

mutations in PPAR-γ in humans have been shown to cause partial lipodystrophy, marked insulin resistance, diabetes, and hypertension [10], whereas a polymorphism of PPAR-γ2, Pro12Ala, which impairs receptor activity, is associated with lower BMI and enhanced insulin sensitivity [3, 26]. In addition, strong genetic proof indicating that PPAR-γ contributes to glucose homeostasis *in vivo* has been provided by Rangwala et al. [97], who found that mice with the S112A mutation in PPAR-γ (this mutation prevents the phosphorylation at Ser-112) were protected from obesity-associated insulin resistance [97].

Since TZDs, drugs known to reduce insulin resistance, are high-affinity PPAR-γ ligands [71], it is most likely that PPAR-γ is involved in insulin sensitization. For example, overexpression of PPAR-γ and its activation by TZDs stimulate adipose differentiation [121], resulting in increased numbers of small adipocytes, which are more insulin sensitive than large adipocytes [92]. However, the exact mechanisms by which TZDs improve insulin sensitivity are still incompletely understood. Reports indicating a role of TZDs in insulin sensitization are not universally accepted and there is evidence to the contrary. Miles et al. [82] have shown that mice with a single copy of the *Pparg* gene (the $Pparg^{+/-}$ gene knockout mouse model) display greater insulin sensitivity than the wild type. Thus a genetic reduction in *Pparg* gene expression may result in augmented insulin sensitivity, implying that normally, full PPAR-γ activity may participate in the development of states of insulin resistance. However, this hypothesis should be verified through further studies on the mechanisms by which PPAR-γ promotes insulin sensitivity.

IMPORTANCE OF WAT FOR TZDs' ANTIDIABETIC ACTION

Support for a role of WAT as an important target of TZDs' action comes from Chao's data, which revealed that TZD (rosiglitazone and troglitazone) treatment of lipoatrophic A-ZIP mice had no effect on improving their hyperglycemia or hyperinsulinemia [21]. In addition, transplantation of wild-type adipose tissue into these mice, which were unresponsive to TZD treatment [21], led to lower plasma glucose and insulin levels [39], demonstrating that diabetes in A-ZIP mice is caused by the lack of adipose tissue and that transplantation of wild-type fat into lipoatrophic mice reverses their diabetic phenotype. Furthermore, mice lacking adipose PPAR-γ exhibit progressive lipodystrophy, steatosis, and insulin resistance in fat and liver, implying that primary defects of PPAR-γ in fat may produce a series of defects in other important metabolic tissues [43]. Taken together, these findings and the fact that PPAR-γ expression is significantly higher in WAT than in liver and muscle, which are other major insulin target tissues [67, 120, 130, 148], suggest that adipose tissue may be the primary target for TZDs' actions and it may play a key role in directing whole-body glucose homeostasis, probably by regulating the expressions of genes involved in adipose signaling (adipokines) to other tissues. However, there are indications of a direct role of PPAR-γ in the modulation of the insulin signal transduction pathway in adipose tissue by PPAR-γ ligand regulation of the adipocyte genes that encode GLUT4 [140] and c-Cbl associating protein (CAP) [12]. It has been demonstrated that TZDs increase the expression of CAP either in 3T3-L1 adipocytes or Zucker (*fa*/*fa*) diabetic rats, resulting in the stimulation of glucose transport [100]. The induction of CAP expression by TZDs takes place through direct binding of activated PPAR-γ/RXRα heterodimers to a PPRE in the CAP promoter [12]. These results imply a direct link between PPAR-γ activation in adipose tissue and insulin sensitivity and, on the other hand, confirm that adipose tissue is a direct target and a major site of TZD action. In contrast, Burant et al. [19], on the basis of their studies utilizing a partially lipoatrophic mouse model, suggest that skeletal muscles are a major site of the action of TZDs.

EFFECT OF TZDs ON ADIPOKINES

Since adipose tissue is also an endocrine organ, several adipocyte-secreted molecules, including TNF-α, leptin, resistin (also known as FIZZ3, mXCP4, hXCP1), adiponectine, and interleukin (IL)-6, have been implicated in the regulation of insulin sensitivity. Many studies have focused on determining the role of TNF-α in

the pathogenesis of insulin resistance in type 2 diabetes. It has been established in several animal models of obesity that the levels of TNF-α mRNA are increased in adipose tissue [51] and, moreover, the neutralization of TNF-α in obese rats causes a significant increase in the peripheral uptake of glucose in response to insulin [50]. In addition, several lines of evidence from studies on animal models have supported the thesis of a crucial role for TNF-α in obesity-related insulin resistance *in vivo* [68, 127, 129]. TZDs have significant effects on the activity of this cytokine. It has been observed that troglitazone treatment of rats infused with TNF-α prevents the induction of insulin resistance [83], whereas pioglitazone blocks TNF-α-mediated effects, including decreased glucose uptake and LPL mRNA level in adipocytes from a TNF-α-overexpression mice model [109]. TZDs have also been implicated in the regulation of leptin expression either in cultured adipose cells or *in vivo*, causing a reduction of leptin mRNA and protein levels [27, 60]. However, there are some opposing data concerning the role of leptin in human insulin resistance *in vivo*. Müller et al. [88] have shown that leptin impairs the metabolic action of insulin in isolated rat adipocytes, whereas other findings infer that leptin may also increase insulin sensitivity [110, 145].

Resistin was identified as an adipocyte-secreted factor responsible for insulin resistance in both cultured cells and genetically or diet-induced mouse obesity mice [85, 114]. It has been suggested that this protein may participate in a mechanism by which obesity is linked to insulin resistance and diabetes, since its level is decreased by rosiglitazone in 3T3-L1 adipocytes and in WAT of mice fed a high-fat diet [114, 115]. Of particular interest is that there are inconsistent animal findings indicating increased [114, 115] or decreased [73, 81, 133] levels of resistin in obesity and either decreased [42, 111, 114, 115] or increased [37, 133] levels by TZDs. Furthermore, both the expression and secretion of resistin in 3T3-L1 adipocytes is inhibited by TNF-α, which causes insulin resistance [33, 111]. In addition, several human studies have revealed a lack of correlation between obesity or insulin resistance and serum resistin concentrations in humans [34, 69], implying that resistin is not a master hormone linking obesity to diabetes. Moreover, it has recently been shown that resistin is produced primarily by monocytes and macrophages in humans [57]. Thus, the relevance of resistin in the regulation of insulin sensitivity remains unclear and further studies are required to resolve this issue.

Among the adipokines, much attention has been paid to the role of adiponectin, which is induced during adipocyte differentiation [53], in insulin resistance. It has been established that the plasma concentrations of adiponectin in obese subjects are significantly lower than in non-obese subjects [5] and that these reduced concentrations are associated with insulin resistance in different ethnic groups [135]. TZD treatment may cause an increase in adiponectin levels in subjects with normal or impaired glucose tolerance, and in patients with type 2 diabetes [25, 48, 77, 144]. These results are also supported by Yu et al. [146], who demonstrated, based on increased adiponectin levels in normal rats, lean subjects, and nonresponders, that adiponectin alone is not able to evoke TZD-induced insulin sensitivity. In addition, an inverse relationship between adiponectin and insulin levels has been found [146]. Therefore it is apparent that the chronic hyperinsulinemia associated with insulin-resistance can lead to downregulation of adiponectin concentration. It is noteworthy that adiponectin can enhance insulin action in muscle and liver, although the mechanism is still unclear. Adiponectin has also been found to enhance insulin-stimulated tyrosine phosphorylation of the insulin receptor in skeletal muscle [143] and to decrease hepatic expression of the glucogenic enzymes, PEPCK, and glucose-6-phosphatase [24].

IL-6 is another molecule secreted from adipose tissue with signaling potential. It has been demonstrated that adipose tissue is able to secrete a large amount of IL-6, with an increased production in obese subjects [84], and there is positive correlation between circulating IL-6 levels and insulin resistance [11]. However, the role of IL-6 in the molecular mechanism involved in obesity-related insulin resistance is not yet well understood.

IMPORTANCE OF PPAR-γ IN OTHER INSULIN-SENSITIVE TISSUES

The expression of hepatic PPAR-γ is very low [30, 120]; however, it is increased in obese and diabetic model mice [13, 19, 80, 95]. In addition, TZDs induce the expression of several PPAR-γ-responsive genes in the livers of these mice, implying that the effects of PPAR agonists on lipid metabolism and energy balance in obesity and type 2 diabetes may partly be mediated through their effects on PPAR-γ in the liver [80]. The significance of hepatic PPAR-γ in insulin resistance has been high-lighted in many studies utilizing *Pparg*-specific knockout mouse models of the liver [38, 78]. The liver-specific disruption of the *Pparg* gene in *ob*/*ob* mice causes a significant decrease in hepatic triglycerides (TGs). However, TG and free fatty acid levels in plasma are elevated, resulting in an aggravation of insulin resistance [78]. In other studies, Gavrilova et al. [38] showed that mice with a disrupted *Pparg* gene in the liver exhibit impaired hepatic uptake of plasma TGs and TG deposition in adipose tissue and muscle, contributing to the development of insulin resistance. Thus the authors concluded that hepatic PPAR-γ contributes to TR homeostasis, regulating both TG clearance and lipogenesis.

Given that enhanced glucose disposal was observed in cultured muscle cells after TZD treatment [4, 23], skeletal muscle is thought to be an important target of insulin-sensitizing PPAR-γ ligands. Results from genetic models of muscle-specific deletion of the *Pparg* gene have shown that these mice have unchanged insulin-

-stimulated glucose uptake in muscle but increased whole-body insulin resistance due to secondary insulin resistance in the liver [91]. TZD treatment reverses insulin resistance in these animals, supporting the hypothesis that TZDs act directly on tissue other than skeletal muscle, presumably adipose tissue [91]. In contrast, Hevener et al. [47] found insulin resistance in muscle that was not improved by TZD treatment. Nevertheless, an explanation of the differences in muscle insulin sensitivity between these models requires further investigation. Finally, muscle-specific knockout studies have revealed that PPAR-γ is important for the maintenance of normal adiposity, whole-body insulin sensitivity, and hepatic insulin action. In addition, the insulin-sensitizing effects of TZDs on muscle are indirect and can be mediated through a "tissue cross-talk" [91], which is not yet understood. Fat-derived signals most likely participate in this process. It has been shown by several lines of experimental evidence that TZDs stimulate the production of adiponectin, which promotes fatty-acid oxidation and insulin sensitivity in muscles and liver (a decrease in hepatic glucose production and an increase in muscles glucose use) [14, 24, 77, 143].

Findings from mouse models performed in the past few years have confirmed that PPAR-γ in adipose tissue is a major target of TZDs, with several secondary effects in the liver and skeletal muscles, and have also provided new insights into the biological functions of liver and muscle PPAR-γ.

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