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Review

Ligands for the Nuclear Peroxisome Proliferator-Activated Receptor Gamma

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Nuclear receptors are ligand-activated transcription factors, which represent a primary class of drug targets. The nuclear receptor peroxisome proliferator-activated receptor gamma (PPARη) is a key player in various biological processes. PPARη is widely known as the target protein of the thiazolidinediones for treating type 2 diabetes. Moreover, PPARη ligands can induce anti-inflammatory and potentially additional beneficial effects. Recent mechanistic insights of PPARη modulation give hope the next generation of efficient PPARη–based drugs with fewer side effects can be developed. Furthermore, chemical approaches that make use of synergistic action of combinatorial ligands are promising alternatives for providing tailored medicine. Lessons learned from fine-tuning the action of PPARη can provide avenues for efficient molecular intervention via many other nuclear receptors to combat common diseases.

Trends

Fine-tuning PPARγ by ligands leads to specific physiological responses.

Synergistic action of ligands can boost beneficial effects.

The principles for activation of PPARγ are paradigmatic for many nuclear receptors.

General Functions of the PPARγ

In humans, 48 different nuclear receptors with various biological functions and cellular specificity are known. These ligand-dependent transcription factors are involved in development and physiological homeostasis in response to environmental changes [1,2].

PPARγ-based gene regulation comprises both, gene activation and repression events, depending on the molecular context. PPARγ is a key transcriptional regulator for fatty acid and glucose metabolism [3]. Over the past 20 years, PPARγ has become an important target, mainly for treating insulin resistance and type 2 diabetes [4]. However, as was shown over recent years, this nuclear receptor can also contribute to the inactivation of genes involved in inflammation [5].

Two main, very similar isoforms of PPARγ were observed in the human and in the mouse: PPARγ1, that is expressed in high amounts in white and brown adipose tissue and – in many cases to a lower degree – in nearly all tissues and in immune cells such as macrophages; and PPARγ2 that is primarily expressed in white and brown adipose tissue (Figure 1) [6].

Various genetic mouse models, for example isoform- or tissue-specific knockout mice, were decisive to dissect physiological roles of PPARγ [7]. Among others, it could be shown that PPARγ2-null mice develop insulin resistance [8]. Gain-of-function mouse models showed that selective activation of PPARγ in adipocytes was sufficient for achieving insulin sensitization, indicating the importance of targeting PPARγ in fat cells [9]. Notably, various animal models showed striking inter-tissue communication in the regulation of energy metabolism and in the development of metabolic diseases [7]. The application of genetic models revealed mechanistic insights into PPARγ and its ligands *in vivo*. However, even though the effects of ligands can be

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 http://dx.doi.org/10.1016/j.tips.2015.06.010

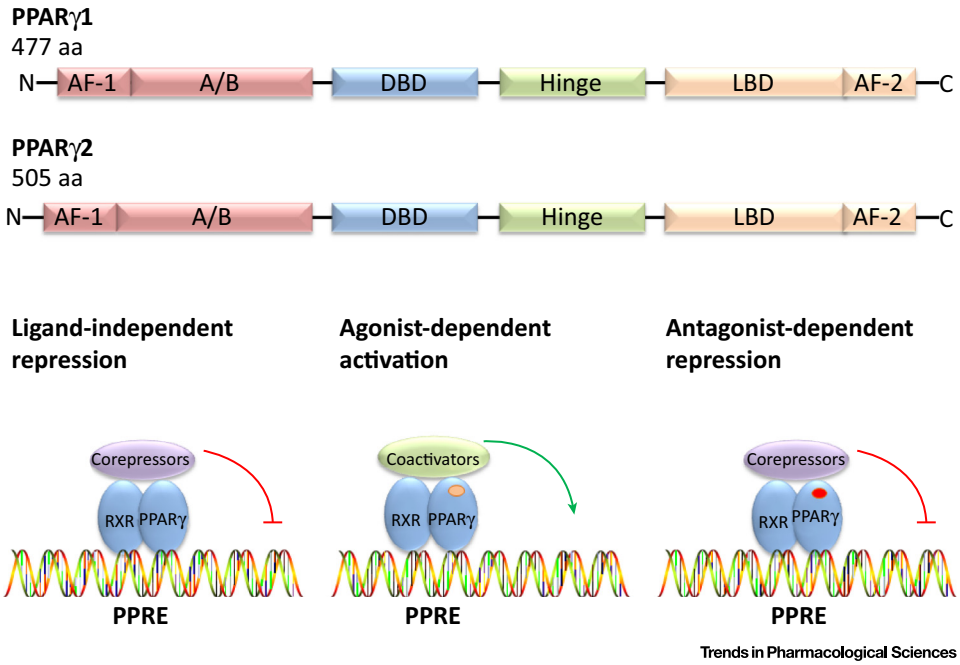

Figure 1. The Main Isoforms of PPARγ and the Mechanisms Modulating Receptor Activity. (Above) Schematic representation of the primary sequences of the two main isoforms of PPARγ are shown. (Below) The three primary mechanisms modulating PPARγ activity. Abbreviations: aa, amino acid; AF-1 A/B, activation function 1 (A/B domain), required for ligand-independent activation; AF-2, activation function 2, required for ligand-dependent activation, ligand-dependent dimerization, coactivator recruitment, and corepressor release; DBD, DNA-binding domain, required for sequence-specific binding to genomic DNA; Hinge, a protein domain required for receptor dimerization; LBD, ligand-binding domain, required for ligand-dependent modulation; PPAR, peroxisome proliferator-activated receptor; PPRE, PPAR response element; RXR, retinoid X receptor.

revealed by such models, careful interpretation is required in the context of compound treatments because overall metabolism and physiology of genetic mouse models can differ strongly from the wild type.

We discuss recent insights on modulation of PPARγ and how the emerging possibilities of synergistic pharmacological approaches provide new avenues to develop a next generation of efficient PPARγ- and other nuclear receptor-based drugs with fewer side effects.

Structural Aspects

Figure 1 illustrates the three major mechanisms of modulation of PPARγ, namely ligand-independent repression, agonist-dependent activation and antagonist-dependent repression. These mechanisms are in a sense paradigmatic for the mode of action of many other nuclear receptors and provide a rough basis for drug design. PPARγ interacts via its ligand-binding domain (LBD) with many structurally-different small molecules. Notably, although several endogenous metabolites with low affinity for PPARγ have been described, high-affinity endogenous ligands are still unknown [10].

In the nucleus, PPARγ forms a heterodimer with the nuclear receptor retinoid X receptor ∝ (RXR∝) to bind to genomic DNA at specific sites (Figure 1) [6]. As observed recently in cultured mouse fat cells, in the absence of exogenous ligands PPARγ occupies a series of genomic sites, but treatment with potent externally supplied ligands such as thiazolidinediones (TZDs) did not induce binding to new sites [11]. However, depending on the conformational change of the LBD triggered by cellular signaling events or small molecules, different coactivating proteins, such as

peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α), or corepressing proteins such as nuclear receptor corepressor 1 (NCoR1), can assemble with PPARγ and modify its activity [12]. These protein complexes are probably regulated by several (in part still unknown) post-translational modifications such as phosphorylation [13], acetylation [14], glycosylation [15], SUMOylation [16], and ubiquitination [17]. Owing to the generally complex mechanisms of nuclear receptors such as PPARγ, the binding affinities and binding sites of small molecules to LBDs can only provide partial information on potential gene activation. Moreover, TZD treatment leads to similar numbers of activated and repressed PPARγ target genes by largely unknown mechanisms, which probably include redistribution of PPARγ cofactors over regulatory sites of the genome [18]. The inherent complexities of modulation of PPARγ thus may render structure–activity relationship (SAR) approaches potentially more challenging than for other biological targets.

PPARγ features a large (1300 $Å^3$) Y-shaped ligand-binding pocket that allows flexible interaction with a ligand. PPARγ ligands display wide structural diversity and, in general, weak affinities (micromolar), but in some cases also higher affinity [19,20]. Structural promiscuity for ligands allows PPARγ to sense and respond to varying environmental changes, such as food intake, to support the adaptive expression of metabolic genes [21]. The LBD of PPARγ consists of 13 α-helices and a small, four-stranded β-sheet [22]. The AF2 domain of PPARγ is defined to encompass the whole LBD and an activating helix H12 domain. Binding of ligands leads to formation of a hydrophobic groove, in general by folding of helix H12 along the LBD core together with helices H3 and H5, resulting in a more compact and rigid conformation. This conformational change can cause recruitment of various cofactors that are required for transcription of genes.

The full PPARγ agonist rosiglitazone stabilizes in particular helix H12 and thereby induces coactivator assembly. In contrast to full agonists, so-called partial agonists and antagonists of PPARγ tend to destabilize helix H12, and instead stabilize helix H3 and the β-sheet region of the binding pocket of the LBD. Stabilization or destabilization of helix H12 is considered as a molecular switch that predefines activation efficiency [22]. Distinct positioning of ligands within the LBD has become a general approach to develop alternatives to TZDs to modulate the activity of PPARγ. For example, ionomycin was recently shown to interact with the PPARγ LBD in a unique binding mode, with epitopes and properties distinct from those of TZDs [23].

To gain a more complete view on relevant PPARγ complexes, Chandra *et al.* presented the structures of intact heterodimers of PPARγ and RXRα bound to DNA, ligands, and coactivator peptides [24]. These authors showed that PPARγ and RXRα form a non-symmetric complex such that the LBD of PPARγ can contact multiple domains in both proteins. PPARγ and RXRα were linked by three interfaces, some of which were DNA-dependent. The LBD of PPARγ cooperates with both DNA-binding domains (DBDs) to enhance response-element (RE) binding. The A/B segments of PPARγ were found to be strikingly dynamic, but lacked folded substructures despite their gene activation properties. This feature may potentially allow flexibility of PPARγ in gene regulation processes.

Selected Physiological Aspects of Therapeutic Interest

PPARγ is a key factor in adipogenesis and crucially required for insulin signaling in peripheral tissues [25]. PPARγ is in particular known as the master regulator of adipocyte differentiation and for inducing storage of fatty acids in mature adipocytes. Major effects of PPARγ ligands stem from gene expression changes in adipose tissue, leading among others to the expression of several metabolic target genes such as those encoding glucose transporters and fatty acid binding proteins [26]. Physiological response to treatment with TZDs seem to derive mainly from activation of PPARγ in adipose tissue, [27]. As said above PPARγ is usually expressed at lower

levels in multiple cells and tissues, which can (unexpectedly) influence the physiological response to PPARγ drug treatments.

Notably, macrophages can infiltrate adipose tissue and thereby contribute to local low-grade inflammation, for example via secretion of tumor necrosis factor ∝ (TNF∝), thus rendering metabolic signaling of adipose tissue more resistant to insulin [28,29]. Activation of PPARγ by TZD-treatment renders proinflammatory M1 macrophages to an alternative, anti-inflammatory M2 state [30]. Many other cell and tissue types have been described to be influenced by TZD treatment, such as other immune cells (such as regulatory T cells), pancreatic β cells, skeletal muscle, liver, and the central nervous system (see also Box 1).

Box 1. Unexpected Roles of PPARγ in the Brain

Depending on the tissue context, nuclear receptors such as PPARγ can exert varying functions. Ligand-based modulation of nuclear receptors can thus lead to tissues-specific gene expression, which can subsequently lead to (unexpected) crosstalk between tissues. We will in particular explore here the roles of PPARγ in the brain (Figure I).

Recently, PPARγ expressed in cells of the central nervous system (CNS) has been implicated in the regulation of energy balance [102,103]. Acute and chronic activation of PPARγ in the CNS, either by drugs such as the TZDs or by hypothalamic overexpression of a PPARγ fusion protein, resulted in positive energy balance in rats [102]. By contrast, inhibiting the endogenous activation of PPARγ in the CNS by pharmacological antagonists, or by reducing its expression with interfering small hairpin (sh)RNA, resulted in negative energy balance. In addition, restored sensitivity to the satiety hormone leptin in rats inhibited the hyperphagic response to oral TZD treatment.

Further, neuron-specific PPARγ knockout mice on a high-fat diet (HFD) showed reduced food intake and increased energy expenditure compared to wild-type mice, resulting in reduced weight gain [103]. Interestingly, when treated with rosiglitazone, neuron-specific PPARγ knockout mice were resistant to rosiglitazone-induced hyperphagia and weight gain. Relative to rosiglitazone-treated control mice, these knockout mice showed only slight improvements in glucose levels. Hepatic insulin-sensitivity induced by rosiglitazone treatment during HFD feeding was completely abolished in neuronal PPARγ knockout mice, including impaired signal transduction by the liver insulin receptor. These data suggested that weight gain induced by HFD feeding depends in part on the effects of activating neuronal PPARγ, which seems to limit thermogenesis and increase food intake. Moreover, neuronal PPARγ signaling is also necessary for the insulin-sensitizing effects of TZDs via the liver.

These results argue for the development of PPARγ ligands taking the specific effects derived from activating PPARγ in different tissues into consideration. PPARγ ligands that specifically act in the adipocyte but which do not pass the blood–brain barrier could provide a means to increase insulin sensitivity in peripheral tissues without necessarily increasing food intake and inducing weight gain [104]. Organ-specific drug action can potentially be achieved by peptide-mediated selective tissue targeting of nuclear hormones (see main text) [86].

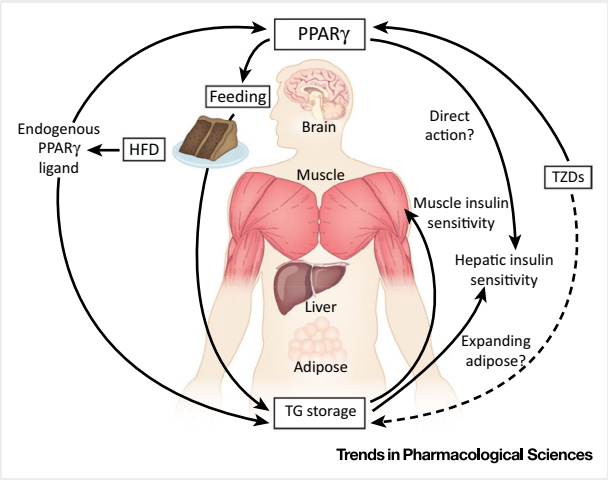

Figure I. Physiological Effects of Activating PPARγ by Small Molecules in the Brain. In brain, activation of PPARγ can mediate the weight gain observed with treatments using compounds such as TZDs by stimulating feeding and increasing adipose tissue mass. PPARγ activity in brain increases food intake in animal models during HFD feeding, potentially via an endogenous PPARγ ligand which could be derived from dietary ingredients. Whereas the contribution of brain PPARγ activity to muscle insulin sensitivity is minor, brain PPARγ seems to contribute to promoting hepatic insulin sensitivity, either via a brain–liver pathway or secondary to the insulin-sensitizing effects of adipose tissue expansion. This figure was reproduced with permission from [104]. Abbreviations: HFD, high-fat diet; TG, triglyceride; TZDs, thiazolidinediones.

Remarkably, expression of PPARγ in multiple tissues can lead to difficult to predict (positive or negative) side effects. For example, in the collecting duct system of the kidney, PPARγ-dependent regulation of sodium transport was found to underlie TZD-induced fluid retention [31]. Future investigation will be necessary to determine exactly the most important molecular pathways and how effects deriving from modulation of PPARγ in different tissues are coordinated *in vivo* [27].

In contrast to metabolic regulation triggered by strong ligands of PPARγ, anti-inflammatory effects of ligand-modulated PPARγ seem to be weaker and involve interconnected mechanisms which are partly still poorly explored (Figure 2) [32]. These mechanisms comprise protein–protein interactions including physical interaction of PPARγ with NF-κB (nuclear factor κ light-chain enhancer of activated B cells) [33] or cofactor competition of both transcription factors [34]. Moreover, regulation of protein localization [35] and post-translational modifications including ubiquitination by an E3 ligase activity of PPARγ and subsequent degradation of NF-κB have been shown [36]. Notably, protein–DNA binding events can also be influenced by SUMOylation of PPARγ, leading to transrepression of NF-κB, or are potentially influenced by other competitive binding events (Figure 2) [37]. Ligands of PPARγ may also contribute to the degradation or inactivation of transcriptional inflammatory regulators. On the other hand, so far unknown factors may, *vice versa*, disturb the action of PPARγ.

In general, PPARγ seems to exert multiple molecular functions, depending on the cellular context. In addition to PPARγ, two other evolutionarily closely related PPAR isotypes are known,

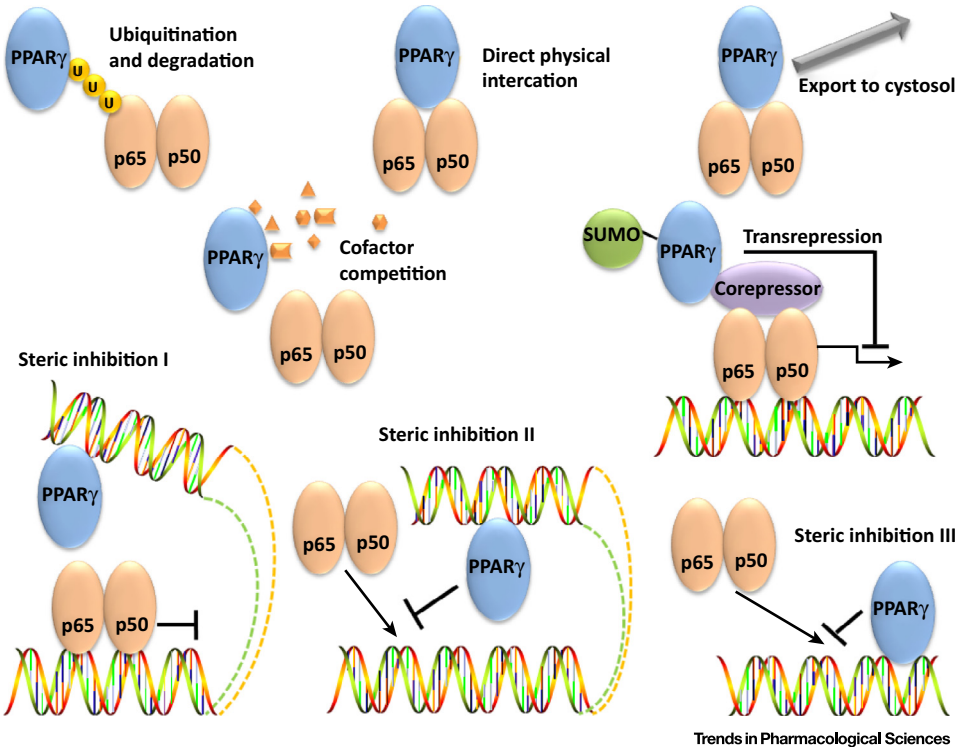

Figure 2. Potential Anti-Inflammatory Mechanisms can be Modulated by PPARγ. Notably, PPARγ can interfere with the nuclear factor κB (NF-κB) pathway, for example by inducing degradation of the NF-κB/p65 complex, direct physical interaction, nuclear export of p65, or by competing for limiting amounts of shared coactivators with NF-κB. Moreover, PPARγ can inhibit in various ways transcriptional initiation of NF-κB by transrepression at transcriptional start-sites of inflammatory mediator genes. Moreover, PPARγ might potentially competitively bind to genomic sites which are specific for inflammation-triggering transcription factors. Long-range genomic interactions might also play a role. Many of these proposed mechanisms were discovered in different cellular models, and can thus not be easily brought into context with each other.

namely PPARα and PPARβ/δ [38]. PPARα was found to be predominantly expressed in the liver and to some degree in other tissues. In liver, PPARα controls fatty acid oxidation, lipoprotein metabolism, gluconeogenesis, and ketone body biosynthesis. PPARβ/δ is expressed ubiquitously to regulate fatty acid oxidation and adaptive thermogenesis, for example in skeletal muscle. PPARs control in part overlapping and in part distinct regulatory metabolic pathways, and can vary in abundance in diverse tissues. Consequently, simultaneous activation of the PPARs by a single compound has been suggested as a synergistic treatment principle for metabolic diseases [39]. Remarkably, some ligands of PPARγ, such as pioglitazone or amorfrutins, also activate other PPARs [40,41]. However, so far it appears that most clinical trials using for example optimized dual PPARγ/PPARα agonists such as aleglitazar or saroglitazar were unsuccessful due to safety concerns [27]. Nevertheless, research on dual PPAR agonists is still actively being pursued in structure-based virtual screening and other discovery approaches to potentially benefit from synergistically activating PPARs in clinical practice [42].

PPARγ-Based Drugs and Other Ligands

PPARγ interacts with multiple compounds that display wide structural diversity (Figure 3 and Table 1). Binding affinities and receptor activation can vary greatly between different compounds. Note that, owing to the above-described complex mechanisms of activation, even weak binders can exert significant PPARγ-driven effects *in vivo*.

PPARγ is generally known as the 'glitazone' receptor [43]. This name refers to synthetic small-molecule activators of PPARγ that contain a thiazole-2,4-dione (thiazolidinedione, TZD) functional group, and these molecules are collectively known as 'glitazones' (Figure 3) [44]. TZDs activate PPARγ by efficiently interacting with the LBD and helix 12 (via residues H449 and Y473) of this nuclear receptor [45].

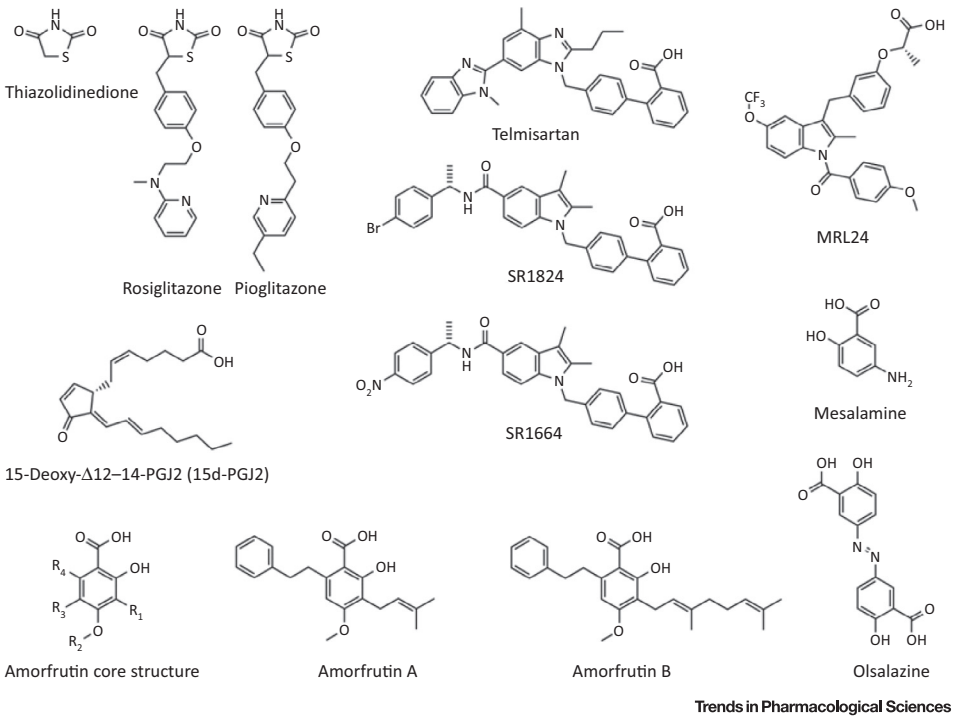

Figure 3. PPARγ Binds to a Large Variety of Structurally Diverse Molecules. Hundreds of natural and synthetic molecules have been described to interact with PPARγ in different ways.

Table 1. Selected Compounds that Interact with PPARγ[a].

PPARγ Ligand	Binding Affinity/EC_{50}	Binding Mechanism	Comments
Rosiglitazone	Very low nanomolar range (both)	Includes helix H3 and in particular stabilization of helix H12. Stabilization is mainly achieved by a direct hydrogen bond between the ligand and amino acid Tyr473 of the LBD, allowing H12 to dock against H3 and H11.	Prominent thiazolidinedione. Full synthetic agonist (used as an insulin-sensitizing drug). Ligand-interaction stabilizes the AF2 surface (helix H3–H4 loop, C-terminal end of H11 and H12) of the receptor to facilitate coactivator interactions. This mechanism leads to highly-efficient activation of PPARγ-dependent gene expression.
Pioglitazone	Mid nanomolar range (both)	Shows similar binding mechanisms to other thiazolidinediones such as rosiglitazone.	Further prominent thiazolidinedione. Strong synthetic agonist (used as insulin-sensitizing drug).
MRL-24	Very low nanomolar range (both)	Includes stabilization of helix H3 and β-sheet. No stabilization of helix H12.	Partial synthetic PPARγ agonist. Less stable ligand-induced H12-conformation results in attenuated coactivator binding. This mechanism leads to less-efficient activation of PPARγ-dependent gene expression compared to full agonists such as rosiglitazone.
Telmisartan	Low micromolar range (both)	Non-canonical, suboptimal hydrogen-bonding network that leads to destabilization of helix 12. Stabilization of other regions such as helix H3.	Originally used as angiotensin II receptor antagonist to reduce blood pressure. Partial synthetic PPARγ agonist.
SR 1664 and analogs such as SR 1824	Binding: low to mid nanomolar range. However, almost no activity induction	Tendency to destabilize H11 and H12 region. Despite the varying mode of binding, SR1664 and rosiglitazone both bind to the same core residues within the PPARγ LBD.	Synthetic PPARγ modulators. Inhibition of the action of the kinase CDK5 that phosphorylates PPARγ at Ser273. This mechanism may contribute to beneficial modulation of PPARγ.
Mesalamine (Mesalazine), Olsalazine	Low to mid-millimolar range (both)	Involves typical mechanisms of partial agonists. Requires further systematic studies.	Mesalamine is a basic member of the 5-aminosalicylic acid class. Compounds such as olsalazine and balsalazide are thought to release mesalamine (5-aminosalicylic acid) in the body as the active compound.
15-Deoxy-Δ12–14-PGJ2	Covalent binding. EC-50: low micromolar range	Covalent binding to Cys285 of LBD. Conformational changes include helices H2, H3, and β-sheet.	Endogenous (natural) ligand. A prominent example of other arachidonic acid ligands that can covalently bind to the LBD of PPARγ.
Amorfrutins (A and B)	Low to mid nanomolar range (both)	Includes stabilization of helix H3 and β-sheet.	Natural products that interact as partial agonists with PPARγ, and in part with other PPAR isotypes.

[a]Data were retrieved from the ChEMBL database (www.ebi.ac.uk/chembl/) and from PubChem (http://pubchem.ncbi.nlm.nih.gov). Ranges of binding affinities and EC_{50} values are presented as indicators (the exact values reported vary significantly between studies, perhaps reflecting differences in experimental procedures).

Some TZDs are strong insulin-sensitizing drugs that have been used in the treatment of insulin resistance and type 2 diabetes, very often as a second-line oral drug [46], in combination with the first-line drug metformin (1,1-dimethylbiguanide). TZDs are unique antidiabetic drugs because they seem to primarily function as insulin-sensitizing agents without increasing pancreatic insulin secretion. Furthermore, TZDs are known as efficient drugs for treating hyperlipidemia to reduce fatty acids in blood [7]. By contrast, metformin primarily suppresses hepatic glucose production [47]. Consequently, combined treatment with metformin and TZDs often results in beneficial complementary effects *in vivo*.

Most effects of TZD-treatment are driven by induction of adipocyte differentiation, thereby increasing the number of glucose transporters such as GLUT4 and inducing lipogenic genes such as *AP2* and *CD36* [7]. However, TZDs also induce the expression of metabolically beneficial genes in mature adipocytes, thereby converting adipose tissue into a metabolically safe reservoir for storing energy as fat [48]. Moreover, TZDs are thought to induce systemic effects by redistributing triglycerides from liver and skeletal muscle to adipose tissue [49]. Recently, some chemical TZD derivatives with antidiabetic effects, such as MSDC-0160 and MSDC-0602, were reported to primarily function via mitochondrial membranes and the pyruvate carriers MCP1 and MCP2, and less via PPARγ [50–52]. The most widely known TZD drugs are rosiglitazone (Avandia®, in general no longer prescribed) and pioglitazone (Actos, currently the only medically-relevant TZD).

Interestingly, some inhibitors of arachidonate 5-lipoxygenase and of cyclooxygenases of the 5-aminosalicylic acid class, such as balsalazide and olsalazine, have been shown to exert their anti-inflammatory potential via interactions with PPARγ at millimolar concentrations [53,54]. *In vivo*, these compounds are rapidly converted to mesalamine (mesalazine), a simple active compound that features a 2-hydroxy benzoic acid structure (Figure 3). 5-Aminosalicylic acids are, for example, presently being used for treating inflammatory bowel disorders such as ulcerative colitis and Crohn's disease. Recently, amorfrutin A (Figure 3, Table 1), which is structurally related to salicylic acid, was shown to exert mild anti-inflammatory effects in inflamed colon cells, at least in part via activation of PPARγ [55]. Ongoing clinical studies are further investigating the effects of various PPARγ-targeting compounds in the treatment of various inflammatory disorders including colitis and rheumatoid arthritis (updates can be found at https://clinicaltrials.gov). Notably, PPARγ ligands such as TZDs have been associated with prevention of many forms of cancer [27]. PPARγ ligands may therefore also have potential for treating cancer [56].

However, antidiabetic TZDs such as rosiglitazone, pioglitazone, and troglitazone have unfortunately generated adverse effects in humans. These include weight gain, fluid retention, cardiovascular complication, bone fractures, bladder cancer, and hepatotoxicity [57]. These adverse effects may reflect overactivation of PPARγ (for example to induce excessive fat storage in adipose tissue), unwanted modulation of PPARγ in various tissues (such as the collecting duct system), or from other compound-specific off-target effects [58,59].

Accumulating observations of adverse clinical effects of TZDs resulted in growing skepticism, leading to provisional retraction of approval of rosiglitazone by the FDA and European authorities, mainly because of cardiovascular side effects [60]. This decision was later abandoned, at least in the USA. Glitazone treatments became possible under restrictions defined by the authorities [61,62]. Nowadays it seems that reported concerns about adverse effects were in part disproportionate. For example, as shown in the ADOPT study, rosiglitazone treatment led to 1.7 kg long-term weight gain, which is less than 3% for adult persons, a value that is considered to be normal value for long-term weight maintenance [63]. Moreover, cardiovascular implications were observed in 1.8% of patients, whereas for the widely applied first-line antidiabetic drug metformin this value was similar (1.5%). Of note, the prospective, randomized

PROactive study focusing on pioglitazone showed positive cardiovascular effects, in particular a reduction of 50% of stroke events after 3 years of treatment [64]. Furthermore, particular usefulness was reported for patients suffering from renal failure [65]. Interestingly, in contrast to common belief, some studies reported that TZDs were not associated with increased risk of myocardial infarction; compared to metformin, pioglitazone was even associated with a significant lower risk of all-cause mortality [66]. In November 2013, the FDA announced it would remove the usage restrictions for rosiglitazone in patients with coronary artery disease. Nevertheless, increasing rates of bone fractures were observed in women treated with rosiglitazone after menopause, indicating a potential side effect that requires stringent medical observation [67]. Recent studies also investigated potential cancerogenic effects of pioglitazone [68], which revealed non-significant minor increases in bladder cancer. On the other hand, pioglitazone was described to potentially prevent more abundant forms of cancer [27].

Clearly, the reported adverse effects of PPARγ must be taken very seriously. However, it seems that the recent skepticism was potentially exaggerated. Based on recent insights into the efficient modulation of PPARγ activity, thereby improving control over physiological response, future avenues for exploiting PPARγ for treating complex diseases may in particular benefit from: (i) fine-tuning ligand development by exploiting molecular mechanisms of PPARγ cofactor modulation; (ii) making use of peptide-conjugate compound chemistries to design PPARγ ligands with synergistic effects; (iii) making use of safe poly-compound mixtures for generating synergistic effects; and (iv) considering varying functions of PPARγ in different tissues, for example in the brain and in liver (Boxes 1 and 2).

These guiding principles explored below may also become useful for developing ligands for other targets of the nuclear receptor family.

Fine-Tuning the Molecular Mechanisms of PPARγ–Ligand Interaction

Deep insights into the (various) biological functions and mechanisms of PPARγ could provide an improved rationale for future ligand development. Targeting small molecules to the LBD of PPARγ can result in specific assemblies with coregulating proteins, and thus in differential target gene expression. Such selective modulation has been proposed as a promising pharmacological approach to increase insulin sensitization in the absence of adverse effects [69].

Compounds that tend to activate PPARγ in general more weakly than the TZDs were termed partial PPAR agonists or selective PPAR modulators (SPPARMs) [70]. SPPARMs were developed to optimize gene expression signatures with a view to generating metabolically beneficial effects while reducing unwanted side effects. Examples include MRL24, INT-131, and dozens if not hundreds of alternative synthetic SPPARMs (Figure 3, Table 1) [71].

Recently, low nanomolar-binding natural products termed amorfrutins were described, which feature properties of SPPARMs [41,72]. These simple 2-hydroxy benzoic acid derivatives (structurally related to salicylic acids) showed a lower increase in the expression of genes involved in fat storage than did rosiglitazone. Significant insulin-sensitizing and anti-inflammatory effects were detected in diabetes mouse models, and the mice did not display the unwanted side effects associated with TZDs (Figure 3, Table 1). Interestingly, first small-molecule libraries of synthetic analogs of amorfrutins and related cajaninstilbene acid derivatives have been generated from a common building block, and this has provided several new PPARγ agonists that open avenues for further optimization [73].

Notably, a widely applied angiotensin receptor blocker, telmisartan (Figure 3), was surprisingly found to directly interact with the LBD of PPARγ as a SPPARM, producing a distinct conformational change compared to a thiazolidinedione. In insulin-resistant diet-induced obese mice,

Box 2. Unexpected Roles of PPARγ in the Liver

Nonalcoholic fatty liver disease (NAFLD) is a severe metabolic disorder that currently affects up to 30% of the adult population in Western societies [105]. However, the underlying molecular mechanisms of this diet-induced disease remain poorly understood. Recently, the dimeric activator protein 1 (AP-1) was discovered as a regulator of NAFLD. Fos-related antigen 1 (Fra-1) and Fos-related antigen 2 (Fra-2) inhibit NAFLD by blocking PPARγ signaling in the liver, thereby reducing steatotic effects [106].

Surprisingly, even fully established NAFLD and liver damage can be reversed by inducing expression of Fra-1 specifically in the liver, indicating that Fra-1 might prevent and potentially fully reverse NAFLD *in vivo* [106]. Strikingly, c-Fos/c-Jun dimers promote PPARγ expression, while Fra/c-Jun dimers repress PPARγ promoters (Figure I). Notably, JunD appeared to be essential for PPARγ signaling and for inhibiting the development of NAFLD. The (antagonistic) regulation of PPARγ by distinct AP-1 dimers observed at the transcriptional level establishes AP-1 as a key regulator linking obesity, hepatic lipid metabolism, and NAFLD. Additional activation of PPARγ in the liver by small molecule ligands might thus potentially boost unwanted steatohepatitis.

Future ligand development may benefit from the here described mechanisms and resulting effects in liver. Interestingly, it has been reported that amorfrutins (partial PPARγ agonists) were surprisingly efficient in inhibiting the development of fatty liver [41]. In general, specific compound-based modulation of PPARγ in fatty liver may inhibit the unwanted action of various mentioned transcriptional regulators, such as c-Fos/c-Jun dimers or JunD, to reverse metabolic deregulation.

In summary, nuclear receptors such as PPARγ can exert various functions depending on the tissue context. Tissue communication may play an (unexpected) role when targeting the same nuclear receptor in different tissues, which thus requires careful investigation of new PPAR-based ligands.

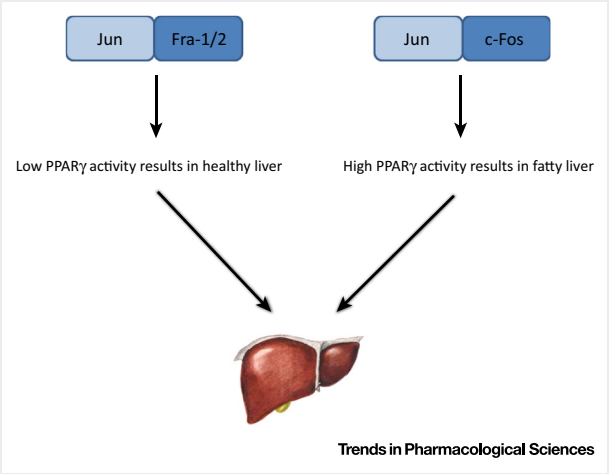

Figure I. Differential Mode of Action of PPARγ in Liver. Fos-related antigen 1 and (Fra-1/2) can inhibit or even reverse diet-induced liver steatosis by impeding PPARγ signaling. By contrast, c-Fos promotes the expression of PPARγ, resulting in accumulation of fat in the liver. The unique antagonistic regulation of PPARγ by distinct activator protein 1 (AP-1) protein dimers occurs at the transcriptional level and establishes AP-1 as a so far unknown link between obesity and lipid metabolism in liver and non alcoholic fatty liver diseases.

telmisartan increased insulin sensitivity in the absence of weight gain [74]. However, so far these promising results could not be adequately confirmed in humans. This is unfortunately also true for several other structurally-diverse synthetic SPPARMs that were developed over the past 15 years.

Most promising compounds SPPARMs based on the TZD core structure and non-TZD PPARg ligands failed during Phase II or III trials. For example, the dual PPARγ- and PPARα-activating compound aleglitazar was recently abandoned due to lack of efficacy and observed toxicity [75,76]. These set-backs have resulted in some reluctance to invest further in the development of PPARγ-based drugs.

However, recent and ongoing basic research has provided new exciting opportunities for developing more efficient PPARγ-based treatment strategies. For example, it was recently suggested that the interaction of PPARγ with cell division protein kinase 5 (CDK5) might play an important role in modulating gene expression activity. CDK5 was shown to be recruited by the key transcriptional corepressor protein NCoR1. Once recruited, CDK5 phosphorylates

PPARγ at serine 273, thereby inactivating this nuclear receptor and lowering the expression of many of its beneficial target genes. Pharmacological interference with the phosphorylation event at serine 273 of PPARγ has been proposed as a new strategy to increase insulin sensitivity because simple inhibition of the kinase CDK5 – that is essential in sensory and other pathways – may be difficult to control physiologically [77]. However, it seems that the underlying phosphorylation event of PPARγ may be difficult to reproduce [72].

Nevertheless, efficient but unfortunately toxic inhibitors of CDK5-dependent phosphorylation of PPARγ at serine 273 have been discovered [77]. These PPARγ-binding compounds, termed SR1664 and SR1824 (Figure 3, Table 1), interfere with PPARγ-controlled gene expression by efficiently blocking phosphorylation of PPARγ at serine 273. Interestingly, SR1664 and SR1824 were structurally very similar to the above-mentioned telmisartan [74], a well-characterized selective modulator of PPARγ (Figure 3, Table 1). A derivative of SR1664, UHC1, was later suggested as a more efficient antidiabetic and anti-inflammatory molecule [78]. Recently, using virtual screening methods tailored for PPARγ [79], further selective agonists that inhibit phosphorylation of PPARγ at serine 273 were shown to produce antidiabetic effects *in vivo*, such as the compounds F12016 [80] and L312 [81].

Notably, knockdown of NCoR1 that interacts with CDK5 in adipose tissue [82], or using PPARγ ligands that result in efficient inhibition of NCoR1 (and CDK5) binding to PPARγ [41], strongly increased insulin sensitivity in diet-induced insulin-resistant obese mice. In general, several PPARγ ligands seem to exert insulin-sensitizing effects via inhibition of the interaction with the repressor NCoR1. Making use of this specific mechanism may hold promise for future development. For example, the above-mentioned antidiabetic amorfrutins were at least as efficient as rosiglitazone in inhibiting the recruitment of NCoR1 peptides to the LBD of PPARγ [41,72].

Screening of potential chemical ligands of PPARγ has generally focused on conventional binding or reporter gene activity assays. However, *in vitro* tests assaying coactivator/repressor peptide recruitment to the LBD of PPARγ have been developed as alternatives for large-scale compound screening to identify hit molecules that activate PPARγ by disturbing interactions with its repressor NCoR1 [72]. Moreover, it becomes evident that PPARγ is modulated by various cellular signaling cascades, including for example mitogen-activated protein kinases (MAPKs) and extracellular signal-regulated kinases (ERK)s and by the resulting post-translational modifications of PPARγ [83]. Inhibiting these signaling pathways, and probably many other still unknown pathways, in adipose or other metabolic target tissues including the brain may provide multiple options for improving insulin resistance [84]. Moreover, the known beneficial effects of ligand-modulated PPARγ on inflammation, cancer, and other complex diseases may deserve further mechanistic studies of signalling pathways including PPARγ that go beyond the metabolic context.

Synergistic PPARγ–Ligand Peptide Conjugates – Discussion of a Promising Pharmacological Approach

Increasing tissue selectivity is a well-known pharmacological approach. In principle, orally administered PPARγ drugs, which are largely inactivated by first-pass liver metabolism, can selectively reach visceral fat including adipocytes and infiltering immune cells without affecting other tissues.

Tissue-targeting of PPARγ ligands in combination with a physiologically-active peptide hormone can potentially reduce side effects and additionally even boost metabolic response. Recently, this emerging synergistic pharmacological approach has been successfully applied to improve metabolic parameters in obese mice by using estrogen attached to modified incretin peptide hormones such as glucagon-like peptide-1 (GLP-1) (Figure 4A–C) [85–87]. This

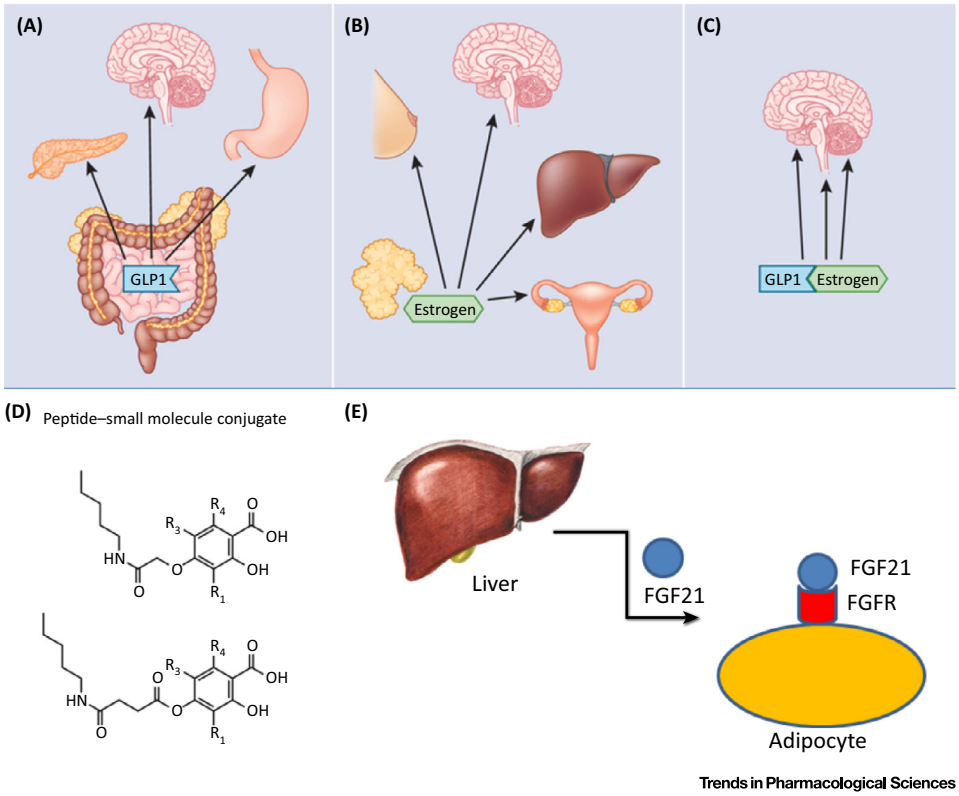

Figure 4. Potential Synergistic Activation of Small Molecule–Peptide Conjugates. This novel chemical approach has initially been demonstrated – using estrogen and a glucagon-like peptide (GLP-1)-derived peptide (see [86]). (A) The hormone GLP1 is produced by the gut, where it primarily acts in addition to pancreas and brain, thereby promoting physiological responses such as increasing insulin sensitivity as well as decreasing appetite and gastric emptying. (B) Estrogen is a gonadal steroid hormone which exerts pleiotropic effects via the activation of its nuclear (estrogen) receptor. Estrogen particularly affects sexual organs and brain function. (C) Combination of the effects of estrogen and GLP1 by forming a coupled compound. This conjugate compound can only be efficiently taken up by cells that express GLP1 receptor. Estrogen can then produce physiological effects through the nuclear estrogen receptor. The expression of receptors for GLP1 and estrogen in similar areas of the brain renders the conjugate selective and synergistic for improving metabolic parameters. Adverse effects associated with estrogen treatment in peripheral organs, such as the uterus and breast, are minimized. The top part of the figure has been reproduced with permission from [87]. (D) In principle, PPARγ ligands can be coupled to peptides using linkers containing ether (stable conjugates) or ester (unstable conjugates for control experiments) functionalities, as exemplified here for an accessible side group of an amorfrutin. (E) FGF21 (fibroblast growth factor 21) is secreted by the liver to communicate with adipocytes via FGFR (fibroblast growth factor receptor). Peptides that interact with FGFR can in principle be used for targeting PPARγ ligands to adipocytes. By linking a PPARγ ligand with an FGFR peptide-based ligand, in part complementary pathways (FGRF1 signaling and PPARγ signaling) could be activated. Future research will evaluate the potential of the emerging pharmacological approach depicted here to improve the action of nuclear receptor ligands and of other drugs.

compound–peptide conjugate approach strongly decreased known side effects of estrogens such as reproductive endocrine toxicity and oncogenicity. Interestingly, synergistic coagonism by the concerted action of GLP-1 and estrogen was observed. Recently, the peptide conjugate approach has been extended to rationally design a tri-agonist for treating obesity and diabetes [88]. Several clinical studies are currently further evaluating this innovative pharmaceutical approach (M. Tschöp, personal communication).

However, peptide-based biopharmaceuticals are in general unstable and can be easily degraded by various proteases. This feature prohibits oral administration, which is a disadvantage for daily application. To improve stability, peptides can be modified to avoid degradation *in vivo*. For example, the peptide part of above-mentioned GLP-1-estrogen conjugates is rapidly

inactivated by the endogenous enzyme dipeptidyl peptidase-4 (DPP-4) [86]. To prevent enzymatic degradation, 2-aminoisobutyric substitution of an alanine at the second position of GLP-1 has been used. Furthermore, resistance to proteolytic cleavage can be achieved by ether-conjugation using standard derivatization (Figure 4D).

Clearly, the compound–peptide conjugate concept can be used for many pharmaceuticals that – during permanent application – might cause adverse off-target effects. One important example in the nuclear receptor field could be steroid-based anti-inflammatory drugs that target the glucocorticoid receptor [89].

In principle, a ligand that would only target PPARγ in adipose tissues, without reaching other tissues, might feature less adverse effects. Depending on the structure, PPARγ ligands can be linked to stable, physiologically active peptides (as, for example, described in patent application WO/2014/177593). A conceivable variation of the above-described peptide–small molecules conjugate theme for targeting PPARγ might consist in linking ligands with (chemically modified) peptide hormones that specifically interact with cell-specific receptors of adipose tissue such as the fibroblast growth factor receptor 1 (FGFR1). For example, stimulation of GLUT1-dependent uptake of glucose while decreasing lipolysis in adipocytes is induced by fibroblast growth factor 21 (FGF21) (Figure 4E) [90] or by agonistic monoclonal antibodies (MAbs) specific to FGFR1 [91]. In general, these FGFR1 MAbs would not be expected to pass the blood–brain barrier, thus predominantly targeting peripheral metabolic target tissues, in particular fat depots in which PPARγ is highly abundant. FGFR1 is strongly expressed in adipose tissue, but at much lower levels in pancreas and kidney, and is almost undetectable in liver. FGFR1 MAbs facilitate dimerization and activation of the receptor, independently of β-Klotho, and synergistically with (endogenous and abundant) FGF21 [91]. Notably, FGFR1 MAbs primarily mediate their effects in adipose tissue and not – as FGF21 – also in liver.

In general, several hormones, growth factors, or cell surface receptors of growth factors of cells of white or brown adipose tissues can be explored as targets for treating type 2 diabetes. Thus, PPARγ ligands might be targeted via physiologically active peptides to tissues such as adipose tissue to boost specific effects and to reduce unwanted side effects. Although, this approach sounds very promising, its successful application for PPARγ ligands remains speculative. Multiple factors such as unknown bioavailability require in-depth investigation in the future.

Phytomedical or Dietary Extracts for Modulating PPARγ

Alternatives to therapeutic approaches for the prevention of metabolic disease [92], for example by using phytomedical or nutritional intervention, are presently a major topic of interest. Complex natural compound extracts exhibit rich resources for synergistically modulating nuclear receptors such as PPARs [93,94]. Clearly, well-developed analytics and standardization are highly important to ensure reproducible treatment effects of plant-derived compound extracts. These requirements will certainly promote general acceptance of plant extracts as well-controlled compound pools. However, highly enriched natural substances with significant physiological effects would probably also entail increased regulatory burdens compared to conventional dietary intervention.

Interestingly, several safe and widely applied phytomedical products from edible plants contain numerous unidentified, potent ligands of PPARγ that display wide structural diversity. For example, insulin-sensitizing chamomile (*Matricaria chamomilla*/*Matricaria recutita*) flowers ([94] and extracts of lemon balm (*Melissa officinalis*) [93] that contain a large fraction of flavonoids were shown to exert cellular effects via activation of PPARγ. For example, chamomile flower extract partially activated PPARγ with a half-maximal effective concentration (EC_{50}) of 86 μg/ml and with a maximal potency of 26% compared to rosiglitazone. The compounds in this extract

also activated at lower efficiency PPARα (EC_{50} = 3750 μg/ml) and PPARβ/δ (EC_{50} = 1204 μg/ml). Knockdown analyses in cell culture further validated PPAR-specific gene expression profiles of the chamomile extract. Typical insulin-sensitizing and liver-protective effects of chamomile extract were observed in insulin-resistant mice fed a high-fat diet [94].

Remarkably, at some stage subfractionation of extracts did not improve but even decreased PPARγ activation, suggesting (beyond potential loss of compounds during fractionation) extinction of typical combinatorial or synergistic effects of compound mixtures [95]. It thus seems that effects derived from poly-pharmacological mixtures can only be partly understood by mechanistic analyses, emphasizing the potential limitations of the key reductionist research paradigm of modern biology and chemistry.

Interestingly, a recent pilot, single-blind, randomized controlled clinical trial in 64 type 2 diabetes patients indicated significant insulin-sensitizing effects and improved serum lipid profiles in patients who consumed chamomile tea (3 g/150 mL hot water) three times per day immediately after meals for 8 weeks [96]. It seems plausible that chamomile tea consumption might also activate PPARs as the above-described chamomile extract, but this requires further evaluation. In summary, evidence gained by molecular insights into the action of various pools of natural products in food and phytomedical products can contribute to set up rational, evidence-based, molecular prevention and treatment strategies.

Concluding Remarks

PPARγ ligands can be optimized to develop drugs that function primarily by increasing insulin sensitivity and by lowering serum lipids. Furthermore, ligand-based modulation of PPARγ can be used for treating and preventing inflammatory disorders, and potentially other diseases such as cancer. Although the clinical application of TZDs has declined markedly, in particular owing to the reported adverse effects of rosiglitazone treatment, it would be a great mistake to dismiss new alternative PPARγ ligands merely because of 'guilt by association' [27].

Recent insights into the detailed biological mechanisms and somewhat surprising physiological roles of PPARγ have significantly enlarged our potential to modulate this nuclear receptor by small molecules while keeping the side effects to a minimum. Combined tissue-targeting and synergistic pharmacological modulation of PPARγ could afford a promising approach for developing efficient drugs and phytomedical or nutritional health-beneficial products.

Preclinical studies of PPARγ ligands will continue to focus on making use of specific activity-modulating mechanisms and on avoiding potential side effects. However, the adverse effects of compounds are difficult to predict, particularly at a systemic level. Recently, transcriptome and in particular proteome and metabolome data gained from rosiglitazone-treated cell and mouse models have provided valuable information on potential side effects such as cardiovascular implications of rosiglitazone treatment in diabetic mice [97–99]. Advanced omics methodologies might thus become powerful predictive tools to select early on the most likely safe and efficient candidates for clinical development [100]. Furthermore, it is known that about a quarter of patients are non-responders to TZDs, while an equal number showed a large response [101]. Genetic variation affecting PPARγ should be taken into consideration in the development of future PPAR-based drugs and in stratification of patients to ensure efficient treatments.

Key nuclear receptors such as PPARγ will remain important pharmacologic targets by making use of recently discovered physiological roles and activation mechanisms, as well as by the application of innovative treatment strategies (see Outstanding Questions). Future research will probably soon provide us with a next generation of efficient PPARγ-modulating ligands for treating and preventing metabolic and other common diseases.

Outstanding Questions

Will new insights into the activation mechanisms and physiological roles of PPARγ lead to efficient ligands to potentially treat diseases?

PPARγ has been a prime target for metabolic diseases over the past 20 years. Will recent insights into the roles of this nuclear receptor also become useful for treating other conditions, for example inflammatory disorders?

Will synergistic approaches, for example those based on compound–peptide conjugates, lead to more efficient activation of PPARγ with fewer side effects compared to conventional ligands? Can this emerging pharmacological compound–peptide conjugate concept be shown not only in mammalian disease models but also, most importantly, in human patients?

Evidence is emerging that some phytotherapeutic products can indeed inhibit metabolic diseases via activating nuclear receptors such as PPARs. However, can this traditional synergistic approach in the near future be evaluated by more-convincing and well-designed large-scale human studies to eventually develop new prevention strategies?

Prevention strategies depend on powerful diagnostic tools for the early detection of physiological deregulation and for monitoring the possibly marginal effects of mild interventions such as phytotherapeutics. Will such tools become available at low cost, potentially along with efficient tools for personalized treatment?

Will innovation in the field be patentable and economically transferable to foster large-scale investment in the development of PPARγ-based drugs or health-beneficial products? In other words: is research on PPARγ modulation likely to raise significant commercial interest in the future?

Acknowledgments

I thank Sophia Bauch and Luise Fuhr for help with some figures, and Magdalena Kliem for additional support. This work has been funded by the German Ministry for Education and Research (BMBF, grants 0315082, 01EA1303), the National Genome Research Net (NGFN, grant 01 GS 0828), and the European Commission (FP7/2007–2013 under grant agreement 262055 ESGI).

References

1. Mangelsdorf, D.J. *et al.* (1995) The nuclear receptor superfamily: the second decade. *Cell* 83, 835–839
2. Fu, M. *et al.* (2005) A Nuclear Receptor Atlas: 3T3-L1 adipogenesis. *Mol. Endocrinol.* 19, 2437–2450
3. Lazar, M.A. (2005) PPAR gamma, 10 years later. *Biochimie* 87, 9–13
4. Rosen, C.J. (2010) Revisiting the rosiglitazone story – lessons learned. *N. Engl. J. Med.* 363, 803–806
5. Barish, G.D. *et al.* (2005) A Nuclear Receptor Atlas: macrophage activation. *Mol. Endocrinol.* 19, 2466–2477
6. Lehrke, M. and Lazar, M.A. (2005) The many faces of PPARgamma. *Cell* 123, 993–999
7. Medina-Gomez, G. *et al.* (2007) Adipogenesis and lipotoxicity: role of peroxisome proliferator-activated receptor gamma (PPARgamma) and PPARgammacoactivator-1 (PGC1). *Public Health Nutr.* 10, 1132–1137
8. Medina-Gomez, G. *et al.* (2005) The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-gamma2 isoform. *Diabetes* 54, 1706–1716
9. Sugii, S. *et al.* (2009) PPARgamma activation in adipocytes is sufficient for systemic insulin sensitization. *Proc. Natl. Acad. Sci. U.S.A.* 106, 22504–22509
10. Schupp, M. and Lazar, M.A. (2010) Endogenous ligands for nuclear receptors: digging deeper. *J. Biol. Chem.* 285, 40409–40415
11. Haakonsson, A.K. *et al.* (2013) Acute genome-wide effects of rosiglitazone on PPARgamma transcriptional networks in adipocytes. *Mol. Endocrinol.* 27, 1536–1549
12. Gronemeyer, H. *et al.* (2004) Principles for modulation of the nuclear receptor superfamily. *Nat. Rev. Drug Discov.* 3, 950–964
13. Hu, E. *et al.* (1996) Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPARgamma. *Science* 274, 2100–2103
14. Qiang, L. *et al.* (2012) Brown remodeling of white adipose tissue by SirT1-dependent deacetylation of Ppargamma. *Cell* 150, 620–632
15. Ji, S. *et al.* (2012) O-GlcNAc modification of PPARgamma reduces its transcriptional activity. *Biochem. Biophys. Res. Commun.* 417, 1158–1163
16. Ohshima, T. *et al.* (2004) Transcriptional activity of peroxisome proliferator-activated receptor gamma is modulated by SUMO-1 modification. *J. Biol. Chem.* 279, 29551–29557
17. Kilroy, G.E. *et al.* (2009) PPAR-gamma AF-2 domain functions as a component of a ubiquitin-dependent degradation signal. *Obesity* 17, 665–673
18. Step, S.E. *et al.* (2014) Anti-diabetic rosiglitazone remodels the adipocyte transcriptome by redistributing transcription to PPARgamma-driven enhancers. *Genes Dev.* 28, 1018–1028
19. Kliewer, S.A. *et al.* (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. *Proc. Natl. Acad. Sci. U.S.A.* 94, 4318–4323
20. Krey, G. *et al.* (1997) Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol. Endocrinol.* 11, 779–791
21. Sauer, S. (2014) Amorfrutins: a promising class of natural products that are beneficial to health. *Chembiochem* 15, 1231–1238
22. Bruning, J.B. *et al.* (2007) Partial agonists activate PPARgamma using a helix 12 independent mechanism. *Structure* 15, 1258–1271
23. Zheng, W. *et al.* (2013) Identification of the antibiotic ionomycin as an unexpected peroxisome proliferator-activated receptor gamma (PPARgamma) ligand with a unique binding mode and effective glucose-lowering activity in a mouse model of diabetes. *Diabetologia* 56, 401–411
24. Chandra, V. *et al.* (2008) Structure of the intact PPAR-gamma–RXR-alpha nuclear receptor complex on DNA. *Nature* 456, 350–356
25. Tontonoz, P. and Spiegelman, B.M. (2008) Fat and beyond: the diverse biology of PPARgamma. *Annu. Rev. Biochem.* 77, 289–312
26. Rizzo, G. and Fiorucci, S. (2006) PPARs and other nuclear receptors in inflammation. *Curr. Opin. Pharmacol.* 6, 421–427
27. Soccio, R.E. *et al.* (2014) Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. *Cell Metab.* 20, 573–591
28. Hotamisligil, G.S. *et al.* (1996) IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 271, 665–668
29. Gregor, M.F. and Hotamisligil, G.S. (2011) Inflammatory mechanisms in obesity. *Annu. Rev. Immunol.* 29, 415–445
30. Prieur, X. *et al.* (2011) Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. *Diabetes* 60, 797–809
31. Zhang, H. *et al.* (2005) Collecting duct-specific deletion of peroxisome proliferator-activated receptor gamma blocks thiazolidinedione-induced fluid retention. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9406–9411
32. Ricote, M. and Glass, C.K. (2007) PPARs and molecular mechanisms of transrepression. *Biochim. Biophys. Acta* 1771, 926–935
33. Chung, S.W. *et al.* (2000) Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor-gamma and nuclear factor-kappa B. *J. Biol. Chem.* 275, 32681–32687
34. Ruan, H. *et al.* (2003) Troglitazone antagonizes tumor necrosis factor-alpha-induced reprogramming of adipocyte gene expression by inhibiting the transcriptional regulatory functions of NF-kappaB. *J. Biol. Chem.* 278, 28181–28192
35. Kelly, D. *et al.* (2004) Commensal anaerobic gut bacteria attenuate inflammation by regulating nuclear-cytoplasmic shuttling of PPAR-gamma and RelA. *Nat. Immunol.* 5, 104–112
36. Hou, Y. *et al.* (2012) PPARgamma is an E3 ligase that induces the degradation of NFkappaB/p65. *Nat. Commun.* 3, 1300
37. Nielsen, R. *et al.* (2008) Genome-wide profiling of PPARgamma: RXR and RNA polymerase II occupancy reveals temporal activation of distinct metabolic pathways and changes in RXR dimer composition during adipogenesis. *Genes Dev.* 22, 2953–2967
38. Evans, R.M. *et al.* (2004) PPARs and the complex journey to obesity. *Nat. Med.* 10, 355–361
39. Pourcet, B. *et al.* (2006) Selective PPAR modulators, dual and pan PPAR agonists: multimodal drugs for the treatment of type 2 diabetes and atherosclerosis. *Expert Opin. Emerg. Drugs* 11, 379–401
40. Sakamoto, J. *et al.* (2000) Activation of human peroxisome proliferator-activated receptor (PPAR) subtypes by pioglitazone. *Biochem. Biophys. Res. Commun.* 278, 704–711
41. Weidner, C. *et al.* (2012) Amorfrutins are potent antidiabetic dietary natural products. *Proc. Natl. Acad. Sci. U.S.A.* 109, 7257–7262

42. Maltarollo, V.G. *et al.* (2015) Structure-based virtual screening and discovery of new PPARdelta/gamma dual agonist and PPARdelta and gamma agonists. *PLoS ONE* 10, e0118790
43. Lehmann, J.M. *et al.* (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J. Biol. Chem.* 270, 12953–12956
44. Lalloyer, F. and Staels, B. (2010) Fibrates, glitazones, and peroxisome proliferator-activated receptors. *Arterioscler. Thromb. Vasc. Biol.* 30, 894–899
45. Nolte, R.T. *et al.* (1998) Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* 395, 137–143
46. Natali, A. and Ferrannini, E. (2006) Effects of metformin and thiazolidinediones on suppression of hepatic glucose production and stimulation of glucose uptake in type 2 diabetes: a systematic review. *Diabetologia* 49, 434–441
47. Phielix, E. *et al.* (2011) The role of metformin and thiazolidinediones in the regulation of hepatic glucose metabolism and its clinical impact. *Trends Pharmacol. Sci.* 32, 607–616
48. Ye, J.M. *et al.* (2004) Direct demonstration of lipid sequestration as a mechanism by which rosiglitazone prevents fatty-acid-induced insulin resistance in the rat: comparison with metformin. *Diabetologia* 47, 1306–1313
49. Hauner, H. (2002) The mode of action of thiazolidinediones. *Diab. Metab. Res. Rev.* 18 (Suppl. 2), S10–S15
50. Colca, J.R. *et al.* (2013) Clinical proof-of-concept study with MSDC-0160, a prototype mTOT-modulating insulin sensitizer. *Clin. Pharmacol. Ther.* 93, 352–359
51. Divakaruni, A.S. *et al.* (2013) Thiazolidinediones are acute, specific inhibitors of the mitochondrial pyruvate carrier. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5422–5427
52. Feinstein, D.L. *et al.* (2005) Receptor-independent actions of PPAR thiazolidinedione agonists: is mitochondrial function the key? *Biochem. Pharmacol.* 70, 177–188
53. Rousseaux, C. *et al.* (2005) Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. *J. Exp. Med.* 201, 1205–1215
54. Dubuquoy, L. *et al.* (2006) PPARgamma as a new therapeutic target in inflammatory bowel diseases. *Gut* 55, 1341–1349
55. Fuhr, L. *et al.* (2015) Amorfrutins are natural PPARγ agonists with potent anti-inflammatory properties. *J. Nat. Prod.* 78, 1160–1164
56. Khandekar, M.J. *et al.* (2011) Molecular mechanisms of cancer development in obesity. *Nat. Rev. Cancer* 11, 886–895
57. Home, P.D. *et al.* (2009) Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial. *Lancet* 373, 2125–2135
58. Tolman, K.G. (2011) The safety of thiazolidinediones. *Expert Opin. Drug Saf.* 10, 419–428
59. Kean, S. (2010) Planned study of Avandia in doubt after FDA review. *Science* 329, 375
60. Woodcock, J. *et al.* (2010) Regulatory action on rosiglitazone by the U.S. Food and Drug Administration. *N. Engl. J. Med.* 363, 1489–1491
61. McCarthy, M. (2013) US regulators relax restrictions on rosiglitazone. *BMJ* 347, f7144
62. Mitka, M. (2013) FDA eases restrictions on the glucose-lowering drug rosiglitazone. *JAMA* 310, 2604
63. Kahn, S.E. *et al.* (2006) Glycemic durability of rosiglitazone, metformin, or glyburide monotherapy. *N. Engl. J. Med.* 355, 2427–2443
64. Dormandy, J.A. *et al.* (2005) Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet* 366, 1279–1289
65. Schneider, C.A. *et al.* (2008) Effect of pioglitazone on cardiovascular outcome in diabetes and chronic kidney disease. *J. Am. Soc. Nephrol.* 19, 182–187
66. Tzoulaki, I. *et al.* (2009) Risk of cardiovascular disease and all cause mortality among patients with type 2 diabetes prescribed oral antidiabetes drugs: retrospective cohort study using UK general practice research database. *BMJ* 339, b4731
67. McDonough, A.K. *et al.* (2008) The effect of thiazolidinediones on BMD and osteoporosis. *Nat. Clin. Pract. Endocrinol. Metab.* 4, 507–513
68. Vallarino, C. *et al.* (2013) Comparing pioglitazone to insulin with respect to cancer, cardiovascular and bone fracture endpoints, using propensity score weights. *Clin. Drug Invest.* 33, 621–631
69. Tobin, J.F. and Freedman, L.P. (2006) Nuclear receptors as drug targets in metabolic diseases: new approaches to therapy. *Trends Endocrinol. Metab.* 17, 284–290
70. Balint, B.L. and Nagy, L. (2006) Selective modulators of PPAR activity as new therapeutic tools in metabolic diseases. *Endocr. Metab. Immune Dis. Drug Targets* 6, 33–43
71. Doshi, L.S. *et al.* (2010) Discovery and development of selective PPAR-gamma modulators (SPPAR-gamma Ms) as safe and effective antidiabetic agents. *Expert Opin. Invest. Drug* 19, 489–512
72. Weidner, C. *et al.* (2013) Amorfrutin B is an efficient natural peroxisome proliferator-activated receptor gamma (PPAR-gamma) agonist with potent glucose-lowering properties. *Diabetologia* 56, 1802–1812
73. Aidhen, I.S. *et al.* (2015) A common building block for the syntheses of amorfrutin and cajaninstilbene acid libraries toward efficient binding with peroxisome proliferator-activated receptors. *Org. Lett.* 17, 194–197
74. Schupp, M. *et al.* (2005) Molecular characterization of new selective peroxisome proliferator-activated receptor gamma modulators with angiotensin receptor blocking activity. *Diabetes* 54, 3442–3452
75. Benardeau, A. *et al.* (2009) Aleglitazar, a new, potent, and balanced dual PPARalpha/gamma agonist for the treatment of type II diabetes. *Bioorg. Med. Chem. Lett.* 19, 2468–2473
76. Loke, Y.K. *et al.* (2011) Comparative cardiovascular effects of thiazolidinediones: systematic review and meta-analysis of observational studies. *BMJ* 342, d1309
77. Choi, J.H. *et al.* (2011) Antidiabetic actions of a non-agonist PPARgamma ligand blocking Cdk5-mediated phosphorylation. *Nature* 477, 477–481
78. Choi, S.S. *et al.* (2014) A novel non-agonist peroxisome proliferator-activated receptor gamma (PPARgamma) ligand UHC1 blocks PPARgamma phosphorylation by cyclin-dependent kinase 5 (CDK5) and improves insulin sensitivity. *J. Biol. Chem.* 289, 26618–26629
79. Guasch, L. *et al.* (2012) Identification of PPARgamma partial agonists of natural origin (I): development of a virtual screening procedure and in vitro validation. *PLoS ONE* 7, e50816
80. Jiang, W. *et al.* (2014) SIRT1 protects against apoptosis by promoting autophagy in degenerative human disc nucleus pulposus cells. *Sci. Rep.* 4, 7456
81. Xie, X. *et al.* (2015) L312, a novel PPARgamma ligand with potent anti-diabetic activity by selective regulation. *Biochim. Biophys. Acta* 1850, 62–72
82. Li, P. *et al.* (2011) Adipocyte NCoR knockout decreases PPARgamma phosphorylation and enhances PPARgamma activity and insulin sensitivity. *Cell* 147, 815–826
83. Banks, A.S. *et al.* (2015) An ERK/Cdk5 axis controls the diabetogenic actions of PPARgamma. *Nature* 517, 391–395
84. Thaler, J.P. *et al.* (2012) Obesity is associated with hypothalamic injury in rodents and humans. *J. Clin. Invest.* 122, 153–162
85. Finan, B. *et al.* (2013) Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci. Transl. Med.* 5, 209ra151
86. Finan, B. *et al.* (2012) Targeted estrogen delivery reverses the metabolic syndrome. *Nat. Med.* 18, 1847–1856
87. Dietrich, M.O. and Horvath, T.L. (2012) A marriage made to last in drug design. *Nat. Med.* 18, 1737–1738
88. Finan, B. *et al.* (2015) A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. *Nat. Med.* 21, 27–36

89. Stahn, C. *et al.* (2007) Molecular mechanisms of glucocorticoid action and selective glucocorticoid receptor agonists. *Mol. Cell. Endocrinol.* 275, 71–78

90. Kharitonenkov, A. (2009) FGFs and metabolism. *Curr. Opin. Pharmacol.* 9, 805–810

91. Wu, A.L. *et al.* (2011) Amelioration of type 2 diabetes by antibody-mediated activation of fibroblast growth factor receptor 1. *Sci. Transl. Med.* 3, 113ra126

92. Ezzati, M. and Riboli, E. (2012) Can noncommunicable diseases be prevented? Lessons from studies of populations and individuals. *Science* 337, 1482–1487

93. Weidner, C. *et al.* (2014) Lemon balm extract causes potent antihyperglycemic and antihyperlipidemic effects in insulin-resistant obese mice. *Mol. Nutr. Food Res.* 58, 903–907

94. Weidner, C. *et al.* (2013) Antidiabetic effects of chamomile flowers extract in obese mice through transcriptional stimulation of nutrient sensors of the peroxisome proliferator-activated receptor (PPAR) family. *PLoS ONE* 8, e80335

95. Wagner, H. and Ulrich-Merzenich, G. (2009) Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomedicine* 16, 97–110

96. Rafraf, M. *et al.* (2015) Effectiveness of chamomile tea on glycemic control and serum lipid profile in patients with type 2 diabetes. *J. Endocrinol. Invest.* 38, 163–170

97. Lamb, J. *et al.* (2006) The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science* 313, 1929–1935

98. Meierhofer, D. *et al.* (2013) Protein sets define disease states and predict in vivo effects of drug treatment. *Mol. Cell. Proteomics* 12, 1965–1979

99. Meierhofer, D. *et al.* (2014) Integrative analysis of transcriptomics, proteomics, and metabolomics data of white adipose and liver tissue of high-fat diet and rosiglitazone-treated insulin-resistant mice identified pathway alterations and molecular hubs. *J. Proteome Res.* 13, 5592–5602

100. Sauer, S. (2013) Protein set analyses: how could this impact the clinic? *Expert Rev. Proteomics* 10, 305–307

101. Sears, D.D. *et al.* (2009) Mechanisms of human insulin resistance and thiazolidinedione-mediated insulin sensitization. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18745–18750

102. Ryan, K.K. *et al.* (2011) A role for central nervous system PPAR-gamma in the regulation of energy balance. *Nat. Med.* 17, 623–626

103. Lu, M. *et al.* (2011) Brain PPAR-gamma promotes obesity and is required for the insulin-sensitizing effect of thiazolidinediones. *Nat. Med.* 17, 618–622

104. Myers, M.G., Jr and Burant, C.F. (2011) PPAR-gamma action: it's all in your head. *Nat. Med.* 17, 544–545

105. Michelotti, G.A. *et al.* (2013) NAFLD, NASH and liver cancer. *Nat. Rev. Gastroenterol. Hepatol.* 10, 656–665

106. Hasenfuss, S.C. *et al.* (2014) Regulation of steatohepatitis and PPARgamma signaling by distinct AP-1 dimers. *Cell Metab.* 19, 84–95