Factors Modulating Fibrates Response: Therapeutic Implications and Alternative Strategies

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Abstract: Peroxisome proliferator-activated receptor alpha (PPARα) regulates transcription of genes involved both in lipid and glucose metabolism as well as in inflammation. Fibrates are PPARα ligands used to normalize lipid and glucose parameters and exert antiinflammatory effects. In fact, fibrates have already been demonstrated to benefit metabolic syndrome, type 2 diabetes and cardiovascular diseases. This article reviews the mechanism of action and the functional roles of fibrates, emphasizing the factors modulating their capacity to activate PPARα and affecting their effectiveness. These factors may possibly explain the findings obtained in animal studies and clinical trials with fibrates which showed either untoward effects and/or inefficient hypolipidemic action of PPARα activation. We also discuss briefly the natural and synthetic agonists of PPARα which are currently being developed and supposedly display greater effectiveness and fewer adverse effects than fibrates.

Key Words: Peroxisome proliferator-activated receptor, fibrates, natural PPAR agonists, synthetic PPAR activators, metabolic syndrome, type 2 diabetes, cardiovascular disease.

GENERAL ASPECTS OF PPARα

Together with the receptor for thyroid hormone, retinoids, steroid hormones and vitamin D, peroxisome proliferator-activated receptors (PPARs) belong to the nuclear hormone receptor superfamily [1]. PPARs are ligand-dependent transcription factors that regulate diverse aspects of energy homeostasis, lipid and lipoprotein metabolism, glucose homeostasis, amino acid metabolism, urea synthesis, and inflammatory and immune responses. PPARs were first identified in 1990, as receptors for fibrates, a class of hypolipidemic drugs used in humans since the late 1960s [2]. The name of PPARs was initially chosen because of their ability to induce the proliferation of peroxisomes in rodents [2]. Three PPAR isotypes have been identified: PPARα (NR1C1), PPARβ (also called –δ, NR1C2), and PPARγ (NR1C3) [1], which exhibit distinct tissue distribution reflecting their biological functions. PPARα expression is highest in tissues exhibiting high rates of fatty acid catabolism such as liver, kidney, heart, skeletal muscle and duodenum [3]. PPARγ is highly expressed in tissues characterized as lipid stores, such as adipose tissue, and PPARβ is ubiquitously expressed. Moreover, PPARs are expressed in vascular and immune cells.

The ligand-binding domain (LBD) of PPARs harbors a "large" pocket which allows it to accommodate many types of natural and synthetic ligands [4]. *Natural ligands*: In general, all PPAR isoforms present a higher selectivity to omega-3 (ω-3) and ω-6 long-chain polyunsaturated fatty acids (PUFA) than to saturated or monounsaturated fatty acids [5]. These fatty acids bind all three PPAR, with PPARα exhibiting the highest affinity. Saturated fatty acids are poor PPAR ligands, whereas branched-chain and isoprenoid-derived fatty acids efficiently bind PPARα [5]. Thus, eicosanoids derived from arachidonic acid *via* lipoxygenase pathway, leukotrienes and oxidized fatty acids, all involved in inflammatory processes, are also natural ligands for PPARα [5]. *Synthetic ligands*: PPARs are also activated by peroxisome proliferators, a large class of structurally diverse compounds that include hypolipidemic drugs, plasticizers, herbicides and solvents. These hypolipidemic agents are fibrates such as clofibrate, fenofibrate, bezafibrate, gemfibrozil and, an experimental compound, Wy-14,643. Other synthetic compounds that bind to PPARα include the 5,8,11,14 eicosatetraynoic acid, an arachidonic acid analog [5], and non-steroidal antiinflammatory drugs, which also appear to activate PPARγ [6]. Fibrates preferentially bind PPARα [2], whereas thiazolidinediones (TZDs) are a class of antidiabetic drugs which selectively bind PPARγ [7]. It also seems that retinoid X receptor (RXR) (NR2B) agonists, such as the natural ligand 9-cis retinoic acid, can induce PPAR:RXR heterodimers and activate PPAR target genes [8].

MECHANISM OF ACTION AND FUNCTIONAL ROLES

The biological and therapeutic activities of PPAR are the result of the combination of two distinct mechanisms (Fig. (**1**)): i) one ligand-dependent transactivation controls metabolic effects, and ii) ligand-dependent transrepression, which controls vascular effects. Since the present article is concerned with fibrates, which mainly act on PPARα, we will focus on the mechanism of action of this PPAR subtype.

TRANSCRIPTIONAL ACTIVATION

The typical function of PPAR is to activate transcription in a ligand-dependent manner, following direct binding to DNA response elements in the promoter or enhancer regions of target genes. Transcriptional regulation by PPARs requires heterodimerization with RXR, which belongs to the same receptor superfamily. The PPAR:RXR complex, acti-

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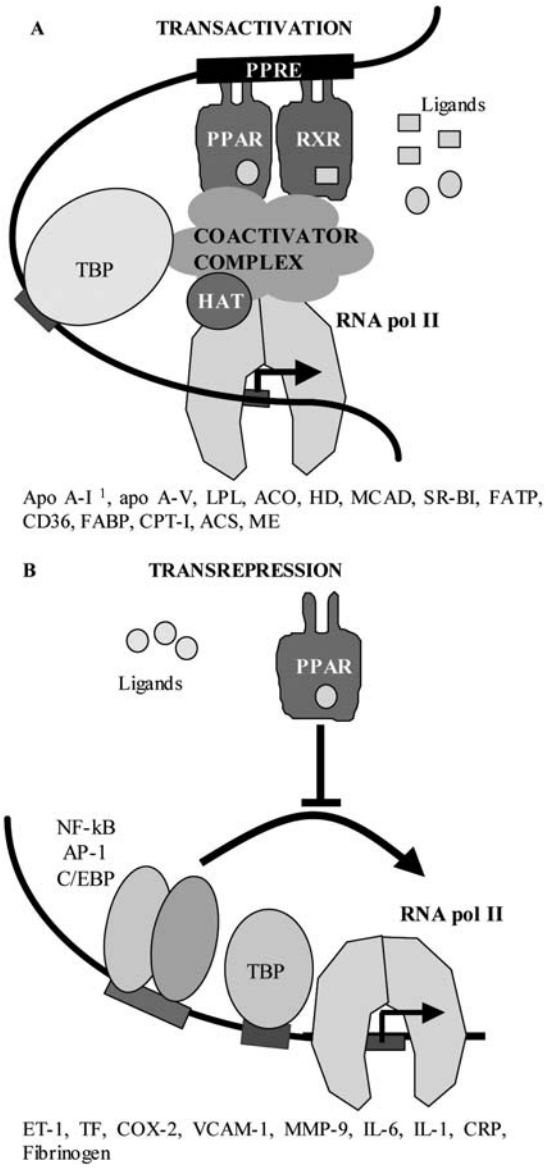

Fig. (1). Mechanisms of PPARα signalling. A) Transactivation: PPARα regulates transcription of its target genes by heterodimerization with RXR. The PPARα/RXR heterodimer binds to PPRE located in the promoter of target genes. The activated PPARα/RXR heterodimer associates with cofactors containing histone acetyltransferase (HAT), modifying nucleosome structure, contacting general transcription factors and allowing gene transcription. Some of the target genes activated through PPARα are shown. **B**) Transrepression: PPARα may repress gene transcription by several different mechanisms (see text) that are independent of DNA binding. This mechanism includes interference by PPARα with other transcriptional factors, which modulate the expression of genes involved in inflammatory pathways. TBP: TATA binding protein; RNA pol: RNA polymerase. [1] Different effect in humans and rodents.

vated by ligand, modulates transcription *via* binding to a specific DNA sequence element, peroxisome proliferator response element or PPRE. This response element is generally composed of two half-sites that occur as a direct repetition of the consensus sequence AGGTCA with a single nucleotide spacing between the two repeats. Hence, it is named DR-1. Nevertheless, the ability of nuclear receptors to initiate or suppress the transcription process relies on their interaction with negatively or positively acting cofactors. These cofactors serve as a bridge between transcription factors and the basic transcription machinery (Fig. (**1A**)) and, more importantly, contain several enzymatic activities controlling gene expression by specifically modifying chromatin and DNA structure. Acetylation of hystones is one critical regulatory mechanism by which gene expression is regulated. In general, increased levels of histone acetylation loosen chromatine packaging and have been correlated with transcriptional activation, whereas decreased activity of histone acetylase is associated with transcriptional repression [9]. In the absence of a ligand, PPAR:RXR heterodimers associates with corepressors, containing histone deacetylase activity [10]. The deacetyled state of histone inhibits transcription [11]. On the other hand, ligand binding induces conformational change in the PPAR, resulting in the release of corepressors and recruitment of coactivators. Some coactivators present histone acetylase activity [11]. Interaction of nuclear receptors with these coactivators results in the binding of the heterodimer to PPRE in the promoter, modification of the chromatine structure and either the activation or supression of the transcription of the target gene. This model implies that chromatine is flexible enough to allow looping (Fig. (**1A**)).

GENE REPRESSION

PPAR can also negatively regulate gene expression in a ligand-dependent manner by inhibiting the activities of other transcription factors (Fig. (**1B**)), such as members of nuclear factor–kappa B (NF-κB), activator protein-1 (AP-1) families [12,13], signal transducer and activator of transcription (STAT), CCAAT/enhancer binding protein (C/EBP) [14], hepatocyte nuclear factor 4 alpha (HNF4α) and others [15]. This mechanism does not involve binding to typical receptor specific response elements in the DNA molecule. However, despite the large number of studies carried out, the mechanisms by which PPARs inhibit these signal transduction pathways are not completely understood. Ricote *et al.* [16] have recently reviewed the molecular mechanisms that may account for gene-specific transrepression by PPAR ligands: direct protein-protein interactions between PPAR and other transcription factors; modulation of kinase activity; competition for the coactivators; and the corepressor-dependent model. Moreover, one of these models of transrepression has established that PPARα upregulates expression of the NF-kB repressor, inhibitor of kappa B (IκB), which sequesters the NF-κB subunits and prevents their translocation to the nucleus and, consequently, their DNA binding activity [17]. Thus, PPARα can inhibit genes induced by NF-kB. On the other hand, the interference of PPARα with the C/EBP and HNF4α signalling pathways can be explained, as PPARα decreases the expression of HNF4α [18] as well as that of C/EBP [19].

EFFECTS IN LIPID METABOLISM

Effects in Very Low Density Lipoprotein (VLDL) Catabolism

Lipoprotein lipase (LPL), a key enzyme in lipoprotein metabolism, hydrolyzes the triacylglycerol (TG) moiety of chylomicrons and VLDL particules. Studies *in vivo* indicate

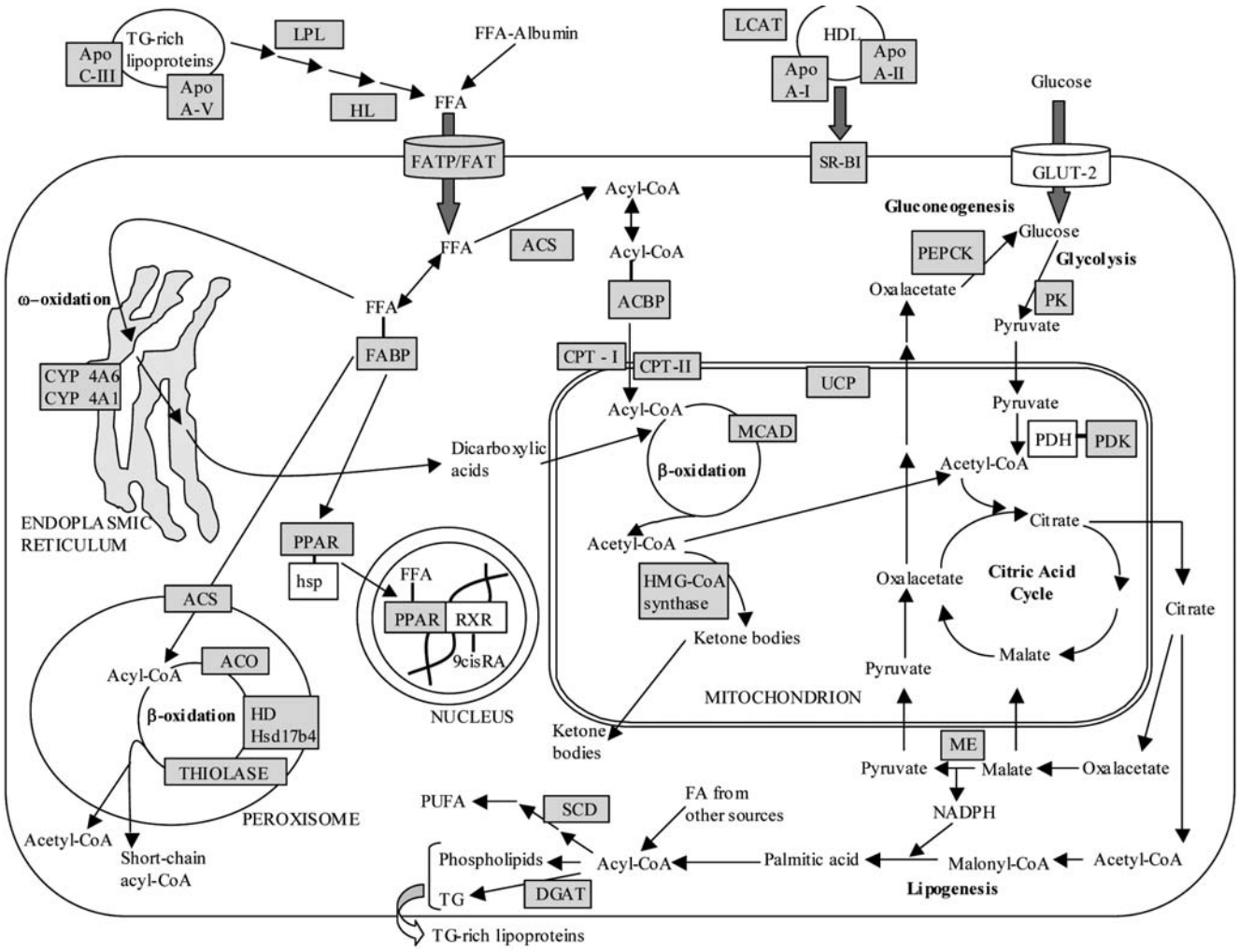

Fig. (2). Main lipid and glucose metabolic pathways regulated by PPARα. Genes encoding the proteins indicated with a grey box are regulated by PPARα. GLUT: glucose transporter; HMG-CoA: 3-hydroxy-3-methylglutaryl-CoA; FFA: free fatty acids; 9cis-RA: 9-cis retinoic acid.

that whereas fibrates and fatty acids clearly induce LPL mRNA in liver (Fig. (**2**)), contradictory results have been found in adipose and other extrahepatic tissues [15, 20, 21]. Thus, the effect of PPARα agonists on plasma TG-rich lipoprotein removal seems to be mainly due to changes in the hepatic expression of inhibitors (apolipoprotein (apo) C-III) and activators (apo A-V) of LPL activity (Fig. (**2**)) [15]. In fact, apo C-III levels appear to correlate positively with plasma TG concentrations. On the other hand, the recently discovered apo A-V has been shown to play an important role in determining TG levels in humans and mice, and represents the first described apolipoprotein gene in which overexpression lowers TG levels. Thus, PPARα activators enhance the catabolism of VLDL, which results in changes in low density lipoprotein (LDL) composition, increasing its removal by the tissues.

Effects in VLDL Production

Any process that may limit the liver availability of fatty acids for conversion to TG will affect VLDL production. First of all, cellular uptake of long-chain fatty acids is facilitated and regulated by fatty acid transporters, such as the fatty acid transporter protein (FATP) and the fatty acid translocase (FAT), also called CD36. The expression of both fatty acid transporters in rat liver is enhanced by fibrates or fatty acids (Fig. (**2**)) [22]. The conversion of free fatty acids (FFA) to acyl-CoA derivatives by acyl-CoA synthetase (ACS) prevents efflux from the cell, and activates FFA for utilization in both catabolic and/or anabolic pathways. ACS gene expression and activity are induced by fibrates and fatty acids in a variety of tissues and cells (Fig. (**2**)) [23]. Once in the cytoplasm, FFA bind to transport proteins, such as the fatty acid binding protein (FABP) and acyl-CoA binding protein (ACBP). Hypolipidemic drugs are also able to induce expression of FABP *via* PPARα (Fig. (**2**)) [24].

A process that may limit the fatty acid availability for conversion to TG and subsequent conversion as VLDL is fatty acid catabolism [15]. The critical role of PPARα agonists in the regulation of oxidation of fatty acids has been well documented. As already commented, peroxisome proliferation was one of the first effects described in rodents after exposure to synthetic PPARα activators, and it has been

related to an increase of the peroxisomal fatty acid β-oxidation activity [25]. This pathway is responsible for the metabolism of long-chain fatty acids and sequentially involves the enzymes acyl-CoA oxidase (ACO), enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase (or L-bifunctional enzyme, HD) and 3-ketoacyl-CoA thiolase [26]. The L- and D-hydroxy intermediates, generated by the first step of the β–oxidation, can be metabolized by either HD protein or D-bifunctional protein (Hsd17b4) [27]. The activities of all these enzymes are stimulated in response to PPARα agonists, due to changes in the transcription rates of their genes (Fig. (**2**)) [28]. Thus, for example, Corton *et al.* [29] showed that Hsd17b4 expression was augmented by peroxisome proliferators in rodents. Moreover, trypsin domain containing 1 (Tysnd1) is a protease whose expression is induced by the PPARα agonist bezafibrate. Recently, Kurochkin *et al.* [30] have proposed a model suggesting that Tysnd1 mediates the processing of the peroxisomal enzymes (ACO, Hsd17b4, and thiolase) which promotes their assembly into a supramolecular complex to enhance the rate of β-oxidation.

Nevertheless, fatty acid metabolism is mainly focused on its mitochondrial oxidation. The first limiting step in mitochondrial β–oxidation is the entry flux of fatty acids into the mitochondria. Carnitine palmitoyl transferase I (CPT-I) catalyzes the formation of fatty acyl-carnitine for translocation across the inner mitochondrial membrane. The CPT-I gene expression is upregulated by fatty acids and peroxisome proliferators (Fig. (**2**)) [25]. PPARα further regulates the mitochondrial β–oxidative spiral by modulating the expression of the medium-chain acyl-CoA dehydrogenase (MCAD) gene (Fig. (**2**)) [31]. Mitochondrial β–oxidation greatly contributes to energy production *via* oxidative phosphorylation generating ATP. However, PPARα appears to increase energy expenditure by inducing the expression of uncoupling proteins (UCPs) (Fig. (**2**)) [32], such as UCP1, UCP2 and UCP3, mitochondrial transporters localized in the inner mitochondrial membrane that act to dissipate the proton gradient and increase thermogenesis while reducing the efficiency of ATP synthesis. On the other hand, the cytochrome monooxygenase system plays a central role in the oxidation of a wide variety of endogenous as well as exogenous compounds. The microsomal CYP4A enzymes participate in the system as a distinct group of the cytochrome P450 superfamily. They catalyze the ω–hydroxylation of fatty acids and eicosanoids, such as leukotriene LTB4. In fact, ω–hydroxylation is the first step in the neutralization of LTB4, which is then completely degraded through β–oxidation in the peroxisomes [25]. At least two of the CYP4A genes, CYP4A1 and CYP4A6, respond both *in vivo* and in cell culture to PPAR activators (Fig. (**2**)) [33, 34].

Finally, PPARα is also involved in fatty acid synthesis. It has been shown that the lipogenic malic enzyme (ME) gene is upregulated by peroxisome proliferators *via* PPARα (Fig. (**2**)) [35]. The reaction catalyzed by the ME consists of the oxidative decarboxylation of cytosolic malate, which generates pyruvate and leads to the formation of NADPH, which is required for lipid synthesis. With regard to the expression of genes more directly involved in TG synthesis, PPARα has recently been described as stimulating diacylglycerol acyltransferase (DGAT) gene expression (Fig. (**2**)) [15]. However, PPARα agonists have been shown to increase DGAT activity in cytoplasm and to decrease it in endoplasmic reticulum, possibly diverting TG from incorporation into VLDL to the accumulation in cytosolic droplets [15].

Effects in High Density Lipoprotein (HDL) Catabolism

PPARα appears to be the major isoform implicated in HDL metabolism [15]. Fibrates affect the expression of the enzymes and receptors regulating HDL metabolism. In rats, fibrates decrease the production of HDL-remodelling enzymes, such as hepatic lipase (HL) and lecithin:cholesterol acyltransferase (LCAT) (Fig. (**2**)) [36, 37]. Murine scavenger receptor I (SR-BI) and its human homologue CLA-I have been identified as HDL receptors which bind HDL with high affinity. They mediate the selective uptake of cholesteryl esters by liver and steroidogenic tissues [38, 39], and may also promote cholesterol removal from peripheral cells, including macrophages. Treatment of human macrophages with PPARα activators resulted in the induction of CLA-I expression, suggesting that PPARα promotes cholesterol efflux to HDL in macrophages [15]. Furthermore, SR-BI is induced in aortas of apo E deficient mice when treated with a PPAR agonist. On the contrary, fibrates through PPARα reduce SR-BI gene expression in rodent liver (Fig. (**2**)) [15].

EFFECTS IN GLUCOSE METABOLISM

PPARs also play an important role in glucose homeostasis. In fact, PPARα agonists, by up-regulating fatty acid oxidation and ketone body production, are able to spare glucose. Accordingly, PPARα null mice present hypoglycemia during starvation due to a diminished capability to oxidize fatty acids and a reduced capacity for hepatic gluconeogenesis [40]. Furthermore, several studies have indicated a beneficial effect of PPARα activation on insulin sensitivity [41, 42]. Thus, hyperinsulinemia and hyperglycemia observed in both mice subjected to a high-fat diet and genetic insulin resistant rodents [41], were sharply lowered by treatment with fibrates.

More specifically, the glycolitic enzyme, liver-type pyruvate kinase (L-PK) plays a key role in hepatic glucose and lipid metabolism. L-PK gene transcription is inhibited by PUFA ω–3 and activators of PPARα (Fig. (**2**)). Moreover, PUFA and Wy-14,643 interfere, although by different mechanisms, with glucose-stimulated pyruvate kinase gene transcription both *in vivo* and in rat primary hepatocytes [43]. On the other hand, pyruvate dehydrogenase catalyzes the transformation of pyruvate into acetyl-CoA, and therefore controls both the transformation of pyruvate to different metabolic compounds such as fatty acid or amino acids, and its complete oxidation. The pyruvate dehydrogenase kinase 4 (PDK4) inactivates the pyruvate dehydrogenase complex *via* phosphorylation, thus guiding the utilization of pyruvate to gluconeogenesis rather than its oxidation to acetyl-CoA. PPARα exerts a direct role in the regulation of gluconeogenesis *via* stimulation of the expression of PDK4 (Fig. (**2**)) [44]. In fasted PPARα null mice, glucose synthesis from lactate is strongly reduced, demonstrating that PPARα also influences substrate utilization for glucose production in the liver [45]. However, hepatic glucose production was surprisingly higher in the PPARα null mice than in the wild type

animals at the end of a fasting period, due to increased glucose production from glycerol in these animals [45]. These data reveal that the severe hypoglycemia observed in the PPARα null mice during fasting is not due to reduced glucose production but to increased glucose disposal [46]. Another enzyme of the glucose biosynthesis pathway regulated by PPARα is phosphoenolpyruvate carboxykinase (PEPCK), which catalyzes a rate-limiting step in gluconeogenesis. However, there are conflicting results regarding the consideration of PEPCK as a PPARα target gene in liver (Fig. (**2**)) [47], since expression of the PEPCK gene is stimulated by a fasted state in both wild-type and PPARα-null mice [40]. PEPCK gene expression is responsive to fatty acids in adipocytes but not in hepatocytes [48].

As regards insulin-sensitizing effects, genetic ablation of PPARα has no effect on insulin sensitivity [49]. Thus, PPARα null mice on normal chow did not demonstrate signs of insulin resistance in spite of having markedly increased circulating fatty acids and TG [50]. Moreover, PPARα null mice have also been shown to be protected from developing insulin resistance when on a high-fat diet or more simply, during ageing [49]. On the other hand, other authors, using a hyperinsulinemic-euglycemic clamp, have proposed that PPARα deficiency does not protect against high-fat diet-induced insulin resistance [50]. Curiously, Knauf *et al.* [51] activated brain PPARα in wild-type mice by infusing Wy-14,643 into the lateral ventricle and showed that whole-body glucose use was reduced. These same authors reexpresssed PPARα in the liver of knockout mice using recombinant adenoviruses, however, the whole-body glucose use rate remained elevated [51]. These findings would suggest that, although PPARα is involved in the regulation of insulin sensitivity, it is done by mechanisms that do not require PPARα expression in the liver.

EFFECTS IN VASCULAR AND HEPATIC INFLAMMATION

The role of inflammation in atherosclerosis is well established [14]. The vascular lesions of atherosclerosis are not only the result of lipid accumulation but also the result of vascular injury leading to activation of specific cellular and molecular responses in the vascular wall and the immune system. PPAR controls vascular biology, including cell recruitment and activation, the local inflammatory response, vascular constriction and cell migration and thrombosis [4, 14]. PPARα inhibits genes of inflammatory markers induced by NF-κB (Fig. (**1B**)), such as vascular cell-adhesion molecule-1 (VCAM-1), cyclooxygenase-2 (COX-2) and interleukins (IL-6 and IL-1), providing a molecular basis for the antiinflammatory effect of PPARα ligands *in vivo*. Moreover, similar to synthetic PPARα agonists, LPL-treated VLDL represses cytokine-induced VCAM-1 expression in wild-type but not in PPARα-deficient endothelial cells (ECs) [52]. PPARα also inhibits the production of endothelin-1 (ET-1) and tissue factor (TF) in ECs, vascular smooth muscle cells (VSMCs) and macrophages [13, 53], and increases the expression of endothelial nitric oxide synthase (eNOS) in vascular cells [54]. All these effects improve endothelial function and increase nitric oxide production [54], therefore producing a protective vasodilatatory effect. Furthermore, Delerive *et al.* [55] have demonstrated that aortas from PPARα null mice display an exacerbated inflammatory response to lipopolysaccharide (LPS) stimulation demonstrating that the antiinflammatory activity of PPARα agonists requires the nuclear receptor expression *in vivo*. On the other hand, PPARα agonist treatment increased LPS-induced plasma tumor necrosis factor (TNFα), a proinflammatory agent. Such effect was significantly decreased in PPARα null mice [56], suggesting a proinflammatory role of PPARα. In contrast, Poynter *et al.* have shown that splenocytes from PPARα null mice produce higher levels of IL-6, supporting a PPARα-dependent antiinflammatory action [57]. Matrix metalloproteinase 9 (MMP-9) [58] and TF [53] are macrophage proteins that promote lesion instability and coagulation, respectively. *In vitro*, PPARα agonists inhibit the expression of these proteins in macrophages, thereby providing a possible mechanism by which PPARα agonists might stabilize atheromas and reduce thrombogenesis. The molecular mechanisms explaining the interaction of PPARα at several levels of inflammatory signalling pathway have recently been reviewed [59].

Since PPARα is highly expressed in liver, a role of PPARα in regulating inflammatory response at the hepatic level was expected [59]. The acute phase response is an important inflammatory process for the initiation of defense mechanisms, but may become deleterious if chronic activation is reached. IL-6 and IL-1 stimulate the production of acute-phase proteins such as C-reactive protein (CRP), fibrinogen, and serum amyloid A (SAA), which are markers of cardiovascular disease. Fibrates are shown to be negative regulators of all these positive acute-phase proteins (Fig. (**1B**)) [60] and others [61]. Interference of PPARα with the C/EBP signalling pathway has been suggested as the molecular basis for the inhibition of IL-6 induced fibrinogen-α and β and of SAA expression [19]. A similar mechanism which interferes with the NF-κB signalling, has been described for the fibrate-mediated inhibition of IL-1 induced expression of CRP [62]. Therefore, expression of acute-phase proteins is downregulated by PPARα activators, and their plasma levels are lowered after fibrate treatment in humans. In fact, fenofibrate treatment of dyslipidemic patients decreases the plasma concentrations of fibrinogen, SAA, IL-6, and CRP, whereas levels of albumin, a negative acute phase response protein, are augmented [63].

MECHANISMS CONTROLLING PPARα ACTIVATION (AND AFFECTING ITS EFFECTIVENESS)

There are several factors which may modulate PPAR activity: its cellular content, the nature of ligand, the cross-talk signalling and the structure of the PPRE (Table **1**). In addition, posttranslational modifications are important regulatory controls: phosphorylation, sumoylation and ubiquitination, among others [14]. Those factors modulating PPARα agonists response might be responsible for the variable, sometimes inefficient, drug-induced hypolipidemic action, and the mixed results yielded in clinical cardiovascular trials (Table (**1**)) [64].

RELATIVE CELLULAR CONTENT OF PPARα

To date, there has been very little research related to the regulation of PPARα expression. Studies have reported that the regulation of PPARα expression is carried out either by

Table 1. Mechanisms Controlling PPARα Activation (and Affecting its Effectiveness)

Factors which may Modulate PPAR Activity	Examples in which Fibrates Response is Affected
PPAR cellular content is modified by: ✓ Glucocorticoids, growth hormone, insulin, fatty acids and fibrates. ✓ DNA methylation and protein ubiquitination. ✓ Translocation to the nucleus. ✓ Polymorphisms.	• Human apo A-I PPARα +/- [75]. • Ageing [64,76]. • Interindividual variation [64,75]. • PPARα G/C intron 7 polymorphism [77].
The nature and/or cellular content of ligand(s) may be modulated by: ✓ Enzymatic pathways that generate ligands locally. ✓ Lipid binding proteins that select the ligand to be shuttled to PPARα. ✓ Coenzyme Q10. ✓ Competition between ligands by PPARα and/or FABP.	• Tissue specificity [4,86]. • Interspecies differences [89]. • Animals receiving a hypercholesterolemic diet [87]. • Hypertriacylglycerolemic rats [87,88,90]. • Animals receiving a high-fat diet [86]. • Late pregnant rats [21,67]. • Human subjects affected by massive hypertriacylglycerolemia [92]. • Clinical trials [98,99].
Cross-talk signalling, due to: ✓ DR-1 is also recognized by RAR:RXR, RXR:RXR, HNF-4 and COUP-TF. ✓ RXR is the dimerization partner of PPAR and other nuclear receptors. ✓ Coactivators and/or corepressors are recruited by different nuclear receptors. ✓ Competition is thought to occur among PPAR isotypes. ✓ Dimerization of the orphan LXRα with either PPARα or RXRα. ✓ PPAR:RXR complex also recognizes an ERE.	• Interspecies differences or between diverse cell types [72]. • Human apoA-II transgenic mice [103]. • Concomitant treatment with estradiol [110,111,113]. • Late pregnant rats [67].
Structure of the PPRE and/or the conformation of the PPAR DBD: ✓ Sequence of the regulatory element and the context of the promoter. ✓ Unidentified PPRE in promoter of putative target genes. ✓ Target genes containing non-functional PPRE.	• Apo A-V gene regulation: interspecies difference [116]. • Apo A-I expression: interspecies difference [80,104,105]. • PPARα-humanized mouse [117]. • PPARα Leu162Val polymorphism [119-121]. • Clinical trials [4,63,64,77,122].
Posttranslational modifications: ✓ Phosphorylation, sumoylation, S-nitrosylation.	• Cross-talk with kinases and phosphatases [123-125]. • Cross-talk with statins [123,126].

glucocorticoids [65], insulin or, even by fatty acids and fibrates [66, 67]. PPARα mRNA and protein levels change in parallel with the circadian rhythm of circulating glucocorticoids. Stress or fasting, which induce an increase in the level of plasma glucocorticoids, also result in enhanced synthesis of PPARα [65]. Although conflicting results regarding the regulation of PPARα expression by insulin exist [68, 69], it seems that the down-regulatory effect of insulin is only observed when the expression of PPARα is already augmented. This counterregulatory effect of insulin has been described both *in vitro* by Steineger *et al.* [69] and *in vivo* by us [66]. Our studies *in vivo* in rat during perinatal development showed that PPARα mRNA and protein levels varied profoundly, reaching a high value during the suckling period and later declining in adult rats [70]. This difference was clearly related to both the milk intake, rich in lipids, and the low insulinemia [66]. In fact, it was observed that an oral load of fat was able to markedly increase hepatic PPARα expression, unless plasma insulin levels were high [66]. Moreover, an upregulation of PPARα gene expression by its own ligands, fibrates or FFA, which would suggest an autoregulatory feedback loop of this nuclear receptor (Fig. (**2**)), has been found both in rat cells [68] and *in vivo* studies in adult rat liver [67], and confirmed in PPAR null mice [71].

More specifically, the nuclear content of PPARα will be a determinant in its capacity for gene expression regulation. The unbound form of PPARα is generally present in cytosol, in a complex with a cytoplasmic chaperone. After binding an agonist, the complex is dissociated and PPARα migrates to the nucleus (Fig. (**2**)) [72]. In fact, it has been shown that PPARs are able to bind with both heat shock protein (hsp) 72-kDa [73] and hsp90 [74].

A better understanding of how PPARα expression is regulated is important since PPARα protein levels determine the response to PPARα agonists. Duez *et al.* [75], using human apo A-I transgenic mice on a PPARα +/- background, demonstrated that the fibrates were less effective in reducing

plasma lipids and modulating liver mRNA levels than in human apo A-I PPARα +/+. These findings showed that PPARα is required for the response to fibrates in a gene dosage-dependent manner [75]. Another situation where PPARα expression levels might mediate different responses to PPARα agonists in gene expression is in ageing. Old rats show a profound reduction in the expression and activity of hepatic PPARα [76]. They are resistant to the hypotriacylglycerolemic effect and to increasing the liver expression of PPARα target genes when treated with either gemfibrozil or bezafibrate at doses that are effective in young animals [64]. Possibly related to that, negative results were found in several clinical trials with fibrates, the population over 65 years of age being the one unresponsive to fibrate administration [64]. In fact, a common observation in fibrate-treated patients is the considerable variation in induced lipid changes. It has been reported that human liver PPARα mRNA levels vary >2-fold among individuals, therefore it is likely that in humans, the response to fibrate treatment differs between individuals because of differences in PPARα expression levels [75]. Moreover, in a 3 year study with fenofibrate, patients were divided into high responders and low responders in their plasma TG. In the high responders group, there was a prevalence of PPARα intron 7 GG homozygotes when compared to the low responders [77]. The mechanisms by which the intron 7 variant influences the TG reduction in response to fenofibrate treatment are unknown. There are many examples of regulatory regions being situated in introns, and therefore affecting the expression of the mRNA and the protein levels. However, it is unlikely that the intron 7 variant is itself functional as it is not close to the splice branch point sequence [77]. Whether the G/C intron 7 polymorphism is directly involved in the genetic control of PPARα level remains to be elucidated.

Interestingly, the Holden and Tugwood hypothesis [78] has also speculated that relative amounts of PPARα might mediate different responses in gene expression. Thus, the expression of peroxisome proliferation genes (i.e. ACO, PPAR itself) would be sensitive to PPAR ligands when a high level of PPARα is present. However, the expression of genes related to lipid and/or carbohydrate metabolism (i.e. PEPCK, LPL) would be responsive to activators even when the PPARα amount was low. Such explanation would justify the different sensitivity of PPARα gene expression to nutritional changes found by us in the liver of suckling rats (displaying a high PPARα level) and adult rats (having a low PPARα level) [47]. Moreover, it has been suggested that the low levels of PPARα expression in human liver, compared to mouse liver, may be responsible for human unresponsiveness to peroxisome proliferation [79]. In humans, the low amount of PPARα would be limiting and only the genes related to hypolipidemia may be induced upon exposure to ligand. In rodents, the high level of PPARα would be sufficient to activate both the genes associated to lipid metabolism and the genes related to peroxisome proliferation. In fact, in rodents, the peroxisomal ß-oxidation pathway is much more induced than the mitochondrial ß-oxidation pathway by PPARα agonists treatment. On the contrary, in man, the mitochondrial ß-oxidation pathway is strongly induced, but the peroxisomal ß-oxidation is not [80].

NATURE OF THE LIGAND

Data relied on *in vitro* assays with direct addition of fatty acids at high concentrations showed that a wide variety of saturated and unsaturated fatty acids bind to PPARα, but with relatively low affinity. Nevertheless, it seems that circulating diet-induced changes in blood levels of unsaturated fatty acids are sufficient to slightly modify PPAR activity [14]. Due to the relatively high concentration of endogenous ligands present within the cell, it is difficult to establish which molecule is the true endogenous physiologic PPARα ligand [4]. Therefore, an alternative strategy has been to evaluate enzymatic pathways that could locally generate ligands. Thus, it was demonstrated that LPL releases fatty acids from TG-rich lipoproteins that activate PPARα in ECs, although other lipases equally effective at generating FFA were unable to activate PPARα [52], suggesting differences in the intracellular localization of those FFA or a selective regulation of the receptor. In this same sense, it has been shown that *de novo* synthesized fatty acids regulate PPARα activity, whereas the fatty acids released from adipocytes are less active [81]. One possibility is that lipid binding proteins select the fatty acids to be shuttled to PPARα [82]. The role of FABP and other cytoplasmic transporter proteins guiding the ligands to the nuclear receptors and mediating in the interaction of PPAR with its agonists has been suggested [82-84] and already confirmed, at least, for retinoic acid.

In a way similar to that shown for natural ligands, fibrates that are low affinity ligands for PPARα, must generally be used at high doses to achieve efficacious lipid-lowering activity [85]. Moreover, fibrates appear to act preferentially in the liver, whereas high-affinity ligands, such as Wy-14,643, are suspected of acting more efficiently in peripheral tissues [4], indicating tissue specificity in the response to PPARα activation [86]. Moreover, different fibrates may have different, and even opposite, effects. In one old study, it was observed that in animals receiving a standard diet, fenofibrate and gemfibrozil decreased the TG level, whereas in animals receiving a hypercholesterolemic diet, fenofibrate, but not gemfibrozil, lowered plasma TG [87]. A similar situation was found in animals fed a hypertriacylglycerolemic diet, where the diminution in plasma TG levels provoked by fenofibrate was not observed with gemfibrozil [87]. In a study using bezafibrate and gemfibrozil, both drugs lowered plasma TG to about the same extent in both chow-fed and hypertriacylglycerolemic rats. However, gemfibrozil lowered LDL-cholesterol and elevated HDL-cholesterol, whereas bezafibrate produced the opposite effects [88]. Furthermore, changes in liver TG concentrations in hypertriacylglycerolemic rats produced by these drugs were opposing [88].

To increase the complexity, species differences in ligand activation of PPARα cannot be discarded. For example, fibrates are more effective than linoleic acid in promoting PPARα activation in cultured rat hepatoma cells, whereas linoleic acid is more effective than fibrates in human hepatoma cells [89]. Furthermore, it has been suggested that alteration of gene transcription by fatty acids and fibrates is often disconnected in different target genes. Accordingly, it has been suggested that not all of the fatty acid effects can be

assigned to PPAR activation [48]. For example, in hepatocytes, lipoxygenase inhibitors impair activation of CPT-I gene expression by fibrates, but do not affect the induction by fatty acids. Peroxisome proliferators, but not long-chain fatty acids, induce CPT-II in fetal rat hepatocytes (Fig. (**2**)). The enzyme stearoyl-CoA desaturase 1 (SCD1), which catalyzes Δ9 desaturation of saturated fatty acids, is upregulated by fibrates but downregulated by PUFA (Fig. (**2**)) [48 and references therein].

Interestingly, fatty acids can displace high-potency synthetic PPARα ligands from expressed PPARα protein in cell-free assays [52]. In fact, long-chain fatty acids can also compete or displace peroxisome proliferators for binding to FABP [83], which would be influenced by the relative intracellular concentration of both agonists. Possibly, such competition between fibrates and FFA would make the regulation of both overlapping and different sets of genes possible, resulting in specific and common biological responses [21, 67]. Therefore, FFA could decrease the availability of the fibrates to their corresponding PPAR site, substantially reducing the capability of fibrates to activate PPARα and, consequently, its metabolic effects. This fact could explain those studies describing fibrates failing, or not sufficiently correcting, hypertriacylglycerolemia under certain conditions. That would be the case of animals receiving a high-fat diet along with fibrate [86]. In our hands, in late pregnant rats whose FFA levels are high, fenofibrate was ineffective in reducing their hypertriacylglycerolemia. In pregnant rats the triacylglycerolemia increases over time. Thus, although plasma TG decreased during the first 2 days of fenofibrate treatment in pregnant rats, the effect disappeared on day 3, and plasma TG were even enhanced on day 4. On the contrary, in non-pregnant rats, fenofibrate decreased plasma TG throughout the experiment [21]. In a similar experiment, short-term treatment with fenofibrate to hypertriacylglycerolemic Zucker obese rats markedly raised TG in comparison to untreated obese control rats [90]. Another study showed how in fatty Zucker non-treated rats, plasma TG had increased after 4 weeks in comparison to the initial day of the experiment. Interestingly, semichronic treatment with fenofibrate was only able to prevent the increase in TG observed in control Zucker rats, whereas rosiglitazone, a PPARγ ligand, was able to reduce the hypertriacylglycerolemia efficiently [91]. Related to the competition between fibrates and FFA to activate PPARα, subjects affected by massive hypertriacylglycerolemia have turned out to be hyporesponsive to fibrates. Curiously, coenzyme Q10 improved the efficacy of fenofibrate in massive hypertriacylglycerolemia patients not responding to fenofibrate alone, possibly as a result of a direct effect on mitochondria to increase fatty acid oxidation with a consequent reduction of circulating FFA levels [92].

A permanent elevation of plasma FFA is the dominating factor in obese related metabolic syndrome. According to the original hypothesis of Randle *et al.* [93], increased fatty acid availability competitively inhibits glucose oxidation in the muscle, inducing a diminution of glucose uptake and insulin resistance. PPARα activation favors fatty acid oxidation and is one of the reasons why fibrates have lipid-lowering effects and why PPARα ligands could, in some situations, improve insulin sensitivity by reducing lipid accumulation in tissues [94]. However, only a few clinical trials have reported an improvement of glucose homeostasis after fibrate treatment [95, 96]. Furthermore, these beneficial effects of fibrates on glucose control may not be attributable alone to enhanced fatty acid oxidation related to PPARα activation, because some of those compounds have also modest PPARγ activity [97]. Thus, the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study did not reveal any fenofibrate effect on glucose parameters in diabetic patients [98], and a recent report concluded that administration of fenofibrate for 3 months did not affect insulin sensitivity in obese subjects with type 2 diabetes [99].

CROSS-TALK SIGNALLING AND STRUCTURE OF PPRE

DR-1 elements present in PPRE are also recognized by other nuclear receptors such as RAR:RXR, RXR homodimers, HNF-4 and chicken ovalbumin upstream promoter–transcription factor (COUP-TF). In fact, HNF-4 and COUP-TF homodimers can displace PPAR:RXR from its binding site and thus compete with PPAR signalling [100, 101]. The effectiveness of these interactions depends on the sequence of the regulatory element and the context of the promoter, suggesting cross-talk with other nuclear receptors that may generate different agonistic/antagonistic actions and influence metabolic control [4, 102]. Moreover, on the one hand, RXR is the indispensable dimerization partner of the PPAR and other nuclear receptors such as RAR, thyroid hormone receptor, and vitamin D receptor; and on the other hand, as commented above, there are multiple types of cofactor proteins (coactivators and/or corepressors) which are recruited by many different nuclear receptors, PPAR included, in order to assemble the transcriptional machinery at the target gene promoter [16]. Thus, a competition between nuclear receptors exists. Differences in both the expression of these factors and cofactors and the interactions of one or several of these proteins upon addition of ligands could modulate the capacity of PPAR to regulate the expression of target genes. This might explain the differences found in the response to PPARα agonists interspecies or between diverse cell types [72].

For example, in the human apoA-II transgenic mice, which share some similar phenotypic characteristics with PPARα null mice, fibrate-induced PPARα activation did not correct the combined hyperlipidemia. Thus, after two weeks of treatment with fenofibrate, human apoA-II transgenic mice presented an unexpected increase in plasma TG, mainly due to an impaired VLDL catabolism and PPARα signalling [103]. As PPARα liver gene expression was demonstrated, its functional impairment could result from a defective recruitment of the coactivators that modulate its activity on several critical gene targets. On the other hand, several mechanisms have been suggested to explain the effects of PPARα activation on apo C-III gene regulation: first, a competition between PPAR and HNF-4 for binding to the apo C-III promoter and a substitution of the strong activator HNF-4 by a less active PPAR:RXR complex may explain a reduction of apo C-III expression which depends on the relative abundance of PPARα and HNF-4. Second, apo C-III repression by fibrates might occur *via* PPARα-dependent enhanced expression of a negative regulator of transcription. Thus,

PPARα activators induce the hepatic expression of Rev-erbα, a nuclear orphan receptor which is a strong repressor of transcription [104, 105]. Third, PPARα might repress transcription of apo C-III by physically interacting with other transcription factors, leading to the formation of inactive complexes and thereby limiting the induction of apo C-III expression. This seems to be the case for the positive transcription factor forkhead box O1 (FoxO1) [106].

Moreover, competition is thought to occur among PPAR isotypes [107]. The subtype ß of PPAR has been reported as a physiological human antagonist of PPARα [107], and therefore, a higher expression of the PPARß isoform in human cells than in cells of rodent origin could imply an impairment of PPARα activity in human cells [72]. However, other authors have proposed that the functions of PPARs α and β may be redundant as transcriptional regulators and have even suggested that the beta-subtype might compensate for deficiency of PPARα [108]. In a similar way, PPAR function could be antagonized by dimerization of the orphan LXRα with either PPARα or RXRα, leading to a complex that cannot bind to DNA responsive elements [80]. In addition, the PPAR:RXR complex also recognizes an estrogen response element (ERE) [109]. The possibility of a hormonal cross-talk through ERE exists [109] and genes might be found to be coregulated in an opposite manner by the estrogen receptor (ER) and PPAR:RXR. Thus, for example, mixed studies carried out either in wild-type, LDL receptor-deficient or ovariectomized mice have suggested the involvement of estrogen in the regulation of obesity and hypertriacylglycerolemia by fenofibrate [110]. In a recent study with ovariectomized mice, the concomitant treatment with fenofibrate and estradiol showed that the hormone reversed the effects of fibrate on plasma lipids and hepatic PPARα target gene expression [111]. Related to that, in late pregnant rats treated with fenofibrate, there may be a competition between fenofibrate and estradiol, whose levels during late pregnancy are extremely high [112], to be the "conductor" of the lipid metabolism [67, 113]. Furthermore, a marked dichotomy in the response to atorvastatin, a hypolipidemic statin known to induce the hepatic expression of PPARα in rats [114], was found between old male and female rats. Senescent females were practically resistant to the effects of atorvastatin on lipid metabolism, probably as a result of the inhibitory activity of high estrogen levels on PPARα transactivating activity [115].

On the other hand, human primary hepatocytes treated with Wy-14,643 or fenofibrate displayed a strong induction of apo A-V mRNA [102] through activation of PPARα and binding to a functional PPRE. These findings demonstrate that apo A-V is a highly responsive PPARα gene in humans [102]. However, a PPRE has not yet been identified in the rat apo A-V promoter, probably indicating an additional interspecies difference [116]. This is similar to what occurs with the apo A-I, one of the major protein constituents of HDL. The human apo A-I gene contains functional PPRE, whereas it is not functional in rodents due to a difference in three nucleotides between rodent and human PPRE, which prevent PPARα binding to the rodent PPRE [105]. Activation of PPARα by fibrates induces human apo A-I expression, increasing HDL, whereas in rats fibrates suppress apo A-I expression and decrease HDL levels (Fig. (**2**)) [80]. In rodents, Rev-erbα binds to a negative response element adjacent to the TATA box, present only in the rat apo A-I promoter [105]. Since fibrates induce Rev-erbα expression [104], rodent apo A-I expression may be repressed *via* this mechanism.

Sometimes, the difference is not in the structure of the PPRE but in the conformation of the PPARα DNA-binding domain (DBD). For example, PPARα null mice expressing human PPARα in their livers functionally responded to Wy-14,643 and fenofibrate in a manner similar to wild-type mice, by controlling the expression of known target genes [117]. However, mice having human PPARα did not exhibit peroxisomal proliferation nor hepatocarcinoma, which was observed in mice having murine PPARα, despite the fact that the expression levels of PPARα were comparable in both types of mouse [117]. Human and rat PPARα are not exactly identical in their DNA- and ligand-binding domains and it has even been reported that a single amino acid change in some of these domains of PPARα profoundly alters its transcriptional activity [118]. Related to that, polymorphisms in the PPARα gene may also contribute to the different responses to fibrate treatment. In fact, a significant genotype-dependent response to gemfibrozil [119], as well as to fenofibrate [120], treatment in lipidemia with the PPARα Leu162Val polymorphism has been found. This leucine to valine (Leu162Val) substitution at the PPARα gene is functional and affects ligand-transactivation activity of PPARα. The Leu162Val polymorphism affects the DBD of the receptor, and therefore this mutant receptor may not bind to PPREs as efficiently as the wild-type receptor. However, in this mutant form of the receptor, the low activation found with low doses of ligand is overcome at high doses [121].

Altogether these factors modulating PPARα agonists response would perhaps explain the mixed results yielded by prospective clinical cardiovascular trials with these agents. In some clinical trials with cardiovascular endpoints, fibrates have yielded either beneficial effects restricted to some types of patients or even negative outcomes [recently reviewed by Sanguino, 2007] [64]. Thus, in the Helsinki Heart Study (HHS), cardiovascular disease risk reduction upon gemfibrozil treatment was most pronounced in obese patients with metabolic syndrome or diabetes and atherogenic dyslipidemia [63]. In the Bezafibrate Infarction Prevention (BIP) trial, reduction in coronary events with bezafibrate was observed only in patients with elevated serum TG concentrations. In the Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial (VA-HIT), the significant beneficial effects of gemfibrozil were shown in diabetics or in non-diabetics with high insulin levels [4]. Furthermore, in the FIELD study, although fenofibrate lowered TG as well as LDL cholesterol and hardly elevated HDL cholesterol, it did not reduce the risk of coronary events. Actually, due to the heterogeneity of the study subjects, many of those conclusions were obtained by analyzing results from a special subgroup of the study population of patients [64]. In other cases, the interpretation of the study was complicated by concomitant drug use; for example, in the FIELD study, the use of

statins throughout the study in both groups (placebo and fenofibrate) [63]. As commented above, the influence of the ageing process in the manifestation of fibrates effects cannot be discarded. The analysis *a posteriori* of data from FIELD and Low Extremity Arterial Disease Event Lowering in Diabetes (LEADER) studies show a marked reduction in cardiovascular events in the patient population younger than 65 years, indicating that the population older than 65 years was the one unresponsive to fibrate administration, and the one responsible for the negative outcome of these assays [64]. Perhaps, similar conclusions could be obtained if the association between PPARα gene variants and response to fibrate treatment was examined in those studies. For example, in the St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention (SENDCAP) study, a greater reduction of total cholesterol in response to bezafibrate treatment was observed in the PPARα Val162 allele carriers than in the Leu162 carriers [77]. Moreover, the association between PPARα Leu162Val polymorphism and the risk of a cardiovascular event, as well as response to gemfibrozil therapy was greatest in those patients with either insulin resistance or diabetes mellitus in VA-HIT [122]. Besides, the older fibrates (gemfibrozil and bezafibrate) used in the previous studies are less specific for PPARα than fenofibrate and so may be acting, at least in part, through PPARγ and/or PPARβ [77]. Finally, it seems to be that compounds related to the β-carotene could inhibit the activation of PPARα by their agonists [52]. As already noted, coenzyme Q10 also affects the effectiveness of fenofibrate in correcting dyslipidemia [92]. Therefore, the results of clinical trials with fibrates might be affected by the supplementation used: β-carotene, coenzyme Q10, and so on.

PHOSPHORYLATION AND OTHER POSTTRANSLATIONAL MODIFICATIONS

The phosphorylation-dephosphorylation process is an important regulatory mechanism of transcriptional activity of nuclear receptors. In fact, PPARα is a phosphoprotein and its function is affected by cross-talk with kinases and phosphatases [123]. Therefore, PPARα activity is dependent on its phosphorylated state [124]. Thus, it is possible that the activation by ligand-independent mechanisms of PPARα is associated with kinase-dependent processes [123]. Activators of protein kinase A can increase PPARα activity in the absence and the presence of receptor agonists [123]. Nevertheless, it has been observed that the phosphorylation of PPARα may be enhanced by treatment with peroxisome proliferators like ciprofibrate. In addition, cell treatment with phosphatase inhibitors decreases the levels of ciprofibrate-induced gene expression [124]. Insulin also increases the phosphorylation of PPARα, enhancing its transcriptional activity [125]. On the other hand, PPARα activity has also been shown to be modulated by mitogen-activated protein kinase (MAPK). Thus, the inhibition of MAPK reduces PPARα activity [123]. Further, inhibitors of HMG-CoA reductase, such as cerivastatin, acting through the prenylation of Rho A small G protein, can regulate MAPK signalling which, in turn, modulates the PPARα transcriptional activity [126]. This is an important cross-talking between fibrates and statins [123]. Eventually, modification of the phosphorylation state of PPAR affects ligand affinity [80].

PHARMACOLOGICAL AND NATURAL ACTIVATORS OF PPARα

Metabolic syndrome, type 2 diabetes mellitus and dyslipidemia are increasing worldwide. However, many of these patients are not receiving appropriate treatment [127, 128]. Generally, fibrates, PPARα agonists, have been accepted as the most promising treatment. However, as already commented, a number of animal and human studies have revealed potential adverse effects or failure of PPARα activation by fibrates, underlining the need to search for alternative or complementary therapies [52] ([129, 130] for a review). Furthermore, fibrates are weak agonists of PPARα, and high doses are required for effective treatment [85]. In addition, selective PPARα agonists with higher potency and specificity are supposed to display greater effectiveness and fewer adverse effects. These alternative therapies are currently being developed and proving useful [14, 129, 131]. In this article, we briefly review of the most noticeable agents (Table **2**).

On the other hand, whichever PPARα therapeutics is used, it should be based on a better understanding of PPAR biology, the nature of endogenous PPAR agonists, and how these molecules are generated. Moreover, these PPAR agonists-producing pathways, when defective, might generate inadequate PPAR ligands which may contribute to pathological situations [52]. Whether this should be the case, an alternative strategy would be either to promote the production of the "appropriate" endogenous PPAR agonists or the design of agents which might compete with and displace the "inadequate" PPAR ligands for binding to PPAR. A similar behaviour has been suggested for the PPARγ agonists, TZDs [129, 132].

DUAL PPARα/γ AGONISTS, DUAL PPARα/δ AGONISTS AND PAN-AGONISTS

Glitazars are dual PPARα/PPARγ agonists that are being studied in the treatment of diabetes and metabolic syndrome. Among others, there are naveglitazar, muraglitazar, ragaglitazar, tesaglitazar, and chiglitazar [133]. All of them reduce TG and raise HDL levels and improve insulin sensitivity. Besides, ragaglitazar, tesaglitazar and muraglitazar improve fatty acid and lipoprotein metabolism by decreasing apo B and apo C-III plasma levels and inducing activity of hepatic LPL and CPT-I [131]. In addition, treatment with ragaglitazar significantly reduced blood pressure in spontaneous hypertensive rats and improved endothelial function in Zucker fatty rats when compared with pioglitazone treatment [134]. Due to excellent findings in animal models, clinical trials in humans were initiated. A phase III clinical trial with type 2 diabetic patients compared treatment with muraglitazar versus pioglitazone, both in combination with metformin [135]. Muraglitazar plus metformin showed significantly greater improvement in glycemic control, reduction in plasma TG and an increment in plasma HDL than pioglitazone plus metformin. However, it was also found that death, non-fatal myocardial infarction, or stroke occurred in a higher number of patients treated with muraglitazar, as compared to patients treated with pioglitazone or placebo. Therefore, the clinical study with muraglitazar was recently discontinued due to higher incidence of edema and heart failure [136]. Further-

Table 2. Alternative Strategies for PPARα Activation

Classes of Drugs and/or Nutrients	Examples of Natural and Synthetic Agonists
✓ Dual PPPARα/γ Agonists	• Naveglitazar, muraglitazar, ragaglitazar, tesaglitazar, and chiglitazar [131,133-136]. • DRF 2519 [137]. • LSN862 [138].
✓ Dual PPARα/δ Agonists	• T659 [139].
✓ Pan-agonists	• Netoglitazone and PLX 204 [133,141].
✓ Selective PPAR Modulators (SPPARMS)	• Metaglidasen [133]. • FK614 [143].
✓ Nutraceuticals	• Omega-3 fatty acids [145]. • Conjugated linoleic acid (CLA) [146,147]. • Fatty acids with ethanolamine (FAE) [148-150]. • Antioxidant therapy, such as vitamin E [57,80]. • Isohumulones [151,152]. • Genistein, daidzein and other soy isoflavones [153-155]. • Beta-carotene [52]. • Pterostilbene, piceatannol and resveratrol [156].

more, in clinical trials, tesaglitazar was shown to elevate blood creatinine levels in patients, indicating potential kidney toxicity. Therefore, these two promising compounds were discontinued in May 2006, and at present, new molecules are being tested [136]. In the same sense, although ragaglitazar showed an excellent profile in clinical trials, due to some incidence of bladder tumor in rodents, the trials have been discontinued [133].

On the other hand, DRF 2519, an analogue of the TZDs, has also shown dual activation of PPARα and γ. In an insulin-resistant *ob/ob* mouse model, DRF 2519 showed better alleviation of insulin resistance and dyslipidemia than rosiglitazone. In fatty Zucker rats, DRF 2519 showed greater reduction in plasma insulin, TG and FFA levels and enhanced aortic smooth muscle relaxation than rosiglitazone [137]. In high-fat fed Sprague Dawley rats, DRF 2519 improved plasma lipid profiles more than fenofibrate or rosiglitazone. These results indicate that DRF 2519 could be an interesting candidate in the management of metabolic disorders and associated complications [137]. Another novel PPARα/PPARγ dual agonist is LSN862, which shows improvements in glucose and lipid levels in rodent models of type 2 diabetes and dyslipidemia. In Zucker diabetic fatty rats, LSN862 showed a higher glucose and TG lowering activity than rosiglitazone. In addition, LSN862 in *db/db* mice demonstrated better antidiabetic efficacy compared with rosiglitazone. In the humanized apo A-I transgenic mouse, LSN862 and fenofibrate reduced VLDL levels, whereas rosiglitazone increased them [138]. Recently, T659, a PPARα/δ dual agonist was shown to increase HDL levels in primates [139]. This is interesting as there are very few therapeutic drugs available to increase HDL-cholesterol (HDL-C) concentrations in dyslipidemic patients, since fibrates cause slight increases in HDL-C concentration, and TZDs, like statins, have little or no effect on HDL-C.

The most well-known pan (α, β and γ)-PPAR activator is bezafibrate, with more than a quarter of a century of safe therapeutic use. Bezafibrate improves both insulin sensitivity and blood lipid profile in patients with metabolic syndrome [140]. Therefore, bezafibrate-based clinical studies support the use of pan-PPAR ligands as therapeutic agents against the metabolic syndrome. Nevertheless, since bezafibrate is a weak PPAR ligand, more powerful new pan-PPAR compounds should be developed for the treatment of diseases in which lipid and glucose metabolism disorders coexist. Thus, novel PPAR pan-agonists such as netoglitazone and PLX 204 are under investigation. In the near future, they may be potent therapeutic agents used in the treatment of diabetes associated with cardiovascular complications [133]. In fact PPAR pan-agonists have displayed antidiabetic actions without the usual weight gain associated with PPARγ agonists [133]. Thus, netoglitazone has been shown to decrease plasma fatty acid, glucose, and TG levels and increase insulin sensitivity in obese and diabetic rats [141]. Nevertheless, given the multiple sites of action of PPAR pan-agonists, which involve the transcription of a vast array of genes, more information regarding toxicity in humans is required [133].

SELECTIVE PPAR MODULATORS (SPPARMS) AND PARTIAL AGONISTS

The concept of Selective PPAR Modulators (SPPARMs) was suggested by analogy to Selective Estrogen Receptor Modulators (SERM), which proposes that each different ligand can have different agonist or antagonist properties depending on the cell context and the specific target gene in question. PPAR ligands with different chemical structures

(SPPARMs) bind to the LBD of the receptor, inducing distinct conformational changes that lead to different binding affinities for the various cofactors and, thus, activate PPAR in distinct ways provoking differential gene expression and biological responses. Nevertheless, it should be kept in mind that, unlike classical steroid hormone receptors, PPAR receptors have a large ligand-binding pocket which is not fully filled with the ligand [142]. Thus, the working hypothesis is that the best therapeutic strategy is to select a desired PPAR-mediated action in one cell type without inducing an adverse PPAR-mediated effect in another [80]. The greater part of SPPARMs that have been developed to date act on PPARγ, and have been shown to selectively modify gene expression and reduce insulin resistance without causing weight gain [133]. Thus, metaglidasen decreased insulin and glucose concentrations, increased adiponectin concentrations, and improved plasma lipid profile without causing weight gain in diabetic mice [133]. A SPPARM with differential properties in the regulation of fat cell function is FK614. This compound behaves as a partial agonist in inducing the interaction of PPARγ with both transcriptional coactivators CBP and SRC-1, but as a full agonist with both PBP and PRIP, which are required for PPARγ-mediated adipogenesis. Therefore, in differentiating 3T3-L1 adipocytes, but not in mature adipocytes, FK614 induces adipocyte fatty acid-binding protein (aP2) mRNA expression and TG accumulation, whereas TZDs produce the same effects at the two stages of adipocyte differentiation. Since FK614 behaves as SPPARM with stage-dependent selectivity, it may contribute to ameliorate insulin resistance without stimulating fat accumulation in adipocytes [143]. On the other hand, gemfibrozil, a clasical PPARα agonist, has recently been considered a SPPARM [75]. Accordingly, gemfibrozil is structurally unique as compared with other fibrates. Thus, whereas fenofibrate behaves as a full agonist, gemfibrozil appears to act as a selective modulator of PPARα, due to a differential recruitment of coactivators to the PPARα:RXR-DNA complex [75]. Such distintive interaction between PPARα and its cofactors may transmit signals that result in a unique gene regulatory activity [85]. These observations would explain the differences found between fenofibrate and gemfibrozil in the apoA-I expression and plasma levels [75].

Interestingly, Feige *et al.* [144] have clearly established the difference between selective PPAR modulators and partial PPAR agonists, two terms that are very often interchangeable [75,133]. SPPARMs induce conformational changes different from full agonists, which produce a selective induction of corregulator recruitment, target gene induction, and physiological effects. Indeed, SPPARMs differ from partial agonists, as they promote selective gene regulation by differentially affecting target gene transcription in a gene-specific manner. Thus, some genes are induced to similar levels than those obtained with a full agonist, whereas others exhibit restricted activation. In contrast, when compared with full agonists, partial agonists exhibit a global decrease in the activation of all target genes [144].

NUTRACEUTICAL APPROACHES

We should remember that circulating diet-induced changes in blood levels of PUFA are sufficient to modestly modify PPAR activity [14]. Moreover, natural ligands of PPARα such as eicosapentaenoic acid and docosahexanoic acid are often found in over-the-counter fish oil or ω-3 supplements [133]. Interestingly, PUFA, especially those of the ω-3 class, are known to affect all the metabolic nuclear receptors that modulate TG levels, LXR and HNF-4α, as well as the three PPARs [145]. In fact, the efficient hypotriacylglycerolemic effect of ω-3 fatty acids has been explained by their coordinated action on these different nuclear receptors.

Conjugated linoleic acid (CLA) is found naturally in animal tissues and food sources. Although the action mechanism(s) of CLA is/are not clearly understood, it has been proposed that activation of PPARα and/or PPARγ may account, in part, for the beneficial effects of CLA on atherogenesis, diabetes and immune function, as reported in animal studies [146,147].

Amides of fatty acids with ethanolamine (FAE) are produced naturally in mammalian cells through the concerted action of two enzymes. FAE are a family of natural active lipid mediators, present in circulating blood and tissues, which participate in a variety of biological functions including the regulation of feeding [148] and improvement of myocardial function in conditions of cardiomyopathy [149]. The monounsaturated FAE oleylethanolamide (OEA) stimulates both adipose tissue lipolytic activity and hepatic fatty acid oxidation through the activation of PPARα [150].

Reductions in PPARα gene expression might contribute to the pro-oxidant state observed in aged animals, possibly due to a deficiency in the modulation of the cellular redox state. Aged mice have been shown to express reduced mRNA levels of PPARα and target genes, such as peroxisomal ACO. Reciprocally, that pro-oxidant state may be a cause of the reduced expression of PPARα found in old rats [76]. Interestingly, supplementation of old mice with vitamin E caused elevations in PPARα and ACO transcripts to the levels seen in young animals [57], suggesting that balancing the cellular redox state may positively affect the transcriptional regulation of the PPARα gene. Moreover, since some of the adverse effects of PPARα action appear to be due to the compounds generated in oxidized fatty-acid metabolism, co-administration of a antioxidant therapy, such as vitamin E, with PPAR ligands might alleviate or prevent the undesirable effects of PPAR agonists [80].

There have been few studies concerning the biological activities of the isohumulones found in beer, but some of them have reported that isohumulones can activate PPARα and PPARγ [151]. It has been proposed that isohumulones improve insulin resistance in diabetic mice [151] through their effect of enhancing PPARγ expression, It has also been proposed that isohumulones raise plasma HDL-cholesterol levels and reduce liver cholesterol and TG in mice, through their effect of enhancing PPARα expression [152].

Substantial data from epidemiological and nutritional studies in humans and animals have indicated that soy protein intake reduces serum total and LDL cholesterol and TG as well as hepatic cholesterol and TG [153]. Soy protein is unique among the plant-based proteins because it is associated with isoflavones. Thus, Mezei *et al.*, using PPARα null mice fed diets containing different levels of soy isoflavones, determined that significant improvements in serum TG levels

are provided by the intake of soy isoflavones *via* both PPARα dependent and PPARα independent mechanisms [154]. Curiously, the chemical structure of isoflavones is similar to that of fibrates, and studies *in vitro* have demonstrated that soy isoflavones, particularly genistein and daidzein, are able to activate both PPARα and PPARγ [154]. On the other hand, Mezei *et al.* showed that consumption of a high isoflavone soy protein diet improved glucose tolerance, insulin resistance, and hepatic cholesterol and TG concentrations in obese Zucker rats [155].

The heterodimer composed of RXR and PPAR, can be activated by the RXR ligand 9-cis retinoic acid, independently of the presence or absence of a ligand for PPAR. Retinoic acid can be formed from the symmetric cleavage of β-carotene. Beta-carotene however, can also undergo asymmetric cleavage to yield a series of molecules known as apocarotenals. It has recently been hypothesized that apocarotenals, such as 9-cis retinoic acid, might also be transcription modulators. Thus, several studies have revealed that β-apo-14' carotenal (apo14) is able to inhibit the activation of PPARα and RXR by their respective agonists. Possibly, apo14 might help to modulate the PPARα activation [52].

Finally, polyphenolic compounds such as resveratrol, found in grapes, wine, and peanuts, have shown lipid and glucose lowering properties mediated by PPARα [156]. Rimando *et al.* investigated whether this compound and three analogues (pterostilbene, piceatannol and resveratrol trimethyl ether) would activate PPARα *in vivo* and *in vitro*. Pterostilbene demonstrated the highest induction of PPARα. Accordingly, in hypercholesterolemic hamsters, pterostilbene lowered plasma LDL cholesterol and glucose and increased plasma HDL cholesterol, as compared to the control group [156].

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ABBREVIATIONS

ACBP	=	Acyl-CoA binding protein
ACO	=	Acyl-CoA oxidase
ACS	=	Acyl-CoA synthetase
AP-1	=	Activator protein-1
aP2	=	Adipocyte fatty acid-binding protein
apo	=	Apolipoprotein
apo 14	=	β-apo-14' carotenal
BIP	=	Bezafibrate Infarction Prevention
C/EBP	=	CAATT/enhancer binding protein
CBP	=	cAMP response element-binding protein (CREB)-binding protein
CLA	=	Conjugated linoleic acid
COUP-TF	=	Chicken ovoalbumin upstream promoter transcription factor
COX	=	Cyclooxygenase
CPT	=	Carnitine palmitoyl transferase
CRP	=	C-reactive protein
CYP4A	=	Cytochrome P450 superfamily
DBD	=	DNA-binding domain
DGAT	=	Diacylglycerol acyltransferase
DR-1	=	Direct repeat spaced by one nucleotide
ECs	=	Endothelial cells
ERE	=	Estrogen response element
ET	=	Endothelin
ER	=	Estrogen receptor
FAE	=	Fatty acids with etanolamine
FABP	=	Fatty acid binding protein
FAT	=	Fatty acid translocase
FATP	=	Fatty acid transporter protein
FIELD	=	Fenofibrate Intervention and Event Lowering in Diabetes
FoxO1	=	Factor forkhead box O1
FFA	=	Free fatty acids
HD	=	Enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase or L-bifunctional enzyme
HDL	=	High density lipoprotein
HHS	=	Helsinki Heart Study
HL	=	Hepatic lipase
HMG-CoA	=	3-hydroxy-3-methylglutaryl-CoA
HNF	=	Hepatocyte nuclear factor
Hsd17b4	=	D-bifunctional enzyme or 17-β-hydroxysteroid dehydrogenase type IV
hsp	=	Heat shock protein
IκB	=	Inhibitor of kappa B
IL	=	Interleukin
LBD	=	Ligand-binding domain
LCAT	=	Lecithin:cholesterol acyltransferase
LDL	=	Low density lipoprotein
LEADER	=	Low Extremity Arterial Disease Event Lowering in Diabetes

LPL	=	Lipoprotein lipase
LPS	=	Lipopolysaccharide
LXR	=	Liver X receptor
MAPK	=	Mitogen-activated protein kinase
MCAD	=	Medium-chain acyl-CoA dehydrogenase
ME	=	Malic enzyme
MMP	=	Matrix metalloproteinase
NF-κB	=	Nuclear factor kappa B
eNOS	=	Endothelial nitric oxide synthase
NO	=	Nitric oxide
OEA	=	Oleylethanolamide
PBP	=	PPAR binding protein
PDK	=	Pyruvate dehydrogenase kinase
PEPCK	=	Phosphoenolpiruvate carboxykinase
PK	=	Pyruvate kinase
PPAR	=	Peroxisome proliferator-activated receptor
PPRE	=	Peroxisome proliferator-response element
PUFA	=	Polyunsaturated fatty acids
PRIP	=	PPAR interacting protein
RAR	=	Retinoid acid receptor
RXR	=	9-cis retinoid X receptor
SAA	=	Serum amyloid A protein
SENDCAP	=	St. Mary's, Ealing, Northwick Park Diabetes Cardiovascular Disease Prevention
SERM	=	Selective estrogen receptor modulator
SCD	=	Stearoyl-CoA desaturase
SPPARM	=	Selective PPAR modulator
SR-BI	=	Murine scavenger receptor I
SRC-1	=	Steroid receptor coactivator 1
STAT	=	Signal transducer and activator of transcription
TG	=	Triacylglycerol
TF	=	Tissue factor
TNF	=	Tumor necrosis factor
Tysnd1	=	Trypsin domain containing 1
TZD	=	Thiazolidinedione
UCP	=	Uncoupling protein
VCAM	=	Vascular cell-adhesion molecule
VA-HIT	=	Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial
VLDL	=	Very low density lipoprotein
VSMCs	=	Vascular smooth muscle cells

REFERENCES

[1] Michalik, L. and Wahli, W. (1999) Peroxisome proliferator-activated receptors: three isotypes for a multitude of functions. *Curr. Opin. Biotechnol.*, **10**(6), 564-570.

[2] Issemann, I. and Green, S. (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferator. *Nature*, **347**(6294), 645-650.

[3] Lemberger, T.; Braissant, O.; Juge-Aubry, C.; Keller, H.; Saladin, R.; Staels, B.; Auwerx, J.; Burger, A.G.; Meier, C.A. and Wahli, W. (1996) PPAR tissue distribution and interactions with other hormone-signaling pathways. *Ann. N. Y. Acad. Sci.*, **804**, 231-251.

[4] Lefebvre, P.; Chinetti, G.; Fruchart, J.-C. and Staels, B. (2006) Sorting out the roles of PPAR alpha in energy metabolism and vascular homeostasis. *J. Clin. Invest.*, **116**(3), 571-580.

[5] Forman, B.M.; Chen, J. and Evans, R.M. (1997) Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ. *Proc. Natl. Acad. Sci. USA*, **94**(9), 4312-4317.

[6] Lehmann, J.; Lenhard, J.M.; Oliver, B.B.; Ringold, G.M. and Kliewer, S.A. (1997) Peroxisome proliferator-activated receptors α and γ are activated by indomethacin and other non-steroidal anti-inflammatory drugs. *J. Biol. Chem.*, **272**(6), 3406-3410.

[7] Lehmann, J.; Moore, L.B.; Smith-Oliver, T.A.; Wilkison, W.O.; Willson, T.M. and Kliewer, S.A. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for receptor proliferator-activated receptor γ (PPARγ). *J. Biol. Chem.*, **270**, 12953-12956.

[8] Keller, H.; Dreyer, C.; Medin, J.; Mahfoudi, A.; Ozato, K. and Wahli, W. (1993) Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. *Proc. Natl. Acad. Sci. USA*, **90**(6), 2160-2164.

[9] Struhl, K. (1998) Histone acetylation and transcriptional regulatory mechanisms. *Genes Dev.*, **12**(5), 6969-6978.

[10] Pazin, M.J. and Kadonaga, J.T. (1997) What´s up and down with histone deacetilation and transcription? *Cell*, **89**(3), 325-328.

[11] Xu, L.; Glass, C.K. and Rosenfeld, M.G. (1999) Coactivator and corepressor complexes in nuclear receptor function. *Curr. Opin. Genet. Dev.*, **9**(2), 140-147.

[12] Devchand, P.R.; Keller, H.; Peters, J.M.; Vázquez, M.; Gonzalez, F.J. and Wahli, W. (1996) The PPARα-leukotriene B_4 pathway to inflammation control. *Nature*, **384**(6604), 39-43.

[13] Marx, N.; Schönbeck, U.; Mitchell, A.; Lazar, A.; Libby, P. and Plutzky, J. (1998) Peroxisome proliferator activated receptor gamma activator inhibit gene expression and migration in human vascular smooth muscle cells. *Circ. Res.*, **83**(11), 1097-1103.

[14] Gervois, P.; Fruchart, J.-C. and Staels, B. (2007) Drug insight: mechanism of action and therapeutic applications for agonists of peroxisome proliferator-activated receptors. *Nat. Clin. Pract. Endocrinol. Metab.*, **3**(2), 145-156.

[15] Duval, C.; Müller, M. and Kersten, S. (2007) PPARα and dyslipemia. *Biochim. Biophys. Acta*, **1771**(8), 961-971.

[16] Ricote, M. and Glass, C.K. (2007) PPARs and molecular mechanisms of transrepression. *Biochim. Biophys. Acta*, **1771**(8), 926-935.

[17] Delerive, P.; De Bosscher, K.; Vanden Berghe, W.; Fruchart, J.C.; Haegeman, G. and Staels, B. (2002) DNA binding -independent induction of IkappaBα gene transcription by PPARα. *Mol. Endocrinol.*, **16**(5), 1029-1039.

[18] Shin, M.; Kim, I.; Inoue, Y.; Kimura, S. and Gonzalez, F.J. (2006) Regulation of mouse hepatic alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase, a key enzyme in the tryptophan-nicotinamide adenine dinucleotide pathway, by hepatocyte nuclear factor 4alpha and peroxisome proliferator-activated receptor alpha. *Mol. Pharmacol.*, **70**(4), 1281-1290.

[19] Gervois, P.; Vu-Dac, N.; Kleemann, R.; Kockx, M.; Dubois, G.; Laine, B.; Kosykh, V.; Fruchart, J.C.; Kooistra, T. and Staels, B. (2001) Negative regulation of human fibrinogen gene expression by peroxisome proliferator-activated receptor alpha agonists *via* inhibition of CCAAT box/enhancer-binding protein beta. *J. Biol. Chem.*, **276**(36), 33471-33477.

[20] Schoonjans, K.; Peinado-Onsurbe, J.; Lefebvre, A.M.; Deeb, S.; Staels, B. and Auwerx, J. (1996) PPAR α and PPAR γ activators direct a distinct tissue-specific transcriptional response *via* PPRE in the lipoprotein lipase gene. *EMBO J.*, **15**(19), 5336-5348.

[21] Soria, A.; Bocos, C. and Herrera, E. (2002) Opposite metabolic response to fenofibrate treatment in the pregnant and virgin rats. *J. Lipid. Res.*, **43**(1), 74-81

[22] Schoonjans, K.; Staels, B. and Auwerx, J. (1996) The peroxisome proliferator activated receptor (PPARs) and their effects on lipid metabolism and adipocyte differentiation. *Biochim. Biophys. Acta*, **1302**(2), 93-109.

[23] Schoonjans,K.; Staels, B.; Grimaldi, P.A. and Auwerx, J. (1993) Acyl-CoA synthetase mRNA expression is controlled by fibric-acid derivates, feeding and liver proliferation. *Eur. J. Biochem.*, **216**(2), 615-622.

[24] Kaikaus, R.M.; Chan, W.K.; Ortiz de Montellano, P.R. and Bass, N.M. (1993) Mechanisms of regulation of liver fatty acid-binding protein. *Mol. Cell. Biochem.*, **123**(1-2), 93-100.

[25] Desvergne, B. and Wahli, W. (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.*, **20**(5), 649-688.

[26] Hashimoto, T.; Fujita, T.; Usuda, N.; Cook, W.; Qi, C.; Peters, J.M.; Gonzalez, F.J.; Yeldandi, A.V.; Rao, M.S. and Reddy, J.K. (1999) Peroxisomal and mitochondrial fatty acid β-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor a and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. *J. Biol. Chem.*, **274**(27), 19228-19236.

[27] Baes, M.; Huyghe, S.; Carmeliet, P.; Declercq, P.E.; Collen, D.; Mannaerts, G.P. and Van Veldhoven, P.P. (2000) Inactivation of the peroxisomal multifunctional protein-2 in mice impedes the degradation of not only 2-methyl-branched fatty acids and bile acid intermediates but also of very long chain fatty acids. *J. Biol. Chem.*, **275**(21), 16329-16336.

[28] Reddy, J.K.; Goel, S.K.; Nemali, M.R.; Carrino, J.J.; Laffler, T.G.; Sperbeck, S.J.; Osumi, T.; Hashimoto, T. and Lalwani, N.D. (2001) Transcription regulation of peroxisomal fatty acyl-CoA oxidase and enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase in rat liver by peroxisome proliferators. *Proc. Natl. Acad. Sci. USA*, **83**(6), 1747-1751.

[29] Corton, J.C.; Bocos, C.; Moreno, E.S.; Merritt, A.; Marsman, D.S.; Sausen, P.J.; Cattley, R.C. and Gustafsson, J.A. (1996) Rat 17 β-Hydroxysteroid dehydrogenase type IV is a novel peroxisome proliferator-inducible gene. *Mol. Pharmacol.*, **50**(5), 1157-1166.

[30] Kurochkin, I.V.; Mizuno, Y.; Konagaya, A.; Sakaki, Y.; Schonbach, C. and Okazaki, Y. (2007) Novel peroxisomal protease Tysnd1 processes. *EMBO J.*, **26**(3), 835-845.

[31] Gulick, T.; Cresci, S.; Caira, T.; Moore, D.D. and Kelly, D.P. (1994) The peroxisomal proliferator-activated receptor regulates mitochondrial fatty acid oxidative enzyme gene expression. *Proc. Natl. Acad. Sci USA*, **91**(23), 11012-11016.

[32] Brun, S.; Carmona, C.; Mampel, T.; Viñas, O.; Giralt, M.; Iglesias, R. and Villarroya, F. (1999) Activators of peroxisome proliferator-activated receptor-α induce expression of the uncoupling protein-3 gene in skeletal muscle. A potential mechanism for the lipid intake-dependent activation of uncoupling protein-3 gene expression at birth. *Diabetes*, **48**(6), 1217-1222.

[33] Muerhoff, A.S.; Griffin, K.J. and Johnson, E. (1992) The peroxisome proliferator-activated receptor mediates the induction of CYP4A6, a cytochrome P450 fatty acid omega-hydroxylase, by clofibric acids. *J. Biol. Chem.*, **267**(27), 19051-19053.

[34] Aldridge, T.C.; Tugwood, J.D. and Green, S. (1995) Identification and characterization of DNA elements implicated in the regulation of CYP4A1 transcription. *Biochem. J.*, **306**(1), 473-479.

[35] Castelein, H.; Gulick, T.; Declercq, P.E.; Mannaerts, G.P.; Moore, D.D. and Baes, M.I. (1994) The peroxisome proliferator activated receptor regulates malic enzyme gene expression. *J. Biol. Chem.*, **269**(43), 26754-26758.

[36] Staels, B.; van Tol, A.; Skretting, G. and Auwerx, J. (1992) Lecithin:cholesterol acyltransferase gene expression is regulated in a tissue-selective manner by fibrates. *J. Lipid. Res.*, **33**(5), 727-735.

[37] Staels, B.; Peinado-Onsurbe, J. and Auwerx, J. (1992) Down-regulation of hepatic lipase gene expression and activity by fenofibrate. *Biochim. Biophys. Acta*, **24**(11), 227-230.

[38] Acton, S.; Rigotti, A.; Landschulz, K.T.; Xu, S.; Hobbs, H. and Krieger, M. (1996) Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science*, **271**(5248), 460-461.

[39] Calvo, D.; Gomez-Coronado, D.; Lasunción, M.A. and Vega, M.A. (1997) CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. *Arterioscler. Thromb. Vasc. Biol.*, **17**(11), 2341-2349.

[40] Kersten, S.; Seydoux, J.; Peters, J.M.; Gonzalez, F.J.; Desvergne, B. and Wahli, W. (1999) Peroxisome proliferator-activated receptorα mediates the adaptive response to fasting. *J. Clin. Invest.*, **103**(11), 1489-1498.

[41] Guerre-Millo, M.; Gervois, P.; Raspé, E.; Madsen, L.; Poulain, P.; Derudas, B.; Herbert, J.-M.; Winegar, D.A.; Willson, T.M.; Fruchart, J.-C.; Berge, R.K. and Staels, B. (2000) Peroxisome proliferator-activated receptor α activators improve insulin sensitivity and reduce adiposity. *J. Biol. Chem.*, **275**(22), 16638-16642.

[42] Chou, C.J.; Haluzík, M.; Gregory, C.; Dietz, K.R.; Vinson, C.; Gavrilova, O. and Reitman, M.L. (2002) WY14,643, a peroxisome proliferator-activated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F-1 mice. *J. Biol. Chem.*, **277**(27), 24484-24489.

[43] Xu, J.; Christian, B. and Jump, D.B. (2006) Regulation of rat hepatic L-piruvate kinase promoter composition and activity by glucose, n-polyunsaturated fatty acids, and peroxisome proliferator-activated receptor α agonist. *J. Biol. Chem.*, **281**(27), 18351-18362.

[44] Wu, P.; Peters, J.M. and Harris, R.A. (2001) Adaptative increased in piruvate dehydrogenase kinase 4 during starvation is mediated by peroxisome proliferator-activated receptor alpha. *Biochem. Biophys. Res. Commun.*, **287**(2), 391-396.

[45] Desvergne, B.; Michalik, L. and Wahli, W. (2004) Be fit or be sick: peroxisome proliferator-activated receptors are down the road. *Mol. Endocrinol.*, **18**(6), 1321-1332.

[46] Walker, C.G.; Sugden, M.C.; Gibbson, G.F. and Holness, M.J. (2007) Peroxisome proliferator-activated receptor alpha deficiency modifies glucose handling by isolated mouse adipocytes. *J. Endocrinol.*, **193**(1), 39-43.

[47] Panadero, M.I.; Herrera, E. and Bocos, C. (2005) Different sensitivity of PPARα gene expression to nutritional changes in liver of suckling and adult rats. *Life Sci.*, **76**(9), 1061-1072.

[48] Duplus, E.; Glorian, M. and Forest, C. (2000) Fatty acid regulation of gene transcription. *J. Biol. Chem.*, **275**(40), 30749-30752.

[49] Guerre-Millo, M.; Rouault, C.; Poulain, P.; Andre, J.; Poitout, V.; Peters, J.M.; Gonzalez, F.J.; Fruchart, J.-C.; Reach, G. and Staels, B. (2001) PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. *Diabetes*, **50**(12), 2809-2814.

[50] Haluzik, M.; Gavrilova, O. and LeRoith, D. (2004) Peroxisome proliferator-activated receptor-alpha deficiency does not alter insulin sensitivity in mice maintained on regular or high-fat diet: hyperinsulinemic-euglycemic clamp studies. *Endocrinology*, **145**(4), 1662-1667.

[51] Knauf, C.; Rieusset, J.; Foretz, M.; Cani, P.D.; Uldry, M.; Hosokawa, M.; Martinez, E.; Bringart, M.; Waget, A.; Kersten, S.; Desvergne, B.; Gremlich, S.; Wahli, W.; Seydoux, J.; Delzenne, N.M.; Thorens, B. and Burcelin, R. (2006) Peroxisome proliferator-activated receptor–α null mice have increased white tissue glucose utilization, GLUT4, and fat mass: role in liver and brain. *Endocrinology*, **147**(9), 4067-4078.

[52] Ahmed, W.; Ziouzenkova, O.; Brown, J.; Devchand, P.; Francis, S.; Kadakia, M.; Kanda, T.; Orasanu, G.; Sharlach, M.; Zandbergen, F. and Plutzky, J. (2007) PPARs and their metabolic modulation: new mechanisms for transcriptional regulation? *J. Int. Med.*, **262**(2), 184-198.

[53] Neve, B.P.; Corseaux, D.; Chinetti, G.; Zawadzki, C.; Fruchart, J.-C.; Duriez, P.; Staels, B. and Jude, B. (2001) PPARalpha agonists inhibit tissue factor expression in human monocytes and macrophages. *Circulation*, **103**(2), 207-212.

[54] Goya, K.; Sumitani, S.; Kitamura, T.; Yamamoto, H.; Kurebayashi, S.; Saito, H.; Kouhara, H.; Kasayama, S. and Kawase, I. (2004) Peroxisome proliferator-activated receptor α agonists increase nitric oxide synthase expression in vascular endothelial cells. *Arterioscler. Thromb. Vasc. Biol.*, **24**(4), 658-663.

[55] Delerive, P.; Gervois, P.; Fruchart, J.-C. and Staels, B. (2000) Induction of Ikappa-Bα expression as a mechanism contributing to the anti-inflammatory activities of the peroxisome proliferator-

activated receptor-α activators. *J. Biol. Chem.*, **275**(47), 36703-36707.

[56] Youssef, J. and Badr, M. (2004) Role of peroxisome proliferator-activated receptors in inflammation control. *J. Biomed. Biotechnol.*, **2004**(3), 156-166.

[57] Poynter, M.E. and Daynes, R.A. (1998) Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor-kB signaling, and reduces inflamatory cytokine production in aging. *J. Biol. Chem.*, **273**(49), 32833-32841.

[58] Shu, H.; Wong, B.; Zhou, G.; Li, Y.; Berger, J. and Wood, J.W. (2000) Activation of PPARalpha or gamma reduces secretion of matrix metalloproteinase 9 but not interleukin 8 from human monocytic THP-1 cells. *Biochem. Biophys. Res. Commun.*, **267**(1), 345-349.

[59] Zambon, A.; Gervois, P.; Pauletto, P.; Fruchart, J.-C. and Staels, B. (2006) Modulation of hepatic inflammatory risk markers of cardiovascular diseases by PPARα activators. Clinical and experimental evidences. *Arterioscler. Thromb. Vasc. Biol.*, **26**(5), 977-986.

[60] Kockx, M.; Gervois, P.; Poulain, P.; Derudas, B.; Peters, J.M.; Gonzalez, F.J.; Princen, M.G.; Kooistra, T. and Staels, B. (1999) Fibrates suppress fibrinogen gene expression in rodents *via* activation of the peroxisome proliferator-activated receptor-alpha. *Blood*, **93**(9), 2991-2998.

[61] Corton, J.C.; Fan, L.Q.; Brown, S.; Anderson, S.P.; Bocos, C.; Cattley, R.C.; Mode, A. and Gustafsson, J.A. (1998) Down-regulation of cytochrome P450 2C family members and positive acute-phase response gene expression by peroxisome proliferator chemicals. *Mol. Pharmacol.*, **54**(3), 463-473.

[62] Kleemann, R.; Gervois, P.; Verschuren, L.; Princen, H.M.G. and Kooistra, T. (2003) Fibrates down-regulate IL-1-stimulated C-reactive protein gene expression in hepatocytes by reducing nuclear p50-NFκB-C/EBP-β complex formation. *Blood*, **101**(2), 545-551.

[63] Keating, G.M. and Croom, K.F. (2007) Fenofibrate. A review of its use in primary dyslipidaemia, the metabolic syndrome and type 2 diabetes mellitus. *Drugs*, **67**(1), 121-153.

[64] Sanguino, E.; Roglans, N. and Laguna, J.C. (2007) Fibrates, PPARα and the ageing process: possible transcendence in human therapeutics, in *New emerging pharmacological targets in metabolic diseases* (Vázquez, M. and Laguna, J.A., Eds.), Transworld Research Network, Kerala, India, pp.119-128.

[65] Lemberger, T.; Staels, B.; Saladin, R.; Desvergne, B.; Auwerx, J. and Wahli, W. (1994) Regulation of the peroxisome proliferator-activated receptor α gene by glucocorticoids. *J. Biol. Chem.*, **269**(40), 24527-24530.

[66] Panadero, M.I.; Vidal, H.; Herrera, E. and Bocos, C. (2001) Nutritionally induced changes in the peroxisome proliferator-activated receptor-alpha gene expression in liver of suckling rats are dependent on insulinaemia. *Arch. Biochem. Biophys.*, **394**(2), 182-188.

[67] Soria, A.; González, M.; Vidal, H.; Herrera, E. and Bocos, C. (2005) Triglyceridemia and peroxisome proliferator-activated receptor-alpha expression are not connected in fenofibrate-treated pregnant rats. *Mol. Cell. Biochem.*, **273**(1-2), 97-107.

[68] Sterchele, P.F.; Sun, H.; Peterson, R.E. and Vanden Heuvel, J.P. (1996) Regulation of peroxisome proliferator-activated receptor-alpha mRNA in rat liver. *Arch. Biochem. Biophys.*, **326**(2), 281-289.

[69] Steineger, H.H.; Sorensen, H.N.; Tugwood, J.D.; Skrede, A.; Spydevold, O. and Gautvik, K.M. (1994) Dexamethasone and insulin demonstrate marked and opposite regulation of the steady-state mRNA level of the peroxisomal proliferator-activated receptor (PPAR) in hepatic cells. Hormonal modulation of fatty-acid induced transcription. *Eur. J. Biochem.*, **225**(3), 967-974.

[70] Panadero, M.; Herrera, E. and Bocos, C. (2000) Peroxisome proliferator-activated receptor-alpha expression in rat liver during postnatal development. *Biochimie*, **82**(8), 723-726.

[71] Shin, M.; Kim, I.; Inoue, Y.; Kimura, S. and Gonzalez, F.J. (2006) Regulation of mouse hepatic alpha-amino-beta-carboxymuconate-epsilon-semialdehyde decarboxylase, a key enzyme in the tryptophan-nicotinamide adenine dinucleotide pathway, by hepatocyte nuclear factor 4alpha and peroxisome proliferator-activated receptor alpha. *Mol. Pharmacol.*, **70**, 1281-1290.

[72] Rodríguez, C.; Noé, V.; Cabrero, A.; Ciudad, C.J. and Laguna, J.C. (2000) Differences in the formation of PPARalpha-RXR/acoPPRE complexes between responsive and nonresponsive species upon fibrate administration. *Mol. Pharmacol.*, **58**(1), 185-193.

[73] Huang, Q.; Alvares, K.; Bradfield, C.A. and Reddy, J.K. (1994) Association of peroxisome proliferator-activated receptor and Hsp72. *J. Biol. Chem.*, **269**(11), 8493-8497.

[74] Sumanasekera, W.; Tien, E.; Davis, J.W. 2nd; Turpey, R.; Perdew, G. and Vanden Heuvel, J.P. (2003) Heat shock protein-90 (Hsp90) acts as a repressor of peroxisome proliferator-activated receptor-alpha (PPARalpha) and PPARbeta activity. *Biochemistry*, **42**(36), 10726-10735.

[75] Duez, H.; Lefebvre, A.M.; Poulain, P.; Torra, I.P.; Percevault, F.; Luc, G.; Peters, J.M.; Gonzalez, F.J.; Ginete, R.; Helleboid, S.; Dzavik, V.; Fruchart, J.-C.; Fievet, C.; Lefebvre, P. and Staels, B. (2005) Regulation of human apoA-I by gemfibrozil and fenofibrate through selective peroxisome proliferator-activated receptor alpha modulation. *Arterioscler. Thromb. Vasc. Biol.*, **25**(3), 585-591.

[76] Sanguino, E.; Ramón, M.; Michalik, L.; Wahli, W.; Alegret, M.; Sánchez, R.; Vázquez, M. and Laguna, J.C. (2004) Lack of hypotriglyceridemic effect of gemfibrozil as a consequence of age-related changes in rat liver PPAR alpha. *Biochem. Pharmacol.*, **67**(1), 157-166.

[77] Foucher, C.; Rattier, S.; Flavell, D.M.; Talmud, P.J.; Humphries, S.E.; Kastelein, J.J.; Ayyobi, A.; Pimstone, S.; Frohlich, J.; Ansquer, J.C. and Steiner, G. (2004) Response to micronized fenofibrate treatment is associated with the peroxisome-proliferator-activated receptors alpha G/C intron7 polymorphism in subjects with type 2 diabetes. *Pharmacogenetics*, **14**(12), 823-829.

[78] Holden, P.R. and Tugwood, J.D. (1999) Peroxisome proliferator-activated receptor alpha: role in rodent liver cancer and species differences. *J. Mol. Endocrinol.*, **22**(1), 1-8.

[79] Palmer, A.; Hsu, M.; Griffin, K.; Raucy, J. and Johnson, E. (1998) Peroxisome proliferator activated receptor-α expression in human liver. *Mol. Pharmacol.*, **53**(1), 14-22.

[80] Vamecq, J. and Latruffe, N. (1999) Medical significance of peroxisome proliferator-activated receptors. *Lancet*, **354**(9173), 141-148.

[81] Chakravarthy, M.V.; Pan, Z.; Zhu, Y.; Tordjman, K.; Schneider, J.G.; Coleman, T.; Turk, J. and Semenkovich, C.F. (2005) "New" hepatic fat activates PPARα to maintain glucose, lipid, and cholesterol homeostasis. *Cell. Metab.*, **1**(5), 309-322.

[82] Wolfrum, C.; Borrmann, C.M.; Börchers, T. and Spener, F. (2001) Fatty acids and hypolipidemic drugs regulate peroxisome proliferator α and γ mediated gene expression *via* liver fatty acid binding protein: A signaling path to the nucleus. *Proc. Natl. Acad. Sci. USA*, **98**(5), 2323-2328.

[83] Bocos, C.; Gottlicher, M.; Gearing, K.; Banner, C.; Enmark, E.; Teboul, M.; Crickmore, A. and Gustafsson, J.A. (1995) Fatty acid activation of peroxisome proliferator-activated receptor (PPAR). *J. Steroid. Biochem. Mol. Biol.*, **53**(1-6), 467-473.

[84] Michalik, L. and Wahli, W. (2007) Guiding ligands to nuclear receptors. *Cell*, **129**(4), 649-651.

[85] Berger, J. and Moller, D.E. (2002) The mechanisms of action of PPARs. *Annu. Rev. Med.*, **53**, 409-435.

[86] Xu, Y.; Lu, L.; Greyson, C.; Rizeq, M.; Nunley, K.; Wyatt, B.; Bristow, M.R.; Long, C.S. and Schawartz, G.G. (2006) The PPAR-alpha activator fenofibrate fails to provide myocardial protection in ischemia and reperfusion in pigs. *Am. J. Physiol. Heart. Circ. Physiol.*, **290**(5), 1798-1807.

[87] Olivier, P.; Plancke, M.O.; Marzin, D.; Clavey, V.; Sauzieres, J. and Fruchart, J.-C. (1988) Effects of fenofibrate, gemfibrozil and nicotinic acid on plasma lipoprotein levels in normal and hyperlipidemic mice. A proposed model for drug screening. *Atherosclerosis*, **70**(1-2), 107-114.

[88] Krause, B.R.; Barnett, B.C.; Essenburg, A.D.; Kieft, K.A.; Auerbach, B.J.; Bousley, R.; Stanfield, R.; Newton, R.S. and Bisgaier, C.L. (1996) Opposite effects of bezafibrate and gemfibrozil in both normal and hypertriglyceridemic rats. *Atherosclerosis*, **127**(1), 91-101.

[89] Akbiyik, F.; Ray, D.M.; Bozkaya, H. and Demirpence, E. (2004) Ligand- and species-dependent activation of PPARalpha. *Cell. Physiol. Biochem.*, **14**(4-6), 269-276.

[90] Olivier, P.; Plancke, M.O.; Theret, N.; Marzin, D.; Clavey, V. and Fruchart, J.-C. (1988) Effects of fenofibrate on lipoprotein metabolism and fatty acid distribution in Zucker rats. *Atherosclerosis*, **74**(1-2), 15-21.

[91] Chaput, E.; Saladin, R.; Silvestre, M. and Edgar, A. (2000) Fenofibrate and rosiglitazone lower serum triglycerides with opposing effects on body weight. *Biochem. Biophys. Res. Commun.*, **271**(2), 445-450.

[92] Cicero, A.F.; Derosa, G.; Miconi, A.; Laghi, L.; Nascetti, S. and Gaddi, A. (2005) Possible role of ubiquinone in the treatment of massive hypertriglyceridemia resistant to PUFA and fibrates. *Biomed. Pharmacother.*, **59**(6), 312-317.

[93] Randle, P.J.; Garland, P.B.; Hales, C.N. and Newsholme, E.A. (1963) The glucose fatty-acid cycle. Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. *Lancet*, **1**(7285), 785-789.

[94] Ferré, P. (2004) The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes*, **53**(1 Suppl), S43-S50.

[95] Steiner, G. (2007) Altering triglyceride concentrations changes insulin-glucose relationships in hypertriglyceridemic patients. Double-blind study with gemfibrozil with implications for atherosclerosis. *Diabetes Care*, **14**(11), 1077-1081.

[96] Tenenbaum, A.; Motro, M.; Fisman, E.Z.; Schwammenthal, E.; Adler, Y.; Goldenbeg, I.; Leor, J.; Boyko, V.; Mandelzweig, L. and Behar, S. (2004) Peroxisome proliferator-activated receptor ligand bezafibrate for prevention of type 2 diabetes mellitus in patients with coronary artery disease. *Circulation*, **109**(18), 2197-2202.

[97] Willson, T.M.; Brown, P.J.; Sternbach, D.D. and Henke, B.R. (2000) The PPARs: from orphan receptors to drug discovery. *J. Med. Chem.*, **43**(4), 527-550.

[98] Keech, A.; Simes, R.J.; Barter, P.; Best, J.; Scott, R.; Taskinen, M.R.; Forder, P.; Pillai, A.; Davais, T.; Glasziou, P.; Drury, P.; Kesaniemi, Y.A.; Sullivan, D.; Hunt, D.; Colman, P.; d´Emden, M.; Whiting, M.; Ehnholm, C.; Laakso, M. and the FIELD study investigators (2005) Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet*, **366**(9500), 1849-1861.

[99] Anderlová, K.; Dolezalová, R.; Housová, J.; Bosanská, L.; Haluzíková, D.; Kremen, J. and Haluzík, M. (2006) The influence of PPAR-agonist fenofibrate on insulin sensitivity and selected adipose tissue-derived hormones in obese women with type 2 diabetes. *Physiol. Res.*, **56**(5), 579-586.

[100] Baes, M.I.; Castelein, H.; Desmet, L. and Declercq, P.E. (1995) Antagonism of COUP-TF and PPAR alpha/RXR alpha on the activation of the malic enzyme gene promoter: modulation by 9-cisRA. *Biochem. Biophys. Res. Commun.*, **215**(1), 338-345.

[101] Winrow, C.J.; Marcus, S.L.; Miyata, K.S.; Zhang, B.; Capone, J.P. and Rachubinski, R.A. (1994) Transactivation of the peroxisome proliferator-activated receptor is differentially modulated by hepatocyte nuclear factor-4. *Gene Expr.*, **4**(1-2), 53-62.

[102] Vu-Dac, N.; Gervois, P.; Jakel, H.; Nowak, M.; Baugé, E.; Dehondt, H.; Staels, B.; Pennacchio, L.; Rubin, E.; Fruchart-Najib, J. and Fruchart, J.-C. (2003) Apolipoprotein A5, a crucial determinant of plasma triglyceride levels, is highly responsive to peroxisome proliferator-activated receptor a activators. *J. Biol. Chem.*, **278**(20), 17982-17985.

[103] Ribas, V.; Palomer, X.; Roglans, N.; Rotlan, N.; Fievet, C.; Tailleux, A.; Julve, J.; Laguna, J.C.; Blanco-Vaca, F. and Escobá-Gil, J.C. (2005) Paradoxical exacerbation of combined hyperlipidemia in human apolipoprotein A-II transgenic mice treated with fenofibrate. *Biochim. Biophys. Acta*, **1737**(2-3), 130-137.

[104] Gervois, P.; Chopin-Delannoy, S.; Fadel, A.; Dubois, G.; Kosykh, V.; Fruchart, J.-C.; Najib, J.; Laudet, V. and Staels, B. (1999) Fibrates increase human REV-ERBα expression in liver *via* novel peroxisome proliferator-activated receptor response element. *Mol. Endocrinol.*, **13**(3), 400-409.

[105] Vu-Dac, N.; Chopin-Delannoy, S.; Gervois, P.; Bonnelye, E.; Martin, G.; Fruchart, J.-C.; Laudet, V. and Staels, B. (1998) The nuclear receptors peroxisome proliferator-activated receptor α and Rev-erb α mediate the species-specific regulation of apolipoprotein A-I expression by fibrates. *J. Biol. Chem.*, **273**(40), 25713-25720.

[106] Qu, S.; Su, D.; Altomonte, J.; Kamagate, A.; He, J.; Perdomo, G.; Tse, T.; Jiang, Y. and Dong, H.H. (2007) PPAR alpha mediates the hypolipidemic action of fibrates by antagonizing FoxO1. *Am. J. Physiol. Endocrinol. Metab.*, **292**(2), E421-E434.

[107] Jow, L. and Mukherjee, R. (1995) The human peroxisome proliferator-activated receptor (PPAR) subtype NUCI represses the activation of hPPARα and thyroid hormone receptors. *J. Biol. Chem.*, **270**(8), 3836-3840.

[108] Muoio, D.M.; MacLean, P.S.; Lang, D.B.; Li, S.; Houmard, J.A.; Way, J.M.; Winegar, D.A.; Corton, J.C.; Dohm, G.L. and Kraus, W.E. (2002) Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. *J. Biol. Chem.*, **277**(29), 26089-26097.

[109] Keller, H.; Givel, F.; Perround, M. and Wahli, W. (1995) Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements. *Mol. Endocrinol.*, **9**(7), 794-804.

[110] Jeong, S.; Kim, M.; Han, M.; Lee, H.; Ahn, J.; Kim, M.; Song, Y.-H.; Shin, C.; Nam, K.-H.; Kim, T.W.; Oh, G.T. and Yoon, M. (2004) Fenofibrate prevents obesity and hypertriglyceridemia in low-density lipoprotein receptor-null mice. *Metabolism*, **53**(5), 607-613.

[111] Jeong, S. and Yoon, M. (2007) Inhibition of the actions of peroxisome proliferator-activated receptor alpha on obesity by estrogen. *Obesity (Silver Spring)*, **15**(6), 1430-1440.

[112] Alvarez, J.J.; Montelongo, A.; Iglesias, A.; Lasunción, M.A. and Herrera, E. (1996) Longitudinal study on lipoprotein profile, high density lipoprotein subclas, and postheparin lipases during gestation in women. *J. Lipid. Res.*, **37**(2), 299-308.

[113] Corton, J.C.; Bocos, C.; Moreno, E.S.; Merrit, A.; Cattley, R.C. and Gustafsson, J.-A. (1997) Peroxisome proliferator alter the expression of estrogen-metabolizing enzymes. *Biochimie*, **79**(2-3), 151-162.

[114] Roglans, N.; Sanguino, E.; Peris, C.; Alegret, M.; Vázquez, M.; Adzet, T.; Díaz, C.; Hernández, G.; Laguna, J.C. and Sánchez, R.M. (2002) Atorvastatin treatment induced peroxisome proliferator-activated receptor alpha expression and decreased plasma non-esterified fatty acids and liver triglyceride in fructose-fed rats. *J. Pharmacol. Exp. Ther.*, **302**(1), 232-239.

[115] Sanguino, E.; Roglans, N.; Alegret, M.; Sanchez, R.M.; Vazquez-Carrera, M. and Laguna, J.C. (2005) Atorvastatin reverses age-related reduction in rat hepatic PPARalpha and HNF-4. *Br. J. Pharmacol.*, **145**(7), 853-861.

[116] Dorfmeister, B.; Brandlhofer, S.; Schaap, F.G.; Hermann, M.; Furnsinn, C.; Hagerty, B.P.; Stangl, H.; Patsch, W. and Strobl, W. (2006) Apolipoprotein AV does not contribute to hypertriglyceridaemia or triglyceride lowering by dietary fish oil and rosiglitazone in obese Zucker rats. *Diabetologia*, **49**(6), 1324-1332.

[117] Cheung, C.; Akiyama, T.E.; Ward, J.M.; Nicol, C.J.; Feiganbaum, L.; Vinson, C. and Gonzalez, F.J. (2004) Disminished hepatocellular proliferation in mice humanized from the nuclear receptor peroxisome proliferator-activated receptor a. *Cancer Res.*, **64**(11), 3849-3854.

[118] Hsu, M.H.; Palmer, C.N.; Griffin, K.J. and Johnson, E.F. (1995) A single amino acid change in the mouse peroxisome proliferator-activated receptor alpha alters transcriptional responses to peroxisome proliferators. *Mol. Pharmacol.*, **48**(3), 559-567.

[119] Bosse, Y.; Pascot, A.; Dumont, M.; Brochu, M.; Prud´homme, D.; Bergeron, J.; Despres, J.P. and Vohl, M.C. (2002) Influences of the PPAR alpha-L162V polymorphism on plasma HDL(2)-cholesterol response of abdominally obese men treated with gemfibrozil. *Genet. Med.*, **4**(4), 311-315.

[120] Brisson, D.; Ledoux, K.; Bosse, Y.; StPierre, J.; Julien, P.; Perron, P.; Hudson, T.J.; Vohl, M.C. and Gaudet, D. (2002) Effect of apolipoprotein E, peroxisome proliferator-activated receptor alpha and lipoprotein lipase gene mutations on the ability of fenofibrate to improve lipid profiles and reach clinical guideline targets among hypertriglyceridemic patients. *Pharmacogenetics*, **12**(4), 313-320.

[121] Sapone, A.; Peters, J.M.; Sakai, S.; Tomita, S.; Papiha, S.S.; Dai, R.; Friedman, F.K. and Gonzalez, F.J. (2000) The human peroxisome proliferator-activated receptor alpha gene: identification and functional characterization of two natural allelic variants. *Pharmacogenetics*, **10**(4), 321-333.

[122] Tai, E.S.; Collins, D.; Robins, S.J.; O'Connor, J.J., Jr.; Bloomfield, H.E.; Ordovas, J.M.; Schaefer, E.J. and Brousseau, M.E. (2006) The L162V polymorphism at the peroxisome proliferator activated receptor alpha locus modulates the risk of cardiovascular events associated with insulin resistance and diabetes mellitus: the Veterans Affairs HDL Intervention Trial (VA-HIT). *Atherosclerosis*, **187**(1), 153-160.

[123] Burns, K. and Vanden Heuvel, J.P. (2007) Modulation of PPAR activity *via* phosphorylation. *Biochim. Biophys. Acta*, **1771**(8), 952-960.

[124] Passilly, P.; Schohn, H.; Jannin, B.; Malki, M.C.; Boscoboinik, D.; Dauça, M. and Latruffe, N. (1999) Phosphorylation of peroxisome proliferator-activated receptor α in rat Fao cells and stimulation by ciprofibrate. *Biochem. Pharmacol.*, **58**(6), 1001-1009.

[125] Shalev, A.; Siegrist-Kaiser, C.A.; Yen, P.M.; Wahli, W.; Burger, A.G.; Chin, W.W. and Meier, C.A. (1996) The peroxisome prolif-

erator-activated receptor α is a phosphoprotein: regulation by insulin. *Endocrinology*, **137**(10), 4499-4502.

[126] Martin, G.; Duez, H.; Blanquart, C.; Berezowski, V.; Poulain, P.; Fruchart, J.-C.; Fruchart-Najib, J.; Glineur, C. and Staels, B. (2001) Statin-induced inhibition of the Rho-signaling pathway activates PPARalpha and induces HDL apoA-I. *J. Clin. Invest.*, **107**(11), 1423-1432.

[127] Nesto, R.W. (2005) Beyond low-density lipoprotein: addressing the atherogenic lipid triad in type 2 diabetes mellitus and the metabolic syndrome. *Am. J. Cardiovasc. Drugs*, **5**(6), 379-387.

[128] Dayspring, T. and Pokrywka, G. (2006) Fibrate therapy in patients with metabolic syndrome and diabetes mellitus. *Curr. Atheroscler. Rep.*, **8**(5), 356-364.

[129] González, M.; Panadero, M.I.; Herrera, E. and Bocos, C. (2007) PPARα as target for pharmacological and nutritional agents affecting lipid metabolism. in *New emerging pharmacological targets in metabolic diseases*; (Vázquez, M. and Laguna, J.A. Eds.) Transworld Research Network, Kerala: India, pp. 71-118.

[130] Das, S.K. and Chakrabarti, R. (2006) Role of PPAR in cardiovascular diseases. *Recent Pat. Cardiovasc. Drug Discov.*, **1**(2), 193-209.

[131] Yumuk, V. (2006) Targeting components of the stress system as potential therapies for the metabolic syndrome: the peroxisome proliferator-activated receptors. *Ann. NY Acad. Sci.*, **1083**, 306-318.

[132] Olefsky, J.M. (2000) Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists. *J. Clin. Invest.*, **106**(4), 467-472.

[133] Chang, F.; Jaber, L.A.; Berlie, H.D. and O´Connell, M.B. (2007) Evolution of peroxisome proliferator-activated receptor agonists. *Ann. Pharmacother.*, **41**(6), 973-983.

[134] Mamnoor, P.K.; Hegde, P.; Datla, S.R.; Damarla, R.K.; Rajagopalan, R. and Chakrabarti, R. (2006) Antihypertensive effect of ragaglitazar: a novel PPARalpha and gamma dual activator. *Pharmacol. Res.*, **54**(2), 129-135.

[135] Kendall, D.M.; Rubin, C.J.; Mohideen, P.; Ledeine, J.M.; Belder, R.; Gross, J.; Norwood, P.; O'Mahony, M.; Sall, K.; Sloan, G.; Roberts, A.; Fiedorek, F.T. and DeFronzo, R.A. (2006) Improvement of glycemic control, triglycerides, and HDL cholesterol levels with muraglitazar, a dual (alpha/gamma) peroxisome proliferator-activated receptor activator, in patients with type 2 diabetes inadequately controlled with metformin monotherapy: A double-blind, randomized, pioglitazone-comparative study. *Diabetes Care*, **29**(5), 1016-1023.

[136] Nissen, S.E.; Wolski, K. and Topol, E.J. (2005) Effect of muraglitazar on death and major adverse cardiovascular events in patients with type 2 diabetes mellitus. *JAMA*, **294**(20), 2581-2586.

[137] Chakrabarti, R.; Misra, P.; Vikramadithyan, R.K.; Premkumar, M.; Hiriyan, J.; Datla, S.R.; Damarla, R.K.; Suresh, J. and Rajagopalan, R. (2004) Antidiabetic and hypolipidemic potential of DRF 2519- a dual activator of PPAR-alpha and PPAR-gamma. *Eur. J. Pharmacol.*, **491**(2-3), 195-206.

[138] Reifel-Miller, A.; Otto, K.; Hawkins, E.; Barr, R.; Bensch, W.R.; Bull, C.; Dana, S.; Klausing, K.; Martin, J.A.; Rafaeloff-Phail, R.; Rafizadeh-Montrose, C.; Rhodes, G.; Robey, R.; Rojo, I.; Rungta, D.; Snyder, D.; Wilbur, K.; Zhang, T.; Zink, R.; Warshawsky, A. and Brozinick, J.T. (2005) A peroxisome proliferator-activated receptor alpha/gamma dual agonist with a unique *in vitro* profile and potent glucose and lipid effects in rodent models of type 2 diabetes and dyslipidemia. *Mol. Endocrinol.*, **19**(6), 1593-1605.

[139] Wallace, J.M.; Schwarz, M.; Coward, P.; Houze, J.; Sawyer, J.K.; Kelley, K.L.; Chai, A. and Rudel, L.L. (2005) Effects of peroxisome proliferator-activated receptor alpha/delta agonists on HDL-cholesterol in vervet monkeys. *J. Lipid. Res.*, **46**(5), 1009-1016.

[140] Tenenbaum, A.; Fisman, E.Z.; Motro, M. and Adler, Y. (2006) Atherogenic dyslipidemia in metabolic syndrome and type 2 diabetes: therapeutic options beyond statins. *Cardiovasc. Diabetol.,* **5**, 20-28.

[141] Upton, R.; Widdowson, P.S.; Ishii, S.; Tanaka, H. and Williams, G. (1998) Improved metabolic status and insulin sensitivity in obese fatty (fa/fa) Zucker rats and Zucker Diabetic Fatty (ZDF) rats treated with the thiazolidinedione, MCC-555. *Br. J. Pharmacol.*, **125**(8), 1708-1714.

[142] Balint, B.L. and Nagy, L. (2006) Selective modulators of PPAR activity as new therapeutic tools in metabolic diseases. *Endocr. Metab. Immune. Disord. Drug Targets*, **6**(1), 33-43.

[143] Fujimura, T.; Kimura, C.; Oe, T.; Takata, Y.; Sakuma, H.; Aramori, I. and Mutoh, S. (2006) A selective peroxisome proliferator-activated receptor gamma modulator with distinct fat cell regulation properties. *J. Pharmacol. Exp. Ther.*, **318**(2), 863-871.

[144] Feige, J.N.; Gelman, L.; Rossi, D.; Zoete, V.; Metivier, R.; Tudor, C.; Anghel, S.I.; Grosdidier, A. and Lathion, C. (2007) The endocrine disruptor monoethyl-hexyl-phthalate is a selective Peroxisome Proliferator-Activated Receptor gamma modulator that promotes adipogenesis. *J. Biol. Chem.*, **282**(26), 19152-19166.

[145] Davidson, M.H. (2006) Mechanisms for the hypotriglyceridemic effect of marine omega-3 fatty acids. *Am. J. Cardiol.*, **98**(4A), 27i-33i.

[146] Bassaganya-Riera, J.; Reynolds, K.; Martino-Catt, S.; Cui, Y.; Hennighausen, L.; Gonzalez, F.; Rohrer, J.; Benninghoff, A.U. and Hontecillas, R. (2004) Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. *Gastroenterology*, **127**(3), 777-791.

[147] Houseknecht, K.L.; Vanden Heuvel, J.P.; Moya-Camarena, S.Y.; Portocarrero, C.P.; Peck, L.W.; Nickel, K.P. and Belury, M.A. (1998) Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. *Biochem. Biophys. Res. Commun.*, **244**(3), 678-682.

[148] Fu, J.; Gaetani, S.; Oveisi, F.; Lo, V.J.; Serrano, A.; Rodriguez de, F.F.; Rosengarth, A.; Luecke, H.; Di, G.B.; Tarzia, G. and Piomelli, D. (2003) Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature*, **425**(6953), 90-93.

[149] Su, H.F.; Samsamshariat, A.; Fu, J.; Shan, Y.X.; Chen, Y.H.; Piomelli, D. and Wang, P.H. (2006) Oleylethanolamide activates Ras-Erk pathway and improves myocardial function in doxorubicin-induced heart failure. *Endocrinology*, **147**(2), 827-834.

[150] Guzman, M.; Lo, V.J.; Fu, J.; Oveisi, F.; Blazquez, C. and Piomelli, D. (2004) Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). *J. Biol. Chem.*, **279**(27), 27849-27854.

[151] Yajima, H.; Ikeshima, E.; Shiraki, M.; Kanaya, T.; Fujiwara, D.; Odai, H.; Tsuboyama-Kasaoka, N.; Ezaki, O.; Oikawa, S. and Kondo, K. (2004) Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor alpha and gamma and reduce insulin resistance. *J. Biol. Chem.*, **279**(32), 33456-33462.

[152] Miura, Y.; Hosono, M.; Oyamada, C.; Odai, H.; Oikawa, S. and Kondo, K. (2005) Dietary isohumulones, the bitter components of beer, raise plasma HDL-cholesterol levels and reduce liver cholesterol and triacylglycerol contents similar to PPARalpha activations in C57BL/6 mice. *Br. J Nutr.*, **93**(4), 559-567.

[153] Torres, N.; Torre-Villalvazo, I. and Tovar, A.R. (2006) Regulation of lipid metabolism by soy protein and its implication in diseases mediated by lipid disorders. *J. Nutr. Biochem.*, **17**(6), 365-373.

[154] Mezei, O.; Li, Y.; Mullen, E.; Ross-Viola, J.S. and Shay, N.F. (2006) Dietary isoflavone supplementation modules lipid metabolism *via* PPARα-dependent and -independent mechanisms. *Physiol. Genomics*, **26**(1), 8-14.

[155] Mezei, O.; Banz, W.J.; Steger, R.W.; Peluso, M.R.; Winters, T.A. and Shay, N. (2003) Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese Zucker rats and murine RAW 264.7 cells. *J. Nutr.*, **133**(5), 1238-1243.

[156] Rimando, A.M.; Nagmani, R.; Feller, D.R. and Yokoyama, W. (2005) Pterostilbene, a new agonist for the peroxisome proliferator-activated receptor alpha-isoform, lowers plasma lipoproteins and cholesterol in hypercholesterolemic hamsters. *J. Agric. Food. Chem.*, **53**(9), 3403-3407.

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