Molecular mechanism of PPARα action and its impact on lipid metabolism, inflammation and fibrosis in non-alcoholic fatty liver disease

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Summary

Peroxisome proliferator-activated receptor α (PPARα) is a ligand-activated transcription factor belonging, together with PPARγ and PPARβ/δ, to the NR1C nuclear receptor subfamily. Many PPARα target genes are involved in fatty acid metabolism in tissues with high oxidative rates such as muscle, heart and liver. PPARα activation, in combination with PPARβ/δ agonism, improves steatosis, inflammation and fibrosis in pre-clinical models of non-alcoholic fatty liver disease, identifying a new potential therapeutic area. In this review, we discuss the transcriptional activation and repression mechanisms by PPARα, the spectrum of target genes and chromatin-binding maps from recent genomewide studies, paying particular attention to PPARα-regulation of hepatic fatty acid and plasma lipoprotein metabolism during nutritional transition, and of the inflammatory response. The role of PPARα, together with other PPARs, in non-alcoholic steatohepatitis will be discussed in light of available pre-clinical and clinical data.

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Introduction

PPARα (NR1C1) is a ligand-activated nuclear receptor highly expressed in the liver, initially identified as the molecular target of xenobiotics inducing peroxisome proliferation in rodents. Beside PPARα, the PPAR subfamily contains two other isotypes encoded by the PPARβ/δ (NR1C2) and PPARγ (NR1C3) genes, each displaying isoform-specific tissue distribution patterns and functions. PPARα expression is enriched in tissues with high fatty acid oxidation, inflammation and fibrosis in non-alcoholic fatty liver disease, identifying a new potential therapeutic area. In this review, we discuss the transcriptional activation and repression mechanisms by PPARα, the spectrum of target genes and chromatin-binding maps from recent genomewide studies, paying particular attention to PPARα-regulation of hepatic fatty acid and plasma lipoprotein metabolism during nutritional transition, and of the inflammatory response. The role of PPARα, together with other PPARs, in non-alcoholic steatohepatitis will be discussed in light of available pre-clinical and clinical data.

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models of NASH. Thus, selective and potent PPAR agonism, improves steatosis, inflammation and fibrosis in rodent models of systemic inflammation, atherosclerosis and starvation. PPAR is a nutritional sensor, which allows adaptation of the rates of fatty acid (FA) catabolism, and ligands, thus contributing to specific PPAR isoform-specific transcriptional regulation. PPAR agonists under development for NAFLD treatment.

### Table 1. Functional analysis of PPARα structural domains.

<table>
<thead>
<tr>
<th>Domain</th>
<th>PTM</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>N-term A/B</td>
<td>MAPK-dependent phosphorylation at Ser 6, 12, and 21</td>
<td>Ligand-dependent/independent activation function, Target gene specificity</td>
</tr>
<tr>
<td>C DBD</td>
<td>PKC-dependent phosphorylation at Ser 179 and 230</td>
<td>Providing NR structure flexibility, Potentiating NCoR recruitment</td>
</tr>
<tr>
<td>D Hinge region</td>
<td>SUMOylation at lysine 185</td>
<td>Interaction with c-Jun, Providing NR structure flexibility</td>
</tr>
<tr>
<td>C-term E/F</td>
<td>SUMOylation at lysine 358</td>
<td>Ligand binding specificity, Interaction with RXR and p65</td>
</tr>
</tbody>
</table>

PPARα displays a classical NR canonical architecture. PPARα domains (from A to F) fulfill distinct functions by providing interaction surfaces with other TFs, co-regulators, and ligands, thus contributing to specific PPARα transcriptional regulation. PPARα undergoes several post-translational modifications (PTM) that markedly impact receptor function (details in the text).

### Acid Oxidation (FAO) Rates

Rates such as liver, skeletal muscle, brown adipose tissue, and kidney, although it is also expressed in many tissues and cells including the intestine, vascular endothelium, smooth muscle and immune cells such as monocytes, macrophages and lymphocytes [3]. PPARα is a nutritional sensor, which allows adaptation of the rates of fatty acid (FA) catabolism, lipogenesis and ketone body synthesis, in response to feeding and starvation [4]. PPARα is a transcriptional regulator of genes involved in peroxisomal and mitochondrial β-oxidation, FA transport and hepatic glucose production, the latter being rodent-specific [5]. PPARα negatively regulates pro-inflammatory and acute phase response (APR) signalling pathways, as seen in rodent models of systemic inflammation, atherosclerosis and non-alcoholic steatohepatitis (NASH) [6,7].

### Dyslipidemia and Chronic Inflammation

Dyslipidemia and chronic inflammation are frequent features of non-alcoholic fatty liver disease (NAFLD), likely explaining the association between cardiovascular disease (CVD) and NAFLD. However, there is currently no approved NAFLD treatment. In patients with atherogenic dyslipidemia, fibres act as synthetic PPARα agonists, lower plasma triglycerides and high density lipoprotein (HDL) particles, and raise high density lipoprotein cholesterol (HDL-C) levels. Fibrates reduce triglycerides and small density lipoprotein cholesterol (LDL-C) particles, and raise high density lipoprotein cholesterol (HDL-C) levels. Fibrates reduce major cardiovascular events, especially in patients with high triglyceride and low HDL-C [8]. Thus PPARα agonists may potentially be useful in the management of NAFLD and co-morbidities such as CVD. PPARα activation, in combination with PPARβ/δ agonism, improves steatosis, inflammation and fibrosis in rodent models of NASH [9]. Thus, selective and potent PPARα modulators (SPPARMs) and dual PPAR agonists constitute promising strategies for the treatment of NAFLD. In this review, novel mechanistic insights into PPARα action, in hepatic lipid metabolism, under different nutritional states, and its role in liver inflammation and fibrosis are presented. We also summarize the (pre)clinical findings on PPAR agonists under development for NAFLD treatment.

### Functional analysis of PPARα structure

**Canonical structure of PPARα**

The human and mouse PPARα genes, respectively on chromosome 22 and chromosome 15, encode 468 amino acid polypeptides with 91% homology. In both species, the coding DNA sequence spans the 3' region of exon 3, exons 4–7, and the 5' extremity of exon 8 [10]. PPARα has a canonical nuclear receptor organization with six domains starting from the N-terminal A/B to the C terminus F domain (Table 1). These domains integrate intracellular signals to control the transcriptional activity of multiple target genes. The A/B domain contains the AF-1 region providing basal, ligand-binding-independent and -dependent activity, which can be potentiated by MAPK phosphorylation of serines 6, 12, and 21 [11]. Comparative studies of chimeric PPARαβ/γ proteins identified the AF-1 region as a determinant of isotype-specific target gene activation [12]. The A/B domain is connected to the DNA binding domain (DBD), harboring two zinc-fingers, which binds PPAR response elements (PPREs), localized in gene regulatory regions and organized as direct repeats of two hexamer core sequences AGG(A/T)CA, separated by one nucleotide (DR-1). PPARαβ/γ bind PPREs uniquely as heterodimers with retinoid X receptor (RXR)αβ/γ [13]. The A/T rich motif upstream of the DR-1 provides a polarization signal of the PPAR-RXR heterodimer, and may confer isotype-binding specificity. Accordingly, PPARs interact with 5’-extended hexamers, whereas RXR binds to the downstream motif of the response element [14]. The hinge region (domain D) is a highly flexible domain linking the DBD (domain C) and the ligand binding domain (LBD). The structural integrity of the hinge region conditions the interaction of PPARα with nuclear receptor corepressors, such as NCoR, in the unliganded conformation [15]. The hinge region is a target for post-translational modifications, such as phosphorylation catalyzed by PKC on serines 179 and 230. SUMOylation also targets the hinge domain of human PPARα at lysine 185 and potentiates NCoR recruitment [16,17]. The C-terminal LBD is the only domain of PPARα whose structure has been solved by X-ray crystallography [18]. Similar to PPARγ and PPARβ/δ, the PPARα LBD is composed of a helical sandwich flanking a four-stranded β-sheet and contains the AF-2 helix. The 1400 Å³ volume of the PPARα ligand binding pocket (LBP) is only slightly different than the total volume of the 1600 PPARγ and 1300 Å³ PPARβ/δ LBPs [19,20]. Nevertheless, the PPARα LBP is more lipophilic and less solvent-exposed than the LBPs of the other PPARs, hence allowing the binding of more saturated FA. In contrast to PPARγ, the PPARα AF-2 helix is more tightly packed against the LBD core when complexed with an agonist [21]. Crystallography identified tyrosine 314 as the main determinant of isotype ligand-specificity [12]. The AF-2 domain undergoes ligand-dependent conformational changes, thereby directing various co-activators such as CBP/p300 and SRC-1, carrying LXXLL motifs (L–leucine, X–any amino acid), to a hydrophobic cleft on
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the PPARx LBD surface, thus promoting the formation of an active transcriptional complex. The AF-2 domain may also play a role in ligand-dependent gene repression. Agonist binding unmasks lysine 358 in the LBD for SUMOylation, hence conferring repressive activity to PPARx [22].

Endogenous and synthetic PPARα agonists

PPARα ligands are FA derivatives formed during lipolysis, lipogenesis or FA catabolism. Substrates of the first rate-limiting peroxisomal β-oxidation enzyme, acyl-CoA oxidase 1 (ACOX1), likely are PPARα agonists. Consistently, disruption of ACOX1 in mice results in increased peroxisome proliferation, hepatocarcinoma and elevated PPARα target gene expression [23,24]. Eicosanoid derivatives, including the chemoattractant LTB4 and 8(S)-HETE, the murine 8-LOX product from arachidonic acid, are thought to be endogenous PPARα agonists [25]. The oxidized phospholipid fraction of oxidized LDL enhances PPARα transcriptional activity and induces its target gene, FATP-1, in human primary endothelial cells [26]. Liver-specific knockout of fatty acid synthase (FAS), an enzyme catalysing the synthesis of FA, resulted in hypoglycemia and liver steatosis when mice were fed a fat-depleted diet, which was reversed by dietary fat or a synthetic PPARα agonist, identifying products of FAS-dependent de novo lipogenesis as PPARα activators [27]. Mass spectrometry analysis on purified hepatic PPARα revealed the presence of 16:0/18:1-GPC bound to its LBD in mice expressing hepatic FAS, but not in liver-specific FAS knockout mice, identifying this phospholipid as a FAS-dependent lipid intermediate and endogenous PPARα ligand [28]. Adipose triglyceride lipase (ATGL)-dependent hydrolysis of hepatic intracellular TG also yields lipid PPARα ligands [29]. In line, overexpression of hepatic hormone-sensitive lipase (HSL) and ATGL triggers PPARα-dependent FAO gene expression and ameliorates hepatic steatosis [30].

A range of synthetic PPARα agonists, differing in species-specific potencies and efficacies, have been identified. Fibrates such as gemfibrozil, fenofibrate and ciprofibrate, are clinically used in the treatment of primary hypertriglyceridemia or mixed dyslipidemia [8]. However, fibrates are weak PPARα agonists with limited clinical efficacy [31]. Moreover, the potency of synthetic PPARα agonists may differ between the human and mouse receptor, as measured by using the PPARα-GAL4 transactivation system, i.e., fenofibrate (mouse receptor, ECSo = 18,000 nM vs. human receptor, EC50 = 30,000 nM), bezafibrate (EC50 = 90,000 nM vs. 50,000 nM, respectively) and Wy14,643 (EC50 = 630 nM vs. 5000 nM, respectively) [32]. This may contribute to interspecies differences in response to PPARα agonists that are detailed in the following sections of this review. Potent and selective PPARα modulators (SPPARMs), such as K-877 (EC50 = 1 nM) and GFT505 (EC50 = 6 nM for PPARα), a dual PPARα/δ agonist, are currently under development for the treatment of atherosclerotic dyslipidemia and NAFLD, respectively [31–33]. The therapeutic potential of novel PPAR agonists on NAFLD is further discussed in this review.

Mechanism of PPARα-dependent transactivation

Formation of transcriptionally active multiprotein PPARα complexes

Ligand-activated PPARα recruits numerous co-activator proteins, including members of the CBP/p300 and SRC/p160 family, which exhibit HAT activity, and other co-activators forming the transcriptionally active PPARα-interacting cofactor complex [34]. Such interactions are not seen with a PPARα AF-2 domain deleted mutant [35]. Disruption of the Pbp/MED1 gene showed its essential role in PPARα-dependent gene regulation. PBP/MED1 stabilizes and directs a large transcription initiation complex containing numerous co-activators and RNA polymerase II to the DNA-bound PPAR-RXR heterodimer [36] (Fig. 1A). However, RXR homodimers may bind DR-1 PPREs independent of PPARα and induce PPARα target gene transcription through a co-activator–dependent mechanism [37]. Recently, using a PPARα mutant (PPARαΔLBD), which lacks PPRE-binding activity but maintains interactions with RXR and transcriptional co-regulators, we showed that PPARα-driven transactivation depends on PPRE binding in vitro, in human hepatoma HepG2 cells and in vivo in Pparα-deficient mice with liver-specific PPARαΔLBD expression [35].

Genome-wide transcriptomic and PPARα chromatin binding maps

Genome-wide localization and activity-occupancy studies revealed that induction of PPARα target gene expression by PPARα agonists is associated with increased binding of PPARα to chromatin, rather than strengthening affinity and stability of existing interactions, than creating de novo ligand-inducible binding regions [38]. Interestingly, almost half of the PPARα-binding regions in human hepatoma cells are located within introns, whereas only 26% of them are localized in close vicinity (<2.5 kb) of the transcription start site [39]. In addition, genome-wide profiling of liver X receptor (LXR), RXR, and PPARα in the mouse liver showed overlapping chromatin binding regions of LXR-RXR and PPARα-RXR heterodimers. Nevertheless, only a few percent of LXR and PPARα binding sites contain consensus DR-4 and DR-1 elements, respectively [38]. De novo motif analysis showed co-enrichment of PPARα-binding regions in C/EBPα and TBP motifs, suggesting that PPARα may influence gene expression through the formation of complexes with other transcription factors [39]. Interestingly, PPARα chromatin binding mapping, combined with transcriptomics in primary human hepatocytes treated with the synthetic PPARα agonist Wy14,643, showed that genes whose promoter regulatory regions are directly bound by PPARα via PPREs, are on average more strongly upregulated than genes in which PPARα binds to the DNA indirectly [40]. Comparative transcriptomic studies in primary hepatocytes treated with Wy14,643 revealed only partial overlap of up (~20%) or downregulated (~12%) genes upon PPARα activation, between humans and mice [41]. Nevertheless, searching for enriched biological themes, in human and mouse sets of regulated genes by gene ontology (GO) classification, showed a 50% conservation in over-represented GO categories, mostly corresponding to lipid metabolic pathways [41]. Importantly, the glycolytic and gluconeogenic pathways were specifically upregulated in mice, whereas xenobiotic metabolism and apolipoprotein synthesis pathways rather in human hepatocytes [41,42].
inflammation. Ligand-activated PPARα represses cytokine-induced IL-6 gene expression via interference with AP-1 and NFκB signalling pathways. PPARα-driven transrepression involves direct physical interactions between PPARα, the p65 Rel homology domain, and the N-terminus JNK-responsive part of cJun (Fig. 1C) [43]. Moreover, synergistic transrepression of NFκB-driven transcription factors c-Jun and p65 to negatively regulate their target genes by a mechanism that is thought to be PPRE-independent. (D) Simultaneous ligand-activation of GR and PPARα leads to the enhanced repression of TNF-induced IL-6 transcriptional activity, by the mechanism that stems from a direct GR-PPARα physical interaction. (E) PPARα downregulates fibrinogen β transcriptional activity via ligand-dependent mechanisms, engaging physical interaction between PPARα and GRIP-1/TIF-2.

Fig. 1. Models of PPARα transcriptional regulation. Several models of PPARα transcriptional regulation have been proposed, via which PPARα modulates expression of its target genes as well as pro-inflammatory transcription factors and acute phase response genes. (A) Formation of the PPRE-dependent ligand-activated transcriptional complex containing PPARα-RXR heterodimer, co-activators, HAT, PBP/MED1 and the transcriptional preinitiation complex (PIC). (B) PPRE-dependent inhibition of NFκB transcriptional activity. Upon ligand activation, DNA-bound PPARα directly interacts with p65 to abolish its binding to an NFκB response element (NRE) in the complement C3 promoter. (C) PPARα directly interacts with pro-inflammatory transcription factors cJun and p65 to negatively regulate their target genes by a mechanism that is thought to be PPRE-independent. (D) Simultaneous ligand-activation of GR and PPARα leads to the enhanced repression of TNF-induced IL-6 transcriptional activity, by the mechanism that stems from a direct GR-PPARα physical interaction. (E) PPARα downregulates fibrinogen β transcriptional activity via ligand-dependent mechanisms, engaging physical interaction between PPARα and GRIP-1/TIF-2.

PPRE-dependent transcriptional repression

Recently, a novel PPRE-dependent model of transcriptional regulation has been proposed, through a negative crosstalk between PPARα and p65, diminishing complement C3 promoter transcriptional activity in a human hepatoma cell line. Ligand-dependent activation of PPARα inhibits TNF-mediated upregulation of complement C3 through the physical interaction between PPRE-bound PPARα and p65, to abolish p65 binding to the upstream...
NFkB response element on the complement C3 promoter (Fig. 1B) [49]. In line with these observations, genome-wide studies revealed the presence of STAT-PPAR binding motifs within ligand-inducible PPARα binding regions of downregulated genes. This suggests a direct negative crosstalk between PPRE-bound PPARα and pro-inflammatory transcription factors [39].

### Key Points 1

**Transcriptional regulation by PPARα**

- Transactivation: PPARα recognizes and binds to PPREs located in the regulatory regions of its target genes
- Transrepression: PPARα directly or indirectly interacts with transcription factors to block their transcriptional activity
- PPARα target genes related to fatty acid oxidation are regulated mainly in a PPRE-binding dependent manner
- Expression of pro-inflammatory genes can be repressed by PPARα either via PPRE-dependent or PPRE-binding independent mechanisms. Further studies are needed to understand the mechanisms of PPRE-independent PPARα activities

**Regulation of fatty acid metabolism by PPARα**

*PPARα-regulated FA transport and oxidation*

FA are transported in cells by membrane-associated FATPs [50], FATP1, which catalyses ATP-dependent esterification of LCFA and VCFAs into acyl-CoA derivatives, is a direct PPARα target gene [51,52]. Another plasma membrane FA transporter, FAT/CD36, is positively regulated by PPARα ligands [53]. Functional PPREs were identified within the promoter of the intracellular lipid trafficking L-Fabp [54]. Direct protein-protein interaction were reported between PPARα and L-FABP, suggesting that L-FABP may channel PPARα ligands to the receptor [55,56]. Consistently, a positive correlation between L-FABP protein and PPRE-driven gene transcription was observed in human hepatoma HepG2 cells, treated with PPARα agonists [57].

PPARα controls gene expression levels of the rate-limiting enzymes of peroxisomal β-oxidation, including ACOX1 and EHHADH, most pronouncedly in rodents [41]. In rodents and primates, FA transport across the mitochondrial membrane is triggered by PPRE-dependent regulation of CPT-I and CPT-II, which proteins are localized in the outer and inner mitochondrial membrane respectively [58–60]. Moreover, PPARα regulates the critical reaction of mitochondrial β-oxidation by directly controlling MCAD, LCAD, and VLCAD expression levels [61,62].

Enhanced expression of peroxisomal genes involved in lipid metabolism is related to the induction of peroxisome proliferation by PPARα agonists, which may contribute to tumorigenesis in rodents [63]. A comparative study between mouse and human PPARα expressed in Pparα-deficient mice revealed that Wt14,643 induces mouse liver peroxisomal proliferation in a receptor species-independent manner [64]. However, long-term Wt14,643 treatment induced liver tumors only in 5% of PPARα humanized mice, whereas the incidence of hepatocellular carcinoma was 71% in wild-type mice [65]. Mechanistically, murine but not human PPARα downregulated the expression of let-7C, an miRNA targeting the c-myc oncogen [66]. Moreover, long-term treatment of hyperlipidemic patients with either gemfibrozil or fenofibrate showed no effect on peroxisomal proliferation and hepatocyte hyperplasia, as assessed by light and electron microscopy of liver biopsies [67,68]. Importantly, a meta-analysis of long-term randomized controlled trials demonstrated neutral effects of fibrate treatment on cancer [69].

**PPARα and ketogenesis**

During fasting, hepatic FAO increases, yielding acetyl-CoA which is further converted into ketone bodies. Ligand-activated PPARα upregulates mitochondrial HMGCS, a rate-limiting enzyme of ketogenesis, which catalyses condensation of acetyl-CoA and acetoacetyl-CoA to generate HMG-CoA and CoA [70]. The mild phenotype of Pparα-deficient mice fed ad libitum became more pronounced during fasting, being characterized by impaired FAO, lipid accumulation in liver and heart as well as hypoglycemia and an inability to augment ketone body synthesis [71,72]. Moreover, high-fat, low-carbohydrate ketogenic diet (KD)-feeding increased hepatic mRNA expression and plasma levels of FGF21, in parallel with PPARα induction [73]. Fgf21 knock-down in KD-fed mice impaired hepatic expression of FAO genes (Acox1, Cpt-I) and ketogenesis (Hmgcs, Bdh), indicating that FGF21 is required for the activation of these metabolic pathways [73]. Further studies identified FGF21 as a direct PPARα target gene, induced, in mice and humans, in response to fasting and upon PPARα ligand administration [73,74].

**PPARα in the regulation of hepatic lipid and plasma lipoprotein metabolism**

*Molecular insights into the lipid normalizing effects of PPARα*

In rodent models, the reduction of plasma TG-rich lipoprotein upon PPARα activation is related to enhanced FA uptake, conversion into acyl-CoA derivatives, and further catabolism via the β-oxidation pathways. Moreover, the TG-lowering action of PPARα is also due to increased lipolysis via induction of lipoprotein lipase (LPL), which catalyses the hydrolysis of lipoprotein TG into free FA and monoacylglycerol. PPARα controlled LPL mRNA through binding to a PPRE in the human and mouse LPL gene promoters [75]. Furthermore, PPARα enhanced LPL activity indirectly by decreasing mRNA levels and secretion of hepatic APO-CIII, an LPL inhibitor [76]. Interestingly, glucose induced APO-CIII transcription in hepatocytes through a mechanism involving the transcription factors ChREBP and HNF-4 [77]. Conversely, hepatic expression of APO-CIII was inhibited by insulin through insulin-dependent phosphorylation of FOXO1, resulting in its displacement from the nucleus and inability to drive APO-CIII transcriptional activity [78]. In hepatocytes, inhibition of APO-CIII transcription by fibrates was the consequence of multiple cooperative mechanisms including PPARα-driven displacement of HNF-4 from the APO-CIII promoter, inhibition of FOXO1 activation of APO-CIII transcription via the insulin-responsive element, and inhibition of glucose-stimulated APO-CIII expression [76,79].

In humans, fibrates increase plasma HDL-C by stimulating the synthesis of its major apolipoproteins, APO-AI and APO-AII.
However, species-differences exist between humans and rodents with respect to apolipoprotein regulation by PPARα. A functional PPRE is present in the human, but not rodent APO-AI promoter, as illustrated by increased human APO-AI production in humanized APO-AI transgenic mice upon treatment with fibrates [80]. In contrast, APO-AI and HDL-C levels are elevated in Ppara-deficient mice and fibrate treatment decreases APO-AI mRNA in wild-type animals [81,82]. In the human and mouse livers, APO-AI expression is induced by PPARα. Hepatic human APO-AI gene transcription is induced by PPARαs through interaction with a PPRE localized within the APO-AI promoter region. A functional PPRE could not be identified within the mouse APO-AI promoter [83]. However, based on available data from genome-wide PPARα binding map, we inspected through promoter regions of hepatic mouse Apo-AII for the presence of PPARα ChIP-seq peaks [38] and identified a PPARα binding also in the mouse Apo-AII proximal promoter, 100 bp downstream of the transcription start site (our unpublished data). Similar species-specific transcriptional regulation modes are observed for APO-AV, which enhances LPL activity, by PPARα [84,85]. Studies using human LPL transgenic/ APO-AV-deficient mice and human APO-AV transgenic/Lpl-deficient mice support the hypothesis that APO-AV reduces TG levels by trafficking VLDL and chylomicrons to proteoglycan-bound LPL for lipolysis [86,87]. In vitro and in vivo studies comparing wild-type versus transgenic humanized APO-AV mice revealed that human, but not mouse APO-AV expression is induced in the liver by PPARα agonists [88,89]. These findings are consistent with the identification of a functional PPRE in the human, but not mouse APO-AV promoter [88,89]. In humans, rare SNPs in the APO-AV promoter region are associated with paradoxical decreases in plasma HDL-C and APO-AI in response to fibrates, whereas SNPs within the APO-AV gene are associated with enhanced lipid response to fibrate and statin therapy [90–93]. Thus, unexpected responses to fibrate treatment in some individuals may be due to genetic variations in PPARα target genes, such as APO-AV.

**PPARα and hepatic lipogenesis**

Besides its ability to orchestrate lipoprotein metabolism, PPARα also controls, directly or indirectly, lipogenic pathways in the liver. Lipogenesis is the metabolic pathway allowing FA synthesis when dietary carbohydrates are abundant. Dietary regulation of hepatic lipogenic genes is under control of the insulin-dependent transcription factors SREBP-1c and ChREBP [94]. PPARα agonists enhance human SREBP-1c transcriptional activity through PPARα interacting with a DR-1 element in the human SREBP-1c promoter. Consistently, PPARα binding to the human SREBP-1c promoter is demonstrated in vitro and in vivo, in human primary hepatocytes [95]. In mouse livers, the SREBP-1c target genes Fas, Acc1, and Scd-1 are positively regulated by PPARα agonists [96,97]. Nevertheless, neither SREBP-1c nor its downstream targets have been identified as direct PPARα target genes in mice, with the exception of Scd-1, which contains a PPRE in its promoter [97]. In mice, fibrates increase the protein levels of the mature hepatic form of SREBP-1c, by increasing the rate of proteolytic cleavage of its membrane-bound precursor form, without changing Srebp-1c mRNA levels [98]. The insulin-dependent enhancement of SREBP-1c transcription requires the participation of LXR and SREBP-1c itself [99]. Moreover, via LXR-binding sites in the human and mouse Srebpa-1c promoter, LXR agonists induce its transcriptional activity [95,100]. PPARα can also indirectly modulate SREBP-1c transcription via cross-regulation with the LXR signaling pathway. In mice, PPARα is required for the LXRα-dependent response of SCD-1 and FAS to insulin in re-fed conditions, suggesting a potential role for PPARα in the synthesis of endogenous LXRα ligands [101]. In human primary hepatocytes, PPARα agonists, cooperatively with insulin and LXR agonists, induce lipogenic gene expression, such as FAS and ACC1 [95].

**Key Points 2**

**PPARα-dependent activities in mice and humans**

- Fatty acid metabolism and ketogenesis are the most conserved PPARα-regulated biological processes between mice and humans
- Regulation of the glycolysis-gluconeogenesis pathway by PPARα agonists occurs in mice, but not in men
- Xenobiotic metabolism and apolipoprotein synthesis pathways are specifically controlled by PPARα agonism in human hepatocytes
- Peroxisomal proliferation genes are induced upon activation of both human and mouse PPARα, however, humans are protected from fibrate-induced tumorigenesis

**Hepatic PPARα activity switches in the fed-to-fasted transition states**

PPARα coordinates different pathways of de novo lipid synthesis in the fed state, to supply FA for storage as hepatic TG, for periods of starvation. During fasting, when the organism switches to the utilization of FA, deriving either from the liver or from peripheral tissues, PPARα also shifts its activity to promote FA uptake and β-oxidation, thus yielding substrates for ketone body synthesis to provide energy for peripheral tissues (Fig. 2). The adjustment of PPARα transcriptional activity in the adaptation to fasting/feeding transition can be potentially brought about by kinases controlled by different nutritional states and phosphorylating PPARα or its regulatory proteins.

Several kinases, including PKA, PKC, and MAPK, have been shown to modify PPARα transcriptional activity (see also Table 1), although many studies were performed in *vitro*, and thus lack physiological translation to the coordinated responses to different nutritional signals in the living organism. However, insulin-activated MAPK and glucose-activated PKC stimulate PPARα transactivation in HepG2 cells [16,102], suggesting that MAPK- and PKC-dependent phosphorylations may promote PPARα activity in the post-prandial state. Conversely, in fasting, glucagon induces cAMP and cAMP-dependent kinase PKA activity [103]. PKA-mediated phosphorylation potentiates ligand-dependent PPARα activation and increases expression of FAO genes in mouse primary hepatocytes [104].

Studies performed in mice hint that mTORC1 also plays a role in switching PPARα activities during the feeding/fasting transition as well as in pathophysiological conditions. In the fed state, when mTORC1 is activated by the insulin-dependent PI3K pathway, NCoR1 is partitionned in the cytoplasm and the nucleus of hepatocytes, thus repressing PPARα target gene expression.
Inhibition of mTORC1 and its downstream effector S6K2 during fasting promotes a cytoplasmic relocalization of NCoR1, hence increasing ketogenesis via PPARα derepression [105,106]. Interestingly, S6K2 phosphorylation is elevated in ob/ob mice, a model of obesity and insulin resistance (IR) [106]. The ability of FAS to synthesize phospholipids, acting as endogenous PPARα ligands, contributes to the maintenance of energy sources for peripheral tissues during prolonged fasting.
ligands, depends on its subcellular localization and post-translational modifications [107]. Insulin-dependent phosphorylation of cytoplasmic FAS by mTORC1 limits PPARα ligand generation, whereas membrane-associated FAS, producing lipids for energy storage and export, is less susceptible to phosphorylation. Conversely, in the fasting state, de-phosphorylated cytoplasmic FAS is in a permissive state, allowing the generation of endogenous PPARα ligands, thus activating PPARα-target genes [107].

Hepatic PPARα activity can also be stimulated by AMPK, a sensor of the intracellular energy state activated by high AMP-to-ATP ratios, i.e., during fasting [108]. In contrast, glucose represses PPARα gene expression via AMPK inactivation in pancreatic β-cells [109,110], although it is unknown whether a similar mechanism occurs in the liver. Adiponectin, an insulin-sensitizing adipokine, increases FAO gene expression via AMPK-dependent expression [111]. Serum adiponectin is decreased in obesity and T2DM [112], which may contribute to an impaired PPARα activity in these pathologies.

**PPARα in acute and chronic liver inflammation**

**PPARα and acute hepatic inflammation**

PPARα exerts anti-inflammatory activities in murine models of systemic inflammation. PPARα agonism specifically attenuates the IL-6-induced APR in vitro and in vivo, by downregulating hepatic expression levels of Saa, Hgf, and Fibo-α, -β, and -γ [6]. Similar inhibitory effects of PPARα agonists on IL-1β- and IL-6-induced APR were observed in mice with liver-restricted Pparα expression [113]. By contrast, treatment with IL-1β decreases expression of liver PPARα and its target genes, suggesting a negative crosstalk between IL-1β-induced inflammation and hepatic FAO regulation [114]. In line with these observations, LPS-induced APR is counteracted by fibrates in Pparα-deficient mice with liver-specific reconstituted Pparα [113]. Interestingly, pretreatment with a PPARα agonist markedly prevents the LPS-induced increase of plasma IL-1, IL-6, and TNF, and the expression of adhesion molecules, such as ICAM-1 and VCAM-1 in the aorta, suggesting that liver PPARα controls, in a yet undefined manner, the systemic inflammatory response [113]. The anti-inflammatory effects of hepatic PPARα may also derive from its ability to upregulate anti-inflammatory genes, such as Il1ra and Icbsz, a cytoplasmic inhibitor of NFκB, suggesting a possible cooperation between PPARα-dependent transactivation and transrepression to turn off anti-inflammatory pathways [115,116].

**PPARα action in pre-clinical models of NAFLD**

NAFLD is a chronic liver disease, which affects 10–24% of the population and is associated with IR and the MetS [117]. The pathology initiates with hepatic steatosis, which in some individuals progresses toward NASH, fibrosis, cirrhosis and finally liver failure. The ability of PPARα to counteract different stages of NAFLD has been studied in animal models, which partially replicate the human pathology [118].

Administration of an methionine choline-deficient diet (MCDD) to rodents leads to the development of steatohepatitis, histologically similar to human NASH. However, MCDD does not induce peripheral IR, normally observed in human NASH. Pparα-deficiency in MCDD-fed mice provokes more severe steatosis and hepatitis [7]. In wild-type mice, PPARα agonism normalizes histological changes by preventing intrahepatic lipid accumulation, liver inflammation, and fibrosis [119]. Pharmacological activation of PPARα increases CYP4A-driven α-oxidation as well as peroxisomal and mitochondrial β-oxidation, leading to enhanced hepatic lipid turnover. Moreover, synthetic PPARα agonists decrease the number of activated macrophages and stellate cells in the liver, and lower the expression of fibrotic markers [7]. In rodents, PPARα appears to be expressed mainly in hepatocytes [120], suggesting that the hepatoprotective effects of fibrates in rodents likely occur via PPARα within liver parenchymal cells (Fig. 3). We showed that the hepato-specific expression of the DNA-binding disabled PPARαSASS protects from MCDD-induced inflammation and liver fibrosis, without affecting FAO genes and lipid accumulation in the liver [35]. Hepatoprotective effects of PPARα agonism can also occur via the regulation of hepatic Vnn1 expression [121], since Vnn1-deficiency links hepatic steatosis in response to fasting and changes the expression of inflammation and oxidative stress genes [122]. The role of ATGL-dependent intracellular TG hydrolysis, to generate endogenous PPARα agonists with anti-inflammatory potential, was recently demonstrated in Atgl-deficient mice [123], which display increased susceptibility to LPS- and MCDD-induced hepatic inflammation due to impaired PPARα signaling. The hepatic phenotype of Atgl-deficient mice is partially improved upon treatment with a synthetic PPARα agonist. The foz/foz (ALMS1 mutant) mouse model of Alström syndrome spontaneously exhibits a strong metabolic phenotype hallmarkmed by severe obesity, hyperinsulinemia and T2DM [124–126]. In this genetic background, PPARα activation reverses HFD-induced hepatocellular injury, liver inflammation and improves insulin sensitivity [127]. Similarly, Pparg- deficiency promotes HFD-induced hepatic TG, macrophage infiltration and elevates plasma levels of ALT and SAA [128]. In contrast to the observation that PPARα activation improves insulin sensitivity [129], Pparα-deficient mice are protected from HFD-induced IR, as assessed by glucose tolerance test and euglycemic-hyperinsulinemic clamps in fasted mice [129,130]. Similar tests performed in non-fasted Pparα-deficient mice, however, show no protection from IR compared to wild-type mice [131]. These contradictions can result from the impaired response to fasting in Pparα-deficient mice, in which the inability to oxidize FA leads to a preferential glucose use and depletion of glycogen stores [132].

The development of early stages of NASH was studied in the humanized APO-E2 knock-in (APO-E2KI) mouse. In this model, the Apo-E gene has been substituted for the human APOE2 allele under the control of the endogenous mouse promoter, faithfully mimicking mouse endogenous APO-E tissue distribution and expression levels. The reduced affinity of hAPO-E2 for the LDL-receptor leads to a plasma lipoprotein profile similar to that occurring in human type III hyperlipoproteinemia [118]. APO-E2-KI mice fed a western diet rapidly develop a phenotype characterized by steatosis and inflammation. Interestingly, macrophage infiltration in the liver precedes lipid accumulation. This is in contradiction with the concept that NASH pathogenesis always stems from initial liver steatosis, which leads to inflammation [133]. In accordance, cladronate liposome-induced depletion of residual liver macrophages (Kupffer cells) reduces hepatic TG content in HFD-fed wild-type mice [114]. Western diet-fed Pparα-deficient/APO-E2KI mice manifest exacerbated liver steatosis and inflammation compared to wild-type APO-E2-KI mice.
indicative of a protective role of PPARα against NASH [134]. Consistently, in primary hepatocytes isolated from APO-E2-KI mice, HFD induces an aberrant histone H3K9me3 and H3K4me3 methylation profile of the promoter of Pparα, which correlates with decreased Pparα mRNA expression [135]. In APO-E2-KI mice expressing PPARα, fibrates inhibit NASH due to their inhibitory effects on pro-inflammatory genes and the increase in lipid catabolism in the liver [133,134]. Among the ROS, hydrogen peroxide is a major agent activating TGFβ and collagen production by hepatic stellate cells [136,137]. The anti-fibrotic action of synthetic PPARα agonists was demonstrated in a rat model of thioacetamide-induced liver cirrhosis. PPARα directly upregulates catalase expression, thus ameliorating hydrogen peroxide detoxification and protecting hepatocytes from oxidative stress [138]. Moreover, fibrates improve endothelial dysfunction and ameliorate intrahepatic hemodynamics in CCl4 cirrhotic rats, at least in part, by reducing COX-1 protein expression [139].

PPARα agonism in NAFLD therapy

Few clinical pilot studies were performed to assess the impact of fibrates, which improve atherogenic dyslipidemia, on the evolution of NASH. Fenofibrate treatment (48 weeks) of 16 patients with biopsy-confirmed NASH reduces the proportion of patients with elevated ALT, AST and γGT plasma levels and histologically-assessed hepatocellular ballooning [140]. However, the grade of steatosis, inflammation and fibrosis is not significantly changed upon fenofibrate treatment, in this relatively small, phenotypically heterogeneous cohort [140]. Short-term bezafibrate treatment (2–8 weeks), combined with diet and exercise, of donors for liver transplantation with steatosis decreases macrovesicular steatosis [141]. Treatment of NASH patients (4 weeks) with gemfibrozil lowers ALT, AST, and γGT plasma levels [142]. Treatment with clofibrate (12 months) of 16 patients with NASH does not improve either ALT, AST and γGT or histologically assessed steatosis, inflammation and fibrosis [143]. However, serum TG does not decrease in these hypertriglyceridemic patients, casting doubts on the treatment efficacy. Larger randomized studies evaluating the action of novel PPARα agonists with SPPARM activity, on a broad spectrum of liver pathologies and combining several methods of NAFLD assessment, are still to be performed to unequivocally assess their efficacy. Moreover, despite numerous reports of beneficial effects of fibrates in mice, species-specific differences may exist in the response to PPARα agonism [32]. The relatively weak potency of the currently used PPARα agonists in humans can be additionally affected by the lower...
expression level of PPARα in the human compared to mouse liver [144,145]. Importantly, we found that hepatic Ppara expression decreases with progressive stages of liver fibrosis in patients with NASH (our unpublished data). Thus, novel PPARα agonists with greater potency and efficacy may prove to be more useful in the treatment of NAFLD. Amongst these, K-877 manifests greater efficacy than fibrates in terms of TG-lowering activity. Moreover, K-877 raises plasma FGF21 levels in Ldlr−/− deficient mice fed a Western diet [31]. Consistently, Phase II clinical trials showed better efficacy of K-877 treatment on fasting plasma TG and HDL-C, in individuals with atherogenic dyslipidemia, in comparison to fenofibrate [31]. These data suggest that K-877 could be a novel treatment option to tackle the residual cardiovascular risk. So far no data are available on the effects of K-877 on NAFLD. Recently, GFT505 was shown to counteract multiple stages of NAFLD, as assessed in several animal models of NASH and fibrosis [9,146], effects likely due to the combined activation of the PPARα and δ receptors. GFT505 exerts preventive effects on liver steatosis and inflammation, induced in ApoE−/− mice by a Western diet and in db/db mice by an MCDD. Furthermore, GFT505 exerts anti-fibrotic activities on CCl4-induced fibrosis in rats [9]. In phase II clinical trials, GFT505 treatment decreases plasma concentrations of ALT, γGT, and ALP, in MetS patients [9]. Considering its ability to improve peripheral insulin sensitivity and lower plasma FFA levels, likely via PPARα activation, in abdominally obese patients, as well as its TG lowering/HDL increasing activity in subjects with combined dyslipidemia, GFT505 is a promising drug candidate for the treatment of diseases linked to IR, such as T2DM and NASH [146,147].

Key Points 3

PPARα activities in NASH and in liver fibrosis

• PPARα deficiency leads to exaggerated lipid accumulation in the liver
• Pharmacological PPARα activation decreases liver steatosis by increasing FAO gene expression
• PPARα agonism diminishes chronic liver inflammation and fibrosis independent of its effect on liver steatosis
• The dual PPARα/δ agonist GFT505 is currently tested in a phase llb trial for the therapy of NASH in metabolic syndrome and type 2 diabetes

Perspectives

Genome-wide approaches have shown that PPARα is a master regulator of FA metabolism and ketogenesis in the liver [41]. The ability of PPARα agonists to counteract steatohepatitis and fibrosis appears prominent in murine models of NAFLD, which can be explained by the fact that PPARα expression is more abundant in the mouse compared to human liver and may further decrease with NASH progression (our unpublished data). Moreover, commonly used fibrates are relatively low activators of human PPARα. Thus potent and highly specific PPARα agonists, such as K-877 and the dual PPARα/δ agonist GFT505, have appeared as promising therapies for CVD or NAFLD, respectively. Nevertheless, further clinical studies are required to determine the effectiveness and safety of such SPPARMs in humans. Since the anti-inflammatory and anti-fibrotic activities of PPARα seem to be dissociable from its effect on liver steatosis in mice [35], more potent, possibly selective transrepression-triggering PPARα agonists could be designed in the future, based on virtual drug screening and transcriptomics. A better understanding of PPARα regulation by different nutritional signals in healthy individuals and in MetS patients will allow the design of specific pharmacological therapies, simultaneously targeting different NASH-triggering factors. Moreover, to improve NASH, dietary strategies, such as n-3 PUFA supplementation may be considered to ameliorate steatosis and inflammation, by a mechanism that may partially rely on PPARα activation [148,149]. However, the efficacy of n-3 PUFA in the treatment of NASH in human subjects remains to be demonstrated.

Conflict of interest

BS is an advisor of Genfit SA.

References

Review


Review


