Review

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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 genome-wide 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.

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 [1]. 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 [2]. PPARα expression is enriched in tissues with high fatty

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; FAO, fatty acid oxidation; FA, fatty acid; APR, acute phase response; NASH, non-alcoholic steatohepatitis; NAFLD, non-alcoholic fatty liver disease; CVD, cardiovascular disease; LDL, low density lipoprotein; HDL-C, high density lipoprotein cholesterol; SPPARM, selective PPAR modulator; AF-1, activation function-1; MAPK, mitogen-activated protein kinase; DBD, DNA binding domain; PPRE, PPAR response element; DR-1, direct repeat-1; RXR, retinoid X receptor; LBD, ligand binding domain; NCoR, nuclear receptor co-repressor; PKC, protein kinase C; SUMO, small ubiquitin-like modifier; AF-2, activation function-2; LBP, ligand binding pocket; CBP, CREB-binding protein; SRC-1, steroid receptor coactivator-1; ACOX1, acyl-CoA oxidase 1; LTB4, leukotriene B4; 8(S)-HETE, 8(S)-hydroxyeicosatetraenoic acid; 8-LOX, 8-lipoxygenase; FATP-1, fatty acid transport protein-1; FAS, fatty acid synthase; 16:0/18:1-GPC, 1-palmitoyl-2-oleoyl-sn-glycerol-3-phosphocholine; ATGL, adipose triglyceride lipase; TG, triglyceride; HSL, hormone-sensitive lipase; EC_{50}, half maximal effective concentration; GAL4, galactosidase 4; HAT, histone acetyltransferase; PBP, PPARα-binding protein; MED-1, mediator subunit 1; $PPAR\alpha_{DISS}$, PPARα mutant with selective transrepression activity; LXR, liver X receptor; DR-4, direct repeat-4; C/EBPα, CCAAT-enhancer-binding protein alpha; TBP, TATA-binding protein; GO, gene ontology; IL-6, interleukin-6; AP-1, activator protein 1; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; JNK, c-Jun N-terminal protein kinase; GR, glucocorticoid receptor; Fib, fibrinogen; SAA, serum amyloid A; Hg, haptoglobin; CRP, C reactive protein; STAT3, signal transducer and activator of transcription 3; Fib-β, fibrinogen-beta; GRIP-1/TIF-1, GR-interactin protein-1/transcription intermediary factor-2; ERR, estrogen-related receptor; SIRT-1, sirtuin-1; ERRE, ERR response element; LPS, lipopolysaccharide; TNF, tumor necrosis factor; ATP, adenosine triphosphate; LCFA, long-chain fatty acid; VCFA, very long-chain fatty acid; FAT/CD36, fatty acid translocase; L-FABP, liver fatty acid-binding protein; EHHADH, L-bifunctional enzyme; CPT, carnitine palmitoyltransferase; MCAD, medium-chain acyl-CoA dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase; VLCAD, very long-chain acyl-CoA dehydrogenase; let-7c, let-7 microRNA precursor; miRNA, microRNA; APO-AI, apolipoprotein-AI; HMGCS, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase; KD, ketogenic diet; FGF21, fibroblast growth factor 21; BDH, beta-D-hydroxybutyrate dehydrogenase; LPL, lipoprotein lipase; APO-CIII, apolipoprotein-CIII; ChREBP, carbohydrate-responsive element-binding protein; HNF-4, hepatocyte nuclear factor 4; FOXO1, forkhead box O1; APO-AII, apolipoprotein-AII; ChIP-seq, chromatin immunoprecipitation-sequencing; APO-AV, apolipoprotein-AV; SNP, single-nucleotide polymorphism; SREBP-1c, sterol regulatory element binding protein-1c; Acc1, acetyl-CoA carboxylase 1; Scd-1, stearoyl-CoA desaturase-1; PKA, protein kinase A; cAMP, cyclic adenosine monophosphate; mTORC1, mammalian target of rapamycin complex 1; PI3K, phosphoinositide 3-kinase; S6K2, protein S6 kinase 2; IR, insulin resistance; AMPK, 5′-AMP-activated protein kinase; T2DM, type 2 diabetes mellitus; ICAM-1, intracellular adhesion molecule-1; VCAM-1, vascular cell adhesion molecule-1; IL-1RA, interleukin-1 receptor antagonist; IκB, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor; MetS, metabolic syndrome; MCDD, methionine choline-deficient diet; CYP4A, cytochrome P450 4A; VNN1, vanin-1; ALMS1, Alstrom syndrome 1; HFD, high-fat diet; ALT, alanine aminotransferase; APO-E2, apolipoprotein-E2; ROS, reactive oxygen species; TGFβ, transforming growth factor beta; COX-1, cyclooxygenase-1; AST, aspartate aminotransferase; γGT, gamma-glutamyl transpeptidase; LDLR, LDL-receptor; ALP, alkaline phosphatase; PUFA, polyunsaturated fatty acid.

Table 1. Functional analysis of PPARα structural domains.

		Domain	PTM	Function
N-term	A/B	AF-1	MAPK-dependent phosphorylation at Ser 6, 12 and 21	Ligand-dependent/independent activation function Target gene specificity
	C	DBD		Binding to PPRE Interaction with cJun
	D	Hinge region	PKC-dependent phosphorylation at Ser 179 and 230 SUMOylation at lysine 185	Providing NR structure flexibility Potentiating NCoR recruitment
C-term	E/F	LBD/AF-2	SUMOylation at lysine 358	Ligand binding specificity Interaction with RXR and p65 Interactions with multiple co-regulators e.g., CBP/p300 and SRC/p160

PPARα displays a classical NR canonical architecture. PPARα domains (from A to F) fulfil 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 such as liver, heart, 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 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, fibrates acting as synthetic PPARα agonists, lower plasma triglycerides and small dense low density lipoprotein (LDL) 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 PPARα/β/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). PPARα/β/γ bind PPREs uniquely as heterodimers with retinoid X receptor (RXR)α/β/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 $Å^3$ volume of the PPARα ligand binding pocket (LBP) is only slightly different than the total volume of the 1600 PPARγ and 1300 $Å^3$ 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 [18]. 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

the PPARα 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 PPARα [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 fatdepleted 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, EC_{50} = 18,000 nM *vs.* human receptor, EC_{50} = 30,000 nM), bezafibrate (EC_{50} = 90,000 nM *vs.* 50,000 nM, respectively) and Wy14,643 (EC_{50} = 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 (EC_{50} = 1 nM) and GFT505 (EC_{50} = 6 nM for PPARα), a dual PPARα/δ agonist, are currently under development for the treatment of atherogenic 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 ($PPAR\alpha_{DISS}$), 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 $PPAR\alpha_{DISS}$ 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 by 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].

Models of PPARα transcriptional repression

PPRE-independent transcriptional repression

PPARα negatively regulates pro-inflammatory signalling pathways via protein-protein interactions, a tethering mechanism extensively studied *in vitro* and in mouse models of acute

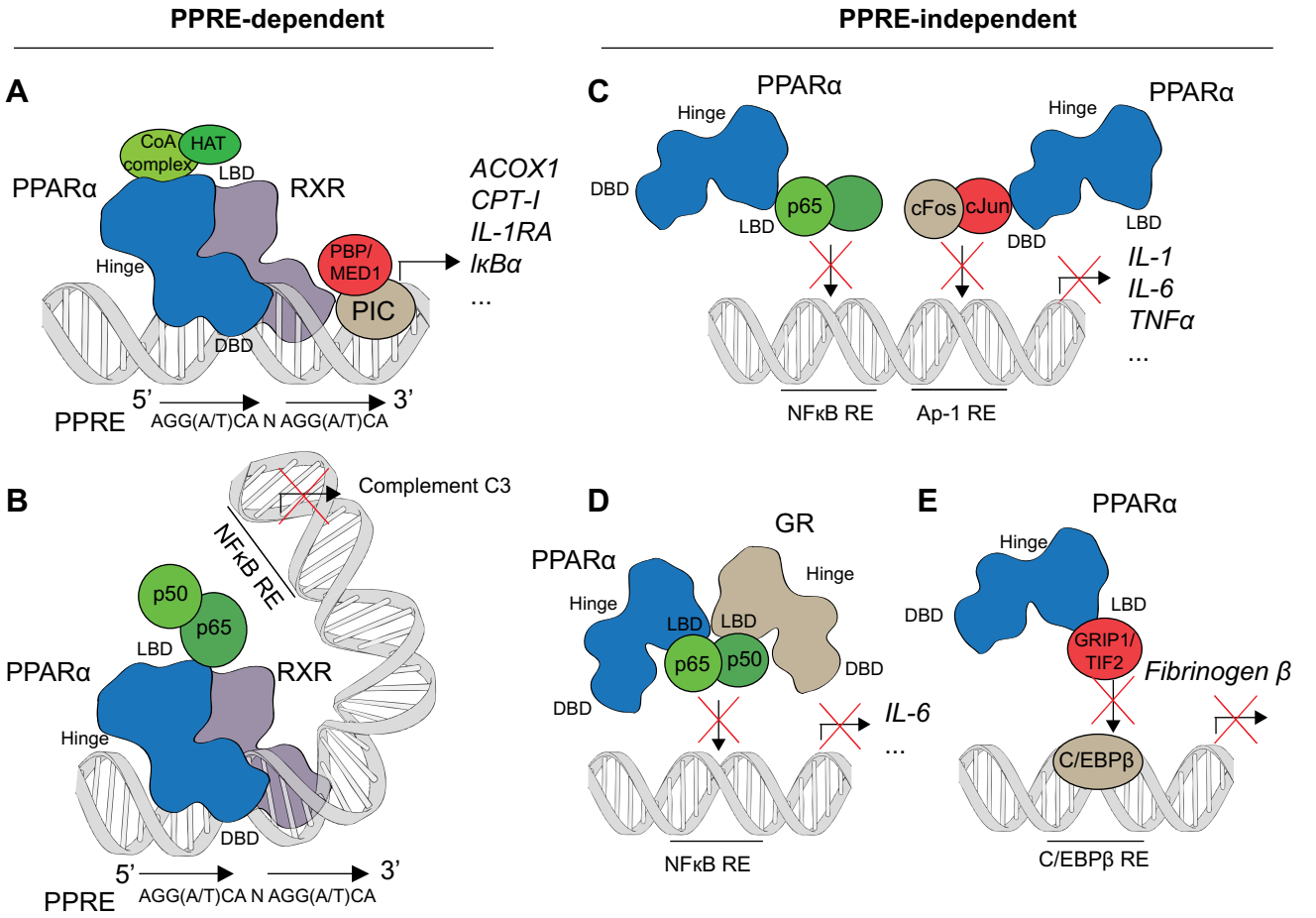

Fig. 1. Models of PPARα transcriptional regulation. Several models of PPARα transcriptional regulation have been proposed, via which PPARα modulates expression of its target genes as well as pro-inflammatory transcription factors and acute phase response genes. (A) Formation of the PPRE-dependent ligand-activated 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.

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 gene expression occurs upon simultaneous activation of PPARα and glucocorticoid receptor (GR), a well-characterized NFκB repressor (Fig. 1D) [44]. However, PPARα and GR transrepress distinct but overlapping sets of genes in vascular endothelial cells [45]. PPARα activation downregulates hepatic APR genes, such as *Fib*, *Saa*, and *Hg* in rodents, and *CRP* in human hepatocytes. Mechanistically, PPARα downregulates mRNA and protein levels of GP80 and GP130, components of the IL6-receptor, thus disrupting the STAT3 and cJun signalling pathways involved in the APR [6]. Similarly, in the liver, fibrates downregulate IL-6-stimulated *Fib-β* expression via PPARα-dependent titration of GRIP-1/TIF-2, thus interfering with C/EBPβ activity (Fig. 1E) [46]. Another mechanism of PPARα-dependent transcriptional repression occurs in the control of ERR-driven mitochondrial respiration and cardiac contraction, where a PPARα-SIRT1 complex binds directly to a single hexameric ERRE motif, thus competitively downregulating ERR target genes [47,48]. Recently, we showed that hepatic PPARα represses cytokine- and LPS-induced inflammatory responses *in vitro* and *in vivo*, independently of direct DNA binding [35].

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

NFκB 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-binding 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 VCFA 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 Wy14,643 induces mouse liver peroxisomal proliferation in a receptor species-independent manner [64]. However, long-term Wy14,643 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 *Pparα*-deficient mice and fibrate treatment decreases *Apo-AI* mRNA in wild-type animals [81,82]. In the human and mouse liver, APO-AII expression is induced by PPARα. Hepatic human *APO-AII* gene transcription is induced by PPARα through interaction with a PPRE localized within the *APO-AII* promoter region. A functional PPRE could not be identified within the mouse *Apo-AII* 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 *Srebp-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

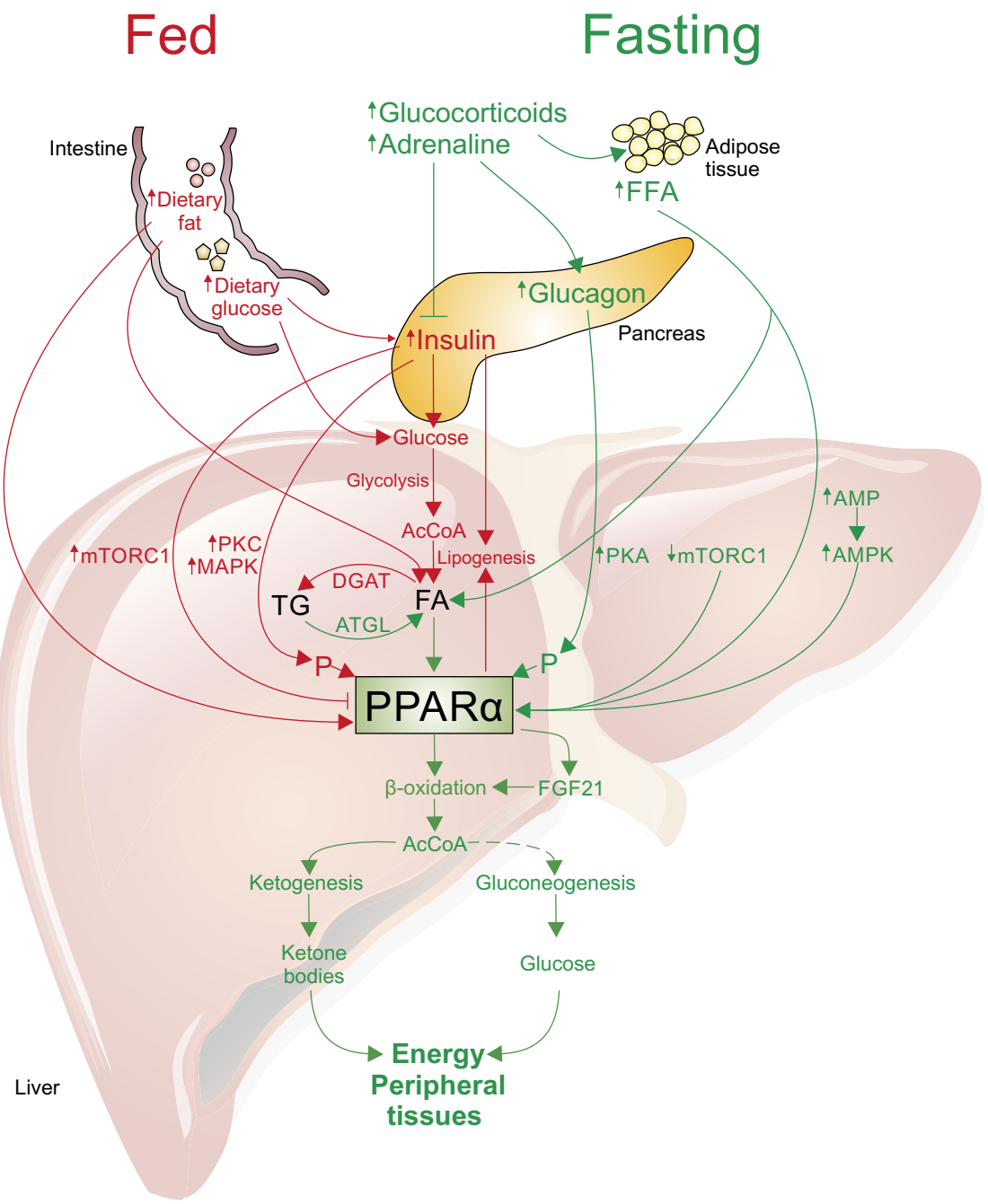

Fig. 2. Molecular switch of PPARα activity in the fed-to-fasted state. Augmented postprandial glucose levels lead to increased production and secretion of insulin by β-cells, which acts on the liver to induce glucose uptake and glycolysis, yielding acetyl-CoA (AcCoA), and enhances FA synthesis. Insulin stimulates PPARα phosphorylation via PKC and enhances its transcriptional activity, whereas insulin-activated mTORC1 blocks PPARα activity by promoting nuclear localization of NCoR. Lipogenesis yields fatty acid-derivatives operating as PPARα ligands. During fasting, stress hormones such as adrenaline and glucocorticoids are synthesized together with glucagon. Glucagon sustains gluconeogenesis through a stimulatory effect on hepatic gluconeogenic precursor uptake as well as on the efficiency of gluconeogenesis within the liver. Moreover, glucagon increases cAMP levels triggering PKA-dependent PPARα phosphorylation and activity. Fasting leads to decreased mTOR1C activation and stimulation of PPARα-dependent FAO and ketogenesis. The lipolytic release of adipose tissue fatty acids raises plasma levels of free fatty acids (FFA) that are subsequently stored in the liver as TG. ATGL-dependent hydrolysis of hepatic intracellular TG provides lipid ligands for PPARα activation. PPARα activation leads to increased β-oxidation rates directly and via FGF21 activation to provide substrates for ketone body synthesis and gluconeogenesis, thus maintaining energy sources for peripheral tissues. During prolonged fasting, high intracellular AMP levels induce AMPK to stimulate energy production by PPARα-driven FAO.

[105]. 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, 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 PPARα activation [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*, *Hg*, and Fib-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 *Il-1ra* and *IκBα*, a cytoplasmic inhibitor of NFκB, suggesting a possible cooperation between PPARα-dependent transactivation and transrepression to turn on 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 ω-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 $PPAR\alpha_{DISS}$ 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 *Atlg*-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 hallmarked 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, *Pparα*-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-E2*KI) 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, clodronate 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-E2*-KI mice manifest exacerbated liver steatosis and inflammation compared to wild-type *APO-E2*-KI mice,

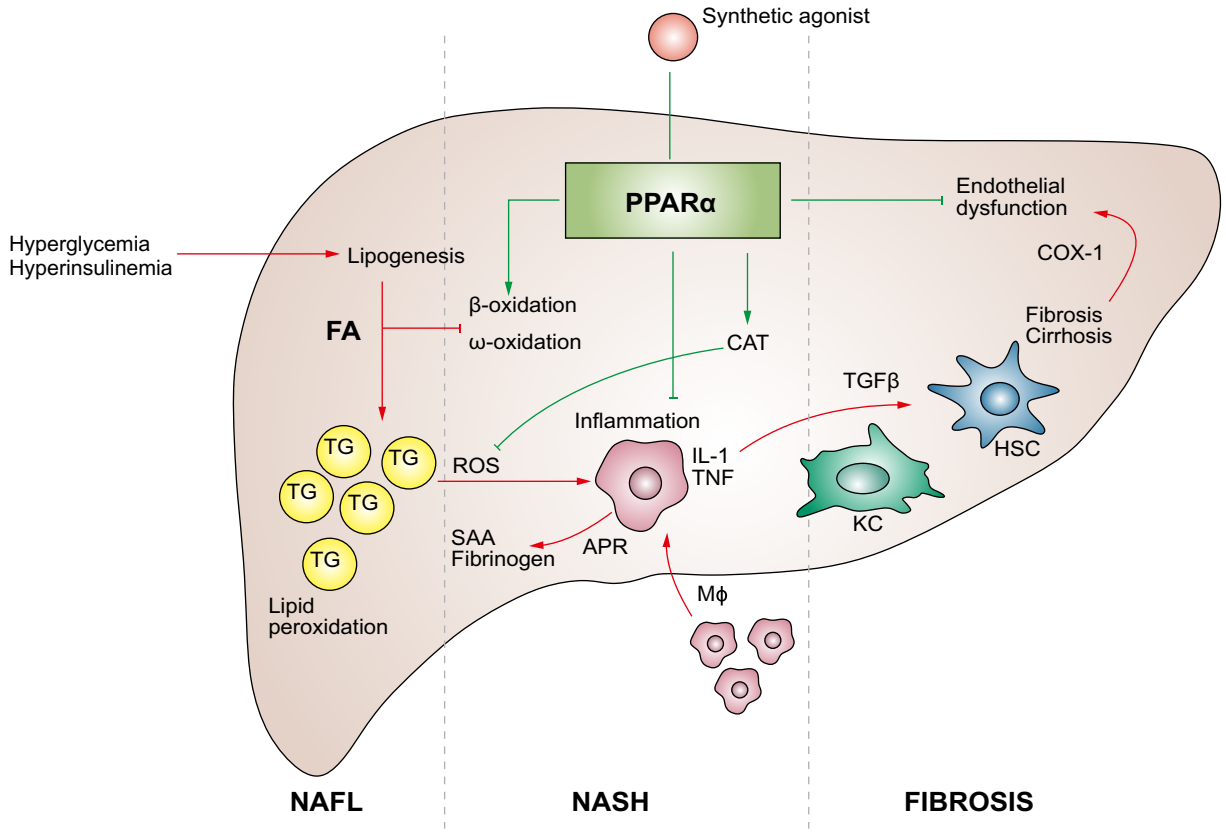

Fig. 3. Hepatoprotective effects of fibrates: examples from rodent models of NAFLD. Development of NASH is provoked by different risk factors, such as Western-type diet, physical inactivity and genetic predispositions that often lead to insulin resistance and T2DM. Exaggerated food intake leads to FA synthesis via hepatic lipogenesis pathways. Enhanced TG storage in the liver (steatosis) provokes uncontrolled lipid peroxidation that generates reactive oxygen species (ROS) and cytotoxic aldehydes. Hepatocyte damage leads to increased inflammatory signaling (IL-1, TNF), acute phase response (APR) and recruitment of circulating (Mφ) and residual macrophages (KC). All of these mechanisms can directly induce apoptosis, necrosis and TGFβ-dependent activation of hepatic stellate cells (HSC) that are the main source of extracellular matrix protein in liver, thus contributing in fibrosis progression. In several mouse models of NAFLD, fibrate-activated PPARα counteracts different stages of NAFLD by promoting FAO and hampering pro-inflammatory response. Moreover, fibrate treatment induces catalase (CAT) expression thus diminishing H2O2 levels in the liver. Hepatic cirrhosis is associated with endothelial dysfunction and impaired intrahepatic hemodynamics that may lead to liver failure. Fibrates improve and ameliorate hepatic vascular resistance by reducing cyclo-oxygenase-1 (COX-1) protein expression.

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 CCl_4 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 NAFLD 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 *Pparα* 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-887 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 *APOE2*-KI mice by a Western-diet and in *db/db* mice by an MCDD. Furthermore, GFT505 exerts anti-fibrotic activities on CCl_4-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 PPARδ 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 IIb 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.

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