

Integrated physiology and systems biology of PPAR α



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ABSTRACT

The Peroxisome Proliferator Activated Receptor alpha (PPAR α) is a transcription factor that plays a major role in metabolic regulation. This review addresses the functional role of PPAR α in intermediary metabolism and provides a detailed overview of metabolic genes targeted by PPAR α , with a focus on liver. A distinction is made between the impact of PPAR α on metabolism upon physiological, pharmacological, and nutritional activation. Low and high throughput gene expression analyses have allowed the creation of a comprehensive map illustrating the role of PPAR α as master regulator of lipid metabolism via regulation of numerous genes. The map puts PPAR α at the center of a regulatory hub impacting fatty acid uptake, fatty acid activation, intracellular fatty acid binding, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, triglyceride turnover, lipid droplet biology, gluconeogenesis, and bile synthesis/secretion. In addition, PPAR α governs the expression of several secreted proteins that exert local and endocrine functions.

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Keywords PPAR α ; Liver; Transcriptional networks; Lipid metabolism; Expression profiling; Metabolic homeostasis; Systems biology

1. INTRODUCTION

PPAR α was the first member to be cloned of a small subfamily of nuclear receptors called Peroxisome Proliferators Activated Receptors [1]. The other members of this subfamily are PPAR δ , also referred to as PPAR β , and PPAR γ [2]. Peroxisome proliferators encompass a diverse set of synthetic compounds that cause peroxisome proliferation and liver cancer in mice. Contradicting their name, PPAR δ and PPAR γ are not activated by peroxisome proliferators, in contrast to PPAR α . The PPAR subfamily is part of the larger family of nuclear receptors that also includes receptors for fat soluble vitamins, steroid hormones, and sterols [3]. Nuclear receptors share a conserved modular structure consisting of a N-terminal domain involved in transcriptional activation, a DNA-binding domain containing a zinc-twist structure, a short hinge region, and a relatively spacious ligand binding domain, which accommodates the lipophilic ligands and also harbors a transcriptional activation function at the far C-terminus [4].

PPARs bind to DNA as a heterodimer with the Retinoid X Receptor RXR, and together they recognize specific DNA sequences in and around target genes referred to as PPAR response elements [5]. These PPAR response elements or PPREs consist of a direct repeat of the consensus hexanucleotide AGGTCA spaced by a single nucleotide. Agonist ligands for PPARs may promote the physical association of the PPAR–RXR heterodimer to DNA, but substantial binding of PPARs to DNA already occurs in the basal state [6]. In contrast to other nuclear receptor–RXR pairs, PPAR–RXR heterodimers are “permissive”, which means that they can be activated by either an RXR-selective ligand (“rexinoid”) or a PPAR ligand [7–9]. Binding of ligand leads to the dissociation of co-repressor proteins and the association of co-activator proteins, which can recruit or have intrinsic histone

deacetylase and histone acetyltransferase activity, respectively, necessary for the assembly of the transcription initiation complex [10]. Readers are referred to another review for more detailed information on co-activators in PPAR-dependent gene regulation [11].

In addition to via direct binding to PPREs, PPARs and PPAR ligands also regulate gene expression by altering the activity of other transcription factors via direct protein–protein interactions. This action of PPARs generally inhibits the function of the other transcription factor and is referred to as transrepression. Transrepression predominantly accounts for the inhibitory effect of PPARs on inflammation related genes [12].

The most abundant natural ligands for PPARs encompass different types of (dietary) fatty acids and fatty-acid derived compounds, including various eicosanoids [13]. In addition, numerous dietary plant bioactive compounds have been suggested to serve as PPAR agonist, although the *in vivo* relevance of PPAR activation by these compounds remains uncertain. Finally, PPARs are the molecular target of different classes of drugs used in the treatment of diabetes and dyslipidemia. All three PPARs are expressed in a variety of tissues [14,15]. Expression of PPAR γ is most restrictive, showing high expression in white and brown adipocytes, macrophages and colonocytes, with lower expression in skeletal muscle and many other tissues. PPAR δ is expressed in virtually all tissues and cell types examined, while expression of PPAR α is highest in liver and brown adipose tissue, followed by small intestine, heart and kidney (<http://biogps.org/>). This review will concentrate on PPAR α .

Since its discovery, PPAR α has evolved from an intracellular receptor for synthetic peroxisome proliferators into one of the key transcriptional regulators of intermediary metabolism and an intermediate in the pathogenesis of numerous diseases [16]. Although most of our

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knowledge on PPAR α is connected to its presence in the liver, studies on PPAR α in other tissues, including heart [17,18], and small intestine [19] have indicated that the role of PPAR α in metabolic homeostasis is relatively well conserved between different cell types. The first part of this review addresses the role of PPAR α in intermediary metabolism in liver. A distinction is made between the metabolic function of PPAR α upon physiological, pharmacological, and nutritional PPAR α activation. As a transcription factor, PPAR α governs biological processes by altering the expression of numerous target genes. Accordingly, the functional role of PPAR α is directly related to the biological function of its target genes. In the second part of the review, a comprehensive overview is provided of metabolic genes targeted by PPAR α , organized into specific metabolic pathways.

2. REGULATION OF INTERMEDIARY METABOLISM BY PPAR α UPON PHYSIOLOGICAL, NUTRITIONAL AND PHARMACOLOGICAL ACTIVATION

2.1. Physiological PPAR α activation during fasting

2.1.1. Regulation of the adaptive response to fasting by PPAR α

Throughout our ancestral history, fasting and starvation were common occurrences that posed a major threat to the survival of the human species. As a consequence, humans have developed an adaptive response mechanism to fasting that relies on two main pillars: 1) generation of a strong hunger sensation that triggers food-seeking behavior and 2) a shift in fuel utilization to exploit the abundant triglyceride stores in the adipose depot. A key event during fasting is activation of adipose tissue lipolysis, contributing to a gradual shift in whole-body fuel utilization from glucose and fatty acids in the fed state to almost exclusively fatty acids after a day of fasting [20]. The shift in fuel utilization is governed by changes in the production of the metabolic hormones insulin and glucagon, as well as by altered secretion of several gut- and adipose-tissue derived hormones. Within this complex

network of metabolic adjustments, the liver plays a key role through its exclusive ability to synthesize glucose and catalyze the formation of ketone bodies [20]. Ample provision of glucose and ketone bodies is necessary to meet the needs of the metabolically active brain, which unlike other tissues and organs is unable to utilize fatty acids as fuel. Research in the past two decades has shown that PPAR α is a master regulator of hepatic nutrient metabolism during fasting [21–23]. Specifically, PPAR α induces hepatic fatty acid oxidation and ketogenesis and regulates hepatic glucose production, which are key events in the adaptive response to fasting (Figure 1). Additionally, PPAR α governs hepatic amino acid metabolism. Elucidation of the role of PPAR α during fasting has benefited immensely from the availability of PPAR α -/- mice, which exhibit a striking fasting-induced phenotype [21–23].

2.1.2. Regulation of hepatic lipid homeostasis during fasting by PPAR α

As indicated above, a crucial event during fasting is activation of adipose tissue lipolysis, resulting in elevated circulating levels of glycerol and free fatty acids (FFA) and elevated flux of fatty acids into many tissues including liver. Increased hepatic uptake of fatty acids during fasting is associated with activation of a number of pathways, most prominently the activation of hepatic fatty acid oxidation and concomitant production of ketone bodies (Figure 2A). Metabolic data have unequivocally demonstrated the crucial role of PPAR α in stimulation of hepatic fatty acid oxidation and ketogenesis during fasting [21–25]. Indeed, the increase in plasma ketone body levels during fasting is largely abolished in fasted mice lacking PPAR α [26] (Figure 2B, Table 1), which is caused by blunted fasting-induced upregulation of numerous PPAR α target genes involved in fatty acid oxidation (e.g. Cpt1a, Cpt2, Acadvl, Hadha) and ketogenesis (Hmgcs2, Hmgcl, Acat1). Furthermore, plasma levels of long chain acyl-carnitines are increased in fasted PPAR α -/- mice, whereas plasma levels of medium and short chain acyl-carnitines and free carnitine are decreased, reflecting impaired fatty acid oxidation (Table 1) [21,27]. Decreased rates of hepatic fatty acid oxidation and

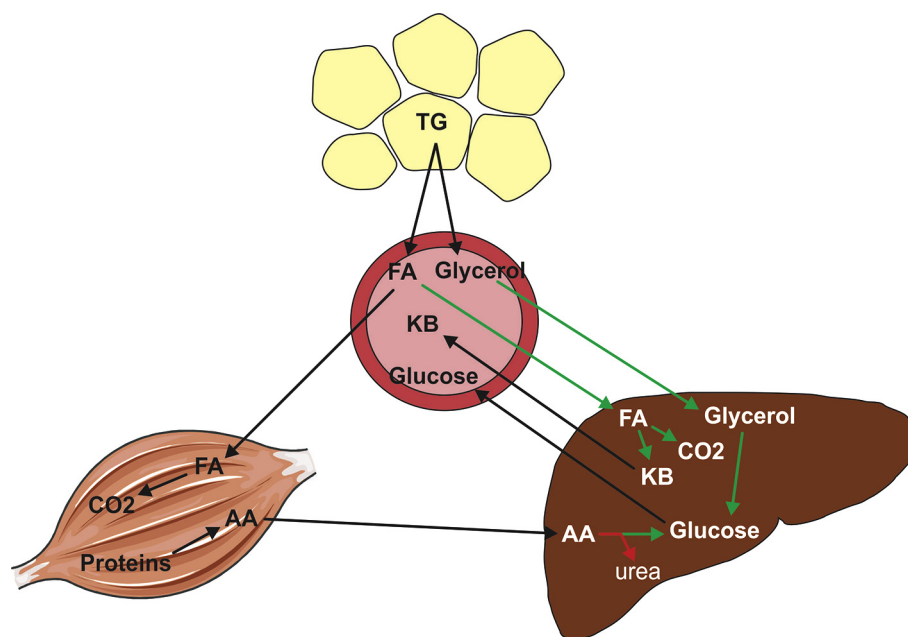


Figure 1: Overall role of PPAR α in the adaptive response to fasting. Fasting is associated with activation of adipose tissue lipolysis, leading to the release of free fatty acids and glycerol into the circulation. Free fatty acids taken up by the liver are partially oxidized and converted into ketone bodies, or completely oxidized to CO₂. Glycerol is converted into glucose, as are amino acids coming from skeletal muscle. The processes induced by PPAR α are indicated by green arrows. The processes suppressed by PPAR α are indicated by red arrow. TG = triglycerides, FA = fatty acids, KB = ketone bodies, AA = amino acids.

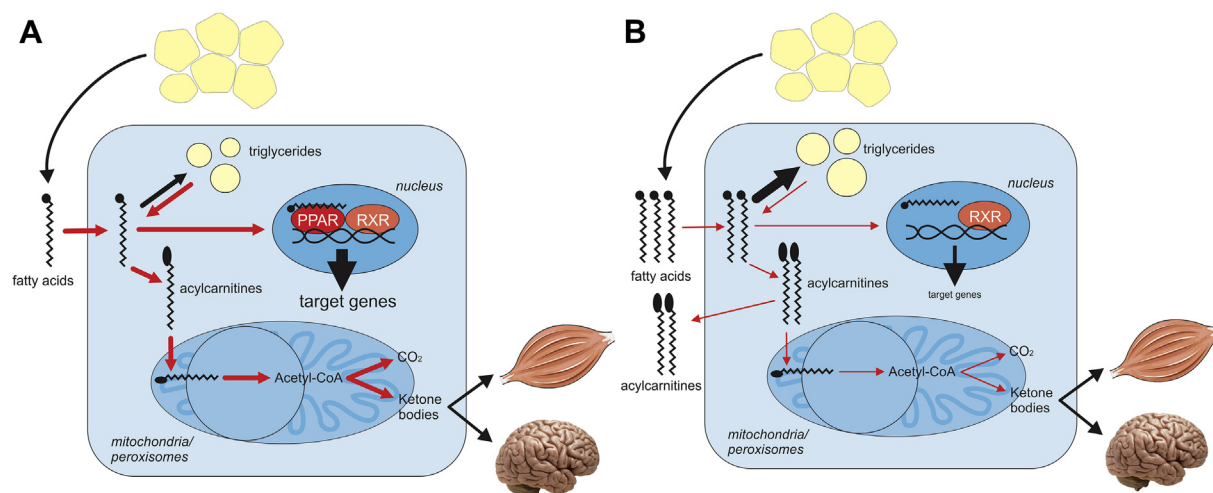


Figure 2: Role of PPAR α in hepatic lipid metabolism. Free fatty acids released by adipose tissue lipolysis are taken up by hepatocytes and either converted into triglycerides or, after conversion to acyl-carnitine, are oxidized in the mitochondria. The metabolic steps or pathways under transcriptional control of PPAR α are indicated in red. A) The normal situation in fasted wildtype mice. B) Consequences of PPAR α deletion on metabolic pathways and specific intra- and extracellular metabolites in the fasted state.

ketogenesis were confirmed in primary hepatocytes of PPAR α -/- mice compared with wildtype mice [28]. Due to a reduced capacity for fatty acid oxidation, a large share of the incoming fatty acids is diverted towards triglyceride synthesis via a pathway that does not seem to be noticeably affected by PPAR α deletion. Consequently, liver triglyceride levels are markedly elevated in fasted PPAR α -/- mice compared with fasted wildtype mice [21,22,26] (Figure 2, Table 1). In addition, plasma and liver concentrations of FFA are elevated in fasted PPAR α -/- mice because of impaired fatty acid oxidation [22,26,29]. For reasons that are unclear, plasma triglyceride levels are elevated in PPAR α -/- mice only in the fed but not fasted state [21,26]. An overview of the metabolic changes in plasma and liver of PPAR α -/- mice is provided in Table 1. The markedly disturbed response to fasting in PPAR α -/- mice thus illustrates the profound importance of PPAR α in governing hepatic lipid metabolism during fasting.

2.1.3. Regulation of hepatic glucose homeostasis during fasting by PPAR α

Besides leading to dramatic alterations in lipid metabolism, deletion or knock-down of PPAR α also causes severe fasting-induced hypoglycemia and reduces intra-hepatic glucose levels, indicating a major impact of PPAR α on glucose homeostasis [21–23,29–31]. Several explanations for the hypoglycemia have been put forward. First, hepatic glycogen level are lower in (re)-fed PPAR α -/- mice [22,29,31–33], likely leading to reduced glycogen breakdown and glucose release during early fasting. Metabolic changes detected in the liver of PPAR α -/- mice are consistent with reduced glucose storage and glucose production by gluconeogenesis (Table 1) [29]. The lower glycogen levels may be due to reduced expression of Gys2, leading to a diminished rate of glycogen formation upon (re) feeding [25,31,32]. Alternatively, it was suggested that impaired fatty acid oxidation and resultant ATP and NADH shortage in livers of PPAR α -/- mice may disable gluconeogenesis [22]. However, the stimulatory effect of fatty acid oxidation on gluconeogenesis appears to be minimal [34]. Another potential explanation for the fasting hypoglycemia in PPAR α -/- mice is decreased hepatic gluconeogenesis from glycerol, uptake and conversion of which is under direct control of PPAR α [24]. However, tracer studies have not supported this notion. One tracer study found that total hepatic glucose production and hepatic glucose production from glycerol

were increased in PPAR α -/- mice in the fasted state, whereas hepatic glucose production from lactate was reduced [31]. Another study reported that after a short-term fast the gluconeogenic flux in PPAR α -/- mice is directed more towards glycogen, leading to a decrease in hepatic glucose output [32]. Despite the ambiguity emerging from tracer studies, the observation that the fasting-induced increase in expression of several genes involved in the gluconeogenic pathway (Pcx, Fbp1, Gpd1, Gyk, Slc16a1), many of which represent PPAR α target genes, is abolished in PPAR α -/- mice provides strong evidence that gluconeogenesis is directly targeted by PPAR α [35]. This notion is further supported by reduced rates of glucose synthesis from lactate/pyruvate in primary hepatocytes from PPAR α -/- mice compared to wildtype mice [28]. Other gluconeogenic genes that have been reported to be reduced in PPAR α -/- mice include G6pc (glucose 6 phosphatase) and Pck1 (phosphoenolpyruvate carboxykinase) [31,32,36]. However, others have not been able to confirm these findings. Finally, apart from changes in glucose production, there is ample evidence that the fasting hypoglycemia is at least partially caused by elevated peripheral glucose utilization, likely as a result of impaired fatty acid oxidation and a reduced supply of ketone bodies [36,37].

Intracellular	
Increased	Decreased
Triglycerides	Short chain acyl-carnitines
Long chain acyl-carnitines	Free carnitine
Free fatty acids	Glucose
Glycogen (fasted state)	Glycogen
Blood plasma	
Increased	Decreased
Triglycerides (in fed state)	Short chain acyl-carnitines
Long chain acyl-carnitines	Free carnitine
Free fatty acids	Ketone bodies
Urea	Glucose
	Arginine
	Tyrosine
	Alanine

Table 1: Metabolites changes in PPAR α -/- mice.

2.1.4. Regulation of hepatic amino acid metabolism during fasting by PPAR α

PPAR α not only is the master regulator of hepatic lipid and glucose homeostasis but also has a profound impact on amino acid metabolism. Specifically, PPAR α downregulates the expression of numerous enzymes involved in amino acid degradation, transamination and the urea cycle (Got1, Prodh, Gls2, Pah, Sds, Asl, Ass1, Cps1) [35,38]. In agreement with enhanced amino acid degradation and urea synthesis, plasma urea levels are elevated in PPAR α -/- mice, whereas plasma levels of several amino acids (arginine, tyrosine, alanine) are reduced [27,38]. These data demonstrate that during fasting PPAR α minimizes amino acid degradation and ureagenesis, which together with induction of fatty acid oxidation is part of an adaptive mechanism aimed at relying maximally on stored triglycerides as fuel, as opposed to breaking down valuable muscle tissue and oxidizing amino acids [27]. The molecular mechanisms underlying downregulation of amino acid metabolism by PPAR α remain elusive.

2.1.5. Mechanism of activation of PPAR α during fasting

Induction of most of the above described genes by fasting is dependent on PPAR α , which suggests that PPAR α in liver is activated during fasting. Activation of PPAR α during fasting is supported by experiments using in vivo PPAR-reporter mice [39]. Since fatty acids are ligands for PPAR α [40], and plasma FFA increase during fasting, it has been tempting to attribute the activation of hepatic PPAR α during fasting to increased plasma FFA uptake into the liver [22]. However, studies suggest that plasma FFA cannot activate PPAR α in liver [41,42]. An alternative mechanism for PPAR α activation during fasting is via induction of PPAR α mRNA [22]. In line with major regulation at the mRNA level, PPAR α mRNA follows a pronounced circadian rhythm that is inversely related to feeding status [43,44]. In addition, circadian transcription of genes encoding acyl-CoA thioesterases may lead to rhythmic changes in the intracellular level of fatty acid ligands [44]. Other potential mechanisms for PPAR α activation during fasting include induction of co-activator proteins such as PGC1 α [42], and intracellular generation of PPAR α ligands via lipolysis of locally stored triglycerides [45,46]. Recently, evidence was provided that induction of the PGC1 α -PPAR α -target genes axis during fasting in liver is mediated by the basic helix-loop-helix (bHLH) leucine zipper transcription factor TFEB, which itself is induced by fasting [47].

2.2. Pharmacological and toxicological PPAR α activation

2.2.1. Fibrates are high affinity PPAR α activators

Besides binding a range of endogenous ligands, PPAR α also serves as receptor for a diverse array of synthetic compounds collectively referred to as peroxisome proliferators that include phthalates, insecticides, herbicides, surfactants, organic solvents, and hypolipidemic fibrate drugs [1,48]. The latter group encompasses the compounds Wy14643, fenofibrate, clofibrate, ciprofibrate, bexafibrate and gemfibrozil. Synthetic PPAR α agonists cause hepatomegaly and peroxisome proliferation in mice but not in humans [49] (Table 2). In humans, fibrates raise plasma HDL and reduce plasma triglycerides and therefore carry therapeutic value in the treatment of dyslipidemia [50]. Accordingly, most of the literature on fibrates is related to their clinical and pre-clinical effects on circulating cardiovascular disease risk factors and lipid metabolic parameters [51]. Chronic administration of peroxisome proliferators to rodents leads to hepatic carcinogenesis via PPAR α [52], which has led to concern about the industrial use of peroxisome proliferators and the potential carcinogenicity of these compounds in humans [53].

Feature	Rodent	Human
Peroxisome proliferation in response to PPAR α agonists	+	-
Hepatomegaly in response to PPAR α agonists	+	-
Plasma TG lowering by PPAR α agonists	+	+
Effect PPAR α agonist on liver transaminase	↓	↑
Highest expression of PPAR α	Liver, heart, brown adipose tissue, heart	Liver, heart, brown adipose tissue?, small intestine
Robust common target genes	Cpt1a, Fgf21, Acox1 Hmgcs2, Angptl4	CPT1A, FGF21, ACOX1 HMGCS2, ANGPTL4
Mouse-specific targets	Mgil, Bbox1, Decr1, Fbp2	
Human-specific targets		PCK1, MBL2, APOA2

Table 2: Differences in PPAR α biology between rodents and humans

2.2.2. Rexinoids activate PPAR α -RXR heterodimers

Consistent with PPAR α -RXR functioning as a permissive heterodimer, RXR activation using the synthetic rexinoid LG1069 leads to upregulation of a large number of PPAR α target genes in liver and heart [54,55], a response that is abolished in mice lacking PPAR α [56,57]. Comparison of the effect of LG1069 and WY14643 in rodent liver reveals a marked resemblance in gene regulation by the two compounds, with many genes being induced by both agonists, including well established PPAR α targets such as Acox1, Acaa1, Ehhadh, Cpt2 and Plin2 [35,55]. By contrast, we found that LG1069 activates a distinct set of genes in primary human hepatocytes as compared with fenofibrate and WY14643, with minimal overlap (our unpublished data).

2.2.3. Regulation of hepatic lipid homeostasis by synthetic PPAR α agonists

Numerous in vitro studies in cultured hepatocytes have demonstrated a stimulatory effect of synthetic PPAR α agonists on peroxisomal and mitochondrial fatty acid oxidation [58-61]. Similarly, treatment of rodents with synthetic PPAR α agonists markedly induces peroxisomal and mitochondrial fatty acid oxidation in liver homogenates and isolated hepatocytes, concurrent with an increase in size and number of peroxisomes, as well as increased activity of fatty acid oxidative enzymes such as carnitine palmitoyl-transferase, 2,4-dienoyl-CoA reductase, peroxisomal 3-ketoacyl-CoA thiolase, and acyl-CoA dehydrogenase [61-69]. Induction of fatty acid oxidative enzyme activity by PPAR α agonists is paralleled by marked upregulation of the corresponding genes Cpt1a, Decr2, Acaa1a, and Acadvl [35,70-72], as well as numerous other genes participating in fatty acid oxidation. The stimulatory effect of in vivo fibrate treatment on fatty acid oxidation and oxidative enzymes can be detected in isolated mitochondria and peroxisomes [73-76]. Besides mitochondrial and peroxisomal fatty acid oxidation, fibrates also stimulate hepatic microsomal omega-hydroxylation of fatty acids [75,77-79]. In addition, fibrates markedly induce ketogenesis in cultured hepatocytes and rodent livers [68,73,80-82]. Stimulation of fatty acid oxidation likely accounts for the beneficial effect of synthetic PPAR α agonists on fatty liver in rats and mice [83-91], and may contribute to lowering of VLDL-triglyceride secretion [83], giving rise to their hypotriglyceridemic effect. It should be mentioned that in humans synthetic PPAR α agonists primarily lower circulating triglycerides by stimulating plasma triglyceride clearance [83,92]. Intriguingly, pharmacological PPAR α activation also stimulates hepatic de novo lipogenesis and chain elongation, as determined using stable isotopes [93]. Induction of lipogenesis by (chronic) fenofibrate treatment depends on sterol regulatory element-binding protein 1c (SREBP-

1c) but not carbohydrate response element-binding protein (ChREBP) [93]. In contrast to de novo lipogenesis, which is thus regulated via an indirect mechanism, several genes involved in fatty acid elongation are under direct transcriptional control of PPAR α . (see below).

2.2.4. Regulation of hepatic glucose homeostasis by synthetic PPAR α agonists

There is limited evidence linking pharmacological PPAR α activation to regulation of glucose homeostasis. Whereas clofibrate lowers fasting glucose and insulin level and improves glucose tolerance and utilization in rodents [86,94–96] and humans [97–100], indicating enhancement of insulin sensitivity, fenofibrate does not have any effect on insulin sensitivity in humans [92,101–104]. It can thus be argued that the effect of clofibrate may be independent of PPAR α activation. Alternatively, the differential impact of clofibrate and fenofibrate on glucose homeostasis may be linked to selective PPAR modulation (SPPARM), based on the notion that different PPAR agonists may induce differential gene expression patterns via selective receptor–coregulator interactions.

At the intracellular level, fenofibrate treatment was found to reduce the contribution of glycolysis to acetyl-CoA production [93], which may be mediated by PPAR α -mediated induction of pyruvate dehydrogenase kinase 4 (Pdk4) [105]. Pdk4 phosphorylates and inactivates pyruvate dehydrogenase, thereby limiting carbon flux through glycolysis. Additional information about the impact of pharmacological PPAR α activation on intracellular glucose metabolism in liver and relevant mechanisms is lacking.

2.2.5. Regulation of hepatic amino acid metabolism by synthetic PPAR α agonists

Pharmacological PPAR α activation has a significant influence on amino acid metabolism. Specifically, hepatic gene expression of numerous enzymes involved in amino acid metabolism and urea synthesis is markedly decreased upon treatment of rodents with the PPAR α agonists Wy14643 or perfluorooctanoic acid (PFOA), including Got1, Asl, Ass1, Gls2, and Otc [38,106]. Downregulation of the corresponding proteins by synthetic PPAR α agonists in mice [107], rats [108], and dogs [109], is supported by proteomics analysis. In agreement with impaired ureagenesis, plasma ammonia levels were elevated in rats fed Wy14643 [110]. For reasons that are unclear, plasma urea levels were increased by Wy14643 as well [110].

In contrast to what is observed in rodents, PPAR α agonists directly upregulate expression of aspartate aminotransferase (Got1) and alanine aminotransferase (Gpt) in human primary hepatocytes and human HepG2 hepatoma cells (Table 2) [111–113], which may explain the elevated serum aspartate and alanine aminotransferase activities in PPAR α agonists-treated subjects that do not show further signs of liver injury. In fact, there is evidence that Gpt is a direct PPAR α target in human hepatocytes [112]. So far, the molecular basis for differential regulation of aminotransferases between mouse and human has remained elusive.

2.3. Nutritional PPAR α activation

2.3.1. Fatty acids and various fatty acid derivatives serve as PPAR α agonists

Several studies have demonstrated that PPAR α is able to bind fatty acids with a general preference for long-chain poly-unsaturated fatty acids (PUFAs) [40,48,114–120]. Furthermore, numerous fatty acid-derived compounds and compounds showing a structural resemblance to fatty acids, including oxidized fatty acids, eicosanoids, endocannabinoids,

and phytanic acid, are able to activate PPAR α [121–126]. In addition, biochemical studies indicate that fatty acyl-CoAs can bind PPAR α . Interestingly, it was found that they oppose the effects of WY14643 and fatty acids on PPAR α conformation, DNA binding, and co-activator interaction, suggesting fatty acyl-CoAs serve as PPAR α antagonists. However, the in vivo relevance of acyl-CoAs as PPAR α ligands remains uncertain [125,127,128]. The combined evidence strongly suggests that PPAR α serves as general fatty acid sensor with a limited degree of ligand selectivity. In addition, there are reports postulating that PPAR α may be activated by specific phospholipid species, including the phosphatidylcholines PC(16:0/18:1) or PC(18:0/18:1) [129,130]. Because phosphatidylcholines are abundant in any cell, and likely fluctuate very little, it is unclear how activation of PPAR α by phosphatidylcholines fits into the notion of PPAR α being a metabolic sensor.

Activation of PPAR α by fatty acids and fatty acid derivatives not only depends on their absolute concentration in the cell but also by the abundance of fatty acid-binding proteins such as FABP1. FABP1 was found to transfer fatty acids to the nucleus and co-localize with PPAR α [131,132]. Subsequent studies revealed that FABP1 physically interacts with PPAR α and enhances PPAR α -dependent gene regulation [133–135].

2.3.2. Activation of PPAR α by high fat ketogenic diets

Studies using in vivo PPAR-reporter mice have suggested that PPAR is activated by chronic high fat feeding [39]. Gene expression analysis of PPAR α target genes has confirmed modest activation of PPAR α in response to high fat feeding [136–140]. A complicating factor is that chronic high fat feeding also invariably causes hepatic steatosis, which is associated with induction of PPAR γ and may therefore lead to induction of PPAR target genes via PPAR γ [137,141–143]. An extreme form of high fat diet that potently induces expression of PPAR α target genes in liver, including Fgf21, Cd36, Pdk4, Acadm, and Pex11a, is the ketogenic diet [144,145]. A ketogenic diet is almost entirely devoid of carbohydrate and elicits a metabolic state of low insulin, high plasma free fatty acids, and enhanced ketogenesis, that resembles fasting. Similar to what is observed during fasting, the metabolic phenotype of PPAR α -/- mice becomes much more prominent upon feeding a ketogenic diet, illustrated by pronounced hepatic steatosis, hypoglycemia and elevated plasma FFA [144]. Overall, the extent of PPAR α activation by high fat feeding will likely depend on three main variables: the amount of fat in the diet in comparison with the control diet, the amount of sucrose in the diet in comparison with the control diet, and the fatty acid composition of the diet. The lower the amount of sucrose and the higher the proportion of poly-unsaturated fatty acids, the stronger the activation of PPAR α .

2.3.3. Preferential activation of PPAR α by long chain poly-unsaturated fatty acids

As indicated above, diets rich in poly-unsaturated fatty acids lead to more pronounced PPAR α activation in liver compared to diets rich in saturated or mono-unsaturated fatty acids, as determined by measurement of enzyme activity or mRNA expression of target genes. Strongest activation of PPAR α is observed when feeding fish oil [90,146–150]. The potency of fish oil towards PPAR α is corroborated by oral lipid loading experiments in which mice received a single bolus of triglyceride composed of one single fatty acid [119]. A single oral fat load, which mimics a high fat meal low in carbohydrate, markedly and very specifically activates PPAR α in liver, with docosahexanoic acid being the most potent fatty acid, followed by linolenic acid, linoleic acid and oleic acid. The dietary fatty acids mostly copy the effects of high affinity synthetic PPAR α agonists, except that they are much less

potent [119]. These findings demonstrate that dietary fatty acids are able to activate hepatic PPAR α after their delivery to the liver as part of chylomicron remnants.

The large hydrophobic binding cavity allows PPARs to interact with a variety of structurally related and unrelated compounds [151]. Indeed, apart from fatty acids, numerous other dietary components have been proposed to serve as PPAR α agonists, including but not limited to the flavanoid cyanidin [152], the carotenoid astaxanthin [153], the plant triterpenoid ursolic acid [154], the plant stilbenoid pterostilbene [155], isohumulones in hops [156], soy isoflavones [157], and conjugated linoleic acid [158]. However, it is highly questionable whether intake of any of these compounds is high enough to lead to PPAR α activation in vivo, especially taking into account the abundance of fatty acids in our diet and in the cell.

3. TARGET GENES AND PATHWAYS OF PPAR α

3.1. Overview of PPAR α -mediated gene regulation

3.1.1. Pharmacological versus physiological PPAR α target genes

PPAR α governs biological processes by altering the expression of a large number of target genes. Gene expression profiling studies have indicated that PPAR α target genes number in the hundreds [35,119,159], which is similar to other liver-enriched nuclear receptors such as LXR [160]. Accordingly, the functional role of PPAR α is directly related to the biological function of its target genes. However, it should be realized that the influence of PPAR α on gene regulation is dependent on whether PPAR α is activated pharmacologically, physiologically, or nutritionally. Fibrates not only bind to PPAR α more avidly compared with fatty acids [48,161], but likely also cause a slightly different conformational change in the PPAR α protein as dictated by the SPPARM concept [119], together leading to slightly altered and generally more robust induction of target genes by fibrates. Furthermore, the endocrine profile and thereby the general chromatin and transcriptional landscape supporting PPAR α -dependent gene regulation can be different depending on feeding status. Nevertheless, for the most part the genes induced by fibrates are the same genes that are reduced in PPAR α -/- mice in the fasted state [35] (Figure 3). The similarity in gene regulation by PPAR α upon pharmacological and physiological activation is well illustrated by gene set enrichment analysis (GSEA), showing large overlap in enriched gene sets between the two conditions (Figure 4). Major exceptions are genes involved in bile synthesis and secretion, many of which are markedly down-regulated in PPAR α -/- mice in the fasted state yet are not induced by fibrates (Figure 3). The same is true for several genes involved in retinoid metabolism. The exclusive regulation of retinol metabolism and bile secretion by PPAR α during fasting is confirmed by GSEA (Figure 4). It is possible that this pattern of gene regulation may reflect indirect regulation, e.g. genes that are reduced in fasted PPAR α -/- mice but that are not induced by synthetic PPAR α agonists may not be direct targets of PPAR α . The opposite is also observed: a number of genes are significantly induced by fibrates but are not decreased in PPAR α -/- mice in the fasted state [42]. Several potential explanations for this type of regulation can be put forward. A) Induction of the gene by fibrates reflects regulation in Kupffer cells [162]. In contrast to hepatocytes, PPAR α in Kupffer cells is most likely not activated by fasting. For example, LPL is specifically expressed in Kupffer cells and is markedly induced by fibrates, yet expression is unchanged by PPAR α deletion in the fasted state. B) The elevated plasma FFA levels in fasted PPAR α -/- mice lead to induction of other transcription factors, such as PPAR δ , obscuring a potential role of PPAR α in regulation of these genes in the

fasted state [42]. Genes in this category include Lpin2, Plin3, and Pdk4. C) As mentioned above, high affinity activation of PPAR α may cause differential recruitment of co-factors (SPPARM) compared with physiological PPAR α activation, resulting in subtle differences in gene regulation. D) Certain genes are relatively weak targets of PPAR α characterized by low affinity PPRES and therefore require a fully activated PPAR α to show any mRNA induction.

3.1.2. Detailed map of PPAR α -mediated gene regulation

Gene expression analysis of livers of PPAR α -/- mice at the level of individual genes or the entire genome via transcriptomics has indicated that PPAR α governs many aspects of hepatic lipid metabolism, providing a molecular basis for the pronounced metabolic disturbances in fasted PPAR α -/- mice and explaining the marked impact of synthetic PPAR α agonist on lipid metabolism [163,164]. To capture the scope of PPAR α activity in liver and to illustrate the role of PPAR α in transcriptional regulation of liver (lipid) metabolism, a detailed regulatory map was created of metabolic genes upregulated by PPAR α , separated according to metabolic pathway (Figure 3). A distinction is made between genes regulated by PPAR α during fasting (in green), by synthetic PPAR α agonist (in blue), or both (in red). Many of the genes shown in Figure 3 and described in the text below are direct PPAR α target genes based on the presence of a functional PPRE, as determined by transactivation assay, gel shift, and/or genomic DNA binding by chromatin immunoprecipitation. However, this criteria was not applied very strictly as assigning PPAR α target gene status, under the assumption that genes showing strong functional resemblance to known PPAR α targets and that are significantly upregulated upon PPAR α activation and/or significantly downregulated upon PPAR α deletion most likely represent direct target genes, despite the fact that a functional PPRE has not (yet) been located. Genes in that category include Acadvl, Hadha, and Etfhd. Because of these relatively loose criteria, it cannot be excluded that some of the genes shown are indirectly regulated by PPAR α . Indeed, several putative targets of PPAR α are also regulated by CREB3L3, a transcription factor that is under strong transcriptional control of PPAR α , including Fgf21, Gys2, Elovl2, Elovl5, Bdh1, G0s2, Mgl1, Decr2, and Cidec [159,165]. Some of these genes have previously been identified as direct PPAR α targets based on the presence of a functional PPRE, including Fgf21, Gys2, and G0s2 [33,166–168], suggesting possible dual regulation by PPAR α and CREB3L3. In fact, recent data suggest that CREB3L3 and PPAR α form a transcriptional complex, which binds to an integrated CRE-PPRE binding motif in the FGF21 gene promoter [169]. Whether CREB3L3 is more broadly involved in target gene regulation by PPAR α is a very intriguing question that deserves further exploration. Other transcription factors that cooperate with PPAR α or are under transcriptional control of PPAR α , and thus may mediate effects of PPAR α on gene expression include KLF11 (Cpt1a, Cyp4a10, Cyp4a14, Acadm) and KLF10 (Cyp4a14, Gpam, Acot3, Retsat) [159,170,171]. In the above situations, PPAR α indirectly upregulates gene expression without binding to a canonical PPRE. Other PPRE-independent mechanisms of gene regulation by PPAR α include physical interaction with other transcription factors, which can either be stimulatory or inhibitory (transrepression), and physical interactions with co-activators leading to reduced availability of those co-activators for other transcription factor pathways (squenching, inhibitory) [172].

3.1.3. PPAR α cistrome

The PPAR α cistrome is defined as the in vivo genome-wide location of PPAR α binding-sites, as determined using ChIP-SEQ and ChIP-on-ChIP. Interestingly, these analyses have found relatively little overlap

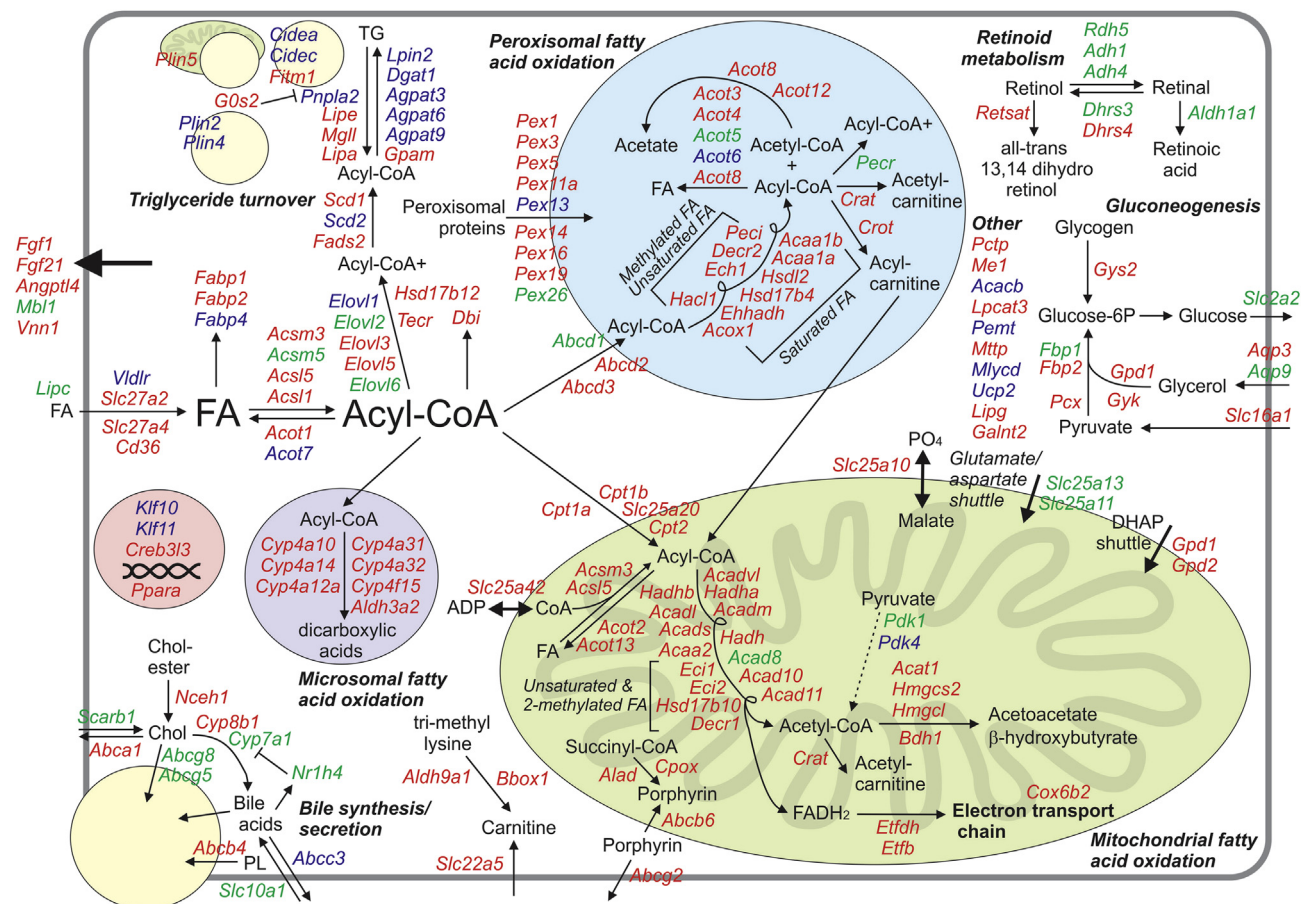


Figure 3: Detailed overview map of metabolic genes upregulated by PPAR α in mouse liver. The map is based on the published literature combined with transcriptomics analysis of liver of wildtype and PPAR α -/- mice. Colors indicate whether expression of a gene is governed by PPAR α during fasting (In green: significantly decreased expression in fasted PPAR α -/- mice compared with fasted wildtype mice, representing physiological regulation), and/or by synthetic PPAR α agonists (In blue: significantly increased expression in wildtype mice treated with Wy14643 or fenofibrate compared with vehicle-treated wildtype mice, representing pharmacological/toxicological regulation). Genes shown in red are upregulated by PPAR α during fasting and are upregulated by Wy14643/fenofibrate. Genes were excluded if they were only upregulated upon chronic administration of Wy14643/fenofibrate, which likely reflects indirect regulation. Genes in this category included Mecr, Fads1, and Lpl.

between induction of gene expression by the PPAR α agonist GW7647 and the GW7647-induced binding of PPAR α to a PPRE in the proximity of the gene, at least in primary human hepatocytes and HepG2 cells [173,174]. These data suggest significant uncoupling between gene induction by PPAR α and the binding of PPAR α to the gene, thus pointing at other mechanisms of gene (up)regulation by PPAR α . However, this conclusion may be partially confounded by the likely flawed assumption that binding of ligand enhances PPAR α binding to DNA and that PPAR binding sites are located within a certain distance of the transcriptional start site. Profiling of genomic binding sites of PPAR α in human HepG2 cells by ChIP-on-CHIP analysis raised the possibility that PPAR α may be recruited to certain gene promoters via direct physical interaction with other transcription factors [174]. In agreement with this notion, ChIP-SEQ revealed extensive overlap in genomic binding sites of LXR and PPAR α [175]. The consequences of the LXR and PPAR α overlap with respect to gene regulation seem to be determined by the context of the genomic binding site(s) and the activity of the individual receptors at the particular sites, suggesting that cross talk is context dependent and probably not due to competition for RXR [175].

Despite the overall usefulness of information on PPAR α cistrome towards enhancing our understanding of the determinants of genomic binding by PPAR α , its value for specific gene regulation by PPAR α and assigning PPAR α target gene status has so far been limited.

3.2. Target genes of PPAR α in hepatic lipid metabolism

3.2.1. Fatty acid uptake, binding and activation

The mechanism of fatty acid uptake into hepatocytes remains poorly defined but a (partial) role for the fatty acid translocase Cd36 is likely. Cd36 is upregulated by PPAR α [176], as are the putative fatty acid transporters Fatp2 and Fatp4 [35,176,177]. Because they are localized to the ER and thus cannot mediate membrane fatty acid transport directly, Fatp2 and Fatp4 seem to stimulate fatty acid uptake into hepatocytes indirectly via their acyl-CoA synthetase activity [178]. Acyl-Co synthetase activity is necessary to convert fatty acids to their acyl-CoA derivatives via thio-esterification, which is an essential step for directing fatty acids towards specific metabolic pathways. Several cytosolic acyl-Co synthetases are under transcriptional control of PPAR α , including Acsl1 and Acsl5 [35,179–181]. Interestingly, expression of the cytosolic Acyl-coenzyme A thio-esterases Acot1 and Acot7, which catalyze the reverse reaction and together with acyl-Co synthetases determine the balance between cytoplasmic concentrations of acyl-CoAs, free CoA and fatty acids, is also upregulated by PPAR α [182].

The FABP gene family comprises a group of high-affinity intracellular fatty acid-binding proteins. Fabp1 (L-FABP) was one of the first PPAR α target genes identified [183–185], and is highly abundant in liver. Fabp1 is likely involved in partitioning of fatty acids to specific lipid

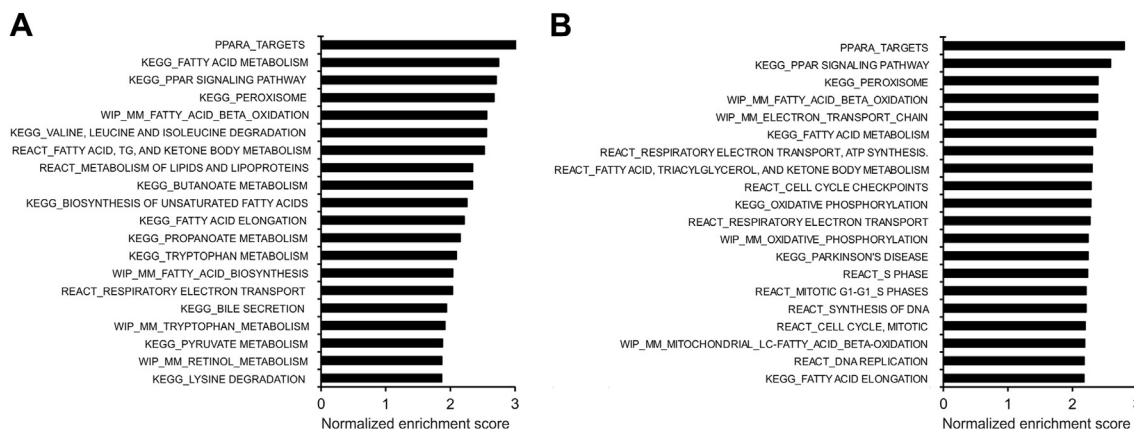


Figure 4: Pathways upregulated by PPAR α during fasting or by Wy14643. Gene set enrichment analysis was performed on transcriptomics data comparing livers from 24 h fasted PPAR α +/- and 24 h fasted PPAR α -/- mice (A), or livers from wildtype mice treated with Wy14643 or treated with vehicle for 5 days (B). The top 20 most significant positively enriched (upregulated) gene sets are shown ranked according to normalized enrichment score.

metabolic pathways [186]. Other Fabp genes induced by PPAR α activation in mouse liver include Fabp2 (I-FABP) and Fabp4 (A-FABP, aP2), whose role in liver is uncertain.

3.2.2. Peroxisomal fatty acid oxidation

The first identified target gene of PPAR α was Acyl-CoA oxidase (Acox1), which encodes the first enzyme in peroxisomal long-chain

fatty acid oxidation [2,5]. Peroxisomes are involved in many lipid metabolic pathways, including synthesis of bile acids and plasmalogens, synthesis of cholesterol and isoprenoids, alpha-oxidation, glyoxylate and H₂O₂ metabolism, and β -oxidation of very-long-straight-chain or branched-chain acyl-CoAs. Many aspects of peroxisomal fatty acid metabolism are under transcriptional control of PPAR α including peroxisomal fatty acid uptake (Abcd1, Abcd2 and Abcd3)

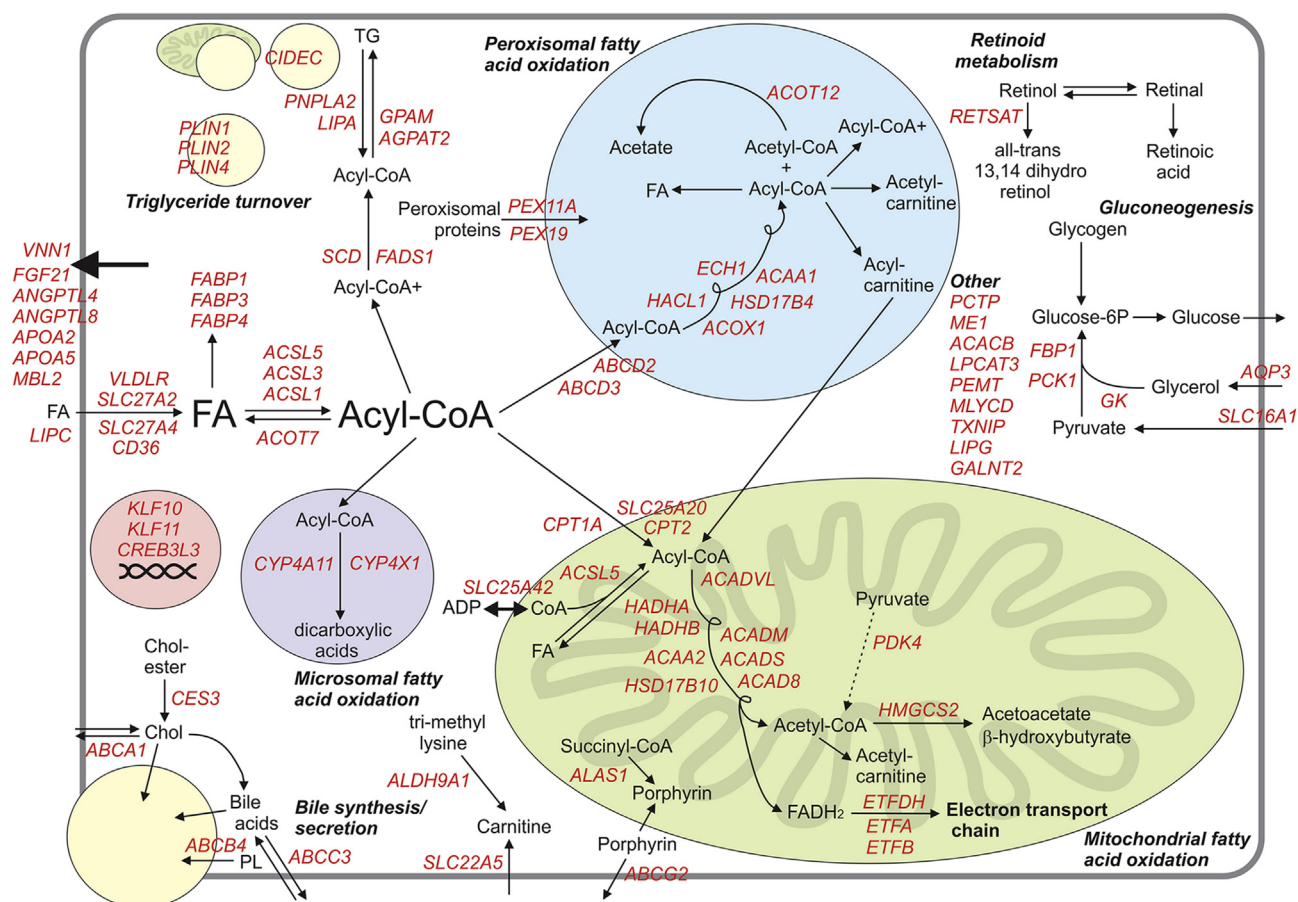


Figure 5: Detailed overview map of metabolic genes upregulated by PPAR α in human hepatocytes. The map is based on the published literature, including transcriptomics analysis of primary human hepatocytes treated with synthetic PPAR α agonists.

[187–189], conversion of acyl-CoA/acetyl-CoA to acyl-carnitine/acetyl-carnitine (Crot/Crat) [35], and conversion of acyl-CoAs back to fatty acids via numerous thioesterases (Acots) [35,190]. Thioesterase and acylcarnitine transferase activities are likely required for transporting fatty acids of various chain length out of the peroxisomes for further oxidation in mitochondria. Apart from Acox1, the enzymes operating downstream from Acox1 in peroxisomal β -oxidation of acyl-CoAs are also all target genes of PPAR α . These enzymes carry enoyl-CoA-hydratase and 3-hydroxyacyl-CoA dehydrogenase activity (L-bifunctional enzyme, Ehhadh; D-bifunctional enzyme, Hsd17b4), and peroxisomal 3-ketoacyl-CoA thiolase activity (Acaa1a, Acaa1b) [179,191–193]. Finally, expression of numerous genes involved in import of peroxisomal proteins and thus peroxisome assembly, referred to as the Pex family, are transcriptionally regulated by PPAR α [159,194]. Induction of the above mentioned genes accounts for the massive proliferation of peroxisomes observed in rodents exposed to synthetic PPAR α agonists.

3.2.3. Mitochondrial fatty acid oxidation

Almost every single enzymatic step in mitochondrial uptake and subsequent oxidative breakdown of acyl-CoAs to acetyl-CoA is regulated by PPAR α . Specifically, PPAR α stimulates acyl-CoA import into the mitochondria by upregulating expression of carnitine palmitoyl-transferases 1a, 1b and 2 (Cpt1a, Cpt1b, Cpt2), and the acyl-carnitine translocase (Slc25a20) [195,196]. Furthermore, PPAR α upregulates expression of genes involved in cellular uptake and biosynthesis of carnitine (Aldh9a1, Bbox1, Slc22a5) [35,197,198]. Decreased expression of carnitine biosynthetic genes explains the reduced total carnitine levels in plasma and liver of PPAR α -/- mice [27].

In the first step of β -oxidation, acyl-CoA is dehydrogenated in a PPAR α -induced reaction catalyzed by a family of four closely related and chain length-specific enzymes, the acyl-CoA dehydrogenases (Acadvl, Acadl, Acadm, Acads) [179,199]. The three subsequent steps in β -oxidation leading to chain shortening and release of acetyl-CoA are catalyzed by the mitochondrial trifunctional enzyme (Hadha and Hadhb). Upon progressive chain shortening, two other enzymes (Hadh, Acaa2) take over the function of trifunctional enzyme, both of which are PPAR α targets, as are the enzymes required to convert unsaturated and 2-methylated acyl-CoAs into intermediates of β -oxidation (Eci1, Eci2, Decr1, Hsd17b10) [119,179]. Other highly suspected PPAR α target genes linked to fat oxidation include a set of poorly characterized acyl-CoA dehydrogenases (Acad8, Acad10 and Acad11), and electron transfer flavoprotein beta (Etfb) and electron transfer flavoprotein dehydrogenase (Etfhd), which shuttle electrons from acyl-CoA dehydrogenases to the electron transport chain [35,200].

Under certain metabolic conditions such as fasting, excess acetyl-CoA generated via β -oxidation feeds into ketogenesis, a pathway reliant on four enzymes (Acat1, Hmgcs2, Hmgcl, Bdh1). All are transcriptional targets of PPAR α , with Hmgcs2 as the best described representative [201].

3.2.4. Microsomal fatty acid oxidation

Cytochrome P450 monooxygenase (CYP4A) enzymes involved in microsomal ω -hydroxylation of fatty acids are among the most sensitive target genes of PPAR α [202,203]. Studies using PPAR α -/- mice have shown that hepatic expression of Cyp4a genes is almost completely abolished in the absence of PPAR α (Cyp4a10, Cyp4a12, Cyp4a14 in mice, Cyp4a11 in human) [137]. The Cyp4A family members exhibit highest activity for lauric acid, with activity decreasing with increasing fatty acid chain length, whereas the Cyp4F family is active towards eicosanoids and xenobiotics [204].

3.2.5. Lipid storage and hydrolysis

Activation of fatty acid oxidation by PPAR α is well established but the stimulatory effect of PPAR α on several other lipid metabolic pathways is less well recognized. Compelling evidence from microarrays indicates that pharmacological and/or physiological activation of PPAR α leads to upregulation of numerous genes involved in fatty acid elongation (Elovl family) and desaturation (Scd1) [35,205]. In addition, PPAR α induces expression of a number of enzymes in the triglyceride biosynthesis pathway (Gpam, Lpin2, Dgat1), albeit primarily upon pharmacological activation of the receptor [35,42]. Concurrent with induction of triglyceride synthesis, enzymes required for intracellular triglyceride hydrolysis are upregulated by PPAR α as well, including monoglyceride lipase Mgl1, the triglyceride lipase Pnpla2 and the Pnpla2 inhibitor G0s2 [35,168,206,207].

Finally, several proteins that are physically and functionally associated with lipid droplets are direct targets of PPAR α in liver. These include Plin2 [208,209], Plin4 [42,210], Plin5 [211,212], Fitm1 [213], Cidea [214], and Cidec [35,173,215]. Most of the genes encoding lipid-droplet associated proteins are specifically induced by pharmacological PPAR α activation and show no regulation by PPAR α during fasting. Detailed insight into the biochemical role of the various lipid-droplet associated proteins is currently lacking, rendering assessment of the functional impact of their regulation by PPAR α challenging.

It is well recognized that loss of PPAR α worsens hepatic steatosis, particularly during fasting and high fat feeding [21,22,216,217], whereas PPAR α activation reduces steatosis [83–91]. In general, it is difficult to tie the diverse effects of PPAR α on lipases, lipid droplet associated proteins, elongases, and enzymes involved in triglyceride synthesis together into a coherent functional model that aligns with the PPAR α -induced changes in intracellular lipid storage. Furthermore, the overarching physiological rationale for activation of these pathways by PPAR α remains ambiguous. It can be hypothesized that induction of genes involved in triglyceride synthesis such as Gpam by PPAR α during fasting may reflect a broader role of PPAR α in metabolizing and neutralizing large amounts of incoming adipose tissue-derived free fatty acids.

3.2.6. Bile synthesis and secretion

Numerous genes involved in bile synthesis and secretion are reduced in fasted PPAR α -/- mice compared with fasted wildtype mice. This includes genes involved in phospholipid synthesis (Lpcat3) [218,219], secretion of cholesterol and phospholipids into bile (Abcg5, Abcg8, Abcb4) [35,220,221], genes mediating bile acid uptake (Slc10a1 = Ntcp) and excretion (Abcc3 = Mrp3), and genes promoting bile acid synthesis (Cyp7a1, Cyp8b1, Nr1h4 = Frx) [168,220,222]. Furthermore, PPAR α regulates cholesterol uptake and export via Scarb1 and Abca1, respectively [220,223]. Most of these genes are uniquely regulated by PPAR α during fasting, with the exception of Nceh1, Abca1 and Abcb4, which are also induced upon pharmacological PPAR α activation. Despite being a direct PPAR α target gene in macrophages [224,225], Scarb1 protein and mRNA levels are significantly decreased by fibrates in liver [226].

Expression of Cyp7a1, encoding the purported rate-limiting enzyme in bile acid synthesis, is markedly downregulated in PPAR α -/- mice during fasting [222]. Paradoxically, Cyp7a1 mRNA and activity are also markedly reduced by synthetic PPAR α agonists in rodents and humans [227–232]. Consistent with an important role of PPAR α in the regulation of many genes implicated in bile acid metabolism, plasma and liver levels of bile acids are elevated in PPAR α -/- mice when challenged with dietary cholic acid [233]. A detailed review of the impact of PPAR α on bile acid homeostasis is provided elsewhere [234]. Overall, what is clear is that unlike for the peroxisomal and mitochondrial fatty

acid oxidation pathway, the direction of the impact of PPAR α on genes involved in bile acid homeostasis is highly dependent on the mode of PPAR α activation.

3.2.7. Retinoid metabolism

Conversion of retinol to retinal and retinoic acid is catalyzed by a number of enzymes, of which the corresponding genes are subject to PPAR α stimulation during fasting (Dhrs3, Dhrs4, Adh1, Adh4, Aldh1a1) [235]. 9-cis retinoic acid serves as a ligand for the PPAR α permissive binding partner RXR, suggesting that stimulation of retinoic acid synthesis by PPAR α may further increase transcriptional activation by PPAR α . Conversion of retinol to all-trans 13,14 dehydroretinol is catalyzed by retinol saturase (Retsat), a direct target gene of PPAR α [236]. Currently, the actual function of Retsat in liver is not clear.

3.3. Target genes of PPAR α in other metabolic pathways

3.3.1. Glucose metabolism

As described previously, PPAR α activation and deletion are associated with major changes in glucose metabolism. A number of genes in the hepatic gluconeogenesis pathway are known or very likely PPAR α targets, including pyruvate carboxylase (Pcx), and fructose bisphosphatase 1 [35,237,238]. Phosphoenol pyruvate carboxykinase (Pck1) appears to be specifically regulated by PPAR α in human but not mouse hepatocytes [24,159].

Adipose tissue lipolysis leads to elevated circulating levels of glycerol, which is processed in the liver. Importantly, numerous genes involved in the metabolic conversion of glycerol in liver such as Gpd1, Gpd2, Gyk, Aqp3, and Aqp9, are targets of PPAR α [24].

Besides governing glucose production, PPAR α may also alter glucose utilization in numerous tissues via induction of pyruvate dehydrogenase kinase isoform 1 and 4 (Pdk1, Pdk4) [25,239]. Finally, glycogen synthesis in liver is targeted by PPAR α via regulation of Gys2, the liver and adipose tissue-specific glycogen synthase isoform [33].

3.3.2. Secreted factors

Apart from direct regulation of genes encoding key enzymes in fatty acid oxidation and ketogenesis, it has been suggested that PPAR α may partially stimulate these pathways indirectly via upregulation of the sensitive PPAR α target and hormone FGF21 [144,166,167]. Importantly, many classical PPAR α targets, including Acot1-4, Ehhadh, Cpt1a, Cpt1b, Cpt2, Hmgcs2, and Hadha, can be induced by PPAR α activation in cultured mouse liver slices in the absence of any changes in FGF21, indicating that FGF21 is not essential for induction of genes involved in fatty acid oxidation and ketogenesis by PPAR α . How FGF21 induces these pathways remains unclear but its effects are thus clearly distinct from direct gene regulation by PPAR α and likely involve extra-hepatic tissues [240,241]. Other secreted factors that may mediate extra-hepatic actions of PPAR α during fasting include Angiopoietin-like 4 (ANGPTL4), Mannose Binding Lectin (MBL2), and Fibroblast Growth Factor (FGF1) [242–244]. Fgf1 was recently identified as target of PPAR γ in adipose tissue but is also transcriptionally regulated by PPAR α in liver [242]. ANGPTL4 is an endogenous inhibitor of lipoprotein lipase that regulates tissue uptake of plasma TG-derived fatty acids [245], whereas MBL2 is a soluble mediator of innate immunity and primary component of the lectin branch of the complement system [244,246].

3.3.3. Vanin-1

One of the most highly induced genes upon PPAR α activation in liver is Vanin-1 (Vnn1), which encodes a glycosylphosphatidylinositol-linked membrane-associated pantetheinase that catalyzes the hydrolysis of

pantetheine into pantothenic acid (vitamin B5) and cysteamine. Multiple lines of experimentation clearly demonstrate that Vnn1 is a direct PPAR α target gene [164,247]. Vanin-1 is present in plasma and determination of Vanin-1 activity and protein abundance was found to accurately reflect PPAR α activation in liver, indicating that serum Vanin-1 may be used as a reliable reporter of hepatic PPAR α activity in mice and humans [247,248].

3.4. Similarity in gene regulation by PPAR α between mouse and human

Befitting their name, peroxisome proliferators and other synthetic PPAR α agonists cause proliferation of peroxisomes and hepatomegaly in rats and mice in a PPAR α -dependent manner [49]. In contrast, neither response is observed in humans (Table 2), which together with the observed lack of effect of PPAR α agonists on peroxisomal fatty acid oxidation in humans [249], has led to suggestions that the function of PPAR α is fundamentally different between mice and humans, and, partly due to the presumed low expression of PPAR α in human liver, that the role of PPAR α in human liver is relatively insignificant [250]. Subsequent studies have dispelled those notions, showing that a) PPAR α expression is similar in mouse and human liver, b) regulation of lipid metabolic pathways and genes, including peroxisomal fatty acid oxidation, is well conserved between mice and humans [159,173]. In addition, the clinical efficacy of synthetic PPAR α agonists attests to the functional importance of PPAR α in governing lipid metabolism in human. Nevertheless, a number of metabolic genes appear to be specifically regulated by PPAR α in rodents and not humans (Figure 5). Putative rodent-specific PPAR α target genes include Mgl1, Bbox1, Decr1, and Fbp2 [159]. It has been suggested that the first PPAR α target Acyl-CoA oxidase is also a rodent-specific PPAR α target, presumably due to the absence of a functional PPRE in the human ACOX1 gene [251]. However, gene expression profiling studies indicate that ACOX1 is one of the mostly significantly induced genes in human hepatocytes treated with synthetic PPAR α agonists [159,173]. Conversely, a very small number of genes may be exclusively induced by PPAR α in human liver cells, including PCK1, MBL2, APOA2 and possibly APOA5 [244,246,252]. With respect to APOA5, the human APOA5 gene has clearly been demonstrated to be a direct PPAR α target characterized by a functional PPRE in its promoter [253]. Consistent with this finding, APOA5 mRNA is induced by PPAR α activation in human and monkey hepatocytes, and APOA5 plasma levels are increased by treatment of monkeys with a synthetic PPAR α agonist [173,253,254]. In contrast, ApoA5 upregulation is not observed in mouse liver or in cultured mouse hepatocytes, presumably due to a degenerate and non-functional PPRE in the mouse ApoA5 promoter [255]. Surprisingly, we found that ApoA5 mRNA is markedly and reproducibly induced by Wy14643 in mouse liver slices (our unpublished data), suggesting that ApoA5 may be a PPAR α target in mouse after all, perhaps via the use of an alternative PPAR response element. Full understanding of the differences in PPAR α -mediated gene regulation between mice and humans is hampered by practical limitations in the form of lack of (fibrate-treated) human livers. One of our future ambitions is to perform a whole genome comparison of the response to PPAR α agonist in mouse and human cultured precision-cut liver slices, under the assumption that cultured liver tissue slices are superior to primary human hepatocytes for studying liver metabolism and gene regulation.

4. CONCLUSION

According to the traditional view, PPAR α governs fatty acid oxidation and ketogenesis in liver. However, gene expression analysis at the

level of individual genes or the entire genome via transcriptomics has indicated that the role of PPAR α extends to numerous other metabolic pathways, providing a molecular basis for the pronounced metabolic disturbances in fasted PPAR α -/- mice and explaining the marked impact of synthetic PPAR α agonists on lipid metabolism in rodents and humans. It is now evident that PPAR α functions at the center of a regulatory hub impacting fatty acid uptake, fatty acid activation, intracellular fatty acid binding, mitochondrial and peroxisomal fatty acid oxidation, ketogenesis, triglyceride turnover, lipid droplet biology, gluconeogenesis, and bile synthesis/secretion. In addition, PPAR α governs the expression of several secreted proteins that exert local and endocrine functions. Hence, PPAR α can aptly be described as a master regulator of hepatic lipid metabolism.

CONFLICT OF INTEREST

None declared.

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