Liver PPARα is crucial for whole-body fatty acid homeostasis and is protective against NAFLD

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ABSTRACT
Objective Peroxisome proliferator-activated receptor α (PPARα) is a nuclear receptor expressed in tissues with high oxidative activity that plays a central role in metabolism. In this work, we investigated the effect of hepatocyte PPARα on non-alcoholic fatty liver disease (NAFLD).

Design We constructed a novel hepatocyte-specific PPARα knockout (Pparα hep−/−) mouse model. Using this novel model, we performed transcriptomic analysis following fenofibrate treatment. Next, we investigated which physiological challenges impact on PPARα.

Moreover, we measured the contribution of hepatocytic PPARα activity to whole-body metabolism and fibroblast growth factor 21 production during fasting. Finally, we determined the influence of hepatocyte-specific PPARα deficiency in different models of steatosis and during ageing.

Results Hepatocyte PPARα deletion impaired fatty acid catabolism, resulting in hepatic lipid accumulation during fasting and in two preclinical models of steatosis. Fasting mice showed acute PPARα-dependent hepatocyte activity during early night, with correspondingly increased circulating free fatty acids, which could be further stimulated by adipocyte lipolysis. Fasting led to mild hypoglycaemia and hyperthermia in Pparα hep−/− mice when compared with Pparα−/− mice implying a role of PPARα activity in non-hepatic tissues. In agreement with this observation, Pparα−/− mice became overweight during ageing while Pparα hep−/− remained lean. However, like Pparα−/− mice, Pparα hep−/− fed a standard diet developed hepatic steatosis in ageing.

Conclusions Altogether, these findings underscore the potential of hepatocyte PPARα as a drug target for NAFLD.

INTRODUCTION
Precise control of fatty acid metabolism is essential. Defective fatty acid homeostasis regulation may induce lipotoxic tissue damage, including hepatic steatosis.1 Peroxisome proliferator-activated receptors (PPARs) are transcription factors that serve as fatty acid receptors and help regulate gene expression in response to fatty acid-derived stimuli.2 PPARs act as ligand-activated receptors, controlling target gene transcription. The three PPAR isotypes, PPARα, PPARβ/δ and PPARγ, display specific tissue expression patterns and control different biological functions,3 but all bind lipids and control lipid homeostasis in different tissues, including the liver.2

A healthy liver does not accumulate lipids, but it plays central roles in fatty acid anabolism and export to peripheral organs, including white
adipose tissue for energy storage. During dietary restriction, hepatic fatty acid catabolism is also critical for using free fatty acids (FFAs) released from white adipose tissues. PPARα is the most abundant isotype in hepatocytes and is involved in many aspects of lipid metabolism, including fatty acid degradation, synthesis, transport, storage, lipoprotein metabolism and ketogenesis during fasting. In addition, PPARα controls glycerol use for gluconeogenesis as well as autophagy in response to fasting. Moreover, PPARα regulates the expression of the fibroblast growth factor 21 (FGF21) during starvation. In turn, FGF21 acts as an endocrine hormone targeting various functions including metabolic control. Finally, PPARα helps repress the acute-phase response and inflammation in the liver.

Obesity can lead to organ and vascular complications. Non-alcoholic fatty liver disease (NAFLD), which are considered the hepatic manifestation of metabolic syndrome, range from benign steatosis to severe non-alcoholic steatohepatitis (NASH), potentially further damaging organs. Sustained elevation of neutral lipid accumulation (mostly triglycerides in hepatocyte lipid droplets) initiates early pathological stages. Different fatty acid sources contribute to fatty liver development, including dietary lipid intake, de novo lipogenesis and adipose tissue lipolysis. In NAFLD, 60% of fatty acids accumulated in steatotic liver are adipose-derived.

Preclinical and clinical studies highlight that PPARα influences NAFLD and NASH. Mice lacking PPARα develop steatosis during fasting, suggesting the importance of PPARα activity for FFA release from adipocytes. However, PPARα is expressed and active in many tissues, including skeletal muscles, adipose tissues, intestines, kidneys and heart, which all contribute to fatty acid homeostasis. Therefore, it remains unknown whether the increased steatosis susceptibility in mice lacking PPARα depends on PPARα activity only in hepatocytes or also in other organs.

Here we investigated consequences of hepatocyte-specific Ppara deletion, focusing on effects on fatty acid metabolism in NAFLD, ranging from steatosis to steatohepatitis. We report the first evidence that adipocyte lipolysis correlates with and stimulates NAFLD when hepatocytes are lacking PPARα. Our data establish that hepatocyte-restricted Ppara deletion is sufficient to promote steatosis, emphasising this receptor’s relevance as a drug target in NAFLD.

MATERIALS AND METHODS

Animals
Generation of floxed-Ppara mice and of Ppara hepatocyte-specific knockout (Pparaflox/−) animals is described in online supplementary file 1.

In vivo experiments
In vivo studies followed the European Union guidelines for laboratory animal use and care, and were approved by an independent ethics committee.

Detailed experimental protocols are provided in online supplementary file 1.

Plasma analysis
Plasma FGF21 and insulin, respectively, were assayed using the rat/mouse FGF21 ELISA kit (EMD Millipore) and the ultrasensitive mouse insulin ELISA kit (Crystal Chem) following the manufacturer’s instructions. Aspartate transaminase, alanine transaminase (ALT), total cholesterol, LDL cholesterol and HDL cholesterol were determined using a COBAS-MIRA+ biochemical analyser (Anexplo facility).

Circulating glucose and ketone bodies
Blood glucose was measured using an Accu-Chek Go glucometer (Roche Diagnostics). β-Hydroxybutyrate content was measured using Optium β-ketone test strips with Optium Xceed sensors (Abbott Diabetes Care).

Histology
Paraformaldehyde-fixed, paraffin-embedded liver tissue was sliced into 5 μm sections and H&E stained. Visualisation was performed using a Leica DFC300 camera.

Liver lipids analysis
Detailed experimental protocols are provided in online supplementary file 1.

Gene expression studies
Total RNA was extracted with TRIzol reagent (Invitrogen). Transcriptomic profiles were obtained using Agilent Whole Mouse Genome microarrays (4×44k). Microarray data and experimental details are available in the Gene Expression Omnibus (GEO) database (accession number GSE73298 and GSE73299). For real-time quantitative PCR (qPCR), 2 μg RNA samples were reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Online supplementary file 2 presents the SYBR Green assay primers. Amplifications were performed using an ABI Prism 7300 Real-Time PCR System (Applied Biosystems). qPCR data were normalised to TATA-box-binding protein mRNA levels, and analysed with LinRegPCR.v2015.3.

Transcriptomic data analysis
Data were analysed using R (http://www.r-project.org). Microarray data were processed using Bioconductor packages (http://www.bioconductor.org, v 2.12) as described in GEO entry GSE26728. Further details are provided in online supplementary file 1.

Statistical analysis
Data were analysed using R (http://www.r-project.org). Microarray data were processed using bioconductor packages (http://www.bioconductor.org) as described in GEO entry GSE38083. Genes with a q value of <0.001 were considered differentially expressed between genotypes. Gene Ontology (GO) Biological Process enrichment was evaluated using conditional hypergeometric tests (GOstats package). For non-microarray data, differential effects were analysed by analysis of variance followed by Student’s t-tests with a pooled variance estimate. A p value <0.05 was considered significant.

RESULTS
Generation of hepatocyte-specific PPARα knockout mice
Progeny carrying the Pparaflox/− alleles (figure 1A), referred to as floxed, were backcrossed in the C57Bl/6J background, and then crossed with albumin-Cre mice in the same genetic background, generating a hepatocyte-specific PPARα knockout (Pparaflox/− albumin-Cre−/−) referred to as Pparaflox/− (figure 1B). PPARα mRNA was not detected in livers from Pparaflox/− mice when compared with floxed and C57Bl6J mice (figure 1C), suggesting that most hepatic PPARα expression is from hepatocytes. PPARα absence in hepatocytes did not alter mRNA expression of other PPAR isotypes (figure 1C).
Hepatocyte-autonomous effect of fenofibrate on PPARα activity

To determine whether PPARα response was hepatocyte-autonomous, we challenged wild-type (WT), floxed (Pparαfloxed+) and Albumin-Cre (Albumin-Crefl+/−) genes from mice that are liver wild-type (WT), (Pparαfloxed+/−) or liver knockout (Pparαfloxed−/−) for Pparα using DNA extracted from different organs. (C) Relative mRNA expression levels of Ppara, Pparβδ and Pparγ from liver samples of WT, liver WT (Pparαfloxed+/−), Pparα liver knockout (Pparαfloxed−/−) and Pparα knockout (Pparα−/−) mice (n=8 mice per group). Data represent mean±SEM. ***p≤0.005. FA, floxed allele; Flp, flippase; FRT, flippase recognition target; LoxP, locus of X-overP1; nd, not detected; PparαΔ, Pparα deletion; WT, the Albumin-Crefl−/− allele.

Hepatocyte PPARα activity is context-specific

The Pparafloxed+ model was used to determine whether PPARα could drive hepatic regulations both in fasting-induced fatty acid catabolism as well as fatty acid anabolism during refeeding. The
fasting–refeeding experimental design was validated by measuring glycaemia (figure 3A) and expression of fatty acid synthase (Fasn), which encodes the rate-limiting enzyme in lipogenesis (figure 3B). Both were low during fasting, intermediary in ad libitum–fed animals, and high in refeed animals. Cyp4a14 (a well–known PPARα target) expression was low or undetectable in Pparα hep−/− animals, and strongly upregulated with fasting in WT mice (figure 3C).

Next we evaluated the hepatic transcriptome expression pattern using microarrays. We performed hierarchical clustering (figure 3D). Most PPARα–dependent changes were observed in fasted mouse livers. Venn diagrams were used to show nutritional status–related PPARα–dependent changes (figure 3E). Among the significant DEGs, 3048 were related to fasting, 390 to ad libitum–fed animals and 156 to refeed mice, suggesting context–specific PPARα activity. The results further highlighted

that fasting, rather than feeding or refeeding, triggered the broader PPARα-dependent hepatocytic response, with most upregulated genes related to metabolism (figure 3E). However, the expression of several genes was identified as PPARα dependent regardless of the nutritional condition tested (fasting, but also feeding and refeeding). These genes are mostly downregulated in the absence of PPARα and pathway analysis highlights their involvement in mitochondrial fatty acid catabolism (see online supplementary file 3).

Biological function analyses revealed that both transcriptional activation and repression were PPARα sensitive (figure 3E). The functions of PPARα-sensitive repressions (GO categories up in Pparαhep−/− mice) varied with context, and included GO categories not directly related to metabolism, including acute-phase response.
response (fed), translation (refed) and protein glycosylation (fasted).

**Hepatocyte PPARα is required for liver and whole-body fatty acid homeostasis in fasting**

We next used Pparδ<sup>esp/–/–</sup> mice to determine the contribution of hepatocyte PPARα, and compared it with Ppara<sup>esp/–/–</sup> and WT mice. We measured FFA and β-hydroxybutyrate (ketonaemia) levels in fasted and non-fasted mice (figure 4A). Plasma FFA was elevated in fasting mice of all three genotypes, but was significantly higher in Pparδ<sup>esp/–/–</sup> and Ppara<sup>esp/–/–</sup> mice compared with controls. Fasting strongly increased ketone body levels in WT mice and to a lesser degree in Pparδ<sup>esp/–/–</sup> and Ppara<sup>esp/–/–</sup> mice. This suggests that hepatic PPARα is required for FFA disposal and for β-hydroxybutyrate production. Correspondingly, fasting Pparδ<sup>esp/–/–</sup> and Ppara<sup>esp/–/–</sup> mice showed elevated hepatic triglycerides and cholesterol esters (figure 4B), and substantial centrilobular steatosis (figure 4C), confirming that hepatic PPARα expression is required for fasting-induced FFA catabolism. PPARα absence led to defective expressions of PPARα target genes (figure 4D), including those involved in fatty acid catabolism and processing in lipid droplets (figure 4E). As a consequence of PPARα deficiency in hepatocytes, Pparδ<sup>esp/–/–</sup> mice exhibit a distinct fasting-induced fatty acid profile with a significant increase in oleic acid (C18:1n-9) and linoleic acid (C18:2n-6) when compared with WT mice (see online supplementary file 4).

**Hepatocyte-specific Ppara deletion impairs constitutive and fasting-induced FGF21 expression**

FGF21 is a hepatokine mainly produced by the liver. We examined liver Fgf21 mRNA expression (figure 5A) and plasma FGF21 levels (figure 5B) in fed and fasted animals. We identified a constitutive expression peak during the day (ZT8) in both groups, and a fasting-triggered night-time peak (ZT16). In Pparδ<sup>esp/–/–</sup> mice, we examined whether fasting-induced FGF21 expression-production was strictly dependent on PPARα hepatic activity. Ppara<sup>esp/–/–</sup> and Pparδ<sup>esp/–/–</sup> mice showed very low plasma FGF21 protein at ZT8 or at ZT16 with fasting (figure 5C).

Since FGF21 has been shown to reduce steatosis and lipotoxic lipids<sup>3 10</sup>, we questioned whether the absence of FGF21 determines fasting-induced steatosis observed in Pparδ<sup>esp/–/–</sup> and Ppara<sup>esp/–/–</sup> mice. FGF21 expression was rescued by adenoviral delivery both in Pparδ<sup>esp/–/–</sup> and in Ppara<sup>esp/–/–</sup> mice (figure 5D). Comparable expression of FGF21 (figure 5E) was obtained in liver of WT, Ppara<sup>esp/–/–</sup> and in Pparδ<sup>esp/–/–</sup> mice. FGF21-sensitive genes such as G6pd and Scd1 showed significantly different expression in response to FGF21 overexpression (figure 5E). However, FGF21 only reduced hepatic triglycerides and cholesterol esters in WT mice, but not in Pparδ<sup>esp/–/–</sup> and in Ppara<sup>esp/–/–</sup> mice (figure 5F, G). These results indicate that the fasting-induced steatosis occurring in Pparδ<sup>esp/–/–</sup> and in Ppara<sup>esp/–/–</sup> mice does not depend on the lack of FGF21. This is in line with our observations that FGF21- and PPARα-sensitive target genes are different (see online supplementary file 5A). Moreover, it is also consistent with the observation that FGF21 overexpression does not rescue the expression of PPARα target genes and conversely that PPARα-sensitive regulations occur in Fgf21<sup>–/–</sup> mice (see online supplementary file 5B, C).

In addition to their defective fatty acid catabolism, Ppara<sup>esp/–/–</sup> mice are hypoglycaemic and hypothermic during fasting. Because FGF21 is important for glucose homeostasis and for thermogenesis,<sup>13</sup> we investigated the role of hepatocyte PPARα in controlling fasting glycemia and body temperature. Both Pparδ<sup>esp/–/–</sup> and Ppara<sup>esp/–/–</sup> mice were hypoglycaemic and hypothermic compared with WT mice during fasting. However, this phenotype was much stronger in fasted Ppara<sup>esp/–/–</sup> mice compared with fasted Pparδ<sup>esp/–/–</sup> mice (figure 5H-I), indicating that extrahepatic PPARα strongly influenced whole-body glucose homeostasis and temperature independent of hepatocytic PPARα activity and FGF21 production.

**Fasting-enhanced hepatocytic PPARα activity is time-restricted and sensitive to adipocyte lipolysis**

We next tested the kinetics of other fasting-induced hepatic PPARα activity in vivo. We used several measures of PPARα activity, including Fgf21 (figure 5A) and Vanin1, Cyp4a10, Cyp4a14 and Fsp27 mRNAs (figure 6A), since these genes were most sensitive to fasting and to fenofibrate, and were strictly PPARα dependent (see online supplementary files 6–10A). Plasma FFA and glucose levels were also measured during fasting (figure 6B). FFA were markedly increased in the early night (ZT14–ZT16). The FFA pattern was correlated with the PPARα mRNA expression profile and expressions of Fgf21, Vanin1, Cyp4a10, Cyp4a14 and Fsp27 (figures 5A and 6A). This strongly suggested that FFA released from adipocytes during fasting-induced hepatic PPARα expression and activity without inflammatory response since hepatic Tnfα mRNA expression was not sensitive to fasting. We further determined that acute treatment of fasted mice with the β3-adrenergic receptor agonist CL316243 enhanced circulating FFA levels in WT and Pparδ<sup>esp/–/–</sup> mice (figure 6C), and increased expressions of Fgf21, Cyp4a14, Vanin1, Cyp4a10 and Fsp27 in WT mice but not Pparδ<sup>esp/–/–</sup> mice (figure 6D) without inducing Tnfα in response to fasting or in response to CL316243 (see online supplementary file 10C and D). These data support a role for acute adipocyte lipolysis as a signal for hepatocyte PPARα activity during fasting.

**Hepatocyte PPARα is required for protection in steatohepatitis**

We next examined whether the hepatocytic PPARα response to chronic lipolysis occurred during methionine-deficient and choline-deficient diet (MCD)-induced weight loss. In rodents, this diet rapidly promotes lipolysis in adipocytes, resulting in steatohepatitis. On the MCD diet, mice of each genotype showed weight loss (figure 7A), steatosis (figure 7B), and increased hepatic triglycerides, cholesterol esters (figure 7C) and plasma ALT (figure 7D). Compared with WT, Ppara<sup>esp/–/–</sup> and Pparδ<sup>esp/–/–</sup> mice showed greater steatosis and liver damage, suggesting a more severe MCD diet-induced phenotype without hepatocyte PPARα. MCD also induced increased expressions of Cyp4a14 and Vanin1 in WT mice, but not Pparδ<sup>esp/–/–</sup> or Ppara<sup>esp/–/–</sup> mice (figure 7E), Fgf21 mRNA (figure 7E) and circulating FGF21 (figure 7F) were increased through a mechanism that is partly dependent on hepatic PPARα. Overall, hepatocyte-specific Ppara deletion aggravated MCD diet-induced liver damage, correlating with defective PPARα-dependent pathway upregulation in response to chronic lipolysis.

Additionally, we questioned whether hepatocyte PPARα may also be required for the protection of the liver during early hits in steatosis such as those occurring in response to short-term exposure to a high-fat diet (HFD). Over 2 weeks of HFD, mouse liver accumulated hepatic triglycerides and cholesterol esters. Importantly, this steatosis was twice higher in Pparδ<sup>esp/–/–</sup> mice than in WT mice, and was further elevated in Ppara<sup>esp/–/–</sup> mice (see online supplementary file 11). Altogether, these data suggest that hepatic PPARα is essential in hepatoprotection.

**Hepatocyte PPARα deficiency leads to steatosis and hypercholesterolaemia but not excess weight gain in ageing mice**

Lastly, we questioned the long-term consequences of hepatocyte-specific *Ppara* deletion during ageing. More specifically, since PPARα is broadly expressed in metabolic tissues, we aimed at clarifying whether the steatosis that develops in aged whole-body *Ppara*−/− mice is due to the hepatic defect in PPARα activity. WT, *Ppara*<sup>fl/fl</sup>−/− and *Ppara*−/− mice were fed a standard diet over 1 year. *Ppara*−/− mice, but not *Ppara*<sup>fl/fl</sup>−/− mice, grew overweight with ageing (figure 8A–C). Both *Ppara*<sup>fl/fl</sup>−/− and *Ppara*−/− mice showed spontaneous centrilobular steatosis (figure 8D), elevated hepatic triglycerides and hepatic cholesterol esters (figure 8E), as well as hypercholesterolaemia (see figure 8F online supplementary file 12) without hyperglycaemia (figure 8G). Overall, hepatocyte-specific PPARα deficiency was sufficient to induce spontaneous steatosis and disrupt whole-body fatty acid as well as cholesterol homeostasis, but did not affect weight gain and diabetes during ageing.

**DISCUSSION**

NAFLD are a spectrum of diseases presenting a major public health concern that is strongly linked with obesity. Most accumulated hepatic fatty acids in NAFLD come from increased non-esterified FFA in the fasting state. Thus, it is essential to define the mechanisms by which the liver adapts to this influx. FFA processing largely involves the fatty acid oxidative pathway, coupled to ketogenesis allowing the liver to use lipids, which is critical during fasting and requires transcriptional regulation of rate-limiting enzymes.

Whole-body *Ppara*<sup>−/−</sup> mice show impaired coping with prolonged fasting, resulting in defective fatty acid oxidation and steatosis, hypoglycaemia and hypothermia. However, PPARα also contributes to metabolic homeostasis through expression in other tissues. Here we investigated the impact of hepatocyte-specific PPARα deletion on liver physiology and lipid metabolism in vivo. To our knowledge, this is the first report that selective PPARα deletion in hepatocytes (*Ppara*<sup>fl/fl</sup>−/−) was sufficient to promote hepatic steatosis.

PPARα is targeted by several fibrate drugs, and by pan-agonists for PPAR isoforms that are currently in clinical trials for NASH treatment. Using *Ppara*<sup>fl/fl</sup>−/− mice, we demonstrated an autonomous transcriptional response of hepatocytes to fenofibrate, indicating that fibrates’ effects on the liver gene expression are largely independent from those in extrahepatic tissues. Moreover, liver gene expression profiles markedly differed between untreated *Ppara*−/− and *Ppara*<sup>fl/fl</sup>−/− mice, suggesting that extrahepatic PPARα activity substantially influenced the hepatic transcriptome.

Food restriction induces PPARα activity, and endogenous PPARα ligand production requires hepatic lipogenesis, which
Hepatocyte and extrahepatocyte peroxisome proliferator-activated receptor \( \alpha \) (PPAR\( \alpha \)) regulate fibroblast growth factor 21 (FGF21), glycaemia and body temperature during fasting. (A and B) Eleven-week-old male mice of the C57Bl/6J background were fed ad libitum or fasted for 24 h, and were killed around the clock from ZT0 to ZT24. (A) \( Fgf21 \) mRNA was quantified by qRT-PCR. (B) Quantification of circulating FGF21 levels by ELISA. (C) Twelve-week-old wild-type (WT), PPAR\( \alpha \)-hepatocyte knockout (\( Ppar\alpha^{hep-/-} \)) and PPAR\( \alpha \) knockout (\( Ppar\alpha^{-/-} \)) male mice were fed ad libitum or fasted for 16 h and blood was collected at ZT8 (ZT8 fed) or at ZT16 (ZT16 fasted). FGF21 plasma level was determined by ELISA. (D–G) Male mice of WT, \( Ppar\alpha^{hep-/-} \) and \( Ppar\alpha^{-/-} \) genotypes were infected with an adenoviral construct containing cDNA of \( Fgf21 \) or an empty vector. Mice were sacrificed after a 24 h fasting period at ZT14. (D) Quantification of circulating FGF21 levels by ELISA. (E) \( Fgf21 \), \( G6pd \) and \( Scd1 \) mRNAs were quantified by qRT-PCR. (F) Quantification of hepatic cholesterol esters and triglycerides. (G) Representative pictures of H&E staining of liver sections. Scale bars, 100 \( \mu \)m. (H) Plasma glucose level was monitored over a 24 h fasting period from ZT0 to ZT24 in WT, \( Ppar\alpha^{hep-/-} \) and \( Ppar\alpha^{-/-} \) mice. (I, J) Plasma glucose (I) and body temperature (J) were determined at ZT0 in fed mice or at ZT0 in mice fasted for 24 h. Data are shown as mean±SEM. *p≤0.05, **p≤0.01, ***p≤0.005.

increases upon feeding. Thus, PPARα may be important during fasting-induced lipid catabolism and in the response to anabolic fatty acid-derived signals. Our data revealed the context dependency of PPARα hepatocytic activity defined by DEGs. This activity was clearly the highest during fasting.

During fasting, hepatocyte-specific PPARα deletion resulted in steatosis, increased plasma FFA and impaired ketone bodies. This supports the concept that FFA released from adipose stores during fasting may activate PPARα for hepatic use. Accordingly, we found that Ppara<sup> hep−/−</sup> mice accumulate high oleic and

Figure 6 Hepatocyte peroxisome proliferator-activated receptor α (PPARα) activity is induced by adipose tissue lipolysis. (A and B) Liver samples were collected from male wild-type (WT) C57Bl/6J mice that were fed ad libitum (black curve) or fasted (blue curve) over 24 h. (A) Hepatic mRNA expression levels of Ppara, Cyp4a14, Vnn1, Cyp4a10, Fsp27 and Tnfa were quantified by qRT-PCR. (B) Plasma glucose and free fatty acids (FFA) were measured. (C and D) WT and PPARα hepatocyte-specific knockout (Ppara<sup> hep−/−</sup>) mice were treated with the β3-adrenergic receptor agonist CL316243 at ZT6 and then killed at ZT14. (C) Quantification of plasma FFA. (D) Relative mRNA expression levels of Fgf21, Cyp4a14, Vnn1, Cyp4a10 and Fsp27 were measured by qRT-PCR. Data are shown as mean±SEM. *p≤0.05, **p≤0.01, ***p≤0.005.

linolenic acids in the liver during fasting (see online supplementary file 4), which is in agreement with the fact that both of them are the main fatty acids stored in the white adipose tissues of mice fed a chow diet. Importantly, we found a high correlation between the kinetics of circulating FFA increase and expression of PPARα and several of its target genes. Moreover, treatment with a β3-adrenergic receptor agonist further enhanced this response in vivo through PPARα but did not induce detrimental FFA-sensitive response driven by toll-like receptor 4 (TLR4). This is likely due to the mixture of FFA released from the adipose stores. Indeed, fatty acids that accumulated in the liver of Pparαhep−/− mice during fasting were mostly oleic (C18:1n–9) and linoleic acids (C18:2n–6), and not only saturated fatty acids such as palmitic acid (C16:0). Interestingly, it has been shown that palmitic acid cannot activate TLR4 in the presence of unsaturated FFA.

Overall, our data highlight hepatic PPARα activity regulation by fatty acids released from adipocytes. This contrasts with the previous evidence that PPARβ/δ rather than PPARα may act as a FFA sensor. However, our data support the possibility that this adipose-derived signal is time-restricted and specifically efficient in early night. Moreover, other pathways likely influence PPARα activity by providing ligands. Several insulin-sensitive signalling mechanisms influence hepatic PPARα, and adipocyte lipolysis is insulin sensitive. Thus, insulin may coordinate hepatic PPARα, both through cell-autonomous mechanisms and adipocyte lipolysis inducing interorgan communication mediated by FFA release. Our findings also correspond with the recent evidence that adipocyte lipolysis may regulate hepatic Fgf21. Circulating FGF21 was strictly dependent on hepatocytic PPARα activation during fasting. Most circulating FGF21 is liver-derived and Pparα−/− mice...

Figure 7  Liver peroxisome proliferator-activated receptor α (PPARα) deficiency aggravates non-alcoholic steatohepatitis in response to a methionine-deficient and choline-deficient diet (MCD). Wild-type (WT), PPARα hepatocyte knockout (Pparαhep−/−) and PPARα knockout (Pparα−/−) mice were fed a MCD or a control diet for 2 weeks and were killed at ZT8. (A) Body weight gain was measured over 2 weeks. (B) Representative pictures of H&E staining on liver sections. Scale bar, 100 μm. (C) Quantification of hepatic triglycerides and cholesterol esters. (D) Alanine transaminase activity level in plasma. (E) Hepatic mRNA expression levels of Cyp4a14, Vnn1 and Fgf21. (F) Plasma levels of fibroblast growth factor 21 (FGF21). Data are shown as mean±SEM. *p≤0.05, **p≤0.01, ***p≤0.005.
show very little FGF21. Other transcription factors can also regulate hepatic Fg21 expression and PPARα is also expressed in extrahepatic tissues. Our findings in Pparaα−/− mice showed very little FGF21 without hepatic PPARα in both fed and fasted states. Pparaα−/− mice are hypoglycaemic and hypothermic during fasting and FGF21 is known for its endocrine effect on glucose homeostasis and thermogenesis. However, compared with fasted Pparaα−/− mice, fasted Pparaα−/− mice showed reduced hypoglycaemia and hypothermia while FGF21 was equally absent in both models. This indicates that extrahepatocytic PPARα strongly influenced whole-body glucose homeostasis and temperature independently of hepatocyte PPARα and FGF21 production during fasting. In addition, while FGF21 prevents steatosis in different mouse models and reduces hepatic lipids in WT mice, its overexpression is not sufficient to protect from lipid accumulation in Pparaα−/− and in Pparaα−/− mice. Therefore, the absence of FGF21 is not the primary cause for the steatosis observed in Pparaα−/− mice.

Lack of hepatic PPARα impairs the liver’s ability to use FFA from acute lipolysis, resulting in steatosis. MCD diet-induced weight loss also correlated with hepatic PPARα activity, suggesting that chronic lipolysis elevates hepatocytic PPARα activity in non-fasted mice. In agreement with the findings in whole-body PPARα-deficient mice, our data demonstrated that the absence of hepatocytic PPARα was sufficient to increase MCD diet-induced liver damage. FGF21 expression/circulating levels increased in steatohepatitis, supporting the possibility that elevated FGF21 may reflect liver stress without fasting. This MCD diet-induced FGF21 increase was not strictly PPARα-dependent, consistent with the findings that amino acid deprivation induces hepatic FGF21 expression through ATF4. PPARα presence led to greater FGF21 increase, and may contribute to hepatoprotection from lipotoxic lipid accumulation.

MCD diet is widely used for preclinical NASH studies. However, it has many limitations, including the important weight loss that occurs in mice fed such diet. Therefore, we also tested the role of hepatocyte PPARα in lipid homeostasis in response to a short-term HFD feeding, which is sufficient to initiate early neutral lipid accumulation that may promote NAFLD. Pparaα−/− mice showed marked increase in hepatic steatosis in response to 2 weeks of HFD feeding (see online supplementary file 11) suggesting that hepatocyte PPARα plays a dual role in exogenous (dietary) as well as in endogenous (released from adipocyte lipolysis) fatty acid homeostasis.

Previous studies have shown that Pparaα−/− mice show a significant alteration of systemic lipid metabolism that leads to hepatic steatosis in ageing mice. Since PPARα is active in skeletal muscle, adipose tissues, intestines, kidneys and heart, which all contribute to fatty acid homeostasis, it is impossible to determine whether the spontaneous steatosis that occurs in ageing Pparaα−/− mice originates from a defect in the hepatocytic PPARα activity. This led us to investigate ageing-related differences between Pparaα−/− and Pparaα−/− mice.
During ageing, Ppara<sup>+/−</sup> mice became overweight and developed steatosis, while Ppara<sup>hep−/−</sup> mice only suffered steatosis. Therefore, neither obesity nor hyperglycaemia, which are both known to promote NAFLD, is responsible for the steatosis observed in mice with hepatocyte-specific PPARα deletion.

Furthermore, both Ppara<sup>+/−</sup> and Ppara<sup>hep−/−</sup> ageing mice were hypercholesterolaemic. This is likely due to the dysregulation of apolipoproteins gene expression as well as cholesterol transport (Abcg8) as revealed in microarray analysis (see online supplementary file 12A). It is also possible that the cholesterol biosynthesis pathway driven by SREBP-2 may be dysregulated in the absence of PPARα since some of the SREBP-2 genes are elevated in Ppara<sup>+/−</sup> and/or in Ppara<sup>hep−/−</sup> mice (see online supplementary file 12B). Therefore, this suggests that drugs that activate hepatic PPARα will likely influence whole-body fatty acid and cholesterol homeostasis.

 Altogether, our extensive analysis performed in Ppara<sup>hep−/−</sup> mice has allowed us to extend the evidence for the central role of PPARα in hepatocyte fatty acid homeostasis (figure 9). PPARα is strikingly essential to many aspects of fatty acid homeostasis including degradation through oxidative pathways. Our work provides the first demonstration that hepatocyte-specific PPARα deletion impairs whole-body fatty acid homeostasis during fasting, MCD and HFD feeding as well as in ageing. These findings underscore the central role of PPARα in the clearance of dietary fatty acids and of FFA released from adipocytes, the major source of lipid accumulation in NAFLD. These data highlight the relevance of PPARα as a drug target for NAFLD treatment.

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