Transcriptional profiling reveals divergent roles of PPARα and PPARβ/δ in regulation of gene expression in mouse liver

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Sanderson LM, Boekschoten MV, Desvergne B, Müller M, Kersten S. Transcriptional profiling reveals divergent roles of PPARα and PPARβ/δ in regulation of gene expression in mouse liver. Physiol Genomics 41: 42–52, 2010. First published December 15, 2009; doi:10.1152/physiolgenomics.00127.2009.—Little is known about the role of the transcription factor peroxisome proliferator-activated receptor (PPAR) β/δ in liver. Here we set out to better elucidate the function of PPARβ/δ in liver by comparing the effect of PPARα and PPARβ/δ deletion using whole genome transcriptional profiling and analysis of plasma and liver metabolites. In fed state, the number of genes altered by PPARα and PPARβ/δ deletion was similar, whereas in fasted state the effect of PPARα deletion was much more pronounced, consistent with the pattern of gene expression of PPARα and PPARβ/δ. Minor overlap was found between PPARα- and PPARβ/δ-dependent gene regulation in liver. Pathways upregulated by PPARβ/δ deletion were connected to innate immunity and inflammation. Pathways downregulated by PPARβ/δ deletion included lipoprotein metabolism and various pathways related to glucose utilization, which correlated with elevated plasma glucose and triglycerides and reduced plasma cholesterol in PPARβ/δ−/− mice. Downregulated genes that may underlie these metabolic alterations included Pklr, Fbp1, Apoa4, Vldr, Lipg, and Pcsk9, which may represent novel PPARβ/δ target genes. In contrast to PPARα−/− mice, no changes in plasma free fatty acid, plasma β-hydroxybutyrate, liver triglycerides, and liver glycogen were observed in PPARβ/δ−/− mice. Our data indicate that PPARβ/δ governs glucose utilization and lipoprotein metabolism and has an important anti-inflammatory role in liver. Overall, our analysis reveals divergent roles of PPARα and PPARβ/δ in regulation of gene expression in mouse liver.

peroxisome proliferator-activated receptors; glucose metabolism; lipids; lipoproteins

DISTURBANCES IN LIPID METABOLISM are at the basis of many chronic disorders, including obesity, diabetes, nonalcoholic fatty liver disease, and atherosclerosis. Regulation of lipid metabolism is mainly coordinated by the liver, which therefore is a key target organ for the pharmacological treatment of the above mentioned diseases. Lipid metabolism is governed via a complex interplay between hormones, transcription factors, and energy substrates, allowing for rapid adaptations to changes in metabolic requirements. An important class of ligand-activated transcription factors involved in regulation of hepatic lipid metabolism are the nuclear hormone receptors, more specifically the farnesoid X receptor (FXR), the liver X receptors (LXR), and the peroxisome proliferator-activated receptors (PPAR) (22, 23). Specific biological processes under control of FXR, LXR, and PPARs in liver include bile acid synthesis and metabolism, lipogenesis, lipoprotein metabolism, and fatty acid degradation. Additionally, FXR, LXR, and PPARs have been implicated in glucose metabolism, although the mechanisms and specific target genes involved remain somewhat ambiguous. FXR, LXR, and PPARs activate gene transcription by forming a complex with the retinoid X receptor, RXR, followed by binding of the heterodimeric complex to response elements in the DNA (8). Binding of ligand to the nuclear receptors results in the recruitment of coactivators and dissociation of co-repressors, leading to chromatin remodeling and subsequent initiation of DNA transcription.

The PPAR group of nuclear receptors can be further separated into three subtypes: PPARα, PPARβ/δ, and PPARγ (3, 8). All three PPARs are activated by fatty acids and by a variety of fatty acid-derived compounds including eicosanoids, oxidized fatty acids, and fatty acid amides (34). PPARα has been shown to be a key regulator of hepatic fatty acid metabolism, a role that is especially prominent during fasting. Indeed, lack of PPARα in fasted mice is associated with pronounced hepatic steatosis, a lack of increase in plasma ketone bodies, decreased plasma glucose, hypothermia, and elevated plasma free fatty acid (FFA) levels (15, 24, 27). These severe metabolic disturbances are the result of decreased expression of a large number of genes involved in hepatic lipid metabolism, many of which have been identified as direct PPARα target genes (11, 29, 41, 53). Despite the relatively low expression level of PPARγ in liver, evidence suggests that PPARγ is critical for development of hepatic steatosis (12, 38, 54). Surprisingly, very limited information is available on the function of PPARβ/δ in liver, even though PPARβ/δ is well expressed in liver (10, 14). PPARβ/δ expression has been shown to be highest in the endothelial cells and hepatocytes, followed by liver resident macrophages (Kupffer cells) (18). Recent studies indicate that PPARβ/δ may influence the inflammatory properties of Kupffer cells (35). Furthermore, recent data suggest that PPARβ/δ is protective against liver toxicity induced by environmental chemicals, possibly by downregulating expression of proinflammatory genes (42, 43). Other studies have linked PPARβ/δ to proliferation of stellate cells and vitamin A metabolism (16, 17). Finally, activation of PPARβ/δ was shown to impact plasma lipoprotein levels (26, 36, 49). However, the overall role of PPARβ/δ in hepatic gene regulation remains poorly defined.

Here we set out to better elucidate the role of PPARβ/δ in hepatic function. To that end, a comparative analysis was carried out between wild-type, PPARα−/−, and PPARβ/δ−/− mice in fed...
and fasted state using a combination of whole genome transcriptional profiling and analysis of plasma and liver metabolites. The results reveal that PPARα and PPARβ/δ have divergent roles in regulation of gene expression in mouse liver. Our data point to a role for PPARβ/δ in hepatic glucose utilization and lipoprotein metabolism. Additionally, they support an anti-inflammatory role of PPARβ/δ in liver.

**MATERIALS AND METHODS**

**Animals.** A breeding colony of pure-bred Sv129 PPARα/–/– mice (129S4/SvJae) and corresponding wild-type mice (129S1/SvImJ) was purchased from Jackson Laboratory (Bar Harbor, ME) and further expanded in our local animal facility. The PPARβ/δ/–/– mice were on a mixed background (Sv129/C57BL/6) and have been previously described (31). Animals were fed regular chow before the fasting intervention.

Male mice (n = 4–5 per group, 2–4 mo of age) were either fed or fasted for 24 h. At the end of the experiment, mice were anaesthetized with a mixture of isoflurane (1.5%), nitrous oxide (70%), and oxygen (30%). Blood was collected by orbital puncture, after which the mice were killed by cervical dislocation. Livers were dissected, snap frozen in liquid nitrogen, and kept at −80°C until further analysis. For RNA analyses, tissue from the same part of the liver lobe was used.

The animal studies were approved by the Local Committee for Care and Use of Laboratory Animals at Wageningen University, the Netherlands, and the University of Lausanne, Switzerland.

**RNA isolation and qRT-PCR.** Total liver RNA was isolated with TRIzol reagent (Invitrogen, Breda, the Netherlands) according to manufacturer’s instructions. RNA concentrations were measured on a NanoDrop ND-1000 spectrophotometer (Isogen Life Science, IJsselstein, the Netherlands). We reverse transcribed 1 μg of total RNA using iScript (Bio-Rad, Veenendaal, the Netherlands). cDNA was amplified on a Bio-Rad MyIQ or iCycler PCR machine using Platinum Taq DNA polymerase (Invitrogen). PCR primer sequences were taken from the PrimerBank (50) and ordered from Eurogentec (Seraing, Belgium). Primer sequences are available upon request.

To compare mRNA expression of PPARα and PPARβ/δ in mouse liver, primers were used that yielded amplicons of equal length. A standard curve was included to confirm amplification efficiency of 100% ± 2 for PPARα and PPARβ/δ and for the 36B4 control gene. PPAR expression was calculated as 1/[2^(CtPPAR-Ct36B4)], allowing for direct comparison between PPARα and PPARβ/δ.

**Affymetrix microarray.** Microarray analysis was performed on individual mouse livers. Total RNA from mouse liver was extracted with TRIzol reagent (Invitrogen) and subsequently purified and DNase treated using the SV Total RNA Isolation System (Promega, Leiden, The Netherlands). RNA quality was measured on an Agilent}

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Fig. 1. Basal gene expression of peroxisome proliferator-activated receptor (PPAR) α and PPARβ/δ and whole genome expression profiling of wild-type, PPARα/–/–, and PPARβ/δ/–/– mice in mouse liver. A: relative expression of PPARα and PPARβ/δ vs. the housekeeping gene 36b4 was measured with qRT-PCR. Error bars represent SE. *Significantly different from fed mice according to Student’s t-test (P < 0.05). B: number of genes that were upregulated in PPARα/–/– vs. PPARβ/δ/–/– mice compared with wild-type mice. C: number of genes that were downregulated in PPARα/–/– and PPARβ/δ/–/– mice compared with wild-type mice. White bars are fed mice (n = 5), black bars are fasted mice (n = 5). D: hierarchical clustering of microarray data from fed and fasted wild-type, PPARα/–/–, and PPARβ/δ/–/– mice. Dendrograms showing clustering of fed and fasted wild-type and PPARα/–/– mice (D) or fed and fasted wild-type and PPARβ/δ/–/– mice (E); n = 5 per group.
2100 Bioanalyzer (Agilent Technologies, Amsterdam, The Netherlands) using 6000 Nano Chips according to manufacturer’s instructions. RNA was judged as suitable for array hybridization only if samples showed intact bands corresponding to the 18S and 28S rRNA subunits, displayed no chromosomal peaks or RNA degradation products, and had an RNA integrity number \( > 8.0 \). Five micrograms of RNA were used for one cycle cRNA synthesis (Affymetrix, Santa Clara, CA). Hybridization, washing, and scanning of Affymetrix NuGO Mouse Arrays (wild-type and PPAR\(\alpha\)/ mice) and Affymetrix GeneChip Mouse Exon 1.0 ST Arrays (wild-type and

Fig. 2. Overlap in genes altered upon PPAR\(\alpha\) and PPAR\(\beta/\delta\) deletion and top 50 of genes most significantly downregulated in PPAR\(\beta/\delta\) mice. Venn diagrams showing overlap in genes significantly altered in PPAR\(\alpha\)/ and PPAR\(\beta/\delta\)/ mice compared with wild-type mice in the fed (A) and fasted (B) state. C: top 50 of genes most strongly downregulated in PPAR\(\beta/\delta\)/ mice vs. wild-type mice in fasted state. Of all genes with \( P < 0.01 \) for comparison wild-type fasted vs. PPAR\(\beta/\delta\)/ mice were selected. For this set of 50 genes, expression level in fed wild-type mice was set at 1 and expression levels in other groups related to this reference condition. The list was subsequently sorted based on fold-change wild-type fasted vs. wild-type fed mice.

D: top 50 of genes most strongly downregulated in PPAR\(\alpha\)/ mice vs. wild-type mice in fasted state; \( n = 5 \) per group. Of all genes with \( P < 0.01 \) for comparison wild type fasted vs. PPAR\(\alpha\)/ fasted, the 50 genes most strongly downregulated in PPAR\(\alpha\)/ mice were selected.

For this set of 50 genes, expression level in fed wild-type mice was set at 1 and expression levels in other groups related to this reference condition. The list was subsequently sorted based on fold-change wild-type fasted vs. wild-type fed mice.
PPARβ/δ−/− mice) were carried out according to standard Affymetrix protocols. The NuGO arrays represent custom designed Affymetrix GeneChip arrays, designed by the European Nutrigenomics Organisation (NuGO) and manufactured by Affymetrix. These NuGO microarray contains in part common probe sets that are also present on the standard Affymetrix arrays and in part newly designed probe sets. To allow for comparison between the PPARα−/− and PPARβ/δ−/− experiment, only genes present on both arrays (15,004 genes in total) were included in the analysis.

Scans of the Affymetrix arrays were processed using packages from the Bioconductor project (13). Arrays were normalized with quantile normalization, and expression levels of probe sets were calculated using the robust multichip average method (6, 20). Differentially expressed probe sets were identified using Limma, and genes were considered to be significantly changed when raw \( P < 0.01 \) (44).

To further explore the role of PPARα and PPARβ/δ in gene regulation during fasting, we determined to what extent genes that are up- or downregulated by fasting in wild-type mice are regulated in a PPARα- or PPARβ/δ-dependent manner, i.e., are still regulated by fasting in PPARα−/− or PPARβ/δ−/− mice. We observed that genes upregulated during fasting were about evenly divided between PPARα-dependent and PPARα-independent regulation, while most of the genes downregulated during fasting were regulated independently of PPARα (Supplemental Fig. S1A).1 Compared with PPARα, fewer genes were upregulated during fasting in a PPARβ/δ-dependent manner (Supplemental Fig. S1B). Surprisingly, a relatively large number of genes was downregulated during fasting in a

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1 The online version of this article contains supplemental material.
Fig. 4. Immunity and inflammation-related pathways are increased in PPARβ/δ−/− mice. A: selected pathways identified by Gene Set Enrichment Analysis. Only pathways are shown that had a false discovery rate q-value <0.15. The normalized enrichment score (NES) reflects the degree to which a gene set is overrepresented at the top (upregulated) or bottom (downregulated) of the ranked gene list and is corrected for gene set size. Gene sets with a positive NES are enriched in PPARβ/δ−/− mice vs. wild type, whereas pathways with a negative NES score are enriched in the wild-type mice. Data are for the fed state.

B: expression of selected inflammation-related and Kupffer cell related genes in wild-type and PPARβ/δ−/− mice. White bars, fed state; black bars, fasted state. Error bars represent SE. *Significantly different from corresponding wild-type mice according to Student t-test (P < 0.05).
PPAR\(\beta/\delta\)-dependent manner, suggesting that PPAR\(\beta/\delta\) may be especially important for downregulation of gene expression.

To examine to what extent PPAR\(\alpha\) and PPAR\(\beta/\delta\) govern the same genes, we created Venn diagrams showing the overlap in genes altered upon PPAR\(\alpha\) and PPAR\(\beta/\delta\) deletion. In the fed state, little overlap is observed between genes altered upon PPAR\(\alpha\) and PPAR\(\beta/\delta\) deletion (Fig. 2A). In the fasted state, however, a relatively large proportion of the genes altered upon PPAR\(\beta/\delta\) deletion was also altered upon PPAR\(\alpha\) deletion, suggesting common regulation (Fig. 2B). This finding is further illustrated by examining the top 50 genes most strongly decreased in PPAR\(\beta/\delta\)\(-/-\) mice compared with wild-type mice in fasted state (Fig. 2C). The changes in expression of this set of 50 genes in PPAR\(\alpha\)-\(-/-\) mice are shown in parallel. Importantly, numerous genes that were downregulated in PPAR\(\beta/\delta\)\(-/-\) mice were also reduced upon PPAR\(\alpha\) deletion, including Lipg, Lgals4, Ech1, Serinc2, Sthd1, and Tlr5, suggesting common regulation. In contrast, few members of the top 50 genes decreased in PPAR\(\alpha\)-\(-/-\) mice compared with wild-type mice in fasted state were also affected by PPAR\(\beta/\delta\) deletion (Fig. 2D). In the fasted state, several of the top 50 genes most strongly downregulated in PPAR\(\beta/\delta\)\(-/-\) mice compared with wild-type mice were also downregulated in PPAR\(\alpha\)-\(-/-\) mice, including Myom2 and Cd207 (Supplemental Fig. S2A). In analogy, in the fed state several of the top 50 of genes most strongly decreased in PPAR\(\alpha\)-\(-/-\) mice were also downregulated in PPAR\(\beta/\delta\)-\(-/-\) mice, including Myom2, Mnrla, and Lpl (Supplemental Fig. S2B). The expression of a number of individual genes representing specific profiles of regulation is illustrated in Fig. 3. Aldh3a2, representing the group of classical PPAR\(\alpha\) target genes, was upregulated during fasting in a PPAR\(\alpha\)-dependent and PPAR\(\beta/\delta\)-independent manner. In contrast, Apor4 was upregulated during fasting in a PPAR\(\alpha\)-independent and PPAR\(\beta/\delta\)-dependent manner. Interestingly, the fasting-induced expression of a number of genes, including Lgals4 and Lipg, was dependent on both PPAR\(\alpha\) and PPAR\(\beta/\delta\). Overall, the data suggest some but not major overlap in gene regulation between the two PPARs in liver in fed and fasted state.

Pathway analysis of microarray data. To investigate the functional role of PPAR\(\beta/\delta\) in mouse liver, we used the pathway analysis tool GSEA, which determines whether an a priori defined set of genes shows statistically significant concordant differences between wild-type and PPAR\(\beta/\delta\)-\(-/-\) mice. The results from fed mice show that PPAR\(\beta/\delta\) deletion is associated with enrichment of gene sets involved in various innate immunity and inflammation-related processes, including antigen processing and presentation, Toll-like receptor signaling pathway, and natural killer cell-mediated cytotoxicity (Fig. 4A), indicating that genes involved in these pathways are induced in PPAR\(\beta/\delta\)-\(-/-\) mice. The anti-inflammatory effect of PPAR\(\beta/\delta\)-\(-/-\) mice was underscored by elevated expression of selected genes that are part of the NF-\(\kappa\)B signaling pathway and by increased expression of Kupffer cell marker genes, including the marker for alternatively activated macrophages Clec7a (Fig. 4B). Consistent with these data, Ingenuity Pathway Analysis, which does not indicate the direction of a change, showed that PPAR\(\beta/\delta\)-\(-/-\) deletion was associated with alterations in numerous pathways of the innate immune system, including cytokine signaling (Fig. 5). This effect was mainly evident in PPAR\(\beta/\delta\)-\(-/-\) mice in fed state.

In agreement with the previously suggested role of PPAR\(\beta/\delta\) in oxidative metabolism (28), GSEA indicated that the electron transport chain and oxidative phosphorylation pathways were decreased in PPAR\(\beta/\delta\)-\(-/-\) mice in fed state (Fig. 4). Importantly, in both fed and fasted state PPAR\(\beta/\delta\) deletion was associated with a decrease in several pathways related to carbohydrate metabolism, including fructose and mannose metabolism, glycogen metabolism, glycglycolysis-gluconeogenesis, and the pentose phosphate pathway, suggesting a role for PPAR\(\beta/\delta\) in governing carbohydrate metabolism (Fig. 4 and data not shown). Simi-
larly, the lipoprotein metabolism pathway was downregulated in PPARβ/δ−/− mice in fed and fasted state (Fig. 4 and data not shown). These changes were corroborated by decreased expression of specific genes within the above mentioned pathways, including liver pyruvate kinase (Pklr), fructose-1,6-bisphosphatase 1 (Fbp1), apolipoprotein A4 (Apoa4), proprotein convertase subtilisin/kexin type 9 and 6 (Pcsk9, Pcsk6), and VLDL receptor (Vldlr) (Figs. 3 and 6). Interestingly, the nicotinate and nicotinamide metabolism pathway was also downregulated in PPARβ/δ−/− mice in fed and fasted state. Within this pathway the gene most significantly downregulated was Sirt5 (Fig. 6).

Metabolic similarities and differences between PPARα and PPARβ/δ. To assess whether changes in expression of genes involved in carbohydrate and lipoprotein metabolism upon PPARβ/δ deletion functionally impacted nutrient metabolism, we studied the metabolic response to fasting. To enable comparison with the role of PPARα, a parallel analysis was performed in PPARα−/− and PPARβ/δ−/− mice. As expected, fasting plasma FFAs were increased in PPARα−/− mice, while fasting plasma levels of β-hydroxybutyrate were dramatically reduced (Fig. 7A, B). No changes in plasma FFA or β-hydroxybutyrate were observed in PPARβ/δ−/− mice, which was paralleled by a lack of change in expression of ketogenic enzymes Hmgcs2 and Hmgcl (Fig. 6). In agreement with reduced expression of numerous genes involved in lipoprotein metabolism, plasma triglycerides were significantly decreased expression of specific genes within the above mentioned pathways, including liver pyruvate kinase (Pklr), fructose-1,6-bisphosphatase 1 (Fbp1), apolipoprotein A4 (Apoa4), proprotein convertase subtilisin/kexin type 9 and 6 (Pcsk9, Pcsk6), and VLDL receptor (Vldlr) (Figs. 3 and 6). Interestingly, the nicotinate and nicotinamide metabolism pathway was also downregulated in PPARβ/δ−/− mice in fed and fasted state. Within this pathway the gene most significantly downregulated was Sirt5 (Fig. 6).

DISCUSSION

In this paper we have used PPARα−/− and PPARβ/δ−/− mice in combination with gene expression profiling and analysis of plasma and liver metabolites to investigate the role of PPARβ/δ in liver. In fed liver, PPARα and PPARβ/δ expression levels are highly similar. Based on the number of genes differentially expressed between wild-type and PPARα−/− or PPARβ/δ−/− mice, it can be argued that PPARα and PPARβ/δ are about equally important in the fed state. Upon fasting, however, the number of genes affected by PPARα deletion grows dramatically, while the number of genes affected by PPARβ/δ deletion remains stable. These data suggest that PPARα is especially important during fasting, whereas PPARβ/δ is about equally important in fed and fasted state. This notion is supported by analysis of several plasma metabolites.

Previously it was shown that PPARβ/δ deletion leads to embryonic lethality due to a placental defect, which was found in three independent PPARβ/δ−/− mouse lines (2, 31, 40). Despite embryonic lethality, breeding colonies could be created from surviving mice, enabling study of the role of PPARβ/δ in adult animals. It is unclear what type of mechanism allows the surviving mice to overcome the placental defects. However, we assume that this mechanism does not

Fig. 6. Effect of PPARβ/δ deletion on specific genes involved in several pathways related to carbohydrate and lipid/lipoprotein metabolism. Expression of selected genes is shown in wild-type and PPARβ/δ−/− mice. White bars, fed state; black bars, fasted state. Error bars represent SE; n = 5 per group. *Significantly different from corresponding wild-type mice according to student t-test (P < 0.05).
confound the data collected in adult PPARβ/δ−/− mice as presented here.

So far the most extensively documented roles of PPARβ/δ are as regulator of cell proliferation, cell differentiation, and inflammation, predominantly in the gastrointestinal tract (reviewed in Ref. 39), and as a critical intermediate in skin wound healing (reviewed in Ref. 47). Research on the metabolic role of PPARβ/δ has focused on regulation of fatty acid oxidation in skeletal muscle. Numerous in vitro studies have shown a stimulatory effect of PPARβ/δ overexpression or activation on genes involved in fatty acid catabolism (7, 9, 19, 30, 48). Forced overexpression of PPARβ/δ in skeletal muscle was shown to alter muscle fiber type characteristics toward more oxidative fibers (28, 52). Consistent with these data, administration of a synthetic PPARβ/δ agonist induced fatty acid oxidation, decreased muscle lipid content, and improved exercise performance (21, 33, 48). Interestingly, a recent study using PPARβ/δ−/− mice does not support a role for PPARβ/δ in fatty acid oxidation in skeletal muscle, at least in normal physiology (4). The reason for this discrepancy is not clear but may reflect differences between the pharmacological and physiological function of PPARβ/δ in muscle. Using gain-of-function models, PPARβ/δ has also been shown to induce fatty acid oxidation in adipose tissue (51). Our data clearly suggest that the key regulator of fatty acid oxidation in liver is PPARα and not PPARβ/δ. Hepatic PPARβ/δ is also not able to compensate for the lack of PPARα in PPARα−/− mice. Instead, the primary metabolic influence of PPARβ/δ in liver is

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**Fig. 7.** Similarities and differences in plasma and liver metabolic parameters between PPARα−/− and PPARβ/δ−/− mice. Plasma and liver metabolites were analyzed in fed and fasted wild type, PPARα−/−, and PPARβ/δ−/− mice. A: plasma free fatty acids; B: plasma β-hydroxybutyrate; C: plasma triglycerides; D: plasma cholesterol; E: plasma glucose; F: liver triglycerides; G: liver glycogen. White bars, fed state; black bars, fasted state; n = 5 per group. *Significantly different from corresponding wild-type mice according to student t-test (P < 0.05).
at the level of carbohydrate and lipoprotein metabolism. We found that PPARβ/δ deletion leads to downregulation of numerous pathways of carbohydrate metabolism, including pentose-phosphate pathway, mannose and fructose metabolism, and especially glycolysis. Genes in the latter pathway that were clearly decreased in PPARβ/δ−/− mice included pyruvate kinase (Pklr) and fructose 1,6 bisphosphatase (Fbp1). Whether Pklr and Fbp1 represent direct PPARβ/δ targets requires further investigation. Decreased flux of glucose through glycolysis might account for the elevated plasma glucose levels in fasted PPARβ/δ−/− mice as well as the reported impaired glucose tolerance (25). Overall, the data are consistent with previous data showing a stimulatory effect of the synthetic PPARβ/δ agonist GW501516 on glucose consumption and the pentose phosphate pathway (25).

In addition to glucose metabolism, PPARβ/δ deletion was associated with decreased expression of a number of genes connected with lipoprotein metabolism, including Apoa4, Lipg, and very low density lipoprotein receptor (Vldlr). Confirming a previous report and in line with the plasma triglyceride-lowering effect of PPARβ/δ agonists in primates (1,36), plasma triglyceride levels were elevated in PPARβ/δ−/− mice, at least in the fed state. In contrast, plasma total cholesterol was reduced in PPARβ/δ−/− mice in the fasted state. Elevated plasma triglyceride levels in PPARβ/δ−/− mice have been suggested to be related to a combination of increased VLDL production and decreased plasma triglyceride clearance, as evidenced by a decrease in postheparin LPL activity and increased hepatic expression of LPL inhibitors Angptl3 and Angptl4 (1). Based on the data presented here, it can be speculated that elevated plasma triglycerides may also be due to decreased expression of VLDL receptor Vldlr and/or changes in production of various apolipoproteins, including Apoa5, Apoa4, and Apoc1. Interestingly, expression of proprotein convertase subtilisin/kexin type 9 (Pcsk9), encoding a proteinase involved in degradation of the LDL receptor, was decreased in PPARβ/δ−/− mice, which may contribute to the lowering of plasma cholesterol levels. Again, whether these genes represent direct PPARβ/δ target genes requires further investigation.

There is emerging evidence supporting a regulatory role for PPARβ/δ in inflammation and immunity (5). Similar to PPARα, PPARβ/δ seems to have mainly anti-inflammatory properties by downregulating expression of proinflammatory mediators and pathways (5,45). However, only limited data are available about the role of PPARβ/δ in hepatic inflammation. It was shown that the PPARβ/δ agonist GW501516 reduced histopathological changes in the MCD model of steatohepatitis and reduced expression of inflammatory cytokines and chemokines (32). Also, recent data suggest that PPARβ/δ protects against liver toxicity induced by environmental chemicals, likely by suppressing proinflammatory genes (42,43). Importantly, Odegaard et al. (35) recently showed that PPARβ/δ is required for the acquisition of the metabolic and immune phenotypes of alternatively activated macrophages in liver. In agreement with these notions, we find marked induction of numerous innate immunity and inflammation-related pathways in PPARβ/δ−/− mice, including antigen processing and presentation, Toll-like receptor signaling, and cytokine signaling. Additionally, expression of Kupffer cell marker genes was increased in PPARβ/δ−/− mice, including the marker for alternatively activated macrophages Clec7a. These latter findings thus support the data by Odegaard et al. and suggest that PPARβ/δ is a modulator of Kupffer cell function. Overall, these data indicate a general anti-inflammatory effect of PPARβ/δ in liver. Although fasting-induced lipid accumulation was not altered in PPARβ/δ−/− mice, it would be of interest to study the impact of PPARβ/δ deletion on liver triglycerides in other models of steatosis and steatohepatitis. Future studies should also address the functional impact of PPARβ/δ deletion or activation under conditions of lipopolysaccharide challenge.

The changes in hepatic gene expression in PPARβ/δ−/− mice reported here are the combined effect of absence of PPARβ/δ in numerous cell types including endothelial cells, hepatocytes and Kupffer cells, all of which express amplex levels of PPARβ/δ (18). It is reasonable to assume that the alterations in glucose and lipoprotein metabolism are likely related to absence of PPARβ/δ in hepatocytes, while changes in innate immunity and inflammation-related pathways are probably explained by the absence of PPARβ/δ in Kupffer cells.

Since PPARα mRNA was about 38% decreased in fasted PPARβ/δ−/− mice compared with fasted wild-type mice (data not shown, P < 0.05), downregulation of several genes in fasted PPARβ/δ−/− mice vs. fasted wild type might reflect indirect regulation via PPARβ/δ. However, since typical, highly sensitive PPARα targets such as Cyp4a14 and Aldh3a2 were completely unaffected by PPARβ/δ deletion during fasting, the overall impact is likely to be modest.

Finally, it should be mentioned that the PPARβ/δ−/− and corresponding wild-type mice used here were on a mixed genetic background, which may have influenced our results. Although these results generally fit with published data, our results and conclusions should be interpreted with some caution.

In conclusion, our data suggest that the roles of PPARα and PPARβ/δ in liver gene regulation only mildly overlap. While PPARα becomes more important during fasting, this does not appear to be the case for PPARβ/δ. Importantly, our study reveals that PPARβ/δ governs hepatic glucose utilization and lipoprotein metabolism, and support an anti-inflammatory role of PPARβ/δ.

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DISCLOSURES

The authors have nothing to disclose.

REFERENCES

ROLE OF PPARβ/δ IN LIVER


