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# PXR and LXR in Hepatic Steatosis: A New Dog and an Old Dog with New Tricks

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**Abstract:** PXR was isolated as a "xenobiotic receptor" that regulates drug-metabolizing enzymes and transporters, whereas LXR is known to promote hepatic lipogenesis by activating the lipogenic transcriptional factor sterol regulatory element-binding protein (SREBP). We have recently shown that PXR can mediate a SREBP-independent lipogenic pathway by activating the free fatty acid (FFA) uptake transporter CD36, PPAR$\gamma$, and several accessory lipogenic enzymes, such as stearoyl CoA desaturase-1 (SCD-1) and long-chain free fatty acid elongase (FAE). More recently, we found activation of LXR also induced the expression of CD36. Promoter analysis established CD36 as a novel transcriptional target of LXR$\alpha$. Moreover, the steatotic effect of LXR agonists was largely abolished in CD36 null mice, suggesting an essential role for CD36 and FFA uptake in LXR-mediated steatosis. We also showed that PPAR$\gamma$, a positive regulator of CD36, is also a transcriptional target of PXR. Thus, PXR can regulate CD36 directly or through its activation of PPAR$\gamma$. Interestingly, PXR- and LXR-mediated CD36 activation and PXR-mediated PPAR$\gamma$ activation are all liver-specific. We conclude that CD36 is a shared target of LXR, PXR, and PPAR$\gamma$. The network of CD36 regulation controlled by LXR, PXR, and PPAR$\gamma$ establishes this FFA transporter as a common target of orphan nuclear receptors in their mediation of hepatic steatosis. It is hoped that the nuclear receptor-mediated CD36 regulation may offer novel targets for the therapeutic management of alcoholic and nonalcoholic steatosis.

**Keywords:** Nuclear hormone receptor; gene regulation; steatosis; lipogenesis; transgenic mice

## 1. Introduction

The liver has an important role in metabolic control and energy conversion during fed−fasting transitions. The liver buffers postprandial triglyceridaemia by temporally storing fatty acids from the circulation as triglyceride (TG), oxidizing these fatty acids, and secreting them as very-low-density lipoprotein (VLDL). In the meantime, adipose tissue, the major long-term fat storage site, releases fatty acids into the blood stream, which are transported as free fatty acids to peripheral tissues. In addition to being used as an energy source for peripheral tissues, circulating fatty acids are also uptaken by hepatocytes for $\beta$-oxidation or conversion to TG.

Liver plays an essential role in lipogenesis. One of the major sources of hepatic lipids is *de novo* fatty acid synthesis and lipogenesis, in which the dedicated lipogenic transcriptional factors sterol regulatory element binding proteins (SREBPs) have an important role. SREBPs coordinately regulate a group of genes required for endogenous cholesterol, fatty acid, triglyceride, and phospholipid synthesis. This regulation is achieved through the binding of SREBP to their cognate binding sites called sterol response elements (SREs).[1] There are three SREBP isoforms, SREBP-1a, SREBP-1c, and SREBP-2, and each has distinct role in lipid synthesis. SREBP-1c preferentially activates genes involved in fatty acid production, whereas SREBP-2 is more specific for the regulation of cholesterol biosynthesis. SREBP-1a seems to be implicated in both pathways. In order to reach the nucleus

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and act as a transcription factor, the N-terminal domain of each SREBP must be released from membrane proteolysis. The N-terminal domain then enters the nucleus and binds to SREs in the promoter region of target genes to activate transcription. Transcriptional targets of SREBP-1c include fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), stearoyl CoA desaturase (SCD), and fatty acid elongase (FAE) for *de novo* fatty acid synthesis and TG formation, whereas SREBP-2 regulates HMG-CoA synthase, HMG CoA reductase, farnesyl diphosphate synthase, and squalene synthase, which are involved in cholesterol biosynthetic pathways.[1]

In addition to *de novo* fatty acid synthesis, another major source of hepatic lipids is the circulating free fatty acids (FFAs).[2] Upon uptake by hepatocytes, FFAs can be converted to triglycerides, especially when intrahepatic FFAs are in excess.[2] Consistent with the importance of FFAs in hepatic lipid accumulation, plasma concentration of FFAs is often significantly increased in various clinical disorders associated with hepatic steatosis.[2]

Nuclear receptors belong to a superfamily of ligand-dependent transcriptional factors that regulate the expression of genes involved in signaling of development and homeostasis, including the homeostasis of lipids.[3–5] The regulation is achieved by binding of nuclear receptors to DNA-responsive elements in enhancer or promoter regions of target genes. A number of nuclear receptors have been linked to steatosis, but this review article will focus on the roles of pregnane X receptor (PXR) and liver X receptor (LXR) in hepatic steatosis.

The topic of this review article is unique in that, although the xenobiotic receptor function of PXR has been extensively reviewed (see section 2.2 for details), this article will focus on the newly revealed endobiotic function of PXR in fatty acid transporter regulation and the implication of this regulation in hepatic steatosis. Similarly, although the lipogenic effect of LXR has been long recognized and extensively reviewed (see section 2.3 for details), this article will focus on a novel LXR-mediated fatty acid transporter regulation, which may have contributed to the steatotic effect of this receptor.

## 2. Cloning and Initial Characterization of PXR and LXR

**2.1. General Signaling of PXR and LXR.** Like many other nuclear receptor superfamily members, both PXR and LXR consist of a N-terminal DNA binding domain (DBD) and a C-terminal ligand-binding domain (LBD). Although the overall domain organization and crystal structure of NRs are quite conserved among family members, individual receptors differ from each other by having unique DNA recognition sequences and ligand preferences. Both PXR and LXR heterodimerize with retinoid X receptors (RXRs) to modulate transcription at the target gene promoters. PXR-RXR heterodimers bind to responsive elements that contains direct repeats (DRs) of the consensus motifs AG(G/T)TC(A/C) separated by three or four base pairs (DR-3, DR-4) or everted repeats separated by six or eight base pairs (ER-6, ER-8).[6–9] IR-0 has also been shown to mediate the PXR transactivation.[10] LXR-RXR heterodimers have been shown to bind to DR-4[11] and IR-0.[12] It is generally believed that in the absence of ligands, PXR and LXR recruit complexes of corepressors, including NCoR, SMRT, RIP140, and SHP, that silence transcriptional activity of target genes.[5] Upon ligand binding, increased transcription is achieved by conformational changes of the receptors that lead to exchanges of corepressors with coactivators, such as the p160 family of coactivators (SRC-1, SRC-2/GRIP1, and SRC3/ACTR/AIB1).[13]

**2.2. Pregnane X Receptor.** PXR was initially identified as a xenobiotic receptor that regulates the expression of the

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cytochrome P450 3A (CYP3A). Subsequent studies have firmly established PXR as a master xenobiotic receptor that plays a central role in the transcriptional control of the mammalian xenobiotic response by regulating the expression of phase I and phase II drug-metabolizing enzymes, as well as drug transporters.[6,7,14–16] Consistent with its identity as a xenobiotic receptor, PXR can be activated by a wide array of structurally diverse chemicals. Reported PXR agonists include the naturally occurring steroids 5α-pregnane-3,20-dione, progesterone, 17α-hydoxyprogesterone, 17α-hydroxypregnenolone and corticosterone, hyperforin (a constituent compound of the herbal medicine St. John's Wort), dexamethasone, the antiglucocorticoids pregnenolone-16α-carbonitrile (PCN) and RU486, Taxol, and the bisphosphonate ester SR12813.[6,17] Selectivity for these compounds also differs across species. For instance, PCN activates the rodent form of PXR, whereas rifampicin binds specifically to the human PXR. The species-specific ligand specificity has led to the creation of hPXR "humanized" mice in which the mouse PXR was genetically replaced with its human counterpart in the liver[15] and later in both the liver and intestine.[18]

Subsequent functional studies using PXR knockout and transgenic mice have revealed an important role for PXR in bile acid and bilirubin homeostasis,[19–21] in conjunction with the constitutive androstane receptor (CAR).[22] A multitude of genes in bile acid metabolism, including sulfotransferase SULT2A, UGT1A1, and the transporters MRP2 and OAPTP2, are under the transcriptional control of PXR and CAR. Mice with combined loss of PXR and CAR exhibited heightened hepatotoxicity after administration of the toxic bile acid lithocholic acid (LCA).[22] Conversely, expression of activated PXR (VP-PXR)[19] or CAR (VP-CAR)[23] in transgenic mice has been shown to be sufficient to confer resistance to LCA toxicity by activating SULT2A and UGT1A1 gene expression. Activation of PXR or CAR has been shown to promote bilirubin clearance.[21,24] Unexpectedly, PXR-null mice showed increased bilirubin clearance,[25] which was explained by an increased expression of bilirubin-detoxifying enzymes and transporters in PXR-null mice, suggesting that PXR has both negative and positive roles in bilirubin clearance. Activation of PXR in the liver also disrupts glucocorticoid and mineralocorticoid homeostasis, which is resulted from altered expression of steroidogenic enzymes in the adrenal gland.[26]

PXR also has been implicated in carbohydrate metabolism and energy homeostasis. Activation of PXR suppresses hepatic gluconeogenesis. This is achieved by attenuation of hepatocyte nuclear factor-4 (HNF-4) signaling by the ligand-activated PXR. PXR competes with HNF-4 for the binding of PPARγ coactivator 1α (PGC-1α).[27] The HNF-4/PGC-1α pathway plays a central role in regulating glucose/energy metabolism by regulating the gluconeogenic glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK) genes.[28–30] Ligand-activated PXR also acts as a corepressor of FOXO1, another positive regulator of gluconeogenic genes, decreasing the transcriptional activity of

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FOXO1 on the insulin response element (IRS),[31] further indicating that gluconeogenesis can be influenced by PXR.

**2.3. Liver X Receptor.** LXRs, both the α and *β* isoforms, were identified as sterol sensors that regulate cholesterol homeostasis.[32] LXR agonists include the endogenous cholesterol metabolites, such as 24(*S*),25-epoxycholesterol, 24(*S*)-hydroxycholesterol, 22(*R*)-hydroxycholesterol,[33] as well as synthetic agonists, such as TO0901317 (TO1317)[34] and GW3965.[35] LXRα is highly expressed in the liver, but its expression is also found in adipose tissues, intestine, macrophages, lung, and kidney. LXR*β* is expressed ubiquitously.[36] The mouse LXRα and LXR*β* are highly conserved, sharing 76% and 78% amino acid sequence homology in their DBD and LBD, respectively (Figure 1). The human and mouse LXR isoforms are also highly homologous to each other (Figure 1). LXRα and LXR*β* share many of the DNA response elements as well as endogenous and exogenous ligands. In rodents, LXRα increases cholesterol catabolism. LXRα-deficient mice exhibited markedly increase in cholesteryl ester accumulation and LDL cholesterol levels in their livers but decrease in HDL cholesterol levels when challenged with a high-cholesterol diet.[37] In contrast, LXR*β*-deficient mice do not display obvious phenotype in the response to high-cholesterol challenge,[38] implying that LXRα plays a more prominent role in hepatic cholesterol metabolism. The first target gene in cholesterol catabolism induced by LXR was identified as the cholesterol 7α-hydroxylase (Cyp7a1), a rate-limiting enzyme that catalyzes the conversion of cholesterol to bile acids.[37] LXRs were later found to increase the expression of the ATP-binding cassette (ABC) superfamily of transporters, including ABCA1, ABCG5, ABCG8 and ABCG1, resulting in cholesterol secretion into the bile and limiting dietary cholesterol absorption.[39] Treatment with LXR ligands, such as TO1317 and GW3965, lowers cholesterol levels and inhibits the development of atherosclerosis in mouse model.[33,40]

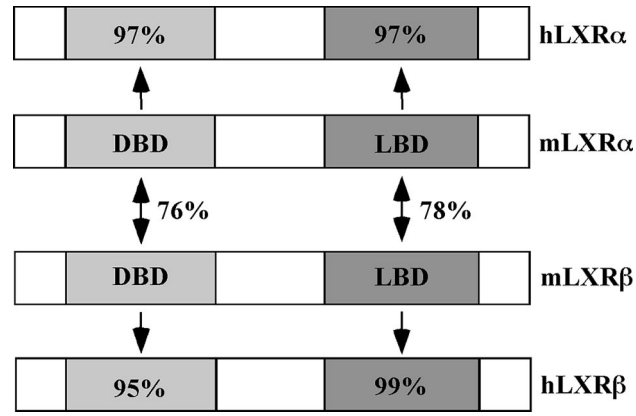


***Figure 1.*** Comparison of the homology between the mouse and human LXRα and LXR*β*. Percentages indicate amino acid identities in the DNA-binding domain (DBD) and ligand-binding domain (LBD) between the two isoforms or between the two species.

Despite their promise as antiatherosclerotic targets, LXRs have also been linked to hepatic lipogenesis.[34,41,42] Treatment of mice with LXR agonists elevates triglyceride levels in the liver as well as in the plasma.[34,41] It was then pro-

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posed that LXR activation increases plasma triglyceride levels through the transcriptional activation of SREBP-1c, a transcriptional factor known to regulate the expression of a battery of lipogenic enzymes, including SCD-1, ACC-1, and FAS.[34,41]

In macrophages, treatment with LXR ligands inhibits inflammatory signaling.[36,43] More recently, we showed that LXR can alleviate bile acid toxicity and cholestasis by activating the bile acid-detoxifying SULT2A gene expression.[12] Results from our laboratory also show that LXR promotes estrogen deprivation and inhibits estrogen-dependent xenograft breast tumor growth by activating the estrogen sulfotransferase (EST).[44]

## 3. A Novel PXR-Mediated and SREBP-Independent Hepatic Lipogenic Pathway

We have recently shown that PXR has a novel role in hepatic steatosis *in vivo*.[18] Compared to the wild type mice, the Alb-VP-hPXR transgenic that express the activated hPXR in the liver had a 79% increase in liver weight when measured as percentage of body weight, displaying marked triglyceride accumulation in the liver. In an independent pharmacological model, treatment with rifampicin (an hPXR agonist) for 5 weeks in hPXR "humanized" mice also showed significantly liver triglyceride accumulation. The lipogenic effect of PXR was independent of the activation of SREBP-1c and its primary lipogenic target enzymes FAS and ACC-1. Instead, PXR-mediated lipid accumulation likely has resulted from increased hepatic free fatty acid uptake by activating the fatty acid transporter CD36. Activation of CD36 was seen in both the VP-PXR transgenic mice and rifampicin-treated humanized mice. Several accessory lipogenic enzymes, such as SCD-1 and FAE, were also induced in PXR-activated mice.

CD36 is a multiligand scavenger receptor present on the surface of a number of cell types, including monocytes/macrophages, endothelial, smooth muscle cells, and at a lower level in the liver.[45] CD36 may contribute to hepatic steatosis by facilitating the high affinity uptake of fatty acids from the circulation.[46] Koonen and colleagues recently showed that CD36 levels in the liver are correlated with

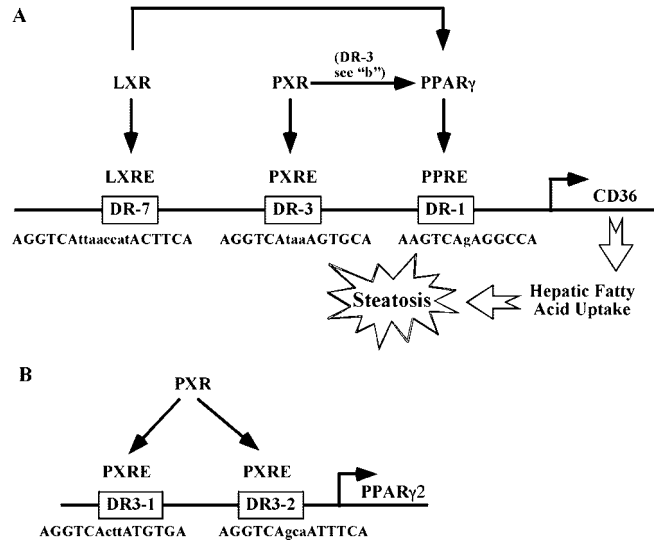


***Figure 2.*** Model of LXR-, PXR-, and PPAR$\gamma$-mediated network of CD36 regulation and steatosis. LXR, PXR, and PPAR$\gamma$ converge to regulate CD36 and subsequently promote free fatty acids (FFAs) uptake and steatosis. The regulation of CD36 by LXR and PXR (A), as well as the regulation of PPAR$\gamma$ by PXR (B), are liver-specific. Key: DR, direct repeat; LXRE, LXR response element; PPRE, PPAR response element; PXRE, PXR response element. The sequences of LXRE, PPRE, and PXRE in the mouse CD36 gene promoter (A) and two PXREs in PPAR$\gamma$2 gene promoter (B) are labeled.

hepatic triglyceride storage and secretion,[47] suggesting that CD36 may play a causative role in the pathogenesis of hepatic steatosis. PPAR$\gamma$, in response to its agonists, such as rosiglitazone, can also up-regulate CD36 gene expression.[48] Our analysis of the mouse CD36 gene promoter revealed that CD36 is a direct transcriptional target of PXR.[18] This regulation is achieved by binding of the PXR-RXR heterodimers to a DR-3 type PXRE found in the CD36 gene promoter (Figure 2A). Electrophoretic mobility shift assay (EMSA), chromatin immunoprecipitation (ChIP) and reporter gene assays showed that this DR-3 element was necessary and sufficient in mediating PXR transactivation. Interestingly, the regulation of CD36 by PXR is liver-specific. In the same study, it was found that activation of PXR also induced the expression of PPAR$\gamma$, another positive regulator of CD36,[48]

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suggesting that PXR can regulate CD36 directly or through its activation of PPAR$\gamma$. Our most recent results showed that the mouse PPAR$\gamma$2 gene promoter is a direct transcriptional target of PXR and two DR-3 type PXREs were found in the PPAR$\gamma$2 gene promoter (see section 4 and Figure 2A for details).

Activation of PXR was also associated with down-regulation of several genes involved in $\beta$-oxidation, including PPAR$\alpha$ and thiolase.[18] However, whether and how the suppression of $\beta$-oxidation contributes to hepatic steatosis remains to be determined. The notion that activation of PXR may suppress $\beta$-oxidation and influence lipogenesis was supported by recent study by Nakamura and colleagues. Nakamura et al. showed that treatment with PXR activator pregnenolone-16$\alpha$-carbonitrile (PCN) down-regulated the mRNA levels of carnitine palmitoyltransferase 1A (Cpt1a) (a key regulatory factor in insulin-dependent repression of $\beta$-oxidation[49]) and mitochondrial 3-hydroxy-3-methylglutarate-CoA synthase 2 (Hmgcs2) (an enzyme important for ketogenesis) in the livers of fasting wild type, but not PXR null, mice.[50] The SCD-1 mRNA was also found to be up-regulated in PCN-treated wild type mice in agreement with our earlier finding.[18] Consistent with the pattern of gene regulation, the levels of hepatic triglycerides and serum 3-hydroxybutylate were increased and decreased, respectively, in the PCN-treated wild type mice.[50] In the same study, it was shown that PXR could cross-talk with the insulin responsive forkhead factor FoxA2 to repress the transcription of Cpt1a and Hmgcs2 genes. PXR directly bound to FoxA2 and repressed its activation of the Cpt1a and Hmgcs2 gene promoters. It was concluded that activation of PXR represses FoxA2-mediated transcription of Ctp1a and Hmgcs2 genes in fasting liver. Several PXR responsive genes implicated in hepatic steatosis are summarized in Table 1.

***Table 1.*** Summary of PXR and LXR Responsive Genes Known To Be Implicated in Hepatic Steatosis

| | target genes | response element | ref |
|---|---|---|---|
| PXR | CD36 | DR3 | 18 |
| | PPAR$\gamma$2 | DR3 | 51 |
| | SCD-1 | unknown | 18 |
| | FAE | unknown | 18 |
| LXR | CD36 | DR7 | 51 |
| | SREBP-1c | DR4 | 34 |
| | FAS | DR4 | 52 |
| | ACC-1 | DR4 | 53 |
| | SCD-1 | DR4 | 54 |
| | PPAR$\gamma$ | DR4 | 55 |
| | ChREBP | DR4 | 56 |
| | LPL | DR4 | 57 |

## 4. Regulation of CD36, a Novel Mechanism by Which LXR Promotes Hepatic Steatosis

It was reported in 2000 that LXRs are key regulators of hepatic lipogenesis.[34,41,42] Treatment of mice with LXR agonists elevates triglyceride levels in the liver as well as in the plasma.[34,41] The lipogenic effect was also observed in transgenic mice that express the activated LXR$\alpha$ (VP-LXR$\alpha$) in the liver.[51] Initially, it was thought that the lipogenic activity of LXRs is mainly medicated by their activation of SREBP-1c, which can subsequently activate lipogenic enzyme genes, such as FAS, SCD-1, and ACC-1.[34] Accumulating evidence suggest that LXRs may also promote steatosis by activating lipogenic genes in a SREBP-independent manner. For example, FAS,[52] ACC-1,[53] and SCD-1[54] can be directly regulated by LXR, instead of having the activation mediated by SREBP. LXRs positively regulate several transcription factors involved in lipogenesis, including PPAR$\gamma$[55] and carbohydrate response element-binding protein (ChREBP),[56] a glucose-sensitive transcription factor and a regulator of conversion to lipids from excess carbohydrates. Activation of LXR has also been reported to induce enzymes in lipoprotein remodeling, such as lipoprotein lipase (LPL),[57] a rate-limiting enzyme for catabolism of triglyceride from VLDL and chylomicrons, which produce much of lipids and apo-lipoproteins.

Recently, we have identified CD36 as a novel LXR target gene, suggesting another novel mechanism by which LXR

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may promote steatosis.[51] Genetic (by using the VP-LXRα transgene) or pharmacological (by using the LXR agonists) activation of LXR induces CD36. Combinations of EMSA, ChIP, and reporter gene assays showed that the CD36 gene promoter contains a DR-7 type LXRE that confers receptor-dependent binding and transactivation (Figure 2A). Interestingly, like its regulation by PXR, CD36 regulation by LXR is also liver-specific. Further studies showed that LXR-promoted steatosis and activation of CD36 was not a simple association, since the effects of LXR agonists on increasing hepatic and circulating levels of triglyceride and free fatty acid were largely abolished in the CD36 null mice,[51,58] suggesting that intact expression and/or activation of CD36 is required for the steatotic effect of LXR agonists. Activation of LXR in the liver was also associated with an increased expression of PPARγ.[51] Several LXR responsive genes implicated in hepatic steatosis are summarized in Table 1.

## 5. Summary and Perspective of Nuclear Receptor-Mediated CD36 Regulation in the Management of Hepatic Steatosis

Recent progresses in PXR and LXR studies have revealed a network of CD36 regulation and the implication of this regulation in hepatic steatosis. The nuclear receptor-mediated CD36 regulation and the crosstalk between receptors are summarized in Figure 2. PXR, LXR, and PPARγ response elements have been identified in the promoter region of the mouse CD36 gene (Figure 2), clustering within a 500-bp in the CD36 gene promoter.[51] According to our current model, PXR can regulate CD36 directly or via its transcriptional activation of PPARγ. LXR can also regulate CD36 directly and possibly by activating PPARγ.

There is potential crosstalk between LXR and PPARγ. Activation of PPARγ has been shown to activate LXR in the macrophages,[59] but it is unclear whether the same regulation occurs in the liver. Seo et al. reported that treatment with LXR agonists induced the expression of PPARγ in the liver and adipose tissues.[55] However, a study by Hummasti et al. showed a lack of LXR effect on the expression of differentiation-linked PPARγ target genes in fat tissues.[60] Our own results suggest that the LXR effect on PPARγ expression is likely to be liver-specific.[51] However, we cannot exclude the possibility that the up-regulation of PPARγ in LXR-activated mice was a secondary effect of increased lipid accumulation.

Our current model also predicts that PXR and LXR may cooperate to promote hepatic steatosis by increasing the expression of CD36. This prediction has been supported by the additive steatotic phenotype in double transgenic mice that express both the activated PXR and activated LXRα in the liver.[51] It is important to note that this network of CD36 regulation is largely liver-specific, further underscoring the unique and previously unnoticed role of CD36 in hepatic steatosis.

Alcoholic and nonalcoholic steatosis is a serious medical problem. The revelation of the role of PXR- and LXR-mediated CD36 regulation in steatosis opens debate on whether these regulatory pathways can be explored as therapeutic targets for steatosis. Nuclear receptors are attractive targets for drug discovery, since their activities can be regulated by small lipophilic molecules. Moreover, in many cases, the nuclear receptor agonistic and antagonistic property of small molecules can be chemically defined and modified. If activation of CD36 is a causative factor in human steatosis, it is tempting to speculate that inhibition of PXR or LXR, or a direct inhibition of the CD36 fatty acid transporter activity may represent novel strategies to prevent or relieve hepatic steatosis.

## Abbreviations Used

ACC-1, acetyl CoA carboxylase 1; ChIP, chromatin immunoprecipitation; EMSA, electrophoretic mobility shift assay; FAS, fatty acid synthase; FAE, long chain free fatty acid elongase; FFA, free fatty acid; LXR, liver X receptor; PCN, pregnenolone-16α-carbonitrile; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; SCD-1, stearoyl CoA desaturase-1; SREBP, sterol regulatory element-binding protein.

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