Nuclear receptors sense bile acid metabolism: A hormonal action of bile acids

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Abstract
Bile acids, the major catabolic products of cholesterol, are essential components in bile and act as a detergent for the intestinal ingestion of lipidsoluble nutrients. In addition to these classical roles, bile acids have more recently been identified as signaling molecules that activate nuclear receptors, ligand-inducible transcription factors. Nuclear receptors, such as liver X receptor α (LXRα), farnesoid X receptor (FXR), pregnane X receptor (PXR), constitutive androstane receptor (CAR) and vitamin D receptor (VDR), function in the regulation
of bile acid metabolism. LXRα, a nuclear receptor for oxysterol, induces the catabolism of cholesterol to bile acids in the rodent liver. FXR, a bile acid receptor, represses bile acid synthesis and import in hepatocytes, stimulate bile acid export from cells, and protects hepatocytes from bile acid toxicity. PXR, VDR, and CAR sense toxic bile acids and induce their elimination through a xenobiotic metabolism pathway. Thus, bile acids, like steroid hormones, function as ligands for nuclear receptors and regulate the transcription of genes involved in bile acid metabolism and other physiological systems.

Introduction

Hormones are molecules that carry a signal from an endocrine cell to target cells through the bloodstream. Steroid hormones (androgens, estrogens, glucocorticoids, mineralocorticoids, and progestins) and thyroid hormones exert many physiological actions by binding to their intracellular receptors, members of the nuclear receptor superfamily [1]. Vitamin D₃ and retinoids are hormone-like messengers that are not secreted by endocrine cells but exhibit biological effects in target cells that express their specific nuclear receptors. Molecular cloning of the nuclear receptors started in the 1980s with steroid hormone receptors such as the glucocorticoid and estrogen receptors. The observation that the subsequently cloned receptors for thyroid hormone and retinoic acid share significant homology with the steroid hormone receptors suggested the existence of additional nuclear receptors [2]. Utilizing this sequence homology, molecular biology techniques led to the identification of additional nuclear receptors with unknown biochemical characteristics and hormone binding specificity. With the reporting of the full drafts of the human, mouse and rat genomes, as many as 48 nuclear receptors have been identified, and one third of them remain orphan receptors without known ligands [3]. The success of ligand hunting efforts allowed several previously orphaned receptors to be "adopted" and led to the discovery of a novel function of nuclear receptors in lipid physiology [4].

Nuclear receptors are transcription factors that regulate many physiological process including cell growth and differentiation, embryonic development and metabolic homeostasis [2]. Nuclear receptor transcriptional activity is modulated by ligands such as steroids, retinoids, and other lipid-soluble compounds. Upon ligand binding, nuclear receptors undergo a conformational change in the cofactor binding site and activation function-2 helix that results in the dissociation of corepressors and recruitment of coactivators [5]. These cofactors form larger-ordered complexes with associated proteins that induce chromatin remodeling and recruitment of the basal transcription machinery, allowing nuclear receptors to modulate the
transcription of specific target genes. The "adopted" nuclear receptors liver X receptor α (LXRα, NR1H3), LXRβ (NR1H2), farnesoid X receptor (FXR, NR1H4), pregnane X receptor (PXR, NR1I2), constitutive androstane receptor (CAR, NR1I3), and the vitamin D receptor (VDR, NR1I1) have been shown to regulate cholesterol and bile acid metabolism by sensing the metabolic environment and regulating the expression of target genes [3].

**Table 1.** Nuclear receptors involved in regulation of bile acid metabolism.

<table>
<thead>
<tr>
<th>Nuclear receptors</th>
<th>Ligands</th>
<th>Regulated proteins</th>
<th>Functions</th>
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<tbody>
<tr>
<td>Liver X receptor α (LXRα, NR1H3)</td>
<td>Oxysterols</td>
<td>‡ Cyp7a1</td>
<td>Increased bile acid synthesis in hepatocytes</td>
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<td>‡ ABCG5/ABCG8</td>
<td>Increased bicanalicular cholesterol efflux from hepatocytes</td>
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<td>Farnesoid X receptor (FXR, NR1H4)</td>
<td>Bile acids</td>
<td>‡ CYP7A1</td>
<td>Decreased bile acid synthesis in hepatocytes</td>
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<td>‡ CYP8B1</td>
<td>Decreased bile acid synthesis in hepatocytes</td>
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<td>‡ BSEP</td>
<td>Increased bicanalicular bile acid efflux from hepatocytes</td>
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<td>‡ MRP2 ?</td>
<td>Increased bicanalicular bile acid efflux from hepatocytes</td>
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<td>‡ NTCP</td>
<td>Decreased bile acid uptake into hepatocytes</td>
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<td>‡ OATP8 ?</td>
<td>Increased bile acid uptake into hepatocytes</td>
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<td>‡ I-BABP</td>
<td>Increased intracellular bile acid transport in enterocytes ?</td>
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<td></td>
<td>‡ Oat1/Oatβ</td>
<td>Increased basolateral bile acid efflux from enterocytes</td>
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<td>‡ MDR3/Mdr2</td>
<td>Increased bicanalicular phospholipid efflux from hepatocytes</td>
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<tr>
<td>Pregnane X receptor (PXR, NR1I2)</td>
<td>Xenobiotics</td>
<td>‡ CYP3A4/Cyp3a11</td>
<td>Increased phase I metabolism of secondary bile acids in hepatocytes</td>
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<td>Secondary bile acids</td>
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<td>‡ MRP3</td>
<td>Increased basolateral bile acid efflux from hepatocytes</td>
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<td>Constitutive androstane receptor (CAR, NR1I3)</td>
<td>Xenobiotics</td>
<td>‡ CYP3A4/Cyp3a11</td>
<td>Increased phase I metabolism of secondary bile acids in hepatocytes</td>
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<td>Secondary bile acids ?</td>
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<td>Increased basolateral bile acid efflux from hepatocytes</td>
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<td>Vitamin D receptor (VDR, NR1I1)</td>
<td>1α,25-Dihydroxyvitamin D3</td>
<td>‡ CYP3A4/Cyp3a11</td>
<td>Increased phase I metabolism of secondary bile acids in hepatocytes</td>
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<td>Lithocholic acid</td>
<td>‡ SULT2</td>
<td>Increased phase II metabolism of secondary bile acids in hepatocytes</td>
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**Bile acid metabolism**

Bile acids are the end products of hepatic cholesterol catabolism, and are essential detergents that are required for the ingestion and intestinal absorption of hydrophobic nutrients, including cholesterol, fatty acids and lipid-soluble vitamins [6]. The primary bile acids cholic acid and chenodeoxycholic acid are derived from cholesterol via several intermediates in the liver and are secreted in bile as glycine or taurine conjugates [7]. Most bile acids are reabsorbed in the intestine and recirculate to the liver through the portal vein in a mechanism called the enterohepatic circulation. Bile acids that escape reabsorption are converted to the secondary bile acids deoxycholic acid and lithocholic acid by intestinal microflora [8]. A portion of the secondary bile acids enter the enterohepatic circulation from the colon. Bile acids produce toxic effects at high concentration, and secondary bile acids are thought to participate in the pathogenesis of liver disease and colon cancer [9].

The synthesis of bile acids from cholesterol is mediated by at least 17 different enzymes [7]. The first step is initiated by 7α-hydroxylation of sterol precursors, and is mediated by two metabolic pathways. In the classic pathway, cholesterol is converted to 7α-hydroxycholesterol by cholesterol 7α-hydroxylase, a microsomal cytochrome P450 enzyme (CYP7A1). Under normal conditions, this pathway is responsible for 50% or more of the production of bile acids in humans. The bile acid pool size in mice deficient in the Cyp7a1 gene is reduced by 75%, a reduction that is not compensated by other bile acid biosynthetic enzymes [10]. Patients with a mutation in the CYP7A1 gene have elevated plasma cholesterol levels and decreased bile acid excretion, and accumulate cholesterol in the liver [11]. In the second pathway, called the alternate or acidic pathway, cholesterol is converted into one of several oxysterols prior to being 7α-hydroxylated by oxysterol 7α-hydroxylase (CY7B1) [7]. The oxysterols 24-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol can serve as substrates for bile acid synthesis. 27-Hydroxycholesterol, which is the most abundant oxysterol in plasma, is synthesized from cholesterol by sterol 27-hydroxylase, a mitochondrial cytochrome P450 (CYP27A1). This enzyme can also hydroxylate cholesterol to 24-hydroxycholesterol and 25-hydroxycholesterol. About 25% of the bile acid pool is derived from oxysterols produced by CYP27A1. Although a microsomal cytochrome P450 (CYP46A1) and a non-P450 microsomal enzyme (cholesterol 25-hydroxylase) participate in the conversion of cholesterol to 24-hydroxycholesterol and 25-hydroxycholesterol, respectively, the significance of these reactions is unclear because genetic disruption of these enzymes in mice did not alter bile acid synthesis. The preferred substrates for 7α-hydroxylation of CYP7B1 are 25-hydroxycholesterol and
27-hydroxycholesterol. Mice deficient in the Cyp7b1 gene have elevated plasma levels of 25-hydroxycholesterol and 27-hydroxycholesterol but not 24-hydroxycholesterol [12]. An additional microsomal cytochrome P450 (CYP39A1) has oxysterol 7α-hydroxylase activity on 24-hydroxycholesterol. The CYP7B1 oxysterol 7α-hydroxylase pathway is likely to synthesize 25% to 30% of total bile acids in mice, and 5% to 10% of the bile acid pool in humans originates from oxysterols, while the contribution of CYP39A1 to the alternate pathway remains unknown [7].

The initial step of 7α-hydroxylation of sterol precursors is followed by the modification of ring structures [7]. 7α-Hydroxylated intermediates derived from cholesterol and the oxysterols are next converted into their 3-oxo, Δ4 forms by a microsomal 3β-hydroxy-Δ5-C27-steroid oxidoreductase. The resulting products take one of two routes in subsequent steps of bile acid synthesis. The reaction by sterol 12α-hydroxylase, a microsomal cytochrome P450 (CYP8B1), initiates one cascade that results in cholic acid. In the absence of CYP8B1, the intermediates are ultimately converted into chenodeoxycholic acid (in rat, human, and hamster), muricholic acid (in mouse), ursodeoxycholic acid (in bear), or hyodeoxycholic acid (in pig). Loss of the Cyp8b1 gene in mice eliminates cholic acid from the bile acid pool and leads to an increase in the synthesis of muricholates [13]. The products of ring modification next undergo oxidation and shortening of the side chain. The first few steps in this pathway are performed by CYP27A1, the same enzyme that initiates bile acid synthesis in the alternate pathway. The side chain oxidation steps catalyzed by CYP27A1 are involved in the synthesis of all bile acids, regardless of their source. As a consequence, disruption of the Cyp27a1 gene in mice has severe damages on bile acid synthesis. Loss of CYP27A1 gene expression in humans results in the neuropathological disorder cerebrotendinous xanthomatosis, which is characterized by the synthesis of abnormal bile alcohols, a reduced synthesis of normal bile acids, and the accumulation of cholesterol and the 5α-reduced derivative of cholesterol, cholestanol, in the blood, brain and other tissues [7]. After oxidation by CYP27A1, bile acid intermediates are next subject to side chain shortening and finally to conjugation with an amino acid, usually glycine or taurine. Conjugation increases the amphipathicity of bile acids and enhances their solubility, which makes them impermeable to cell membranes.

The enterohepatic circulation of bile acids begins in the hepatocyte canaliculus. The canalicular membrane-associated transport systems are required because conjugated and free bile acids cannot cross the cell membrane [14]. Bile salt export pump (BSEP, ABCB11), an ATP-binding cassette (ABC) transporter, is localized in the canalicular membrane of hepatocytes and transports monovalent bile salts, such as taurocholate, glycocholate, taurochenodeoxycholate, and
tauroursodeoxycholate. Mutations in the human \textit{BSEP} gene account for a subtype of progressive familial intrahepatic cholestasis (PFIC-2), characterized by a very low level of bile acid secretion and severe cholestasis. In contrast to humans with a \textit{BSEP} mutation, \textit{Bsep}-deficient mice do not express severe cholestasis and have substantial bile acid secretion [15]. This may be due to the formation of muricholic acid in mice and the induction of an alternative transport system, such as multidrug resistance 1 [16]. In addition to the excretion of monovalent bile salts via BSEP, the canalicular multidrug resistance-associated protein 2 (MRP2, ABCC2) accounts for the transport of divalent bile salts, such as sulfated taur- or glycolithocholate, and other amphipathic conjugates including bilirubin diglucuronide [14]. Mutations of the \textit{MRP2} gene result in Dubin-Johnson syndrome characterized by impaired canalicular excretion of a broad range of range of endogenous and exogenous amphipathic compounds.

As major components in bile, bile acids solubilize dietary lipids and promote their absorption in small intestine. Most conjugated bile acids are reabsorbed in the terminal ileum via a Na\textsuperscript{+}-dependent mechanism mediated by the apical sodium-dependent bile salt transporter (ASBT, SLC10A2) [14]. The ASBT splice variant t-ASBT and MRP3 (ABCC3) are localized basolaterally and may be involved in the efflux of bile salts to the portal blood. Targeted deletion of the \textit{Asbt} gene in mice resulted in a decreased bile acid pool size and increased fecal bile acid excretion, indicating that \textit{Asbt} is essential for the enterohepatic circulation of bile acids [17]. Conversely, \textit{Mrp3\textsuperscript{-/-}} mice have normal bile acid excretion, pool size and fractional turnover under physiological condition [18]. Recently, the mouse organic solute transporter \(\alpha\) (Ost\(\alpha\)) and Ost\(\beta\) were reported to localize on the basolateral membrane of enterocytes and to support apical-to-basolateral transport of taurocholate, as well as other major taurine- and glycine-conjugated bile acids [19]. The final step in the enterohepatic circulation is the uptake of bile acids by hepatocytes from the portal blood [14]. More than 80\% of conjugated bile acids are extracted by hepatocytes predominantly via the Na\textsuperscript{+}–taurocholate cotransporting polypeptide (NTCP, SLC10A1). In addition to Na\textsuperscript{+}-dependent uptake by NTCP, Na\textsuperscript{+}-independent hepatic uptake of bile salts is mediated by the organic anion transporting polypeptides (OATPs). In humans, three liver-specific OATPs, OATP-A (SLC21A3), OATP-C (SLC21A6) and OATP8 (SLC21A8), are involved in basolateral bile salt uptake, while three Oatsps, Oatp1 (Sce21a1), Oatp2 (Sce21a5) and Oatp4 (Sle21a10), serve in bile salt uptake in the rat liver.

Bile acid metabolism is regulated at several levels, including gene transcription, RNA translation and protein stability. Bile acids have been identified as regulatory signals for transcription of genes involved in their synthesis (e.g., CYP7A1, CYP8B1) and transport (e.g., BSEP, NTCP). Nuclear
receptors play an important role in the regulatory network by acting as receptors for the bile acid metabolic environment.

**LXRα**

LXRα, originally identified as an orphan receptor, is chiefly expressed in liver, adipose tissue, intestine, kidney, and macrophages. By examining lipid extracts from a variety of tissues, oxysterols were found to be natural ligands for LXRα, a key discovery in lipid metabolism research [20]. LXRα is activated by intermediate oxysterols in bile acid synthesis, such as 7α-hydroxycholesterol, 24-hydroxycholesterol, 25-hydroxycholesterol and 27-hydroxycholesterol, while the most potent oxysterol for LXRα is 24(S), 25-epoxycholesterol, which is derived from squalene in a shunt pathway of cholesterol biosynthesis [21]. LXRα stimulates the transcription of mouse *Cyp7a1*, the rate-limiting enzyme in the classic bile acid synthetic pathway, by binding to a specific promoter element that consists of two hexanucleotide direct repeat motifs separated by 4 nucleotides (DR4). The ABC transporters ABCG5 and ABCG8 mediate biliary excretion of cholesterol and these proteins are also induced by LXRα activation [22]. Toxic levels of cholesterol accumulate in the livers of *Lxrα*-null mice fed a high cholesterol diet due to a loss of induction of *Cyp7a1*, *Abcg5*, and *Abcg8* expression. A high cholesterol diet increased fecal bile acid expression and bile acid pool size in wild-type mice, but these changes were abolished in *Lxrα*-null mice [21]. Thus, LXRα plays an important role in the feed-forward induction of bile acid synthesis in rodents. Due to sequence variation in the DR4 element, the expression of the human *CYP7A1* gene is not regulated by LXRα.

**FXR**

LXRα heterodimerizes with isoforms of the retinoid X receptor (RXR), RXRα (NR2B1), RXRβ (NR2B2) and RXRγ (NR2B3), the receptors for 9-*cis*-retinoic acid, and heterodimerization allows LXRα to bind to DR4 elements in target gene promoters [3]. The LXRα-RXR heterodimer functions as a permissive heterodimer, in that they can be activated by ligands for either LXRα or RXR [4]. Contrary to the prediction that treatment of mice with an RXR agonist should increase expression of *Cyp7a1*, its expression was dramatically decreased in both wild-type and *Lxrα*-null mice [23]. This unexpected finding led to the hypothesis that RXR heterodimeric partner might respond to bile acids, since bile acids have been known to induce feedback regulation in their synthesis by inhibiting *Cyp7a1* transcription [24]. Indeed, FXR was found to be a bile acid receptor [25]. FXR was originally identified as an orphan receptor that is weakly activated by farnesol, an intermediate in
cholesterol synthesis, and juvenile hormone III, an insect hormone [26]. FXR is expressed in liver, intestine, kidney, and the adrenal gland, heterodimerizes with RXR, recognizes an inverted repeat motif with one spacer nucleotide (IR1), and responds to both primary and secondary bile acids in their free and conjugated forms [25, 27, 28]. Among the major bile acids, chenodeoxycholic acid is the most potent FXR agonist.

FXR regulates bile acid synthesis through an indirect mechanism. The nuclear receptor liver receptor homologue-1 (LRH-1, NR5A2) is required for transcription of the bile acid synthetic enzymes CYP7A1 and CYP8B1 [29, 30]. FXR repressed the expression of these genes by binding to an IR1 element in the SHP promoter and inducing the transcription of small heterodimer partner (SHP, NR0B2), an unusual nuclear receptor that lacks a DNA binding domain [31, 32]. SHP suppresses bile acid synthesis by binding to and inhibiting LRH-1. The hepatic expression of Cyp7a1 and Cyp8b1 requires another nuclear receptor, hepatocyte nuclear factor 4α (HNF4α, NR2A1), and mice deficient in hepatic HNF4α expression have marked decreases in the expression of these enzymes [33]. HNF4α binds to the rat Cyp7a1 and human CYP8B1 promoters and induces their transcription [34, 35]. As in the case of LRH-1, SHP inhibits HNF4α transactivation through a direct interaction [36]. Mice deficient in Fxr demonstrate increased bile acid synthesis, express high levels of Cyp7a1 and Cyp8b1 mRNA, and lack bile acid-induced repression of these enzymes [37]. Disruption of Shp in mice causes abnormal accumulation and increased synthesis of bile acids due to derepression of Cyp7a1 and Cyp8b1 expression [38]. These findings indicate that the FXR-SHP cascade mediates negative feedback regulation of bile acid synthesis. However, significant Cyp7a1 and Cyp8b1 repression is retained in Shp-null mice fed bile acids, indicating the existence of additional, SHP-independent pathways [39]. FXR was found to directly induce the expression of fibroblast growth factor-19 (FGF-19), a secreted growth factor that signals through FGF receptor 4 (FGFR4), a cell-surface receptor tyrosine kinase found in human hepatocytes [40]. FGF-19 suppresses expression of CYP7A1 through a c-Jun N-terminal kinase-dependent pathway. FGF-15, which is the mouse ortholog of FGF-19, is induced by FXR in the small intestine and not the liver, and represses hepatic Cyp7a1 expression through a FGFR4-mediated mechanism [41]. In wild type, but not Shp-null mice, adenoviral FGF-15 overexpression decreased Cyp7a1 expression without changing Shp mRNA levels. This finding suggests the involvement of SHP in FGF-15-mediated Cyp7a1 repression, although the existence of additional SHP-independent mechanisms cannot be ruled out. Promoter analysis of the human CYP27A1 gene demonstrated that HNF4α can transactivate the promoter, an effect that is
inhibited by liganded FXR or SHP [42]. Further studies of mice lacking hepatic expression of HNF4α indicate that the Cyp27a gene is directly regulated by HNF4α in vivo [33]. However, data from Fxr−/− and Shp−/− mice do not indicate that Cyp27a is regulated by the FXR-SHP cascade [37, 38, 43].

The hepatic bile acid transport system is also regulated by FXR. Fxr-null mice exhibit reduced fecal bile acid excretion due to decreased expression of the major hepatic canaliculare bile acid transporter Bsep [37]. The human BSEP gene has an IR1 element in its promoter region that binds directly to the FXR-RXR heterodimer and is activated by chenodeoxycholic acid [44]. While mutations in the BSEP gene account for the syndrome of PFIC-2, another inherited cholestasis syndrome, PFIC-1, is caused by defects in ATP8B1, which encodes familial intrahepatic cholestasis 1 (FIC1) [45]. Both diseases are characterized by low serum γ-glutamyl transpeptidase and cholesterol and by decreased bile salt concentrations. FIC1 is a P-type ATPase with putative activity as an aminophospholipid translocase. PFIC-1 was reported to be associated with decreased expression of hepatic FXR [46]. Down-regulation of FXR results in decreased expression of the FXR targets BSEP and SHP, which probably causes the severe cholestasis seen in PFIC-1, although the mechanism of FIC1-dependent FXR expression remains unknown [47]. The human MRP2 gene has also been reported to have an FXR-binding element in its promoter and to be activated by bile acids [48], but no change was observed in Mrp2 expression in Fxr-null mice [43]. The basolateral bile salt uptake transporter Ntcp gene is negatively regulated by FXR through a SHP-mediated mechanism [37, 38]. Promoter analysis of the rat Ntcp gene revealed induction by the retinoic acid receptor α (RARα, NR1B1)-RXRα heterodimer, an effect which was inhibited by SHP [49]. SHP directly interacts with RARα and RXRα, and inhibits their transactivation activity [31, 36]. Mice deficient in hepatic HNF4α demonstrate the involvement of HNF4α in Ntcp expression [33]. Experiments in Fxr-deficient mice demonstrated that bile acid-dependent repression of Ntcp is mediated by FXR [37]. Although basal expression of Ntcp was increased in Shp-null mice, cholic acid feeding repressed Ntcp expression in Shp-null mice as well as in wild-type mice, suggesting that FXR activation represses Ntcp expression via SHP-independent mechanisms [39]. Recently, human NTCP transcription was reported to be induced by the glucocorticoid receptor (GR, NR3C1) and inhibited by FXR through SHP induction [50]. SHP can inhibit GR-mediated transcription through a direct interaction [51]. An alternative mechanism, in which the forkhead transcription factor HNF3β binds to the human NTCP promoter and is subject to SHP interaction and repression, was also proposed [52, 53]. The FXR-RXR heterodimer was reported to bind to an IR1 element of the human OATP8 promoter and to
transactivate its expression [54], although the physiological relevance of this finding remains unknown.

To summarize FXR function in hepatic bile acid metabolism, FXR activation induces the transcription of transporter genes involved in promoting bile acid clearance and represses genes involved in bile acid biosynthesis and uptake from the portal circulation. The physiological roles of FXR-regulated genes suggest that FXR protects hepatocytes from bile acid toxicity by sensing high cellular bile acid concentrations. The finding that Fxr-null mice have impaired resistance to bile acid-induced hepatotoxicity is consistent with a hepatoprotective function of FXR [37]. In rat models of extrahepatic and intrahepatic cholestasis, treatment with the synthetic FXR agonist GW4064 resulted in reductions in serum alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase, as well as other markers of liver damage [55]. GW4064 treatment also decreased necrosis, inflammatory cell infiltration, and bile duct proliferation. The data indicate that FXR is a promising molecular target in the treatment of cholestatic liver disease.

The ileal bile-binding protein (I-BABP) was the first FXR target gene to be identified [25, 56]. I-BABP, an abundant cytosolic protein in the ileal mucosa, which biological functions are recently emerging, belongs to the fatty acid-binding protein family. Fxr-null mice had no detectable I-babp mRNA in the intestine, and unlike wild-type mice, I-babp transcription remained undetectable after cholic acid feeding [37]. A recent study showed that I-BABP is functionally associated with FXR in the nucleus and with ASBT on the membrane, where it stimulates FXR transactivation activity and ASBT-mediated ileal conjugated bile acid uptake [57]. Fxr-null mice demonstrate efficient intestinal bile salt absorption in the absence of I-babp expression, suggesting that the physiological role of I-BABP in the enterohepatic circulation is limited [43]. LRH-1 binds to and activates mouse Asbt promoter, an effect repressed by chenodeoxycholic acid via the FXR-SHP mediated mechanism in human colon cancer cells [58]. Despite this finding, FXR deficiency did not affect expression of Asbt and t-Asbt in the ileum [43]. Treatment of mice with a synthetic FXR agonist induced Ostα and Ostβ mRNAs in intestine as well as in the kidneys and adrenal glands of wild-type, but not Fxr-deficient mice [59]. The data suggest that FXR facilitates apical-to-basolateral bile acid transport in enterocytes [19].

Chenodeoxycholic acid, which is the most potent FXR agonist among the major bile acids, has been shown to reduce cholesterol gall stone disease and hypertriglyceridemia [6, 7]. Prevention of cholesterol gallstone disease by FXR agonists was recently demonstrated in a mouse model [60]. The main lipids in bile are cholesterol, bile salts and phospholipids. The proper balance of these components is crucial in maintaining cholesterol in a solubilized state.
Supersaturation of bile with cholesterol induces precipitation of cholesterol crystals, causing gallstone formation. Biliary secretion of bile salts, phosphatidylcholine and cholesterol is controlled at the canalicular membrane of hepatocytes by BSEP, MDR3 (ABCB4, corresponding to the murine Mdr2) and ABCG5/ABCG8, respectively. BSEP is the major canalicular bile salt export transporter and is a FXR target [14, 37, 44]. MDR3/Mdr2 functions as a flippase, translocating phosphatidylcholine molecules from the inner to the outer leaflet of the canalicular membrane [14]. Mutations in the MDR3 gene cause PFIC-3, which is characterized by an inability to form bile salt micelles and progressive cholestatic liver injury. Mdr2-null mice are completely deficient in bile phospholipid. FXR transactivates the human MDR3 promoter through an IR1 element [61], and GW4046 treatment induces hepatic Mdr2 expression in wild-type, but not Fxr-null mice [60]. Thus, the transporters for bile salts and phosphatidylcholine are both FXR targets. ABCG5 and ABCG8 function as biliary transporters and intestinal export transporters for sterols, including cholesterol and plant sterols, and these mutations cause sitosterolemia, which is characterized by hyperabsorption of β-sitosterol and other sterols, and markedly reduced secretion of sterols into bile [62]. The expression of ABCG5 and ABCG8 in the liver and intestine is induced by the LXR oxysterol receptors [63]. Although involvement of FXR in the expression of mouse Abcg5 and Abcg8 in the liver was also demonstrated [62], GW4046 treatment did not increase their expression in both wild-type and Fxr-null mice [60]. GW4046 treatment increased bile salt levels, phospholipid concentrations, and the cholesterol saturation index, and prevented cholesterol gallstone formation in an FXR-dependent manner.

Fxr-null mice demonstrate increased serum and hepatic triglyceride levels [37], and treatment of rats with GW4046 decreased serum triglycerides [64], indicating that the effect of chenodeoxycholic acid in reducing hypertriglyceridemia is mediated by FXR. Apolipoprotein C-II (ApoC-II) functions as a cofactor for lipoprotein lipase while ApoC-III inhibits lipoprotein lipase-mediated triglyceride lipolysis. FXR regulation of ApoC-II, ApoC-III and SHP expressions has been proposed as the molecular mechanism. FXR activation induced the expression of the human ApoC-II gene by binding to two IR1 elements in the promoter [65]. Treatment with bile acids decreased serum triglyceride levels, reduced serum ApoC-III, and decreased hepatic apoC-III mRNA expression in wild-type but not Fxr-null mice [66]. Administration of synthetic LXR agonists induces the expression of fatty acid biosynthetic genes, such as acetyl CoA carboxylase, fatty acid synthase, and stearyl CoA desaturase-1, and results in increased liver and blood triglycerides in mice [67]. This is mainly due to LXR-induced expression of the
sterol regulatory element-binding protein 1c (SREBP-1c), a transcription factor that enhances the expression of genes involved in fatty acid biosynthesis. FXR agonists were shown to repress LXR-mediated expression of Srebp-1c by inducing Shp expression, and the effect of FXR agonists in decreasing serum triglycerides was abolished in Shp-null mice [68].

FXR has also been reported to be involved in the regulation of cholesterol and carbohydrate metabolism. Loss of FXR increased atherosclerotic lesions in the apoE-deficient mouse model of atherosclerotic disease and the underlying molecular mechanism is not clear [69]. Activation of FXR improved glucose tolerance in both diabetic and wild-type mice [70] and many additional genes have been reported to be regulated by FXR [71]. These findings suggest an endocrinological role of bile acids in a variety of biological processes, although the possibility of the existence of other natural FXR ligands cannot be ruled out.

**PXR, CAR and VDR**

The secondary bile acids deoxycholic acid and lithocholic acid are produced from primary bile acids by the intestinal microflora. Bacterial enzymes deconjugate and dehydroxylate the conjugated primary bile acids, modifications that lower their hydrophilicity and bacteriostatic properties [8]. Deoxycholic acid and lithocholic acid are absorbed to some extent in the colon and are returned to the liver [6]. In the hepatocyte, deoxycholic acid is conjugated with glycine or taurine and circulates along with the primary bile acids. Deoxycholic acid constitutes about 20% of the bile acids in the bile duct of most adult humans. Lithocholic acid is conjugated with glycine or taurine and is sulfated at the C-3 position. With two chemical modifications, lithocholic acid is excreted into the bile but is poorly reabsorbed from the small intestine. Although deconjugated, and to some extent desulfated, by intestinal bacteria, lithocholic acid is fecally excreted and does not normally accumulate in the enterohepatic circulation [8]. The secondary bile acids, especially lithocholic acid, are toxic and have pathological roles in cholesterol gallstone disease and colon cancer. In abnormal conditions in which secondary bile acids accumulate, such as cholestasis, xenobiotic-sensing nuclear receptors such as PXR are activated and induce the detoxification of toxic bile acids. PXR binds numerous structurally diverse drugs and environmental contaminants, induces expression of a battery of genes encoding phase I and II metabolic enzymes as well as transporters, and plays an important role in the detoxification and clearance of xenobiotics [72]. It is highly expressed in the liver and intestine, and binds to DNA sequences, such as DR3 response elements, as a RXR heterodimer [73, 74]. PXR is activated by the secondary bile acid lithocholic acid and its metabolite 3-ketocholanic acid, and induces the
expression of Cyp3a11 and Oatp2 in mice [75, 76]. CYP3A4, a human ortholog of Cyp3a11, is involved in the metabolism of 50-60% of pharmaceuticals as well as natural compounds such as steroids and herbal supplements [72]. CYP3A4 metabolizes lithocholic acid into 3-ketochohanic acid (3-dehydrolithocholic acid) by 3-oxidation, hydoxychohanic acid by 6α-hydroxylation, and 1β,2β-dihydroxy-5β-chanoic acid by 1β-hydroxylation, while it catalyzes the 3-oxidation and 1β-hydroxylation of deoxychohanic acid [76, 77]. CYP3A4 can also catalyze 3-oxidation of chenochohanic acid, cholic acid and ursodeoxychohanic acid. 3-Ketochohanic acid is a more potent PXR ligand than lithocholic acid, indicating that lithocholic acid enhances detoxification via a PXR-mediated feed-forward mechanism [78]. Oatp2 is involved in bile salt uptake from the hepatocyte basolateral membrane [14]. PXR-binding elements have been identified in the CYP3A4 and OATP2/Oatp2 promoters [79-81]. Sulfation of lithocholic acid is mediated primarily by dehydroepiandrosterone sulfotransferase 2 (SULT2). SULTs catalyze the transfer of a sulfonyl group from the donor molecule 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to hydroxyl or amino groups of lipophilic molecules, forming sulfate or sulfamate conjugates, respectively. The expression of Sult2 and PAPS synthase 2 (Papss2) was demonstrated to be directly regulated by PXR [82]. Although Mrp2 has been reported to be a PXR target gene [48], data from Pxr-null mice did not show PXR-mediated regulation of Mrp2 gene in vivo [83, 84]. While MRP2 localizes to canalicular membranes, MRP1 and MRP3 are involved in basolateral bile salt efflux in hepatocytes [14]. MRP1 and MRP3 transport divalent bile salts such as sulfated taurocholate and taurochenodeoxychohlate, and MRP3 can also transport monovalent bile salts such as taurocholate. Pxr-null mouse studies show that PXR activation induces Mrp3, Cyp3a11 (mouse ortholog of CYP3A4) and Oatp2 [85]. PXR senses abnormal bile acids and enhances their hepatic uptake, metabolic detoxification and basolateral export, resulting in the elimination of toxic bile acids through a xenobiotic metabolic pathway.

CAR is another nuclear receptor that regulates the transcription of genes involved in xenobiotic metabolism and is abundantly expressed in the liver and intestine [86]. CAR and PXR bind to response elements with overlapping specificity, and the two receptors coordinately regulate the expression of xenobiotic enzymes, such as CYP2Bs and CYP3As [72]. Although there is no evidence to date that endogenous bile acids are ligands for CAR, CAR mediated compensatory induction of Cyp3a11 in cholic acid-fed Pxr/Fxr double knockout mice [83]. Comparison of single null (Pxr-/- or Car-/-) mice and Pxr/Car-double knockout mice showed that CAR predominantly mediates induction of Cyp3a11 and Mrp3, whereas PXR is the major regulator of Oatp2
CAR is also involved in the induction of Sult2a and Papss2 [88]. The finding that mice deficient in Mrp3 have normal bile acid excretion, pool size, and fractional turnover rates suggests an alternative mechanism [18]. Mrp4 may compensate for the loss of Mrp3 function in Mrp3-null mice, because human MRP4 can transport bile acids and localizes at the hepatocyte basolateral membrane [89]. CAR activators increased Mrp4 expression in wild-type mice but not in Car-null mice [90]. In a bile duct ligation model of extrahepatic cholestasis, hepatic damage was increased in both Car-null mice and Pxr-null mice [84]. Therefore, PXR and CAR coordinately serve in the elimination of toxic bile acids.

VDR has been thought of primarily as a receptor for 1α,25-dihydroxyvitamin D₃, the active form of vitamin D₃ [91]. Sequence and structure analyses reveal that VDR is the nuclear receptor most closely related to PXR [92-94]. When activated by lithocholic acid, and more effectively by 3-ketocholanic acid [95], VDR induces the expression of Cyp3a11 in mice and CYP3A4 in human cells [96]. SULT2A and MRP3 were also demonstrated to be VDR targets in mouse and human cells [97, 98]. The in vivo function of VDR in bile acid metabolism is currently under investigation. Dietary uptake of vitamin D reduces the risk of colon carcinogenesis [99]. In addition to direct VDR effects on oncogenic mechanisms, VDR-mediated detoxification of lithocholic acid may contribute to colon cancer prevention by vitamin D [91]. A functional VDR was cloned from the lamprey (Petromyzon marinus), an ancient vertebrate lacking a calcified skeleton and teeth [100]. Lamprey VDR is only weakly activated by 1α,25-dihydroxyvitamin D₃ and does not respond to 3-ketocholanic acid [100]. Although lamprey vitamin D physiology and bile acid metabolism are poorly understood, this finding suggests that VDR may have originally evolved as a regulator of xenobiotic metabolism.

**Perspective**

Bile acids function not only as lipid detergents in intestinal digestion but also as signaling molecules that activate nuclear receptors. Furthermore, bile acids were shown to act as ligands for a cell membrane G protein-coupled receptor and to regulate energy metabolism [101]. In male lampreys, bile acids function as sex pheromones and relay signals to females [102]. In the manner of pheromones and hormones, bile acids appear to have evolved signaling function. Elucidation of the regulatory mechanisms of bile acid metabolism should lead to further progress in bile acid physiology, "bile-ology" [103], and in the development of new drugs targeting bile acid regulators.
Reference


