Nuclear Receptor Signaling in the Control of Cholesterol Homeostasis: Have the Orphans Found a Home?

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Abstract—Cholesterol is essential for all mammalian cells. Cellular cholesterol requirements are met through de novo synthesis and uptake of plasma lipoproteins, homeostatic responses that are transcriptionally regulated by the sterol regulatory element-binding proteins (SREBPs). To prevent cytotoxicity attributable to accumulation of excess cholesterol, liver X receptors (LXRs) and the farnesoid X receptor (FXR), together with other members of the nuclear receptor superfamily, promote the storage, transport, and catabolism of sterols and their metabolites. Members of this metabolic nuclear receptor family include receptors for oxysterols (LXRs), bile acids (CAR, FXR, and PXR), and fatty acids (PPARs). Through coordinated regulation of transcriptional programs, these nuclear receptors regulate key aspects of cellular and whole-body sterol homeostasis, including cholesterol absorption, lipoprotein synthesis and remodeling, lipoprotein uptake by peripheral tissues, reverse cholesterol transport, and bile acid synthesis and absorption. This review focuses on the nuclear receptors that are central to the lipid metabolic signaling cascades, communication between lipid metabolites and their receptors, and the role of nuclear receptors in orchestrating the complex transcriptional programs that govern cholesterol and bile acid metabolism. (Circ Res. 2004;95:660-670.)

Key Words: cholesterol ■ bile acids ■ lipid homeostasis ■ nuclear receptors ■ liver X receptors ■ farnesoid X receptor

In mammalian cells, cholesterol is essential for diverse cellular functions. Cholesterol is required for maintenance of membrane fluidity and permeability, regulation of integral membrane protein function, transcriptional regulation, and for lateral domain or lipid raft formation.1 In most tissues, cells meet their cholesterol requirements through de novo synthesis. However, in the presence of plasma lipoproteins, cells principally obtain cholesterol through internalization of exogenous cholesterol.2 Uptake of lipoprotein cholesterol is important for many cell types, including hepatocytes and steroidogenic cells, which use cholesterol as a precursor for bile acids and steroid hormones, and for macrophages that scavenge plasma lipoproteins and recycle cholesterol back to the liver through reverse cholesterol transport pathways.

Cellular and whole-body cholesterol homeostasis is maintained through a network of transcriptional programs. At the cellular level, de novo cholesterol synthesis and uptake of lipoprotein cholesterol are exquisitely regulated at multiple steps through a negative feedback loop that responds to elevations in intracellular cholesterol. This pathway is regulated by a family of membrane-bound transcription factors, designated sterol regulatory element-binding proteins (SREBPs), that directly activate expression of genes involved in the synthesis and uptake of cholesterol, and lipogenesis.3

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Although the SREBP pathway ensures that there is sufficient cholesterol to meet cellular requirements, in the setting of excess free or unesterified cholesterol, activation of SREBP-dependent gene expression is suppressed. To prevent cholesterol accumulation, liver X receptors (LXRs) and the farnesoid X receptor (FXR), together with other members of the nuclear receptor superfamily, promote sterol storage, transport, and catabolism.\(^5\) These metabolic nuclear receptors serve a central role in bidirectional flux of cholesterol between the liver and peripheral tissues, and in hepatic excretion of cholesterol, dietary sterols, and sterol metabolites.

### Regulation of Cholesterol Absorption by Nuclear Receptors

LXRs, LXR\(\alpha\) (NR1H3) and LXR\(\beta\) (NR1H2), are ligand-activated transcription factors that are members of the nuclear receptor superfamily. LXRs preferentially bind with their heterodimeric partner, retinoid X receptor (RXR), to LXR response elements (two hexanucleotide repeats separated by four nucleotides) to activate gene expression.\(^6\) The consensus sequence for the LXR response element (DR-4) is AGGTCA mnn CGTCA. LXR\(\alpha\) is highly expressed in liver tissue, with lower levels present in adipose, intestine, kidney, and splenic tissues.\(^7\) LXR\(\beta\) is ubiquitously expressed.\(^8\) Although cholesterol loading results in activation of genes with LXR response elements, neither free cholesterol nor cholesteryl esters (CEs) appear to be physiological ligands for LXRs. Recent studies have shown that oysterless are specific ligands for the LXRs. The most potent oysterless include 24(S),25-epoxycholesterol, which is produced in hepatocytes and macrophages, 24(S)-hydroxycholesterol, an abundant cholesterol metabolite in brain tissue, and 22(R)-hydroxycholesterol, an intermediate in steroid hormone production.\(^9\)\(^,\)\(^10\) 27-hydroxycholesterol, which is produced in macrophages in response to cholesterol loading, also activates LXRs and has been proposed to be an important physiological ligand.\(^11\)

LXRs respond to elevated cholesterol levels via transactivation of genes involved in sterol transport (ABCA1, ABCG1, ABCG5, and ABCG8), cholesterol efflux and high-density lipoprotein (HDL) metabolism (ABCA1, APOE, CETP, and PLTP), and sterol catabolism (CYP7A1).\(^5\) Additionally, LXRs also play a central role in regulating cellular lipid content through activation of SREBP-1c, which is the master regulator of de novo lipogenesis.\(^13\) SREBP-1c governs genes involved in fatty acid synthesis, such as fatty acid synthase and stearyl-CoA desaturase-1. Stearyl-CoA desaturase-I is responsible for the \(\Delta^\text{7-9}\) desaturation of stearyl-CoA and its conversion to oleyl-CoA, the preferred substrate for acyl-CoA:cholesterol acyltransferase. Coupling the regulation of cholesterol catabolism to de novo lipogenesis enables LXRs to more efficiently dispose of excess sterol and prevent cholesterol-induced cytotoxicity. Under high cholesterol conditions, SREBP-1c-mediated synthesis of fatty acids provides substrate for esterification and intracellular storage of excess free cholesterol. Because of the continual turnover of lipid droplet-associated cholesterol via the CE cycle,\(^12\) storage of cholesterol in this organelle serves only as a temporary buffer for cellular free cholesterol. However, in lipoprotein synthetic tissues, LXR-mediated CE and triglyceride synthesis supplies essential components for lipoprotein assembly and secretion, thereby providing a mechanism for bulk export of sterols. Thus, through dual activation of cholesterol catabolic and efflux pathways, and de novo lipogenesis, LXRs function as sterol sensors to restore cellular cholesterol balance and prevent lipotoxicity.

### Role of LXR in Intestinal Sterol Transport

The ATP-binding cassette (ABC) transporter protein ABCA1 and the ABC half-transporters, ABCG5 and ABCG8, are LXR target genes in the intestine and participate in cholesterol absorption\(^1\)\(^3\)\(^-\)\(^5\) (Figure 1). Administration of synthetic LXR or RXR ligands has been shown to inhibit cholesterol absorption in wild-type mice, but not in LXR\(\alpha\)/\(\beta\) double-knockout mice, suggesting that LXR target genes are key participants in regulation of cholesterol absorption.\(^14\) Although LXR ligands strongly upregulate ABCA1 expression in enterocytes, the role of ABCA1 in cholesterol absorption is less clear in light of conflicting findings from studies with ABCA1-deficient mice.\(^16\)\(^-\)\(^18\) Earlier studies suggested that ABCA1 countered cholesterol absorption via efflux of cholesterol from the enterocyte into the gut lumen. However, recent studies have demonstrated that ABCA1 participates in the efflux of cholesterol from the basolateral membrane, but not the apical membrane, of the enterocyte.\(^19\)\(^,\)\(^20\) Thus, ABCA1 may contribute to cholesterol trafficking in the enterocyte by channeling absorbed cholesterol away from chylomicron synthesis into a secretory pathway, perhaps involving formation of HDL-like particles.\(^21\) Nevertheless, the effect of LXR activation on cholesterol absorption appears to be independent of ABCA1 expression.\(^19\)

However, there is considerable evidence that LXR agonists exert their effect on cholesterol absorption through upregulation of ABCG5 and ABCG8\(^22\) (Figure 1). Mutations in either of these ABC half-transporters results in sitosterolemia, an autosomal recessive disorder characterized by elevated plasma and tissue levels of cholesterol and plant-derived sterols. Whereas mice with targeted deletions of both ABCG5 and ABCG8 show an increase in the fractional absorption of dietary plant sterols and a marked increase in plasma and hepatic cholesterol levels after cholesterol feeding,\(^23\) overexpression of ABCG5 and ABCG8 reduces intestinal absorption of both dietary sterol and cholesterol.\(^24\) Although the mechanism by which accumulation of noncholesterol sterols is prevented is not well-understood, the finding that the rank order for fractional absorption of sterols in the intestines of ABCG5/ABCG8 knockout mice is the same as in wild-type mice indicates that factors other than intestinal expression of ABCG5 and ABCG8 are responsible for preventing whole-body accumulation of noncholesterol sterols.\(^25\) The recent demonstration that hepatic ABCG5 and ABCG8 preferentially secrete noncholesterol sterols into the bile suggest that differential sterol secretion, rather than selective sterol absorption, may be responsible for limiting dietary sterol accumulation.\(^25\) This hypothesis will need to be tested experimentally in mice in which intestinal-restricted and liver-restricted expression of the ABCG5 and ABCG8 transporters
is reconstituted in the ABCG5/ABCG8 knockout background.

Several lines of evidence suggest that ABCG5 and ABCG8 are coordinately expressed through common regulatory sequences. The ABCG5 and ABCG8 genes are oriented in a head-to-head configuration, separated by a common core promoter, and exhibit identical tissue-specific and cell-specific patterns of expression. Furthermore, ABCG5 and ABCG8 assemble as heterodimers and must be co-expressed for appropriate trafficking to the apical surface of intestinal enterocytes and hepatocytes. Although ABCG5 and ABCG8 are targets for LXR, the LXR response element for the ABCG5/ABCG8 gene cluster has not yet been identified. However, a recent study has shown that the ABCG5/ABCG8 intergenic region contains a binding site for the orphan nuclear receptor liver receptor homolog-1 (LRH-1, NR5A2). LRH-1, which is expressed in the intestine and the liver, bidirectionally stimulates expression of ABCG5 and ABCG8. In contrast to LXR, FXR, and PPAR nuclear receptors, LRH-1 does not form a heterodimeric complex with RXR, but binds as a monomer to an extended half-site.

**Figure 1.** Sterol absorption and lipoprotein synthesis and remodeling. Dietary cholesterol (C) and plant sterols (P) are transported into the intestinal enterocyte through an NPC1L1-mediated pathway. Cholesterol and plant sterols are effluxed back into the gut lumen by apical ABCG5/G8 transporters. Cholesterol is effluxed from the enterocyte by the basolateral ABCA1 transporter, and is esterified (CE) for incorporation into chylomicrons (CM). CM remnants (CM-R), generated by lipoprotein lipase (LPL) hydrolysis, are cleared by the hepatic LDL receptor (LDL-R). Lipoprotein cholesterol is re-esterified and repackaged with de novo-synthesized triglycerides (TG) into VLDL, which is remodeled through the actions of LPL and CETP. Dark gray boxes represent LXR target genes; light gray boxes represent PPARα/β target genes; dark-hatched boxes represent FXR/LXR target genes; light-hatched boxes represent PPAR/LXR target genes.

**Cholesterol Import Into the Intestinal Enterocyte**

Although ABCG5 and ABCG8 are required for sterol secretion from the enterocyte, the molecular mechanism for sterol uptake from the lumen is poorly defined. Recently, the Niemann-Pick C1-like (NPC1L1) protein was identified for its role in absorption of dietary cholesterol. NPC1L1-deficient mice exhibit decreased cholesterol absorption and do not show a further reduction in absorption when treated with ezetimibe, a drug that lowers cholesterol absorption, suggesting that NPC1L1 participates in ezetimibe-sensitive pathway for sterol absorption. Because ezetimibe has not been shown to bind to NPC1L1, it is unclear whether this protein is a molecular target of ezetimibe. Given the high degree of homology of NPC1L1 with the Niemann-Pick C1 (NPC1) protein and other sterol-sensing domain containing proteins (eg, HMG CoA reductase, SREBP cleavage activation protein [SCAP] and Patched), and the emerging role of these proteins in sterol-regulated vesicular trafficking, it is interesting to speculate that NPC1L1 may function in an analogous manner for trafficking newly absorbed sterols. The observation that cholesterol/cholate feeding suppresses NPC1L1 expression is consistent with regulation through SREBPs or nuclear receptor transcription factors.

**Nuclear Receptor Control of Lipoprotein Synthesis and Uptake by Peripheral Tissues: Secretion and Remodeling of Triglyceride-Rich Lipoproteins**

In the liver, nuclear receptors exert control over lipid metabolism by several mechanisms. The hepatic nuclear receptor, PPARα, binds fatty acid, eicosanoid, and fibrate ligands, leading to activation of genes involved in the uptake, metabolism, and β-oxidation of fatty acids. Channeling of fatty acids to the β-oxidative pathway decreases the available substrate for triglyceride synthesis, and, ultimately, secretion of very-low-density lipoprotein (VLDL) by the liver. VLDL production is also controlled through the actions of the ubiquitously expressed PPARβ (also referred to as PPARδ). Studies with PPARβ-null mice demonstrate an increased rate of VLDL secretion and a concomitant reduction in hepatic lipid stores, revealing a role for PPARβ in regulating serum triglyceride levels. In addition to affecting lipoprotein secretion, both PPARα and PPARβ contribute to remodeling of apoB-containing lipoproteins (Figure 1). Whereas PPARα influences lipoprotein remodeling by inhibiting expression of apoC-III, an apolipoprotein that inhibits lipoprotein lipase (LPL)-dependent hydrolysis of triglyceride-rich lipoprotein
particles. PPAR\(\beta\) promotes remodeling by suppression of angiopoietin-like proteins 3 and 4, which are known to inhibit LPL activity.

Plasma levels of apoB-containing lipoproteins are also modulated through the action of LXR (Figure 1). Treatment with a synthetic LXR ligand induces expression of hepatic lipogenesis and results in hypertriglyceridemia in mice, primarily through induction of SREBP-1c lipogenic program. LXR activation also induces expression of LPL, which is responsible for the hydrolysis of postprandial triacylglyceride-rich particles, and coordinately upregulates expression of the apoE/apoC-I/apoC-IV/apoC-II gene cluster. ApoC-II is an obligate cofactor for LPL-dependent hydrolysis of lipoprotein-associated triglycerides. Additionally, LXRs affect remodeling of apoB-containing lipoproteins via induction of hepatic expression of cholesterol ester transfer protein (CETP), which facilitates the transfer of CE from HDL to apoB-containing lipoproteins.

**Regulation of Peripheral Uptake and Metabolism of Lipoproteins**

Macrophages play a central role in the development of vascular lesions through the uptake of atherogenic plasma lipoproteins. Macrophages express scavenger receptor A (SR-A) and CD36, which bind and internalize modified lipoproteins, such as oxidized LDL (Figure 2). The importance of these receptors in formation of atherosclerotic lesions is underscored by studies in which SR-A-deficient or CD36-deficient mice were crossed into apoE-deficient mice, resulting in decreased lesion size. Because the scavenger receptors, in contrast to the LDL receptor, are not controlled by the negative feedback loop that regulates SREBP-dependent gene expression, macrophages accumulate massive quantities of lipoprotein-derived lipids. To meet this metabolic challenge, the macrophage responds by activation of feed-forward nuclear receptor-driven pathways.

On internalization, lipoprotein-derived cholesterol is initially delivered to an early endosomal sorting organelle. Studies with cholesterol analogs suggest that early endosomal free cholesterol may be trafficked to the plasma membrane via an endocytic recycling compartment. In contrast, lipoprotein-derived CE is delivered to a late endosomal organelle, where the CE is hydrolyzed to free cholesterol. Delivery of free cholesterol from the endocytic pathway to the plasma membrane and to the endoplasmic reticulum (ER) requires the coordinated actions of the late endosomal Niemann-Pick C1 (NPC1) and lysosomal NPC2 proteins. NPC1-deficient and NPC2-deficient cells accumulate lysosomal free cholesterol and exhibit delayed sterol homeostatic responses attributable to the failure of lipoprotein cholesterol both to suppress SREBP-dependent gene expression and to promote LXR-mediated responses. Recent studies have shown that NPC mutant cells have impaired LDL cholesterol-stimulated production of 25-hydroxycholesterol (25-HC) and 27-hydroxycholesterol (27-HC). Oxysterols, such as 27-HC, are potent suppressors of the SREBP pathway, and are endogenous ligands for the LXR. These findings provide support for an NPC1–NPC2–LXR signaling pathway to prevent cellular cholesterol overload.

In macrophages, LXR activation contributes to sterol homeostasis through upregulation of ABCA1, ABCG1, and apoE expression. ABCA1 and ABCG1 are cell surface transporters that function in cellular cholesterol and phospholipid efflux to extracellular acceptors, such as apoA-I. In

**Figure 2.** Peripheral uptake of lipoproteins. Oxidized LDL is taken-up by CD36 and SR-AI receptors expressed in macrophages. LDL cholesterol (C) is trafficked by the NPC1/NPC2 proteins to acyl-CoA:cholesterol acyltransferase for esterification (CE) and storage in lipid droplets, and to the mitochondrial sterol 27-hydroxylase (CYP27A1) to generate 27-HC. 27-HC activates LXR target genes, including the ABCA1 and ABCG1 transporters that efflux cholesterol to apoA-I and apoE, and is secreted into the plasma for reverse transport. Dark gray boxes represent LXR target genes; light gray boxes represent PPAR\(\gamma\) target genes; dark-hatched boxes represent PPAR\(\gamma\)/LXR target genes; light-hatched boxes represent PPAR\(\alpha\) target genes.
contrast to ABCA1, which interacts poorly with mature forms of HDL, ABCG1 effluxes cholesterol to HDL-2 and HDL-3, but not to lipid-poor apoA-I. ApoE is secreted by macrophages and similarly participates in cholesterol efflux by serving as a cholesterol acceptor, although the relative contribution of apoE, as compared with apoA-1, to overall lipid efflux is not known. ApoE is also found on chylomicron and VLDL remnant particles, and is necessary for hepatic clearance of these particles by the LDL receptor and lipoprotein receptor-related protein (LRP). These observations underscore the importance of LXRs in promoting net cholesterol efflux from peripheral tissues and consequently preventing cholesterol accumulation in tissue wall.

In concert with the LXRs, the nuclear receptor PPARγ plays a critical role in the pathways that regulate macrophage lipid homeostasis. The role of PPARγ in regulation of cellular lipid homeostasis was first suggested by studies identifying CD36 as a PPARγ target gene. Lipid components of oxidized LDL, such as 9-hydroxyoctadecenoic acid (9-HODE) and 13-HODE, have been shown to activate PPARγ and induce CD36 expression. Furthermore, recent studies have confirmed that CD36 is a PPARγ target gene. In theory, treatment with PPARγ ligands, such as thiazolidinediones, could accelerate foam cell formation by stimulating lipid uptake and worsening atherosclerosis. However, several groups have shown that activation of PPARγ does not stimulate lipid accumulation. Rather, PPARγ appears to play an antiatherogenic role, exerting its effects through activation of LXRα. These findings support a feed-forward PPARγ–LXRα signaling cascade, in which lipoprotein uptake potentiates both CD36 expression (promoting further lipid uptake) and ABCA1 expression (promoting lipid efflux).

**Regulation of Reverse Cholesterol Transport by Nuclear Receptors: Peripheral Cholesterol Efflux and HDL Remodeling**

Macrophages function not only in lipoprotein uptake but also in the catabolism and turnover of lipoprotein cholesterol for reverse cholesterol transport. In this process, HDL facilitates delivery of cholesterol from peripheral tissues to the liver for conversion to bile acids or for biliary secretion of free sterol. Removal of cholesterol from the vessel wall, which inhibits formation of atherogenic foam cells and suppresses inflammatory responses, likely accounts for the atheroprotective effect of HDL.

Nuclear receptors exert control over many aspects of reverse cholesterol transport (Figure 3). The initial step in this pathway is the formation of nascent HDL particles. Hepatic synthesis of apoA-I and apoA-II, the two major apolipoproteins in discoidal (pre-β) HDL, is regulated via PPARα activation. ApoA-I expression is also under transcriptional regulation of RORα (NR1F1), a widely expressed nuclear receptor that is activated by cholesterol or cholesterol sulfate ligands. An in vivo role for RORα in HDL metabolism was suggested by studies with RORα-deficient mice, which exhibited hypo-apolipoproteinemia and increased susceptibility to atherosclerosis. The next step in reverse transport from peripheral tissues, lipidation of apoA-I and apoA-II, occurs through selective interaction with plasma-membrane lipid domains formed through the action of ABCA1, the PPARγ/LXR-regulated cholesterol/phospholipid transporter. In addition to apoA-I and apoA-II, the LXR-regulated apolipoproteins (apoE, apoC-I, apoC-II, and apoC-IV) all possess amphipathic α-helices that can serve as acceptors for ABCA1-mediated lipid efflux. Recent studies provide support for direct apolipoprotein–ABCA1 interactions, although a direct link has not been established between apolipoprotein binding and lipid efflux.

After lipidation, free cholesterol in the HDL particles is esterified by lecithin:cholesterol acyltransferase (LCAT) to generate more mature CE-rich HDL. In the plasma, these particles undergo a series of remodeling steps involving two HDL-associated proteins: phospholipid transfer protein (PLTP) and CETP. PLTP is activated by both LXR and FXR. The primary role of PLTP is in the transfer of surface remnants, which contain apolipoproteins and phospholipids originating from triglyceride-rich lipoproteins, to pre-β-HDL. PLTP has also been implicated in mediating fusion of HDL particles to generate pre-β-HDL and CE-rich HDL. CETP, which is transactivated by LXR, promotes both transfer and exchange of hydrophobic lipids, CE, and triglyceride between lipoproteins. The transfer of CE from HDL to other apoB-containing lipoproteins results in loss of core lipids and decrease in HDL particle size. CETP also facilitates exchange of HDL CE with chylomicron or VLDL-associated triglycerides. The net effect of these reactions is hepatic clearance of the HDL-derived cholesterol through LRH-mediated uptake of apoB-containing lipoproteins.

**Farnesoid X Receptors**

FXR (NR1H4) is a ligand-activated nuclear receptor that heterodimerizes with RXR and principally activates transcription of target genes involved in bile acid metabolism. FXR/RXR complexes transactivate genes containing consensus elements referred to as FXR response elements or bile acid response elements. FXR is expressed at high levels in the intestine and liver, tissues involved in bile acid metabolism. Primary bile acids, such as chenodeoxycholic acid and cholic acid, and bile acid intermediates are physiological ligands for FXR. Recently, a novel FXR family member, FXRβ (NR1H5), was identified as an FXR homolog in nonprimate mammals. FXRβ specifically binds the cholesterol precursor lanosterol, suggesting a role for this receptor in regulation of cholesterol synthesis in nonprimates.

In vivo studies identify FXR as an intracellular bile acid sensor that governs bile acid synthesis and transport. Although LXRs activate bile acid synthesis through a feed-forward mechanism, FXR suppresses bile acid synthesis through feedback regulation involving a promoter-specific activator (LRH-1, NR5A2) and a promoter-specific repressor (SHP, NROB2). In response to bile acids, FXR induces expression of the SHP, a nuclear receptor lacking a DNA binding domain. In turn, SHP binds to and inhibits LRH-1, which normally activates target genes that participate in bile acid synthesis. In the absence of bile acids, LRH-1 acts in concert with LXRα to stimulate expression of CYP7A1, a key enzyme in bile acid synthesis. The proposed FXR–SHP-
1–LRH-1 regulatory cascade offers a mechanism to explain how these nuclear receptors serve as both negative and positive modulators of gene expression (Figure 3, inset).4,84

FXR is also central to the signaling pathway through which bile acids modulate triglyceride levels. Whereas bile acids are known to prevent hepatic triglyceride accumulation, reduce VLDL secretion, and lower plasma triglyceride levels,85–87 the mechanism by which bile acids counter hypertriglyceridemia has remained elusive. It is possible that bile acids, which are endogenous FXR ligands, may affect triglyceride homeostasis through transcriptional activation of other nuclear receptors, such as PPARγ.83,88 Alternatively, treatment with bile acids may stimulate feedback inhibition of bile acid synthesis, resulting in increased cholesterol and oxysterol levels, and attenuation of post-transcriptional processing of SREBP-1c. Recent studies investigating the relationship between FXR transcriptional activity and triglyceride metabolism provide support for the former mechanism. Watanabe et al have shown that bile acids reduce triglyceride levels through suppression of SREBP-1c gene expression, and that FXR activation reduces SREBP-1c gene expression via increased SHP-1 expression.89 In other studies, peroxisome proliferator-activated receptor-γ co-activator 1α (PGC1α) has been shown to be a co-activator of FXR.90 Thus, PGC1α and FXR may regulate plasma triglyceride levels in a cooperative manner by reducing hepatic triglyceride synthesis, increasing VLDL clearance (through induction of apo-C-II transcription91), and promoting PGC1α-mediated fatty acid β-oxidation.

FXR is expressed in a wide range of tissues, including the heart, kidney, thymus, and spleen, tissues not usually associated with bile acid metabolism.91 Recent studies have also identified FXR in the vasculature in vivo and in cultured vascular smooth muscle cells.92 Treatment of vascular smooth muscle cells with FXR ligands stimulated cells to undergo apoptosis, which correlated with induction of FXR target genes. These findings indicate that FXR is functional in vascular smooth muscle cells, although further study is needed to understand how FXR may participate in lipid metabolism in the vessel wall.

**Hepatic Catabolism of Lipoprotein Cholesterol**

Hepatic clearance of HDL cholesterol is dependent on expression of the scavenger receptor class B type I (SR-BI). SR-BI knockout mice exhibit elevated plasma HDL, reduced HDL CE clearance, and decreased bile acid secretion.93 Conversely, hepatic overexpression of SR-BI results in re-
duced plasma HDL, increased HDL CE clearance, and increased biliary cholesterol content. Numerous in vitro and in vivo studies have demonstrated the importance of nuclear receptors on regulation of hepatic SR-BI expression. Activation of FXR, LXR, LRH-1, and PPARγ have all been shown to increase SR-BI expression.94–97 However, fibrate activation of PPARα suppresses SR-BI protein, but not mRNA, expression in the liver.98

In the liver, HDL-derived cholesterol serves as a precursor for bile acid synthesis, an important route for cholesterol catabolism (Figure 3). Bile acids are synthesized via “classical” or “alternative” routes.99 In the classical route, bile acid synthesis is initiated by the enzyme cholesterol 7α-hydroxylase (CYP7A1), which catalyzes the rate-limiting step in the biosynthetic pathway. Expression of cholesterol 7α-hydroxylase is positively regulated by oxysterol activation of LXRα.100 Consistent with this finding, several animal studies have shown cholesterol accumulation in the liver of LXRα−/− mice, but not in wild-type or LXRβ−/− mice.100–102 Bile acid synthesis is also regulated via a negative feedback mechanism mediated by the previously described FXR–SHP–LRH-1 regulatory cascade. Accumulation of bile acids stimulates FXR-mediated suppression of cholesterol 7α-hydroxylase and sterol 12α-hydroxylase (CYP8B1), key participants in bile acid synthesis.

The first step in the alternative bile acid pathway is performed by sterol 27-hydroxylase (CYP27A1), which converts cholesterol to 27-HC. This sterol metabolite, which is generated in peripheral tissues (eg, macrophages) and is the most abundant plasma oxysterol species, is secreted into the circulation, and taken-up by the liver.103 27-HC is also generated in situ in the liver through mitochondrial oxidation. In the liver, 27-HC and other peripheral tissue-derived oxysterols, such as 24-hydroxycholesterol and 25-HC, are generated in situ in the liver through mitochondrial oxidation. Expression of cholesterol 7α-hydroxylase is positively regulated by oxysterol activation of LXRα.100 Consistent with this finding, several animal studies have shown cholesterol accumulation in the liver of LXRα−/− mice, but not in wild-type or LXRβ−/− mice.100–102 Bile acid synthesis is also regulated via a negative feedback mechanism mediated by the previously described FXR–SHP–LRH-1 regulatory cascade. Accumulation of bile acids stimulates FXR-mediated suppression of cholesterol 7α-hydroxylase and sterol 12α-hydroxylase (CYP8B1), key participants in bile acid synthesis.

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Secretion and hepatic uptake of oxysterols, therefore, serves as a parallel pathway to HDL-mediated reverse cholesterol transport pathway for the return of sterol from peripheral tissues to the liver to maintain tissue lipid homeostasis.105,106 In humans, defects in CYP27A1 result in cerebrotendinous xanthomatosis (CTX), a disorder characterized by absence of 27-HC, altered sterol regulatory responses, and premature atherosclerosis.107 Although studies in CYP27A1−/− mice demonstrate a nonuniform increase in cholesterol synthesis across tissues, the overall increase in whole-body cholesterol synthesis indicates that 27-HC generation is one of several pathways that contribute to the regulation of whole animal sterol homeostasis.108

**Regulation of Bile Acid and Sterol Secretion in Hepatocytes**

In addition to its central role in bile acid synthesis, FXR also regulates the excretion, re-uptake, and enterohepatic circulation of bile acids (Figure 3). Study of FXR-deficient mice identified the bile salt export pump (BSEP) as an FXR target gene.82,109 BSEP (ABCB11), which is a member of the ABC superfamily of transporters, localizes to the hepatocyte canalicular membrane and facilitates excretion of bile acids into the bile.110,111 Luminal bile acids are taken-up at the apical surface of the intestinal enterocyte in the terminal ileum by the apical sodium-dependent bile salt transporter. Intracellularly, the cytosolic ileal bile acid-binding protein, an FXR target gene, prevents bile acid-induced toxicity by limiting the free concentration of bile acids.78 FXR and LRH-1 also coordinately regulate the expression of multidrug resistance protein 3, an ABC transporter that is expressed in the ileum and functions in the export of bile acids across the basolateral surface of enterocytes into the portal circulation for re-uptake by the liver.112 Thus, under conditions of low dietary cholesterol, conversion of cholesterol into bile acids is reduced, whereas BSEP, apical sodium-dependent bile salt transporter, ileal bile acid-binding protein, and multidrug resistance protein 3 are upregulated, promoting the hepatic export of bile acids, and increased ileal absorption of bile acids and emulsified dietary lipids (eg, cholesterol).

Hepatic cholesterol homeostasis is also maintained by direct excretion of cholesterol into the bile. Cholesterol transport across the apical membrane of the hepatocyte is facilitated by the ABCG5 and ABCG8 half-transporters.23 Similar to intestinal enterocyte, transcription of ABCG5 and ABCG8 is coordinately regulated by the LXR and LRH-1 nuclear receptors.22,23 In ABCG5/ABCG8-deficient mice, biliary cholesterol concentration is extremely low and does not increase even on a high-cholesterol diet.24 Conversely, overexpression of ABCG5/G8 in mice enhances biliary cholesterol secretion.24 In both animal models, there is no effect on biliary phospholipid content, indicating that ABCG5/ABCG8 are specific sterol transporters.

**Prevention of Bile Acid-Induced Toxicity in Hepatocytes**

Protection against bile acid toxicity is mediated by the triumvirate of the pregnane X receptor (PXR, NR12), constitutive androstane receptor (CAR, NR1), and vitamin D receptor (VDR, NR11). These receptors respond to a broad range of compounds that includes drugs, xenobiotics, and bile acid metabolites. PXR is activated by chenodeoxycholic acid, deoxycholic acid, and the toxic bile acid metabolite lithocholic acid, a secondary bile acid generated in the intestine by bacterial 7α-dehydroxylation of chenodeoxycholic acid.113 Both CAR and VDR exhibit ligand specificities that overlap with PXR.114,115 Activation of PXR, CAR, and VDR stimulate transcription of cytochrome P450, sulfotransferase, and transport genes involved in metabolism and excretion of bile acids.116,117 CAR and PXR also contribute to bilirubin metabolism through induction of multiple components of the bilirubin clearance pathway.118,119
PXR, the most well-studied of these receptors, induces expression of the sodium-dependent organic anion transporting peptide and CYP3A, which are involved in the uptake and catabolism of lithocholic acid (Figure 3). In turn, lithocholic acid inhibits bile acid synthesis via suppression of CYP7A1. Recently, the bile acid intermediate 5β-cholostane-3α,7α,12α-triol was identified as an endogenous PXR ligand in mice. Activation of PXR by this triol intermediate results in induction of CYP3A and detoxification of this potentially toxic sterol metabolite, defining an important salvage pathway for hepatic sterol clearance. Interestingly, human PXR is not activated by 5β-cholostane-3α,7α,12α-triol, which is an endogenous CYP27A1 substrate and accumulates to high levels in CTX. This observation is a likely explanation for the failure of cyp27α deficiency to inactivate CYP7A1.113,120 Recently, the bile acid intermediate 5β-cholostane-3α,7α,12α-triol was identified as an endogenous PXR ligand in mice. Activation of PXR by this triol-

Summary

Metabolic nuclear receptors serve a central role in maintaining cellular and whole-body cholesterol homeostasis. These nuclear receptors bind a range of physiological ligands, including fatty acids and cholesterol and their metabolites, and act in concert to regulate in a tissue-specific manner a network of transcriptional programs. As lipid sensors, nuclear receptors respond to excess cellular cholesterol through activation of target genes involved in cholesterol catabolism and efflux. In the whole animal, these receptors orchestrate the bidirectional trafficking of cholesterol between peripheral tissues and the liver, and the biliary excretion of cholesterol, dietary sterols, and sterol metabolites. Understanding the physiological role of nuclear receptors in lipid metabolism will identify potential therapeutic targets for treatment of cholesterol and bile acid disorders.

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