A Role of the Bile Salt Receptor FXR in Atherosclerosis

Jurre Hageman, Hilde Herrema, Albert K. Groen, Folkert Kuipers

Abstract—This study reviews current insights into the role of bile salts and bile salt receptors on the progression and regression of atherosclerosis. Bile salts have emerged as important modifiers of lipid and energy metabolism. At the molecular level, bile salts regulate lipid and energy homeostasis mainly via the bile salt receptors FXR and TGR5. Activation of FXR has been shown to improve plasma lipid profiles, whereas Fxr−/− mice have increased plasma triglyceride and very-low-density lipoprotein levels. Nevertheless, high-density lipoprotein cholesterol levels are increased in these mice, suggesting that FXR has both anti- and proatherosclerotic properties. Interestingly, there is increasing evidence for a role of FXR in “nonclassical” bile salt target tissues, eg, vasculature and macrophages. In these tissues, FXR has been shown to influence vascular tension and regulate the unloading of cholesterol from foam cells, respectively. Recent publications have provided insight into the antiinflammatory properties of FXR in atherosclerosis. Bile salt signaling via TGR5 might regulate energy homeostasis, which could serve as an attractive target to increase energy expenditure and weight loss. Interventions aiming to increase cholesterol turnover (eg, by bile salt sequestration) significantly improve plasma lipid profiles and diminish atherosclerosis in animal models. Bile salt metabolism and bile salt signaling pathways represent attractive therapeutic targets for the treatment of atherosclerosis. (Arterioscler Thromb Vasc Biol. 2010;30:1519-1528.)

Key Words: atherosclerosis ■ bile salt ■ FXR ■ nuclear receptors ■ TGR5

Bile salts are crucial for the proper solubilization and uptake of dietary fats and fat-soluble vitamins in the intestine. Bile salts are synthesized from cholesterol in hepatocytes, and this conversion quantitatively represents the major route for removal of cholesterol from the body. Bile salts can also be synthesized from brain-derived cerebrosterol1 or from oxysterols derived from other tissues, but these appear to be minor pathways.2 It has been demonstrated repeatedly that increasing the turnover of bile salts has a beneficial effect on low-density lipoprotein cholesterol (LDL-C) levels, a major risk factor for the development of atherosclerosis and cardiovascular diseases (CVD). Consequently, bile salt sequestrants (eg, cholestyramine, colestipol, and colesevelam HCl) have been successfully used to reduce LDL-C levels in patients with hypercholesterolemia.

In addition to their role in absorption of dietary fats and fat-soluble vitamins, bile salts have emerged as important endocrine signaling molecules affecting multiple organs beyond the organs constituting the enterohepatic tract. Bile salts have been shown to signal via the farnesoid X receptor (FXR/NR1H4) and the G protein–coupled bile acid receptor 1 (TGR5/GPBAR1). However, (secondary) bile salts have also been shown to activate the constitutive androstane receptor,3 pregnane X receptor,4 and vitamin D receptor.5

In addition to controlling their own metabolism, bile salts influence cholesterol fluxes, inflammation, and vascular tension. Moreover, bile salts are important modulators of glucose, lipid, and energy metabolism. Bile salt signaling pathways could therefore have profound effects on the development of atherosclerosis. The use of genetically modified animal models has greatly contributed to the understanding of the role of bile salt signaling in the development of atherosclerosis. In contrast to humans, however, mice transport the majority of plasma lipids in high-density lipoprotein (HDL) particles, which makes them less prone to atherosclerosis development. In addition, the bile salt composition differs considerably between humans and mice. Besides cholate and chenodeoxycholate (CDCA), present in both species, mice also produce muricholates as primary bile salts. Moreover, whereas mice conjugate the majority of their bile salts to taurine,6 human bile salts are conjugated predominantly to glycine.7
Regulation of Bile Salt Synthesis

Cholesterol conversion to bile salts involves multiple enzymatic conversions, sequentially carried out in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes of hepatocytes. The end products of hepatic bile salt synthesis are the primary bile salts cholate and CDCA, synthesized in the neutral biosynthetic pathway. Rodents synthesize limited amounts of CDCA but do form a series of muricholates (α, β, γ), i.e., highly hydrophilic bile salt species. CDCA can also be synthesized in the so-called acidic biosynthetic pathway. Cholesterol 7α-hydroxylase (CYP27A1) is the first and rate-controlling enzyme in the neutral pathway of bile salt synthesis, whereas sterol 27-hydroxylase (CYP27A1) has this function in the acidic pathway. Sterol 12-hydroxylase (CYP8B1) determines the ratio of cholate to CDCA synthesis in the neutral pathway. Upon conjugation with either taurine or glycine, bile salts are secreted into bile. Structural modifications of bile salt molecules by intestinal bacteria result in the formation of secondary bile salts. Deoxycholic acid is the major secondary bile salt species. Bile salts are efficiently maintained in an enterohepatic circulation; 95% of biliary bile salts are reabsorbed in the terminal ileum. A small fraction of bile salts enters the colon to be converted to secondary bile salts, which are partially reabsorbed. The fraction of bile salts that finally escapes the enterohepatic circulation is lost in the feces. This loss is completely compensated for by de novo synthesis of bile salts from cholesterol. Therefore, in steady state conditions, fecal excretion of bile salts equals hepatic bile salt synthesis. The latter contributes strongly to cholesterol turnover.

Bile salts tightly regulate their own synthesis by activating FXR. FXR binds DNA mainly as a heterodimer with the 9-cis-retinoic acid receptor (RXR/NR2B1). The preferred DNA motifs for FXR/RXR are inverted repeat elements separated by a spacing of a single nucleotide. Farnesol, a derivative of isoprenoid intermediates in the cholesterol biosynthetic pathway, was first identified as endogenous FXR ligand. Later studies identified bile salts, in particular CDCA and deoxycholic acid, as the most potent endogenous FXR ligands for FXR. FXR-dependent repression of bile salt synthesis was evidenced by the use of Fxr−/− mice, which are unable to reduce their bile salt synthesis on bile salt feeding. Activated hepatic FXR reduces the expression of CYP7A1 in a pathway that requires activation of the small heterodimer partner (SHP/NR0B2). SHP binds the nuclear liver receptor homolog 1 (LRH-1/NR5A2) and blocks its transcriptional activity. Initially, in vitro studies identified LRH-1 as a critical transcription factor for Cyp7a1. However, results from in vivo studies questioned the regulatory role of LRH-1 on Cyp7a1 transcription, as liver-specific Lrh-1 gene deletion did not alter the expression of Cyp7a1. Possibly, compensatory mechanisms regulate Cyp7a1 transcription under conditions of Lrh-1 deficiency. Experimental proof for such a compensatory mechanism, however, is still lacking. Clearly, many of the molecular details regarding the bile salt-induced repression of Cyp7a1 repression remain to be resolved.

FXR also regulates hepatic bile salt synthesis via an autocrine mechanism. Activation of FXR in the distal ileum induces the expression of Fgf15 (the mouse ortholog of FGF19), which is subsequently thought to be released into the portal bloodstream. Unlike for FGF19, detection of plasma FGF15 levels has yet to be accomplished. The secreted FGF15 binds the FGFR4/klotho receptor complex expressed on hepatocytes. This induces the activation of various signal transduction routes, including the jun N-terminal kinase/extracellular signal-regulated kinase (JNK/ERK) pathway. Thus, the ability of FXR to sense and act on bile salt concentration both in the liver and in the terminal ileum allows for a potent and elegant feedback machinery to tightly regulate bile salt synthesis from distinct locations within the enterohepatic circulation. Although FXR is considered the major regulator of bile salt synthesis, other pathways likely are involved as well. This, however, is beyond the scope of this review and is discussed in detail elsewhere.

Bile Salt Sequestrants

Stimulating fecal bile salt loss results in increased bile salt synthesis from cholesterol in the liver. This leads to a relative deprivation of hepatic microsomal cholesterol content, which causes upregulation of the low-density lipoprotein receptor (LDLR) as well as 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase (HMGR). Consequently, low-density lipoprotein (LDL) uptake from plasma is increased, which leads to a reduction in plasma LDL-C. Expression levels of LDLR and HMGR are under the control of sterol regulatory element binding proteins (SREBPs) in a sterol-dependent manner, i.e., low sterol levels induce proteolytic cleavage of SREBP2 and translocation into the nucleus, where it induces transcription of its target genes. Via the reverse mechanism, high hepatic cholesterol content leads to reduced expression of LDLR in humans with CYP7A1 deficiency. These patients have reduced clearance of very-low-density lipoprotein (VLDL) particles and increased conversion of VLDL to LDL. In addition, CYP7A1-deficient humans are resistant to HMGR inhibitors, which was hypothesized to be due to altered hepatic compartmentalization of CYP7A1 and HMGR. Interestingly, Cyp7a1-null mice are normolipidemic and do not show hepatic cholesterol accumulation.

Bile salt sequestrants reduce the reabsorption of bile salts from the intestine and, hence, increase their excretion into feces. Although bile salt sequestrants have been shown to be effective in lowering LDL-C, modest increases in plasma triglyceride levels have been reported. Pharmacological inhibition of the intestinal apical sodium-dependent bile salt transporter (ASBT/ISBT/SLC10A2) by SC-435 decreased LDL-C levels in atherosclerosis-susceptible ApoE−/− mice. Interestingly, CDCA increased LDLR expression levels and activity in vitro.
CDCA treatment in humans did not affect LDLR expression levels.40 The exact effect of CDCA treatment on the expression of the LDLR is therefore yet to be defined. In addition, this discrepancy indicates the importance of crosstalk between intestine and liver. Consistent with the role of FXR in repression of bile salt synthesis, treatment of wild-type mice but not Fxr−/− mice with the FXR antagonist guggulsterone reduced hepatic cholesterol levels.41 Nevertheless, concerns have been raised about the specificity of this antagonist.42 Unfortunately, so far there have been no reports on the existence of more specific antagonists.

**FXR and Lipid Metabolism**

Elevated plasma LDL-C and triglyceride levels and reduced HDL cholesterol (HDL-C) levels are associated with an increased risk to develop CVD. Bile salts exert a broad regulatory role in systemic lipid metabolism via FXR. Gallstone patients treated with CDCA showed decreased plasma triglyceride levels and reduced VLDL production.43 These results were confirmed in mouse studies using synthetic FXR agonists.44,45 Both adenovirus-mediated overexpression of FXR and treatment with the potent FXR agonist GW4064 lowered plasma cholesterol levels in mice.44 Moreover, the FXR agonist WAY-362450 was shown to reduce serum cholesterol levels in mouse, rat, and hamster.46 In addition, Fxr−/− mice have increased triglyceride and VLDL levels compared with their wild-type littermates.15,47 Although initially Fxr−/− mice were reported to have increased plasma HDL-C levels only on cholesterol feeding,15 later studies showed that Fxr−/− mice have markedly elevated plasma HDL-C levels on normal chow feeding.48,49 Thus, the expression and activity of FXR seem to be associated with both anti- and proatherosclerotic lipid profile properties. Although the exact molecular bases of these observations are yet to be determined, an increasing body of evidence on the regulatory role of FXR in lipid metabolism is emerging. Bile salts and synthetic FXR agonists were shown to repress the transcription of Srebp-1c, a key regulator in triglyceride synthesis.50 In agreement with this, Srebp-1c and fatty acid synthase (Fas) expression levels were found to be induced in Fxr−/− mice.51 These data suggest that activated FXR inhibits de novo lipogenesis in liver. Conversely, CDCA treatment has been shown to reduce VLDL production without significantly altering the expression levels of Srebp-1c in fructose-fed hamsters.52 FXR-mediated changes in Srebp-1c expression and activity might therefore be species-dependent and are possibly influenced by diet. Indeed, intermediates of the pentose phosphate pathway have been shown to upregulate Fxr expression in rat hepatocytes, thereby providing evidence for a direct regulatory role of glucose on Fxr.53 Moreover, a direct role for FXR in the expression of glucose-regulated genes has been proposed. FXR activation has been shown to repress the induction of the carbohydrate response element–binding protein target genes Lpk, Acc-1, and S14 under high-glucose conditions, whereas in Fxr−/− mice, these genes were induced under similar conditions.54 FXR-mediated modification of carbohydrate response element–binding protein target gene expression may thus alter lipogenic substrate availability and add to the observed alterations in hepatic triglyceride levels in Fxr−/− mice.

Besides its effects on lipogenesis, FXR influences the transcription of genes involved in lipoprotein metabolism. FXR agonists induce the transcription of Apoc2, which encodes the apolipoprotein that activates lipoprotein lipase. In contrast, FXR agonists reduce the transcription of Apoc3, a major constituent of VLDL that inhibits lipoprotein lipase55,56. In addition, the VLDL receptor (VLDLR) was identified as an FXR target gene in the liver.57 VLDLR is highly homologous to LDLR but is present only in trace amounts in the liver. However, this protein is highly expressed in skeletal muscle, heart, adipose tissue, and macrophages, and its expression in liver has been shown to complement the dyslipidemic profile associated with familial hypercholesterolemia.58 FXR also negatively regulates the expression of microsomal triglyceride transfer protein and apolipoprotein B (APOB).59 Microsomal triglyceride transfer protein is involved in the transfer of triglycerides, cholesterol esters, and phospholipids to newly synthesized APOB and thereby plays a critical role in the assembly and secretion of VLDL and chylomicrons. In human hepatic cells, FXR has been shown to induce the expression of peroxisome proliferator-receptor α (PPARα).60 Alterations of lipid metabolism in humans could therefore involve FXR-mediated induction of PPARα. Because the FXRE is not conserved in the murine Ppara promoter, altered lipid profiles in Fxr−/− mice are probably independent of FXR-mediated alterations in Ppara.

Excess cholesterol is transported in HDL particles from the periphery to the liver, where it is secreted into the bile, either as free cholesterol or, upon conversion in the hepatocyte, as bile salt. High HDL levels are generally considered to be cardioprotective.61 Recent data, however, underscore that it is not the HDL level per se but its activity that determines the capacity to transport cholesterol from peripheral tissues to the liver.62,63 The observation that bile salts and GW4064 reduced the expression of APOA1, the major constituent of HDL,48 raises concerns regarding the therapeutic value of FXR agonists. Consistent with this finding, in vivo administration of GW4064 reduced the levels of HDL-C.50 This is consistent with the observation that plasma HDL-C was increased in Fxr−/− mice.48,49

**FXR and Atherosclerosis**

Currently, 3 studies have been published that assessed a direct role for FXR in the initiation and progression of atherosclerosis in mice.47,65,66 These studies were performed in Fxr−/− mice backcrossed on atherosclerosis-susceptible mouse models, ie, Apoe−/− or Ldlr−/− mice.68 Fxr−/− Apo−/− double knockout mice fed a high-fat/high-cholesterol diet showed an increased atherosclerotic lesion size compared with wild-type, Fxr−/−, and Apo−/− mice.65 In agreement with this finding, Fxr−/− Apc−/− double knockout mice showed a more...
atherogenic plasma lipid and lipoprotein profile, eg, increased VLDL-C and LDL-C and reduced HDL-C levels. Surprisingly, atherosclerosis was not detected in FXR−/− mice fed the high-fat/high-cholesterol diet despite tremendously increased plasma VLDL and LDL levels.65

In contrast, 2 other studies have reported that FXR−/− Apoe−/− double knockout mice and FXR−/− Ldlr−/− double knockout mice showed reduced atherosclerotic lesion areas.47,66 In contrast, 1 study reported increased LDL-C and triglycerides levels,66 whereas another reported reduced LDL-C and increased triglyceride levels.47 The explanation for the observed discrepancies between these studies is unclear, and they cannot be ascribed to differences in plasma lipids. Thus, follow-up studies to unravel these discrepancies are urgently needed. Despite these discrepancies, studies on the activation of FXR yielded consistent results. Recently, 2 articles addressing the effects of FXR activation on atherosclerosis have been published.45,69 In 1 study, Apoe−/− mice were fed the synthetic FXR agonist INT-47.69 Activation of FXR reduced aortic plaque formation with efficacy similar to that of rosiglitazone, a potent and specific PPARγ agonist with established antiatherosclerotic effects.69 In another study, both male and female Ldlr−/− and Apoe−/− mice were shown to be protected against diet-induced aortic lesion formation on treatment with the potent synthetic FXR agonist WAY-362450.45 In addition, WAY-362450 prevented increased plasma non-HDL-C levels and hepatic cholesterol contents. Surprisingly, female but not male mice had reduced aortic lesion formation. Although activation of FXR could have important therapeutic value in atherosclerosis, the exact mechanisms involved are still under debate. It is clear that data from animal models that resemble the lipid profiles found in humans (majority of cholesterol in LDL particles) are highly warranted. Guinea pigs could be a suitable small animal model because these animals have LDL and HDL profiles similar to those of humans and are naturally prone to develop atherosclerosis.70 So far, gene knockout is unavailable in these models. Nevertheless, alternative genetic strategies involving short interfering RNA (siRNA)-mediated Fxr knockdown or overexpression of Fxr are technically possible in guinea pigs and could yield results with a higher correlative value for the human situation. In addition, FXR activation strategies such as bile salt or FXR agonist feeding in guinea pigs could yield results that are indicative for FXR activation in the human situation.

**FXR in Vasculature**

Recently, it was shown that FXR is expressed in vascular smooth muscle cells (VSMC) of both normal and atherosclerotic blood vessels.71,72 In addition, FXR is expressed in vascular endothelial cells.73 The use of specific FXR agonists on arterial organ cultures and aortic endothelial cell cultures revealed a role of FXR in these “nonclassical” bile salt target tissues. Administration of GW4064 to organ cultures from the main branches of the superior mesenteric arteries of rabbits provided evidence for a role of FXR in vascular contractility and endothelium-dependent relaxation.74 FXR activation impaired endothelium-dependent relaxation due to a reduced sensitivity of smooth muscle to nitric oxide (NO). In smooth muscle cells, NO induces vasodilatation by increasing the synthesis of cGMP and inhibition of Ca2+ sensitization to contractile elements in VSMC. Thus, FXR stimulation resulted in a hypertensive phenotype, a well-known risk factor in the development of atherosclerosis.75 On the other hand, GW4064 increased eNOS mRNA and protein expression and NO production in isolated endothelial cells, which is suggestive of a hypertensive phenotype.76 It should be noted that the latter study was performed in an endothelial cell line rather than in organ cultures, the latter resembling the in vivo situation more precisely. In line with a correlative hypertensive phenotype, it was found that activation of FXR induced the expression of dimethylarginine dimethylaminohydrolase-1 (DDAH1).77 DDAH1 degrades asymmetrical dimethylarginine, which in turn is a potent nitric-oxide synthase inhibitor. Activation of FXR also resulted in a repression of endothelin 1 (EDN),73 a potent vasoconstrictive peptide.78 Although these data are in agreement with the data obtained from organ tissue cultures,74 it should be noted that the administration of EDN to the GW4064-stimulated in vitro cultured mesenteric arteries did not alter vascular contractility.74 A study in rat aortic smooth muscle cells also linked the activation of FXR to the regulation of vascular tension. Activation of FXR induced vasoconstriction via increased expression of the angiotensin type II receptor (AT2R). AT2R is a key player in the renin-angiotensin system, which regulates blood pressure and inflammation, 2 risk factors for the development of atherosclerosis.79 In contrast, binding of the effector peptide angiotensin II (AT2) to AT2R caused NO-mediated vasodilatation.80 Despite these discrepancies, there is emerging evidence that FXR influences vasomotor tension. Because all of these studies were carried out in either rabbit superior mesenteric arteries, rat endothelial cells or rat aortic smooth muscle cells, the definite proof for the requirement of FXR by means of FXR knockout could not be addressed. Such knockout models are especially required to rule out the possibility that some of the reported effects by the agonist are attributable to off-target effects.

FXR has also been linked to the expression of proteoglycans.81 Proteoglycans are components of the extracellular matrix that regulate cell proliferation, migration, and the activation of growth factors and have been detected at high concentrations in atherosclerotic lesions.82 Proteoglycans are thought to induce retention of lipoproteins within the intima of the vessel wall and could therefore contribute to atherosclerosis.83 An FXR-dependent upregulation of decorin but not of other proteoglycans, such as versican or biglycan, was found in VSMC.84 Interestingly, it was also found that the adenooviral delivery of decorin attenuated atherosclerosis development in Apoe−/− mice.84 The protective properties of decorin on atherosclerosis suggest that it does not act as a retentive molecule, and the interplay of FXR and decorin in the regression of atherosclerosis requires further investigation.
Paraoxonase 1 (PON1) is produced by the liver and secreted into plasma, where it is incorporated in HDL particles.\textsuperscript{85} PON1 exhibits phospholipase A2 activity, which might play a role in the inactivation of proatherogenic inflammatory lipids produced by oxidative modification of LDL. Interestingly, hepatic \textit{Pon1} transcription levels were found to be repressed on bile salt feeding in mice in a fibroblast growth factor 15/19 dependent manner.\textsuperscript{86} This reduction was accompanied by a reduction of HDL-C levels, which is in agreement with previously reported negative correlations between FXR activation and HDL-C levels.\textsuperscript{50}

**FXR and Inflammation**

Inflammation plays a role in almost every aspect of the progression of atherosclerosis, eg, the progression of fatty deposits to advanced lesions, plaque formation, and thrombosis.\textsuperscript{87} Recently, it was shown that inflammation impaired reverse cholesterol transport, ie, cholesterol efflux from lipid-laden foam cells onto circulating HDL for transport to the liver for secretion into bile and feces.\textsuperscript{88} Several studies assessed the impact of bile salt signaling on inflammation in both vessel wall and liver. Activation of FXR in VSMC was shown to inhibit the inflammatory response and the migration of VSMC.\textsuperscript{71,89} In addition, FXR activation in VSMC induced \textit{SHP} expression and down-regulation of \textit{COX}-2 and \textit{iNOS}, important contributors to vascular inflammation and the migration of VSMC.\textsuperscript{89}

Treatment of \textit{C57BL/6} mice with the FXR agonist WAY-362450 attenuated lipopolysaccharide-induced serum amyloid P component and serum amyloid A3 mRNA levels in the liver. This effect was not observed in \textit{Fxr}\textsuperscript{-/-} mice.\textsuperscript{90} Loss of \textit{Fxr} function was associated with increased inflammatory gene expression and extensive aortic plaque formation in \textit{Apoe}\textsuperscript{-/-} mice fed a high-fat, high-cholesterol diet.\textsuperscript{65} These data provide new evidence for direct anti-inflammatory properties of FXR in atherosclerosis.

Because \textit{Fxr}\textsuperscript{-/-} mice display increased liver inflammation,\textsuperscript{91} FXR was suggested as a modulator of this process. Nuclear factor \textit{κB} is considered a major regulator of the inflammatory response and was shown to reduce the expression of the nuclear steroid and xenobiotic receptor (\textit{PXR}).\textsuperscript{92} Interestingly, FXR activation antagonized nuclear factor \textit{κB} signaling, which is suggestive of a crosstalk between nuclear factor \textit{κB} and FXR.\textsuperscript{93} More evidence for crosstalk between inflammatory pathways and FXR signaling came from a study performed in macrophages in which interferon-γ repressed \textit{Fxr} gene expression.\textsuperscript{94} Moreover, expression levels of \textit{Fxr} and the FXR target genes \textit{IBABP} and \textit{SHP} correlated with expression levels of the chemokines interleukin 8 and macrophage inflammatory protein-3α in Barrett epithelium, a condition in which bile salts injure the esophageal mucosa because of chronic gastroesophageal reflux. In vitro activation of FXR was shown to induce recruitment of immune cells in Barrett epithelium.\textsuperscript{95}

**FXR in Macrophages**

A key event in atherosclerosis is apoptosis of lipid-scavenging macrophages or foam cells resulting in the formation of a necrotic core in an atheroma.\textsuperscript{96} Although it was shown that FXR was not expressed in THP-1 cells differentiated into macrophages and peritoneal macrophages,\textsuperscript{56,97} recent studies showed that \textit{Fxr} is expressed in RAW264.7 macrophages and in blood-derived macrophages.\textsuperscript{69} The contribution of FXR to gene regulation in macrophages to atherosclerosis is still unclear. It was found that \textit{Cd36} levels were reduced in macrophages isolated from \textit{Fxr}\textsuperscript{-/-} mice.\textsuperscript{69} \textit{Cd36} is a member of the class B scavenger receptor family of cell surface proteins and is important for the uptake of oxidized LDL and free fatty acids.\textsuperscript{98,99} In contrast, activation of FXR by INT-747 reduced the expression of \textit{Cd36} in \textit{Fxr}-expressing macrophages.\textsuperscript{69} Furthermore, stimulation of FXR in macrophages attenuated lipopolysaccharide-induced generation of the proatherosclerotic cytokines interleukin-1β, interleukin-6, and tumor necrosis factor α. Importantly, this effect was not seen in macrophages from \textit{Fxr}\textsuperscript{-/-} mice.\textsuperscript{69} In addition, INT-747 could rescue the decrease of \textit{Abca1} levels found in macrophages from \textit{Apoe}\textsuperscript{-/-} mice. ABCA1 acts together with ABCG1 to remove cholesterol from macrophages and load it on to APO-A1 containing HDL particles.\textsuperscript{100} Although these data suggest that FXR in macrophages could play a role in “unloading” cholesterol from these cells, the exact contribution of FXR in macrophages remains controversial, particularly as endogenous ligands are unknown. Future studies using a conditional knockout approach to selectively knock out \textit{Fxr} in macrophages, as reported for \textit{Ppar},\textsuperscript{101} could reveal the importance of FXR in macrophages on atherosclerosis.

**Bile Salt Signaling, Obesity, and Energy Metabolism**

Obesity is associated with an increased risk of developing CVD. Excess adipose tissue can lead to dysregulation of adipocytokine production. This can promote a state of low-level systemic inflammation, which has been implicated in the development of atherosclerosis.\textsuperscript{102} A role for FXR in adipocyte function came from the observation that \textit{Fxr}\textsuperscript{-/-} mice have reduced fat cell size.\textsuperscript{103} In addition, activation of FXR promotes adipocyte differentiation and function in vivo and in vitro.\textsuperscript{104}

The progression rate of atherosclerosis in obese people can be significantly reduced by weight loss.\textsuperscript{105} Since bile salts have emerged as important regulators of whole body energy homeostasis, bile salt-activated signaling pathways might serve as an attractive target to increase energy expenditure and, hence, weight loss.\textsuperscript{106} Although in humans the role for bile salts as regulators of energy metabolism is not fully defined, data from mouse studies strongly suggest that bile salts could serve as important modulators of energy metabolism. \textit{Fxr}\textsuperscript{-/-} mice have an accelerated entry into torpor on fasting and cold exposure compared with wild-type littermates. A regulatory role for FXR in thermogenesis was hypothesized to be due to low triglyceride and glycogen stores in the white adipose tissue and livers, respectively, of \textit{Fxr}\textsuperscript{-/-} mice.\textsuperscript{107}

Brown adipose tissue (BAT) is the main regulating organ in energy expenditure in mice. However, both \textit{Fxr}
and Shp could not be detected in BAT,\textsuperscript{107,108} which would exclude a direct role of the bile salt-FXR signaling pathway in energy expenditure. Evidence for a role of bile salts in energy metabolism via FXR-independent means was reported by Watanabe et al, who observed that bile salts could prevent development of obesity and insulin resistance by increasing energy expenditure in BAT during high-fat feeding in mice.\textsuperscript{108} Bile salts are suggested to exert their action on energy metabolism via modulation of local thyroid hormone production in brown adipose tissue (rodents) and muscle (humans) via TGR5.\textsuperscript{109,110} Activation of TGR5 by bile salts induces the conversion of inactive to active thyroid hormones via type 1 and type 2 iodothyronine deiodinase (D1 and D2).\textsuperscript{111} Thyroid hormones are produced by the thyroid gland and act to increase metabolic rate.

In adult humans, muscle is considered the most important thermogenic organ. Nevertheless, there is emerging evidence that a substantial fraction of the adult human population does possess active BAT.\textsuperscript{115} Interestingly, human myocytes were shown to express D2 and TGR5.\textsuperscript{108} In addition, thyroid hormones could be activated by D2 in myocytes.\textsuperscript{116} Studies in which the role of bile salts in energy metabolism in human muscle is assessed are still lacking. Bile salts have been suggested as regulators of metabolic rate by inducing the production of the incretin glucagon-like-peptide 1 (GLP-1) in a Tgr5-dependent manner in vitro.\textsuperscript{117} Recently, it was shown that in vivo stimulation of TGR5 had beneficial effects on glucose homeostasis and increased energy expenditure via, among others, increased incretin secretion in mice.\textsuperscript{118} Incretins control initiation of satiety and glucose-induced insulin secretion. These results suggest that bile salts could serve as important regulators of energy expenditure. Both FXR and TGR5 could serve as attractive targets for weight loss, thereby reducing the development or progression of atherosclerosis.

**FXR Elsewhere**

FXR is expressed in tissues not directly associated with bile salt transport, synthesis, or metabolism, such as the adrenals, kidney, and vasculature.\textsuperscript{71,119} Relatively high FXR expression levels have been detected in the adrenal cortex, as well as the renal tubules of the kidney.\textsuperscript{120} Little is known about the function of FXR in these tissues. The organic solute transporters α and β (OSTα and OSTβ) are direct FXR target genes in both the adrenal gland and the kidney.\textsuperscript{119} OSTα and OSTβ are transporters of bile salts and conjugated steroids localized at basolateral membranes in intestine and liver.\textsuperscript{121} These results are sugges-
tive of a role for FXR in bile salt resorption in the kidney and steroid homeostasis in the adrenal cortex. In line with this, it was found that the enzyme 3β-hydroxysteroid dehydrogenase isomerase type II (HSD3B2), involved in the synthesis of aldosterone and cortisol, is regulated by FXR.122 Aldosterone is a mineralocorticoid synthesized in the adrenal cortex from cholesterol and an indirect modulator of vascular tension by modulating renal salt and water retention. In addition, aldosterone has a direct effect on vascular tension by binding mineralocorticoid receptors on both endothelial and smooth muscle cells. Expression of these receptors was increased in a hypertension-susceptible rat model.123,124 The induction of HSD3B2 could therefore link adrenal FXR function to atherosclerosis. In the vasculature, aldosterone impairs endothelium-dependent relaxations, promotes inflammation, and stimulates vascular remodeling.125 Interestingly, a possible link between atherosclerosis and inflammation induced by aldosterone was supported by the observation that eplerenone, a selective blocker of mineralocorticoid receptors, inhibits atherosclerosis in nonhuman primates.126,127

**Conclusion**

Taken together, the data available to date suggest a potential role of bile salts, the bile salt receptor FXR, and possibly TGR5 in the progression and regression of atherosclerosis. However, the molecular mechanisms and the net effects of bile salts are yet to be determined. From the current data, it is clear that the effects of bile salts and FXR and TGR5 signaling pathways on atherosclerosis represent a multiorgan and multifactorial interplay reaching beyond the enterohepatic circulation (summarized in the Figure).

The preclinical phase of atherosclerosis in humans is long; pathological changes in arteries can develop decades before the disease presents clinically, and they are dependent on a complex interaction between genetic and epigenetic factors. Studies of early (plasma) markers of atherosclerosis have a reduced risk of developing CVD, at least in the premenopausal period.129 Therefore, caution should be taken in extrapolating results obtained from mouse models of atherosclerosis-prone mouse models occurs predominantly in females.128 This is in contrast to humans, where females have a reduced risk of developing CVD, at least in the premenopausal period.129 Therefore, caution should be taken in extrapolating results obtained from mouse models of atherosclerosis to humans. Humanized mouse models such as the CETP.E3L mouse, which displays increased LDL-C and triglyceride levels and decreased HDL-C levels130 are prone to develop atherosclerosis on a Western-type diet and are therefore valuable models to study the effects of bile salt receptor signaling on hallmarks of atherosclerosis. The use of tissue- and organ-specific Fxr−/− and Tgr5−/− mice backcrossed to an atherosclerosis-susceptible mouse strain may provide a means to assess tissue-specific involvement of bile salt receptors in the development or regression of atherosclerosis. Alternatively, the use of short hairpin RNA approaches targeting Fxr or Tgr5 in animal models that naturally match the HDL profile of humans, such as guinea pigs, are a plausible strategy to verify the results obtained using the atherosclerotic transgenic mouse models.

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None.

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