The Farnesoid X Receptor
A Molecular Link Between Bile Acid and Lipid and Glucose Metabolism

Thierry Claudel, Bart Staels, Folkert Kuipers

Abstract—Bile acids are the end products of cholesterol metabolism. They are synthesized in the liver and secreted via bile into the intestine, where they aid in the absorption of fat-soluble vitamins and dietary fat. Subsequently, bile acids return to the liver to complete their enterohepatic circulation. The Farnesoid X receptor (FXR) is a member of the nuclear receptor superfamily and has emerged as a key player in the control of multiple metabolic pathways. On its activation by bile acids, FXR regulates bile acid synthesis, conjugation, and transport, as well as various aspects of lipid and glucose metabolism. This review summarizes recent advances in deciphering the role of FXR in the context of hepatic lipid and glucose homeostasis and discusses the potential of FXR as a pharmacological target for therapeutic applications. (Arterioscler Thromb Vasc Biol. 2005;25:0-0.)

Key Words: bile acid ■ FXR ■ glucose metabolism ■ lipid ■ nuclear receptor

As a direct consequence of the obesity epidemic,1–3 the prevalence of the metabolic syndrome is increasing at an alarming rate.4 Almost 25% of the adult US population is currently affected, and the situation is even worse in people older than 60 years.5 The metabolic syndrome has been defined by the National Cholesterol Education Program as a cluster of at least 3 of 5 criteria: insulin resistance and glucose intolerance, abdominal obesity, hypertension, low high-density lipoprotein (HDL) cholesterol, and hypertriglyceridemia.6 Given the fact that the metabolic perturbation associated with the metabolic syndrome predisposes to cardiovascular diseases and stroke,7 novel and more specific therapeutical strategies are urgently needed. Certain ligand-activated nuclear receptors provide promising new targets for this purpose. In this review, we discuss the biology of the Farnesoid X receptor (FXR, NR1H4), recently identified as a modulator of lipid and glucose homeostasis.

Nuclear receptors are transcription factors that, on ligand binding by specific molecules and cofactor recruitment, regulate the expression of specific target genes.8 FXR is highly expressed in liver and intestine9 and was cloned during a search for new Retinoid X receptor (RXR or NR2B1) heterodimers.9,10 Originally described as a farnesol-activated receptor interacting with RXR, and accordingly named, FXR was later identified as a receptor that is activated by bile acids (Figure 1).11–13 Subsequently, triterpenoids like forskolin were shown to increase FXR activity in a cell-based assay.14 More recently, polyunsaturated fatty acids (PUFA) like arachidonic, linolenic, or docosahexaenoic acid,15 as well as intermediates of the bile acid synthetic pathways,16 were shown to be FXR ligands and modulators in vitro. Bile acid intermediates could be important FXR ligands during cholestasis or inborn metabolic disorders when these compounds can potentially be present in large amounts. In addition, several pharmacological FXR ligands have been generated.17–20

After ligand binding, FXR binds to DNA elements called FXR response elements (FXREs). Interestingly, FXR can bind to and activate or repress through a large variety of FXREs21 either as a monomer22,23 or as a FXR/RXR heterodimer24 (Figure 2). The recent identification of several FXR cofactors,25–28 together with the description of the ligand-binding domain structure,20,29 will help to elucidate how the FXR complex is stabilized and interacts with RNA polymerase II to modulate transcriptional activity.

FXR is expressed from a single gene locus in humans (chromosome 12q23.1). Two alternative promoters, with the presence of an internal cryptic splicing site, lead to the expression of 4 isoforms called FXRα1/FXRα2 and FXRα3/FXRα4 (referred to as FXRβ1/FXRβ2)30 that are not equivalent in term of gene transactivation.30 Interestingly, the organization of the FXR locus is conserved in rodents.30,31 A nuclear receptor activated by lanosterol was also called FXRβ,32 but because it is a pseudo-gene in primates and because its expression pattern in rodents is confined to the reproductive tract, it is not discussed in this review.

FXR Regulates Bile Acid Synthesis, Transport, and Detoxification
Bile acids are synthesized from cholesterol exclusively by the liver. The biosynthetic steps that collectively accomplish the
conversion of water-insoluble cholesterol molecules into more water-soluble compounds also confer detergent properties to the bile acids that are crucial for their physiological functions in bile formation and intestinal fat absorption. On conjugation with glycine or taurine, bile acids are actively secreted by the hepatocytes into the bile canaliculi that drain into intrahepatic bile ducts, stored in the gallbladder, and expelled into the intestinal lumen in response to a fatty meal. In the small intestine, bile acids act as detergents to emulsify and facilitate the absorption of dietary fats and lipid-soluble vitamins. Subsequently, they are reabsorbed from the terminal ileum by specific transporter proteins: 95% return to the liver to be secreted again into the bile, completing the so-called enterohepatic circulation, whereas 5% escape reabsorption and are lost via the feces. The fraction that is lost per cycle is compensated for by hepatic synthesis from cholesterol, which maintains the bile acid pool size constant. Although the fractional loss per cycle is relatively small, daily bile acid synthesis in adult humans amounts up to ~500 mg, which accounts for ~50% of total cholesterol turnover.

Two major pathways, generally referred to as the neutral and the acidic pathway, are involved in bile acid synthesis. CYP7A1 is the first and rate-limiting enzyme of the synthetic pathway. FXR induces the expression of BACS and BAT, which are involved in bile acid conjugation. FXR activates the expression of the bile acid export transporters MRP2 and BSEP and simultaneously represses bile acid import by downregulation of NTCP and possibly OATP-C. FXR induces also the expression of the CYP3A4, UGT2B4, and SULT2A1 enzymes involved in the detoxification of bile acids. Subsequently, sulfated/glucuronidated bile acids are exported by MRP2 into the canalicus. At the intestinal level, FXR induces the expression of IBAB-P, a potential bile acid shuttle, and influences the import of bile acids by interfering with the transcription factor network controlling ASBT.

Figure 1. Structure and activation potency of several bile acids on human FXR.

Figure 2. Various modes of FXR gene regulation. FXR can induce transcription as a monomer (UGT2B4) or a heterodimer with RXR (PLTP). FXR can repress indirectly through the induction of SHP (CYP7A1), or directly as a monomer (apoA-I) or a heterodimer (apo CIII).

Figure 3. Overview of bile circulation between the liver and the intestine. FXR negatively regulates bile acid production by repressing CYP7A1 the rate-limiting enzyme of the synthetic pathway. FXR induces the expression of BACS and BAT, which are involved in bile acid conjugation. FXR activates the expression of the bile acid export transporters MRP2 and BSEP and simultaneously represses bile acid import by downregulation of NTCP and possibly OATP-C. FXR induces also the expression of the CYP3A4, UGT2B4, and SULT2A1 enzymes involved in the detoxification of bile acids. Subsequently, sulfated/glucuronidated bile acids are exported by MRP2 into the canalicus. At the intestinal level, FXR induces the expression of IBAB-P, a potential bile acid shuttle, and influences the import of bile acids by interfering with the transcription factor network controlling ASBT.
Bile acids are conjugated to taurine or glycine, by sequential actions of the enzymes bile acid coenzyme A (CoA) synthetase (BACS) and the bile acid-CoA amino acid N-acetyltransferase (BAT), to increase their hydrophilicity in a process regulated by FXR. Conjugated bile acids require a transporter network to convey bile acids from the basolateral side of the enterocytes to traffic from the apical membrane to the portal vein either by a truncated form of ASBT (tASBT),66 or by the newly described Ost carriers68 (Figure 3).

The uptake of bile salts that return to the liver after intestinal absorption is mainly mediated by the Na+-taurocholate cotransporting polypeptide (NTCP or SLC10A1).69,70 Bile acids downregulate NTCP expression via a FXR-dependent mechanism, but NTCP expression is not changed in FXR-deficient mice compared with wild-type controls.65,71 A potential mechanism involves SHP activation that inhibits RXR/RAR (retinoic acid receptor or NR1B1) transactivation of the promoter72 (Figure 3). Approximately 75% of uptake occurs by this Na+-dependent process; yet, another family of transporters is also involved, the organic anion transporter polypeptides (OATPs). OATP-C (or SLCO1B1) is the most ubiquitously expressed OATP transporter in human hepatocytes, but it is currently unknown whether FXR is involved in its regulation (Figure 3). In mice, it has been reported that bile acids regulate OATP-1 (or Slco1a1) expression,71,73 but the basal expression of OATP-1 was not modified in FXR-deficient mice.65,71 Taken together, FXR activation in hepatocytes will suppress de novo bile acid synthesis, accelerate their biliary excretion and detoxification, and simultaneously limit their reutilization by ileal resection increase levels of HDL cholesterol and of apo A-I levels.22 Furthermore, patients undergoing bile acid sequestrant therapy78–82 or after ileal bypass surgery77 display an enhanced intestinal bile acid absorption despite an extremely low IBAB-P expression64. However, the fact that FXR-deficient mice were found to display enhanced intestinal bile acid absorption despite an extremely low IBAB-P expression65 demonstrates that the real physiological function of IBAB-P remains unresolved. At the basolateral side, bile acids are believed to be secreted into the portal vein either by a truncated form of ASBT (tASBT),66 or by the newly described Ost carriers68 (Figure 3).

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conversion of cholesterol into bile acids is stimulated. The depletion of hepatic (microsomal) cholesterol leads to increased SREBP2 (sterol regulatory element binding protein 2) activity, which gives rise to induction of LDL receptor expression that accounts for the decline in total and LDL cholesterol. However, the increase in HDL cholesterol and triglyceride levels could not be explained by this metabolic adaptation. Therefore, we and other groups started to investigate whether FXR “deactivation” could explain the lipid phenotype that results from interrupting the enterohepatic cycle of bile acids.

**FXR and HDL Metabolism**

HDL carries cholesterol from the peripheral organs to the liver, where it can be excreted into the bile as either free cholesterol or after conversion into bile acids. FXR-deficient mice are hypercholesterolemic because of an increase in HDL cholesterol levels and show elevated plasma apo A-I concentrations. In human apo A-I transgenic mice, FXR activation decreased total cholesterol because of a reduction of HDL cholesterol. In these transgenic mice, feeding of the FXR agonist taurocholic acid reduced mouse as well as human apo A-I gene expression in liver and plasma concentrations of the protein. In vitro, the synthetic FXR agonist GW4064 and natural bile acids reduced apo A-I expression both in human primary and immortalized hepatocytes and show elevated plasma apo A-I concentrations. In human apo A-I transgenic mice, FXR activation decreased total cholesterol because of a reduction of HDL cholesterol. In these transgenic mice, feeding of the FXR agonist taurocholic acid reduced mouse as well as human apo A-I gene expression in liver and plasma concentrations of the protein.

Because several species-differences were reported with respect to the role of LRH-1 in bile acid synthesis regulation, it is unfortunate that the authors used mouse LRH-1 to investigate the regulation of human apo AI. Therefore, although it is possible that LRH-1 and FXR act in a complementary manner to regulate apo A-I transcription, more studies are needed to solve this issue.

FXR induces also the expression of the phospholipid transfer protein (PLTP), an enzyme involved in HDL remodeling (Figure 5). Moreover, bile acid treatment of cerebrotendinous xanthomas (CTX) patients increased cholesterol ester transferase protein (CETP) activity. CETP exchanges cholesterol esters from HDL with triglycerides in triglyceride-rich lipoproteins. Unlike humans, rodents do not express CETP. Low CETP activity in CTX patients with high HDL cholesterol levels might cause accumulation of cholesterol esters in peripheral organs. Intriguingly, bile acids induced or repressed human CETP gene expression in transgenic mice in a sex-dependent manner, i.e., an upregulation in females and a downregulation in males was observed, an

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**Table 1. Metabolic Effects of Bile Acid Sequestrants and Intestinal Resection**

<table>
<thead>
<tr>
<th>Study (ref)</th>
<th>Total Cholesterol</th>
<th>LDL Cholesterol</th>
<th>HDL Cholesterol</th>
<th>Triglyceride</th>
<th>Comments and/or Results</th>
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<td>Shepherd76</td>
<td>−32%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NHLBL type II Levy/Brensicke78,80</td>
<td>−17%</td>
<td>−26%</td>
<td></td>
<td>+8%</td>
<td></td>
</tr>
<tr>
<td>LRC-GPPT158-161</td>
<td>−13%</td>
<td>−20%</td>
<td>+2%</td>
<td>+10%</td>
<td></td>
</tr>
<tr>
<td>Betteridge82</td>
<td>−23%</td>
<td>−31%</td>
<td></td>
<td>+18%</td>
<td>Comparison pravastatin/cholestyramine</td>
</tr>
<tr>
<td>POSCH77</td>
<td>−23%</td>
<td>−37%</td>
<td>+4% apo A-I</td>
<td>+19%</td>
<td>Intestinal resection decreased chol absorption and increased fecal sterol excretion, decreased BA re-absorption</td>
</tr>
</tbody>
</table>

BA indicates bile acid; CAD, coronary artery disease; CHD, coronary heart disease; Chol, cholesterol; VLDL, very-low-density lipoprotein.
effect that could be mediated by LRH-1 (Figure 5). Because the authors did not study FXR-deficient mice, it is not possible to assess the relative importance of FXR and LRH-1 in the regulation of CETP by bile acids, an issue that clearly needs further clarification. Together, these studies have established that FXR provides a link between bile acids and HDL metabolism and the existence of coordination between cholesterol transport in the plasma compartment and its catabolism in the liver.

FXR and Triglyceride Metabolism

Chow-fed FXR-deficient mice are clearly hypertriglyceridemic, suggesting, in line with the clinical studies mentioned, a role for FXR in the control of triglyceride metabolism.

Because serum triglyceride levels reflect the balance between production and clearance of triglyceride-rich lipoproteins such as very-low-density lipoprotein and chylomicrons, and because lipoprotein lipase (LPL) is a key enzyme involved in the lipolysis of these particles, several groups have explored the effects of FXR activation on LPL cofactors. Apolipoprotein (apo) C-III is an inhibitor of LPL activity, whereas apo C-II and apo A-V are LPL activators. FXR activation induces apo C-II expression and human apo A-V promoter activity in liver cells. However, natural and synthetic FXR agonists were found to repress hepatic apo C-III expression in mice and in human primary hepatocytes. Interestingly, promoter analysis, together with chromatin immunoprecipitation assays, revealed that the FXR/RXR heterodimer represses apo C-III expression. Moreover, FXR induces the expression of the very-low-density lipoprotein receptor, a protein that plays a major role in the metabolism of postprandial lipoproteins by enhancing LPL-mediated triglyceride hydrolysis. In addition, expression of syndecan-1, a transmembrane protein that binds remnant particles before their transfer to receptors, was found to be FXR-sensitive. Thus, FXR controls a variety of genes crucially involved in triglyceride metabolism in the blood compartment.

Surprisingly, FXR also regulates the expression of peroxisome proliferator-activated receptor (PPAR)α in humans. PPARα is a nuclear receptor that is activated by fatty acids and by fibrates, a class of synthetic hypolipidemic drugs. Because PPARα activation decreases plasma triglyceride levels, probably by enhancing fat oxidation, but also modulates bile acid composition and synthesis, this implies that FXR controls bile acid and triglyceride metabolism both directly and indirectly and that a coordinated regulation of fatty acid and bile acid metabolism occurs also in humans.

Several studies have shown expression of the FXR target gene CYP7A1 to be reduced in rats and piglets during fasting, i.e., a situation in which PGC1α (PPARγ coactivator 1α) expression is induced. Subsequently, PGC1α was identified as a new FXR cofactor. Intriguingly, whereas 2 groups demonstrated that PGC1α was recruited in a ligand-dependent manner and through the interaction between a charge clamp (helix 3 and 12) on FXR and the LXXLL motif of PGC1α, another group showed that FXR interacts with PGC1α in a ligand-independent manner via its DNA binding domain without requirement of the LXXLL domain of PGC1α. These latter investigators suggested a specific role for FXR in triglyceride metabolism during fasting, because triglyceride levels were increased in fasted FXR-deficient mice instead of being decreased as it is observed in fasted wild-type mice. This was mechanistically ascribed to inhibition of SREBP1c expression because of induction of SHP expression by FXR/RXR/PGC1α. The fact that, according to these authors, PGC1α and FXR interact in a ligand-independent manner implicates that bile acids are not directly involved in the control of these pathways. In contrast, other investigators demonstrated that SREBP1c expression is negatively regulated by bile acids via SHP induction. These discrepancies could be caused by the fact that some investigators studied human whereas others studied murine FXR in vitro systems. In addition, the synthetic agonist used in one of these studies is known not to regulate the same genes as natural bile acids do, probably because of the so-called specific bile acid receptor modulator (SBARM) effect that postulates that FXR adopts a different conformation on binding of chemically distinct ligands. Intriguingly, with respect to the FXR/PGC1α pathway, other groups studying the consequences of fasting in mice showed that Cyp7A1 expression is stimulated by PGC1α. This would suggest that induction of PGC1α during fasting is not linked to FXR signaling. The events that dictate the specificity of PGC1α interactions with FXR or other nuclear receptors in complex situations like fasting need to be addressed in more detail in the future. Taken together, FXR decreases triglyceride levels by: (1) increasing their clearance by modulating LPL activity; (2) inducing PPARα in humans; and probably (3) inhibiting SREBP1c in mice.

Role of FXR in Glucose Homeostasis

A first piece of evidence for a link between bile acid and glucose metabolism came from a short-term study in patients with noninsulin-dependent diabetes mellitus (NIDDM) or type II diabetes. Patients with high LDL cholesterol but normal triglyceride levels, using either glyburide or insulin to control glycaemia, were treated with cholestyramine or placebo. Cholestyramine treatment lowered plasma glucose by 13% and decreased urinary glucose excretion, with a tendency toward lower glycosylated hemoglobin concentrations. At the same time, cholestyramine reduced total and LDL cholesterol and increased triglyceride levels. This study therefore identified bile acid sequestrants, which are not absorbed, as a potential option to treat type II diabetes. It will be of interest to determine whether the ASBT inhibitors, like S 8921 or SC 435, that selectively interfere with bile acid reabsorption also regulate glycaemia in diabetic patients. Nevertheless, because an increase of unbound bile acids entering the colon might have adverse consequences, it will be necessary to evaluate the intestinal effects of this treatment in detail before assessment of their potential impact in terms of metabolism.

More recently, studies demonstrated a link between triglyceride levels and gallbladder diseases. Because FXR controls triglyceride metabolism, because hypertriglyceridemia is associated with type 2 diabetes, and because bile
composition is altered in diabetic patients. Evaluation of a potential link between FXR and glucose metabolism was initiated. First, FXR was identified as a gene positively regulated by glucose. In cultured hepatocytes, glucose was shown to induce FXR gene expression, probably via metabolites of the pentose phosphate pathway, whereas insulin counter-regulated this effect. Moreover, apo CIII gene expression was additively repressed by glucose and the synthetic FXR agonist GW4064 in cultured cells and Cyp7a1 gene expression was inversely correlated with Fxr gene expression in livers of diabetic rats. Given the fact that hypertriglyceridemia is a common feature in diabetes, it is tempting to speculate that FXR dysregulation by glucose participates in the development of the diabetic phenotype or, conversely, that FXR modulation could reverse part of the lipid abnormalities associated with this condition. Interestingly, a patient with homozygous familial hypercholesterolemia (LDL receptor deficiency) was found to have increased synthesis rates of cholesterol and bile acids when fed a normal diet. On a high-glucose diet, this patient showed a decrease in total, HDL, and LDL cholesterol levels, whereas plasma triglyceride levels increased. At the same time, at least one large cutaneous xanthoma disappeared, demonstrating that the decline in plasma cholesterol was not caused by accelerated storage in peripheral organs. Fecal balance studies showed that the high cholesterol and bile acid synthesis rates in this patient decreased on ingestion of the high-glucose diet. Because FXR expression is induced by glucose, because FXR activation decreases HDL cholesterol, and because FXR suppresses bile acid synthesis, it is tempting to speculate that the high-glucose diet increased hepatic FXR expression, which, in turn, was followed by repression of apo AI and CYP7A1 expression. Finally, the fact that not only homozygous familial hypercholesterolemic patients but also normocholesterolemic men on total parenteral nutrition (a diet extremely rich in glucose) display a significant reduction in plasma cholesterol and a decreased excretion of bile acids suggest that glucose interference with FXR signaling is likely to constitute a novel mechanism involved in the control of plasma cholesterol levels.

Recently, in vitro and in vivo data showed that bile acids modulate gluconeogenesis by regulating the expression of the rate-controlling enzyme phosphoenolpyruvate carboxykinase (PEPCK), as well as of glucose-6-phosphatase (G6Pase) and fructose-1,6-bisphosphatase (FBP1). Bile acid treatment in mice reduced gene expression of PEPCK. Subsequently, De Fabiani et al demonstrated that FXR was not required to downregulate PEPCK expression in vitro, because incubation of cells with bile acids reduced hepatic nuclear factor 4α (HNF4α) transactivation of the PEPCK promoter, whereas a synthetic FXR agonist did not. A similar interference of bile acids with HNF4α transactivation potential was already described to explain the negative regulation of CYP7A1 without SHP requirement. HNF4α is a major regulator of PEPCK expression and a master regulator of hepatic gene expression and is also involved in the maturity onset diabetes of the young type 1 (MODY1). More recently, Yamagata et al showed that PEPCK, FBP1, and G6Pase are negatively regulated by bile acid treatment in vivo, effects that they ascribed to induction of SHP, which subsequently interacts with HNF4α, to repress PEPCK and FBP1, or Foxo1 to repress G6Pase, respectively. Nevertheless, the mechanism is still hypothetical because these authors did not eliminate SHP from cells and did not use Shp-deficient mice. Moreover, the physiological consequences of activation of these regulatory pathways under physiological (fasting/feeding transition) or pathophysiological (insulin resistance) conditions of altered glucose homeostasis have not yet been studied.

During the fasting period when gluconeogenesis is induced, Cyp7a1, Pepck, Ppara, and, paradoxically, Fxr gene expression are upregulated. Because Fxr-repressed genes such as Cyp7a1 and Pepck are upregulated, this implies that FXR control is weak in this condition, probably because bile acids are not circulating and are stored in the gallbladder. At the same time, it is important to note that Shp gene expression is not changed during fasting. Therefore, not only is the induction of FXR is important but also is the bioavailability of its ligands crucial to obtain a physiological effect on gene expression.

Finally, it is remarkable that glucose is almost absent in human bile despite the fact that hepatocytes do secrete glucose into bile. Therefore, glucose is effectively re-absorbed from the biliary tract by an active mechanism in a so-called bilihepatic circulation. Because FXR deactivation controls glycemia and glucose controls FXR expression, it will be of interest to determine whether FXR is involved in control of this glucose bilihepatic cycle.

**FXR: Therapeutic Implications**

**Dyslipidemia**
Increasing HDL and lowering LDL cholesterol and triglyceride levels by dietary or pharmacological means remain the most important goals to reach in dyslipidemic patients. Currently, statins are the first choice drugs for the treatment of hypercholesterolemia. Given the fact that “resin effects” (cholestyramine, colestipol, colesevelam) may be partly caused by FXR deactivation, a mechanistic basis for the additive responses on statin and resin combination therapies can now be proposed and may lead to a further enhancement of beneficial effects.

Interestingly, the need for new drugs to treat dyslipidemia in specific patient groups could open new avenues for the development of FXR “mixed agonists.” FXR is at the cross-road of bile acid and lipid metabolism and because FXR modulation changes triglyceride and HDL cholesterol levels in the same direction (Figure 5), a simple FXR agonist or antagonist will have undesired side effects from a therapeutic point of view. The recent description of FXR partial agonists or BARMs that do not activate the entire spectrum of classical FXR targets suggests that such compounds, like AGN-34, can be generated (Table 2). Nevertheless, it is impossible that a single BARM will emerge to cover the wide range of therapeutical goals to treat hypertriglyceridemia, mixed dyslipidemia, or hyperlipidemia, diseases that all have causes that could be solved by FXR modulators.
Recently, much attention has been paid to natural BARM E and Z-guggulsterones, which are the active components of guggulipid, to treat obesity and lipid disorders. In vitro experiments showed that guggulsterone acts as a FXR antagonist and a pregnane X receptor (PXR) agonist. Wild-type mice fed high-cholesterol diet and treated with guggulsterone displayed a decreased hepatic cholesterol content, an effect that was not observed in FXR-deficient mice. Therefore, it was suggested that the hypolipemic properties of guggulsterone were caused by FXR antagonism. However, despite the fact that coactivator assays confirmed that guggulsterone can act as a FXR antagonist, it was shown that guggulsterone is a partial FXR agonist, which induces Bsep and Shp expression but does not downregulate Cyp7a1 or Cyp8b1. Interestingly, guggulsterone raised HDL cholesterol and lowered triglyceride levels in rats, a highly desirable therapeutic goal in humans. Unfortunately, a recent study performed to address guggulsterone effects in humans showed that the compound did not decrease cholesterol levels in patients (Table 2). This discrepancy could be caused by species-specific differences in FXR biology.

Based on available data, however, it is plausible to assume that FXR modulators could provide a new class of drugs for the treatment of specific aspects of the metabolic syndrome and perhaps provide alternatives or complements to existing therapy in the future.

### Treating Cerebrotendinous Xanthomatosis and Inborn Errors in Primary Bile Acid Synthesis

Originally, chenodeoxycholic acid (CDCA), the most potent natural agonist of human FXR (Figure 1), was evaluated to treat either gallstone disease or hypertriglyceridemia (Table 2). However, side effects like liver toxicity and diarrhea led to the withdrawal of the compound from clinical application. Moreover, with respect to the hypertriglyceridemia-lowering effects, CDCA was shown to be only transiently effective, i.e., hypertriglyceridemia was back to initial levels between 6 months and 1 year after the initiation of the treatment. A potential application for CDCA in humans might be in the normalization of lipid levels in cerebrotendinous xanthomatosis (Cyp27A1-deficiency) patients in conjunction with statin treatment. Nevertheless, because FXR induces CYP3A4 expression and because most of the statins are metabolized by CYP3A4, safety issues should be carefully monitored for this combination. It must also be noted that reported improvements of the lipid profile were not translated into gain in brain functions and that the levels of various lipid parameters (cholestrol, lithosterol) remained supraphysiological during treatment. Therefore, it could be of interest to study the influence of more potent FXR agonists than CDCA, like 6-ECDCA (Table 2). Finally, another natural bile acid, cholic acid, was recently tried as an orphan drug (EU/3/02/127) to treat inborn errors in primary bile acid synthesis (Table 2). The first results are promising but will need to be confirmed.

### Treating and Preventing Gallstones

Ursodeoxycholic acid is a weak FXR agonist (Figure 1), and so far the only bile acid with a clear therapeutic application. Ursodeoxycholic acid is used to treat gallstone disease and cholestatic liver diseases (Table 2). Because it was estimated that up to 800,000 patients in the US had gallstones diagnosed during the year 2000 only, the potential market for a better drug to treat this disorder is enormous.

Very recently, Moschetta et al proposed that nonbile acid FXR agonists might be used to prevent or treat patients at risk for cholesterol gallstone disease and acute microlithiasis pancreatitis. This recommendation was based on the presence of supersaturated gallbladder bile in FXR-null mice fed a lithogenic diet and on reduction of biliary cholesterol saturation index in gallstone-prone C57L mice fed the synthetic FXR agonist GW4064. The effects were explained by FXR-mediated induction of BSEP (ABCB11) and MDR3 (ABCB4), allowing for increased bile acid and phospho-

### Table 2. FXR Modulators Approved or in Clinical Development

<table>
<thead>
<tr>
<th>Research Name</th>
<th>Status</th>
<th>Use</th>
<th>Comments and Side Effects</th>
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<td>AGN-34</td>
<td>Phase 3 clinical trial</td>
<td>Hypercholesterolemia or colon cancer</td>
<td>Liver toxicity and metabolism unknown</td>
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<tr>
<td>Guggulsterone</td>
<td>Traditional herbal medicine</td>
<td>Hypercholesterolemia</td>
<td>Efficiency questioned, able to activate several signaling pathways, rash</td>
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<tr>
<td>CDCA (eg, chenofalk)</td>
<td>Not approved in European Union, safety concern, extract from bovine bile</td>
<td>Originally gallstone dissolution, cerebrotendinous xanthomatosis</td>
<td>Liver toxicity, diarrhea, transient effects</td>
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<tr>
<td>6-Ethyl CDCA (INT-647)</td>
<td>?</td>
<td>Liver fibrosis</td>
<td>Side effects and metabolism unknown, tested in rats not in humans</td>
</tr>
<tr>
<td>CA (eg, cholifalk)</td>
<td>Not approved in European Union, safety concern, extract from bovine bile</td>
<td>C27 3beta-hydroxy steroid oxidoreductase deficiency or delta^4-3 o xo-reductase deficiency</td>
<td>Liver toxicity and pruritus</td>
</tr>
<tr>
<td>UDCA (eg, ursofalk, ursodiol)</td>
<td>approved</td>
<td>Gallstone dissolution, cholestatic liver diseases, and PBC</td>
<td>No side effects</td>
</tr>
</tbody>
</table>

CA indicates cholic acid; CDCA, chenodeoxycholic acid; PBC, primary biliary cirrhosis; UDCA, ursodeoxycholic acid.
lipid secretion into bile in the absence of changes in the expression of ABCG5/ABCG8 that control biliary cholesterol secretion. Apart from the fact that the presence of bile supersaturated with cholesterol is a prerequisite for gallstone formation but not by definition leads to gallstone formation, there are a number of issues that require critical evaluation. First, it should be noted that biliary bile acid secretion rates/concentrations are not controlled by BSEP, but rather by the magnitude of the bile acid pool and its cycling frequency. This is illustrated by the facts that bile acid secretion is not impaired in heterozygous BSEP-deficient mice152 or in heterozygous human PFIC2 patients who carry a mutation in ABCB11.153 Furthermore, bile acid secretion is actually impaired in heterozygous BSEP-deficient mice152 or in heterozygous human PFIC2 patients who carry a mutation in ABCB11.153 Furthermore, bile acid secretion is actually increased by 135% in FXR-deficient mice in which BSEP expression is reduced by 40%.65 The latter mice, when fed standard chow diet, were shown to have a 2-fold increase in bile acid pool size and actually a reduced cholesterol/bile acid ratio in comparison to wild-type controls.65 In addition, and most importantly, it is anticipated that treatment of patients with an effective synthetic FXR agonist will reduce the size of the circulating bile acid pool and, thereby, adversely affect bile composition, ie, lead to a relative increase in biliary cholesterol content. A reduced bile acid pool size has frequently been reported in human gallstone patients154–157 and might contribute to gallstone development.157 In view of these issues, together with the reported HDL-lowering effects of FXR agonists, it seems reasonable to state that application of FXR agonists for treatment of gallstone disease is by no means self-evident and will at least require development of highly selective BARMs.

In conclusion, given the various roles exerted by FXR in energy and bile acid metabolism, FXR will be an attractive target to design new drugs to treat dyslipidemia and liver disorders using highly specific modulators.

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