

Invited Review

Bile Acid Metabolism in Liver Pathobiology

John Y. L. Chiang and Jessica M. Ferrell

Department of Integrative Medical Sciences, Northeast Ohio Medical University, Rootstown, OH, USA

Bile acids facilitate intestinal nutrient absorption and biliary cholesterol secretion to maintain bile acid homeostasis, which is essential for protecting liver and other tissues and cells from cholesterol and bile acid toxicity. Bile acid metabolism is tightly regulated by bile acid synthesis in the liver and bile acid biotransformation in the intestine. Bile acids are endogenous ligands that activate a complex network of nuclear receptor farnesoid X receptor and membrane G protein-coupled bile acid receptor-1 to regulate hepatic lipid and glucose metabolic homeostasis and energy metabolism. The gut-to-liver axis plays a critical role in the regulation of enterohepatic circulation of bile acids, bile acid pool size, and bile acid composition. Bile acids control gut bacteria overgrowth, and gut bacteria metabolize bile acids to regulate host metabolism. Alteration of bile acid metabolism by high-fat diets, sleep disruption, alcohol, and drugs reshapes gut microbiome and causes dysbiosis, obesity, and metabolic disorders. Gender differences in bile acid metabolism, FXR signaling, and gut microbiota have been linked to higher prevalence of fatty liver disease and hepatocellular carcinoma in males. Alteration of bile acid homeostasis contributes to cholestatic liver diseases, inflammatory diseases in the digestive system, obesity, and diabetes. Bile acid-activated receptors are potential therapeutic targets for developing drugs to treat metabolic disorders.

Key words: Bile acid synthesis; Metabolic regulation; Nuclear receptor; Takeda G protein receptor 5 (TGR5); Farnesoid X receptor (FXR)

INTRODUCTION

Bile acid synthesis is an important pathway for catabolism of cholesterol and is tightly regulated by a complex but integrated network of mechanisms that are not completely understood. Bile acids are detergent molecules that can be highly toxic if accumulated in high concentrations in the liver and other tissues. It is thus necessary to tightly control bile acid synthesis to maintain bile acids at low and constant levels in the circulation pool, including the liver, gallbladder, and intestine. Recent research in mouse models and human patient studies has revealed that bile acids are signaling molecules that activate nuclear receptors and membrane G protein-coupled bile acid receptors to regulate not only the classic bile acid synthesis pathway but also the alternative bile acid synthesis pathways to maintain lipid, glucose, and energy metabolism in the liver, intestine, and adipose tissue. Alteration of bile acid homeostasis affects hepatic metabolic homeostasis, causes inflammation, and contributes to the pathogenesis

of metabolic diseases such as nonalcoholic fatty liver disease (NAFLD), diabetes, obesity, and inflammatory bowel diseases (IBDs). Bile acids are required for absorption of fats, steroids, and lipid-soluble vitamins in the intestine for metabolism in the liver^{1,2} and are signaling molecules that activate nuclear and membrane bile acid receptors to modulate hepatic lipid, glucose, and energy metabolism²⁻⁴. It has been known for more than half a century that bile acids are antibacterial agents in the gut that control gut bacteria overgrowth and protect intestinal barrier function. More recent studies of the gut-to-liver axis in bile acid metabolism, composition and pool size, and host metabolism have opened a new era for studying bile acid biology and physiology. This review will focus on recent advances in understanding regulation of bile acid synthesis, metabolism, and homeostasis and the emerging concepts on bile acid signaling crosstalk in metabolic regulation and potential therapeutic drug development for treating NAFLD. Most references cited

were published in the last 10 years, and older original references can be found in recent reviews²⁻⁷.

BILE ACID BIOLOGY AND PHYSIOLOGY

Enterohepatic Circulation of Bile Acids

In the liver, conversion of cholesterol to bile acids is the major pathway for catabolism of cholesterol. The liver synthesizes about 0.5 g of bile acids per day (80–90 kg body weight) (Fig. 1). Bile acids are secreted into canaliculi by active transport to form bile. Bile acids are stored in the gallbladder and are released after meal intake into the intestinal tract via the common bile ducts. Small amounts of bile acids are circulated back to hepatocytes via a cholangio-hepatic shunt. In the upper intestine, a small amount of bile acids is passively absorbed, but most bile acids are reabsorbed in the ileum by an active transport system to portal blood circulation and then to the liver. This enterohepatic circulation of bile acids is highly efficient and recovers about 95% of bile acids in the pool. The small amount of bile acid lost in

feces (0.5 g/day) is replenished by de novo synthesis in the liver. Enterohepatic circulation of bile acids occurs on average six to eight times a day to maintain a constant bile acid pool size of ~3 g. Bile acids (~0.5 mg/day) spilled over into the systemic circulation are excreted into urine.

Bile Acid Synthesis

The liver is the only organ that has all the enzymes required for bile acid synthesis (Fig. 2). There are two pathways in the liver: the classic or neutral pathway and the alternative or acidic pathway (all intermediates are cholestenic acids). The classic pathway starts with modifications of the steroid rings of cholesterol by hydroxylation, isomerization, reduction, and dehydroxylation enzymes located in the cytosol and endoplasmic reticulum, followed by steroid side chain oxidation in the mitochondria and oxidative cleavage of the side chain in peroxisomes. The alternative pathway starts with oxidation of the steroid side chain, then modification of the steroid rings and oxidative cleavage of the steroid side chain.

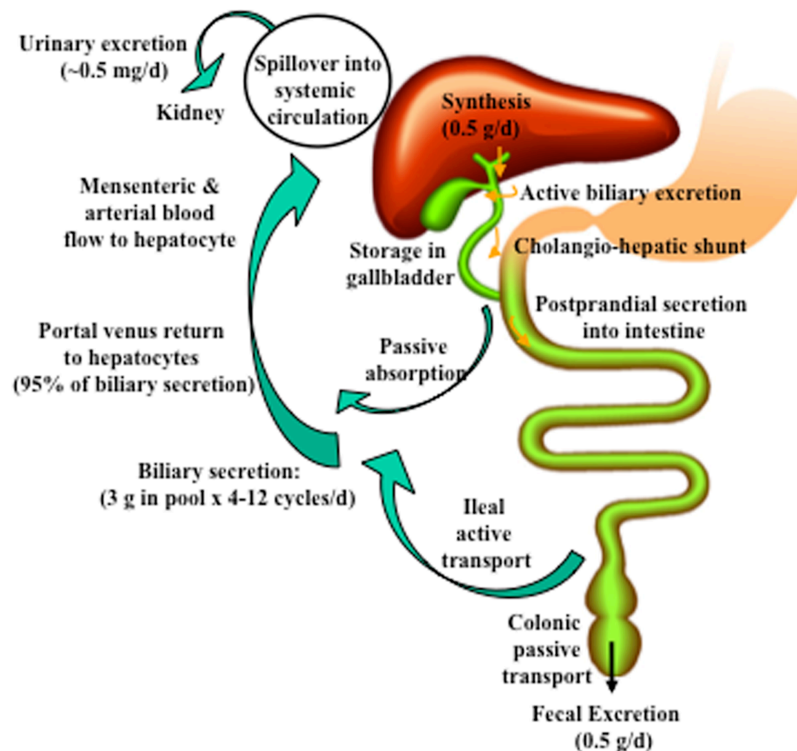


Figure 1. Enterohepatic circulation of bile acids. About 0.5 g of bile acids is synthesized per day in an average adult. Bile acids are secreted into bile and stored in the gallbladder. Small amounts of bile acids are circulated from cholangiocytes to the liver via the cholangio-hepatic shunt. After each meal, bile acids are excreted to the intestinal tract. A small amount of bile acids is passively absorbed in the upper intestine into mesenteric and arterial blood flow to hepatocytes. Most bile acids are reabsorbed in the ileum by active transport via apical sodium-dependent bile salt transporter (ASBT) and transported back to the liver via portal blood circulation. A constant bile acid pool (3 g) is circulated 4 to 12 times a day. Approximately 95% of bile acids in bile are recirculated back to the liver, and about 5% (0.5 g) lost in fecal excretion are replenished by de novo synthesis in the liver. A small amount (0.5 mg/day) of bile acid spillover into systemic circulation is cleared in urine.

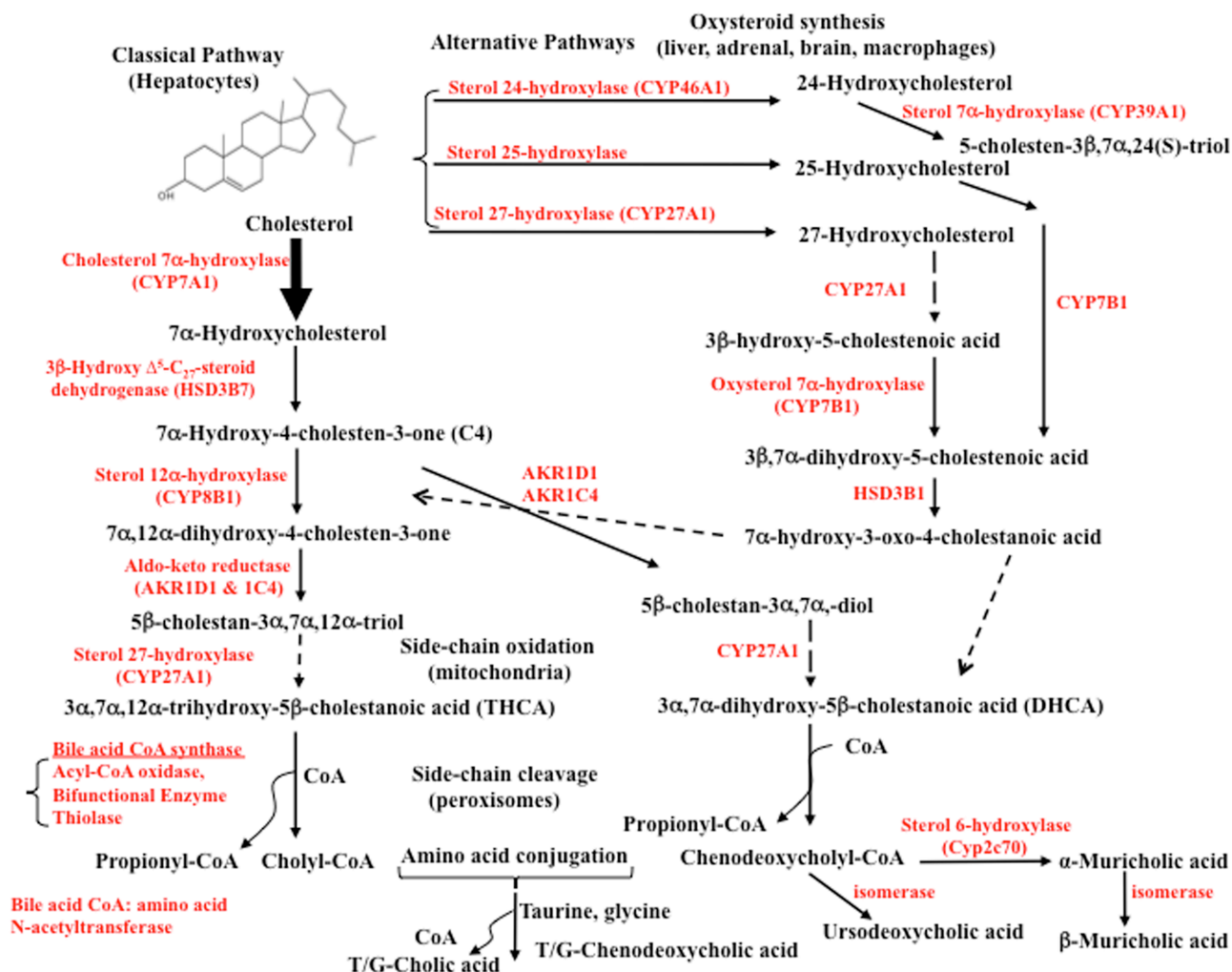


Figure 2. Bile acid synthesis pathways. In the liver, cholesterol 7 α -hydroxylase (CYP7A1) initiates the classical bile acid synthesis pathway by hydroxylation of the steroid rings at 7 α -C for further modifications of the steroid rings, followed by steroid side chain oxidation and cleavage, whereas sterol 27-hydroxylase (CYP27A1) initiates the alternative bile acid synthesis pathway by oxidation of the steroid side chain followed by modifications of the steroid rings and cleavage of the side chain in the classic pathway. CYP27A1 is expressed in most tissues and macrophages. Sterol 25-hydroxylase (a non-CYP450 enzyme) in the liver and sterol 24-hydroxylase (CYP46A1) in the brain also oxidize cholesterol. In the alternative pathways, a nonspecific oxysterol 7 α -hydroxylase (CYP7B1) hydroxylates 27-hydroxycholesterol and 25-hydroxycholesterol, whereas a specific 7 α -hydroxylase (CYP39A1) hydroxylates 24-hydroxycholesterol. Only the liver has all the enzymes required for the synthesis of cholic acid (CA) and chenodeoxycholic acid (CDCA), the two primary bile acids synthesized in humans (shown on the left, see text for details). The oxidized steroid intermediates (oxysterols) produced in the extrahepatic tissues can be used for bile acid synthesis in the liver. Sterol 12 α -hydroxylase (CYP8B1) is required for CA synthesis. Without 12 α -hydroxylation, CDCA is synthesized. Following steroid side chain cleavage, cholyl-CoA and chenodeoxycholy-CoA are conjugated to amino acids, either taurine or glycine. In mice, CDCA is 6 α -hydroxylated to form α -muricholic acid (α -MCA) by a sterol-6 α -hydroxylase (Cyp2c70) catalyzed reaction. The 7 α -OH group in α -MCA is epimerized (isomerized) to a 7 β -hydroxyl group to form β -MCA. The 7 α -HO group in CDCA can be epimerized to 7 β -HO to form ursodeoxycholic acid (UDCA), a highly soluble bile acid in humans and mice.

The Classic Bile Acid Synthesis Pathway

The classic pathway of bile acid synthesis is initiated by the rate-limiting enzyme cholesterol 7 α -hydroxylase (CYP7A1) to specifically hydroxylate cholesterol at the 7 α position, forming 7 α -hydroxycholesterol, which is converted to 7 α -hydroxy-4-cholesten-3-one (named C4) by

3 β -hydroxy- Δ^5 -C₂₇-steroid dehydrogenase (HSD3B7)⁸⁻¹⁰. C4 is the common precursor of cholic acid (CA) and chenodeoxycholic acid (CDCA), two primary bile acids synthesized in human liver. Serum C4 levels reflect the rate of bile acid synthesis in humans^{11,12}. Sterol 12 α -hydroxylase (CYP8B1) is a branch point enzyme for CA

synthesis. CYP8B1 catalyzes 12α -hydroxylation of C4 to 7α , 12α -dihydroxy-4-cholesten-3-one, which is then converted to 5β -cholestan- 3α , 7α , 12α -triol by two aldoketo-reductases (AKR1D1 and AKR1C4). Without 12α -hydroxylation, C4 is converted to 5β -cholestan- 3α , 7α -diol for CDCA synthesis. Mitochondrial sterol 27-hydroxylase (CYP27A1) catalyzes the steroid side chain oxidation of 5β -cholestan- 3α , 7α , 12α -triol and 5β -cholestan- 3α , 7α -diol to $-C_{27}$ -OH, which is subsequently converted to C_{27} -OOH to form 3α , 7α , 12α -trihydroxy- 5β cholestanic acid (THCA) and 3α , 7α -dihydroxy- 5β cholestanic acid (DHCA), respectively. The very long chain acyl-CoA synthase in peroxisome or sterol side chain cleavage occurs in the peroxisomes. Bile acid-CoA synthase (lyase) activates THCA and DHCA to form acyl-CoAs for peroxisomal β -oxidation by acyl-CoA oxidase and a bifunctional enzyme (enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase), and then a propionyl-CoA is cleaved

by thiolase to produce trihydroxychoyl-CoA and dihydroxychoyl-CoA, which are subsequently conjugated to glycine (G) or taurine (T) by bile acid CoA: amino acid N-acyltransferase (BAAT) to form T/G-CA and T/G-CDCA, respectively (Fig. 3A). In mouse liver, most CDCA is converted to α -muricholic acid (α -MCA) by a species-specific sterol 6β -hydroxylase (Cyp2c70), and then the 7α -OH group in α -MCA is epimerized to a 7β -OH group to form β -MCA¹³. MCAs are the primary bile acids synthesized in mouse liver. In mice and humans, the 7α -OH groups in CDCA can be isomerized to 7β -OH to form ursodeoxycholic acid (UDCA).

The Alternative Bile Acid Synthesis Pathways

The alternative bile acid pathway in the liver is initiated by CYP27A1, which converts cholesterol to 27-hydroxycholesterol and then to 3β -hydroxy-5-cholestenic acid. A nonspecific oxysterol 7α -hydroxylase

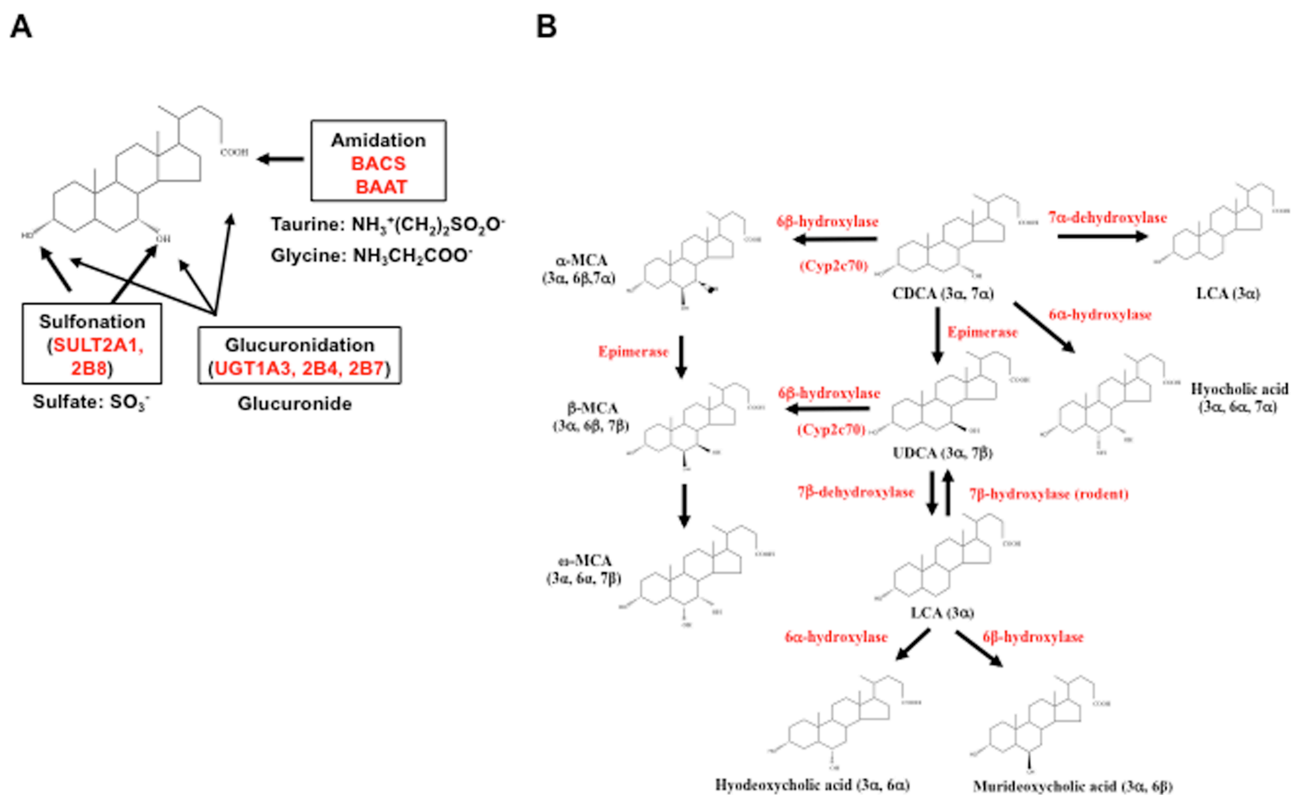


Figure 3. Bile acid conjugation and transformation. (A) Bile acid conjugation. In addition to conjugation to taurine and glycine at the C_{24} -carboxyl group by bile acid Co-A synthase (BACS) and bile acid CoA: amino acid N-acetyltransferase (BAAT), bile acids can be conjugated to sulfate by bile acid sulfotransferase (SULT2A1, SULT2B8) at the C_3 and C_7 positions, or glucuronidated by UDP-glucuronosyl transferase (UGT1A3, 2B4, and 2B7). (B) Bile acid transformation in enterocytes. Primary bile acids, CDCA, CA, and UDCA, can be converted to other bile acids in the liver and intestine. In the liver, CDCA can be converted to α -MCA, β -MCA, and UDCA as described in Figure 2. In the colon, bacterial bile salt hydratases (dehydroxylases) first deconjugate bile acids to free bile acids, and then gut bacteria converts CA to deoxycholic acid (DCA). CDCA can be converted to lithocholic acid (LCA) by 7α -dehydroxylase and hyocholic acid by 6α -hydroxylase. LCA can be converted to hyodeoxycholic acid by 6α -hydroxylase, or murideoxycholic acid by 6β -hydroxylase. In rodents, LCA can be converted to UDCA by 7β -hydroxylase and UDCA can be converted to CDCA. In the colon, α -MCA and β -MCA can be converted to ω -MCA for fecal excretion.

(CYP7B1) hydroxylates this cholestenic acid to 3 β , 7 α -dihydroxy-5-cholestenic acid, followed by HSD3B1/3B2 to synthesize 7 α -hydroxy-3-oxo-4-cholestenic acid in liver, steroidogenic tissues, and macrophages. In mouse and human liver, sterol 25-hydroxylase (CH25H), a non-CYP450 enzyme converts cholesterol to 25-hydroxycholesterol, followed by CYP7B1 to produce 5-cholesten-3 β , 7 α , 25-triol. In the brain, steroid 24-hydroxylase (CYP46A1) converts cholesterol to 24-hydroxycholesterol, which is then converted to 5-cholesten-3 β , 7 α , 24(S)-triol by a specific sterol-7 α -hydroxylase (CYP39A1) in mouse liver. Oxidized steroid intermediates (oxysterols) formed in the extrahepatic tissues can be transported to the liver for synthesis of bile acids. The alternative pathways produce not only CDCA but also CA.

The Classic Versus Alternative Bile Acid Synthesis Pathways

The classic bile acid synthesis pathway is the major pathway that is regulated by CYP7A1, the only rate-limiting enzyme in bile acid synthesis. The alternative pathways synthesize bile acids in the neonate when CYP7A1 is not expressed. After weaning, CYP7A1 is expressed, and the classic pathway becomes the major pathway for bile acid synthesis in adult liver. It has been suggested that the classic pathway is the predominant pathway for synthesis of ~80% of bile acids in human livers, whereas the classic and alternative pathways contribute about equally to bile acid synthesis in rodents. Mutations of the *CYP7A1* gene in male adult patients only caused mild hypercholesterolemia and premature gallstone diseases, indicating that the alternative bile acid synthesis pathways are activated to produce bile acids when the classic pathway initiated by CYP7A1 is defective¹⁴. Mutation of the *CYP7B1* gene in a neonate patient caused severe cholestatic liver injury and accumulation of oxysterol metabolites and monohydroxy bile acids, supporting the notion that the alternative bile acid synthesis pathway is important in the neonate¹⁵.

Bile Acid Conjugation and Transformation

Bile acids form Na²⁺ salts (bile salts), which are ionized at physiological pH. Conjugation of bile acids to glycine and taurine at C₂₄ increases bile acid ionization, amphipathic properties, and solubility (Fig. 3A). Bile acids also can be conjugated to glucuronide by the UDP-glucuronosyltransferases UGT 1A1, 2B4, and 2B7 at the C₃, C₇, or C₂₄ positions. The bile acid sulfotransferases SULT2A1 and SULT2A8 transfer a sulfate group to the C₃ or C₇ position¹⁶. Conjugated bile acids are secreted into bile via the canalicular bile salt export pump (BSEP), forming mixed micelles with cholesterol and phosphatidylcholine

in the gallbladder to prevent precipitation of cholesterol and to protect gallbladder epithelium cells from bile acid toxicity. After a meal, cholecystokinin (CCK) released from the pancreas stimulates gallbladder contraction to release bile acids into the gastrointestinal tract. Some bile acids are passively absorbed in the upper intestine, but most are reabsorbed in the ileum and colon where gut microbial bile salt hydrolase (BSH) deconjugates bile acids to free bile acids, then bacterial 7 α -dehydroxylase activity converts CA and CDCA to deoxycholic acid (DCA) and lithocholic acid (LCA), respectively^{17,18} (Fig. 3B). CDCA can be 6 α -hydroxylated to hyocholic acid. The 7 α -OH groups in CDCA can be isomerized to 7 β -OH groups to UDCA, which can be converted to LCA by 7 β -dehydroxylase in gut bacteria. Rodents can convert LCA to UDCA by 7 α -hydroxylase, to hyodeoxycholic acid by 6 α -hydroxylase, or to murideoxycholic acid by 6 β -hydroxylase (Fig. 3B). In the colon, α -MCA and β -MCA can be converted to ω -MCA. Secondary bile acids formed in the intestine are excreted into feces, and some are reabsorbed in the colon and are circulated with CA and CDCA in portal blood to the liver, contributing to the circulating bile acid pool. A small amount of LCA (~1%) reabsorbed in the liver is efficiently sulfur conjugated and excreted into urine.

Bile Acid Pool and Bile Acid Composition

The bile acid pool is defined as the total bile acids circulating in the enterohepatic circulation, including bile acids in the liver (<1%), intestine (~85%–90%), and gallbladder (~10%–15%). Bile acids spilled over from the liver to systemic circulation are not counted in the pool. Bile acids in gallbladder bile are from newly synthesized bile acids and bile acids returned to the liver via enterohepatic circulation. Thus, bile acid compositions in gallbladder bile more closely represent bile acid composition in the circulating bile acid pool. In the colon, most bile acids are unconjugated DCA, and most bile acids (~95%) in the pool are conjugated bile acids. In humans, the bile acid pool consists of CA (~40%), CDCA (~40%), and DCA (~20%), and the ratio of glycine (G)- to taurine (T)-conjugated bile acids is ~3 to 1, and this bile acid pool is highly hydrophobic. In mice, most bile acids (95%) are taurine conjugated, and the bile acid pool consists of TCA (~60%) and T α -MCA and T β -MCA (~40%) and is highly hydrophilic. Increasing bile acid pool size or bile acid hydrophobicity causes cholesterol gallstone disease and cholestatic liver injury. Feeding of mice with CA increases bile acid pool size and hydrophobicity and causes hepatic steatosis and cholestasis. Blockage of biliary bile acid secretion causes accumulation of bile acids in hepatocytes and liver injury. On the other hand, bile acid sequestrants reduce bile acid pool size, stimulate bile

acid synthesis, and reduce serum cholesterol in hypercholesterolemic patients and animals.

Interestingly, mice with overexpression of a *Cyp7a1* cDNA (*Cyp7a1-Tg* mice) have an enlarged bile acid pool (2.5-fold increase) consisting mostly of TCDCA (60%) and TMCA (40%) and no TCA due to inhibition of *Cyp8b1*¹⁹. In the ileum and colon of *Cyp7a1-Tg* mice, TMCAs are increased and ceramides are reduced²⁰. These mice are resistant to diet-induced obesity (DIO), likely due to activation of hepatic farnesoid X receptor (FXR) by TCDCA to inhibit lipogenesis, and antagonization of intestinal FXR by TMCAs to reduce ceramide synthesis²¹. On the other hand, *Cyp7a1*^{-/-} mice bred to a pure C57BL/6J genetic background have a reduced bile acid pool size (~60% of wild type) with a more hydrophilic bile acid pool containing reduced TCA but increased TMCA²². Interestingly, *Cyp7a1*^{-/-} mice also have improved glucose tolerance and reduced triglycerides and are resistant to Western high-fat diet (HFD)-induced insulin resistance, likely due to a switch to the alternative bile acid synthesis pathway, less TCA to reduce dietary cholesterol absorption, and more TMCA synthesis to antagonize intestinal FXR activity and reduce ceramide synthesis²². In *Cyp8b1*^{-/-} mice, *Cyp7a1* expression is increased to increase bile acid pool size with increased TMCAs, and mice are resistant to HFD (60% fat calories)-induced hepatic steatosis²³. In *Cyp7b1*^{-/-} mice, bile acid pool size and composition are normal, but 25- and 27-hydroxycholesterols are accumulated²⁴. Thus, the existence of two bile acid synthesis pathways is important for switching between two pathways to produce a sufficient bile acid pool size, although bile acid composition may be altered to affect hepatic metabolism.

BILE ACID SIGNALING IN LIVER PATHOPHYSIOLOGY

Enterohepatic circulation of bile acids reabsorbs bile acids in the intestine to control bile acid synthesis in the liver by a negative feedback mechanism and maintain a constant bile acid pool. Emerging research in the last two decades has discovered that bile acids are endogenous signaling molecules that activate the nuclear receptors FXR, vitamin D₃ receptor (VDR), pregnane X receptor (PXR), and constitutive androstane receptor (CAR), as well as the membrane G protein-coupled receptors Takeda G protein receptor 5 [TGR5; aka G protein bile acid receptor-1 (Gpbar-1)] and sphingosine-1-phosphate receptor 2 (S1PR2) in the gastrointestinal tract (Fig. 4). The circulating bile acid pool consists of primary and secondary bile acids from the liver, gallbladder, and intestine. Primary bile acids are efficacious endogenous ligands of FXR and S1PR2 in the liver. In the intestine, TCDCA activates FXR, whereas TLCA/LCA and DCA activate TGR5, PXR, and VDR. TMCAs are FXR antagonists in the intestine.

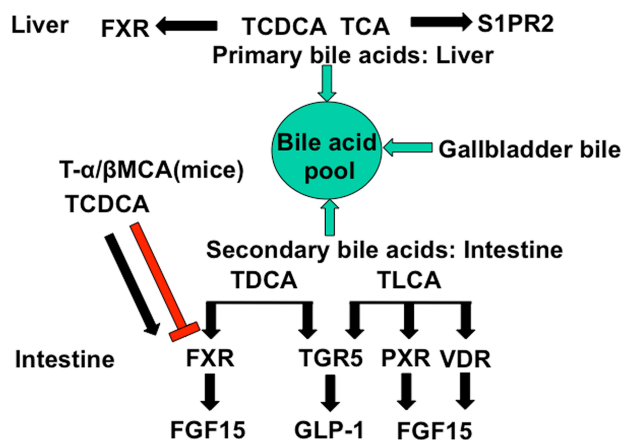


Figure 4. Bile acid-activated receptors in the gastrointestinal system. Bile acid-activated receptors are differentially activated by primary and secondary bile acids in the liver and intestine. The bile acid pool consists both of the primary bile acids TCDCA, TCA, T α -MCA, and T β -MCA, and the secondary bile acids TDCA and TLCA. In the liver, TCDCA activates FXR, whereas TCA activates S1PR2. In the intestine, TCDCA and TDCA activate FXR, whereas T α -MCA and T β -MCA antagonize FXR. TLCA and TDCA activate TGR5, and TLCA activates VDR and PXR.

FXR Signaling

TCDCA is the most potent endogenous FXR agonist with an EC₅₀ of 17 μ M. In mice, TCA and TMCAs are the predominant bile acids in the bile acid pool. However, TCA is a very weak FXR agonist (EC₅₀ = ~590 μ M), while T α -MCA (IC₅₀ = 28 μ M) and T β -MCA (IC₅₀ = 40 μ M) are potent FXR antagonists²³ (Fig. 4). Therefore, FXR may not be activated in mouse hepatocytes under physiological conditions. In the intestine, bile acid concentrations are much higher than in hepatocytes, and TCDCA may activate FXR to induce ileum bile acid-binding protein (IBABP), fibroblast growth factor 15 (FGF15), and sinusoidal bile acid efflux transporter organic solute transporter α/β heterodimer (OST α/β). The secondary bile acids formed in the colon, LCA and DCA, activate TGR5 to stimulate GLP-1 secretion from enteroendocrine L cells, which stimulates insulin secretion from β -cells and improves insulin sensitivity²⁵.

The role of FXR in the regulation of glucose, lipid, and energy metabolism and in improving glucose and insulin sensitivity and obesity has been the subject of many recent reviews^{2,3,26,27}. Contradictory roles of FXR in the regulation of glucose metabolism have been reported recently. Earlier studies have shown that activation of FXR improves glucose homeostasis in diabetic mice²⁸. It has been suggested that activation of FXR induces small heterodimer partner (SHP), which may lower triglycerides by inhibiting liver X receptor (LXR)-induced and steroid response element-binding protein-1c

(SREBP-1c)-mediated lipogenesis²⁹. FXR may inhibit fatty acid synthesis by inhibiting glucose-induced nuclear translocation of carbohydrate response element-binding protein (ChREBP), which induces glycolytic genes involved in the conversion of glucose to fatty acids³⁰. In contrast, activation of FXR has been shown to induce fatty acid synthase, implicating bile acid signaling in the stimulation of hepatic lipogenesis³¹. Furthermore, it was shown that activation of FXR by GW4064 increased body weight and glucose tolerance and reduced energy expenditure in HFD-fed mice (DIO)³², but deficiency of FXR improves glucose homeostasis in DIO mice³³. Several recent studies have reported that inhibition of intestinal FXR activity by the antioxidant tempol remodeled the gut microbiome and reduced obesity in mice³⁴. Consistently, deficiency of intestinal FXR protects mice from DIO and diabetes³⁵, and the intestine-selective FXR inhibitor glycine-MCA improved obesity and diabetes in mice³⁶. In contrast, other recent studies report that selective intestinal FXR agonists stimulate FGF15 to promote adipocyte browning, reduce weight, and improve insulin resistance in DIO mice³⁷, and protect against cholestasis³⁸. It is possible that different intestinal FXR agonists alter the gut microbiome differently and result in opposite effects on hepatic metabolism (see Bile Acids and Gut Microbiota, below).

TGR5 Signaling

TGR5 is a Gαs protein-coupled receptor activated by the secondary bile acids TLCA (EC₅₀=0.3 μM) and DCA (EC₅₀=1 μM). TGR5 is expressed in the epithelial cells of the gastrointestinal system, including intestine, spleen, cholangiocytes, gallbladder, hepatic sinusoidal endothelial cells, and hepatic macrophages (Kupffer cells)^{39–42}. TGR5 may have different functions in different tissues. TGR5 activates cAMP/PKA signaling to stimulate energy metabolism in brown adipose tissue⁴³ and is involved in relaxing and refilling the gallbladder⁴⁴, secreting glucagon-like peptide 1 (GLP-1) from L cells²⁵, and controlling GI motility⁴⁵ (Fig. 4). Activation of TGR5 has been shown to protect the liver from bile acid overload during liver regeneration⁴⁶. Pharmacological activation of TGR5 markedly decreased lipopolysaccharide (LPS)-induced cytokine production in primary macrophages and protected adipose tissue from inflammation and associated insulin resistance through the Akt/mTORC/EBP pathway⁴⁷. Another study reports that activation of TGR5 by a nonsteroidal agonist inhibits LPS-induced TNF-α and IL-12 release in mice and primary human hepatocytes⁴⁸. In contrast, it has been reported that activation of TGR5 in macrophages promotes bile acid- or LPS-induced inflammation^{49,50}. TGR5 is highly expressed in cholangiocytes, and its expression levels are increased in cholangiocarcinoma cells. In *Tgr5*^{-/-} mice, CA feeding- and

chronic bile duct ligation-induced cholangiocyte proliferation was significantly reduced, while in wild-type mice, TLCA and TGR5-selective agonists induced cholangiocyte proliferation through increased reactive oxygen species and ERK1/2 phosphorylation⁵¹. A recent study shows that TGR5 may be involved in the regulation of CYP7B1, a sexually dimorphic and male-predominant gene in the alternative bile acid synthesis pathway⁵².

Mechanisms of Bile Acid-Activated Receptors in the Regulation of Bile Acid Synthesis

A complex network of signaling pathways regulates bile acid synthesis (Fig. 5). Bile acid inhibition of CYP7A1 is a classic feedback mechanism whereby the end products of a metabolic pathway inhibit the first enzyme in the pathway. The mechanism of bile acid feedback regulation has been studied over 50 years, but the molecular mechanism is still not clear. Two mechanisms have been proposed (Fig. 5). In the liver, activation of FXR by agonists induces SHP to inhibit HNF4 and LRH-1 trans-activation of *CYP7A1* and *CYP8B1* gene expression. In the intestine, activation of FXR by agonists induces FGF15 (or human FGF19), which is transported via portal blood circulation to hepatocytes to activate FGF receptor 4 (FGFR4)/β-Klotho complex. The complex inhibits *CYP7A1* and *CYP8B1* gene transcription via an unknown mechanism that may involve the cJun and ERK1/2 pathways of the mitogen-activated protein kinase (MAPK). This intestine-to-liver signaling pathway may be a more physiologically relevant mechanism for bile acid feedback regulation of bile acid synthesis. TCA activation of S1PR2 may stimulate ERK1/2 signaling to inhibit *CYP7A1* and *CYP8B1* gene transcription, but the role of S1PR2 in bile acid feedback regulation has not been studied. Activation of TGR5 may regulate expression of CYP7B1, and TGR5 signaling in the brain may stimulate growth hormone secretion to activate STAT5 signaling in hepatocytes, which induces gene transcription of the male-predominant *CYP7B1* to regulate bile acid composition⁵².

CIRCADIAN RHYTHM IN BILE ACID METABOLISM

Figure 6 illustrates the close linkage between the liver and gut in bile acid synthesis and metabolism, which is modulated by circadian rhythms²⁷. The central circadian clock is located in the hypothalamic suprachiasmatic nucleus (SCN), which generates biological rhythms via a transcription–translation feedback loop of core clock genes encoding transcriptional repressors Period 1/2/3 (*Per1/2/3*) and cryptochrome 1/2 (*Cry1/2*), and transcriptional activators brain and muscle ARNT-like protein 1 (*Bmal1*) and circadian locomotor output cycles kaput (*Clock*). The central clock evolved due to the rotation of

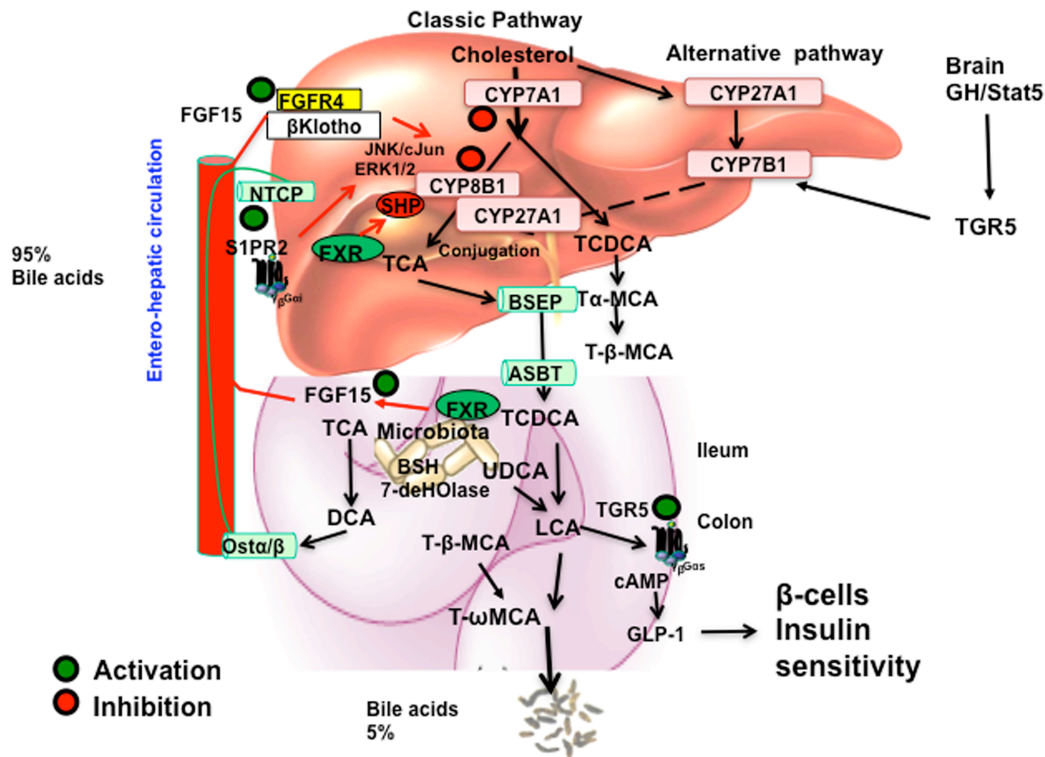


Figure 5. Mechanisms of bile acid regulation of bile acid synthesis. In the liver, activation of FXR by agonists induces SHP to inhibit CYP7A1 and CYP8B1 gene transcription. TCA activates S1PR2 and may inhibit CYP7A1 via the ERK1/2 pathway. In the intestine, TCDCA activates FXR to induce FGF15, which is transported to hepatocytes to activate FGFR4/ β -Klotho receptor, which stimulates the JNK/cJun and ERK1/2 pathways to inhibit CYP7A1 and CYP8B1. In the brain, growth hormone (GH) activates STAT5 signaling to activate TGR5 signaling to induce CYP7B1 in male mice and regulate the alternative bile acid synthesis pathway. In the intestine, TLCA activates TGR5 in L cells to stimulate GLP-1 secretion, which increases insulin sensitivity by stimulating insulin secretion from pancreatic β -cells.

the Earth around the sun every 24 h. The peripheral clocks are synchronized to the central clock via the core clock genes *Per/Cry* and *Bmal1/Clock*, which regulate transcription of thousands of other clock-controlled genes. A secondary regulatory loop of the core clock is composed of reverse-erythroblastosis α (*Rev-erb α*) and retinoid acid-related receptor α (*ROR α*). The peripheral clocks are influenced and entrained by neuronal input to tissues, hormones, physiological activity, and environmental factors such as diets, time of feeding, drugs, and alcohol^{53–59}. *Rev-erb α* and *ROR α* regulate *CYP7A1* and *CYP8B1* in bile acid synthesis to exhibit a distinct circadian rhythm of bile acid synthesis in mice⁶⁰. Fasting and refeeding cycles and nutrients regulate bile acid synthesis. Feeding rapidly induces *Cyp7a1* but represses *Cyp8b1*, whereas fasting represses *Cyp7a1* but induces *Cyp8b1*. In rodents, *Cyp7a1* expression peaks 2 h into the dark phase and rapidly decreases in the light phase²⁷. In humans, bile acid synthesis shows a distinct diurnal rhythm, which peaks at 1:00 pm and 9:00 pm, and declines at midnight⁶¹. Bile acid synthesis varies in individuals, is lower in females compared to males, and is correlated to serum triglycerides

and circulating FGF19 levels⁶². Circulating FGF19 has a pronounced diurnal rhythm, peaking 90–120 min after the postprandial rise of serum bile acids⁶³. Activation of intestinal FXR induces FGF15 during the late postprandial state⁶⁴. Circadian disruptions, such as shift work or chronic sleep disorders, contribute to cardiovascular diseases⁶⁵, digestive diseases⁶⁶, and metabolic syndrome^{67–69}. A recent study in mice shows that even short-term sleep disruption of 6 h during the day for 5 days significantly suppressed core clock gene (*Clock* and *Per1/2*, *Rev-erb α*), the genes involved in hepatic lipid metabolism (*Srebp1*, hepatocyte nuclear factor 4 α), *Cyp7a1* regulators (hepatocyte nuclear factor 4 α and D-site-binding protein), and *Cyp7a1* expression⁶⁹. Disruption of circadian rhythms alters bile acid homeostasis, causes liver and intestine inflammation, and alters the gut microbiome and contributes to cholestatic liver injury, NAFLD, diabetes, and IBDs and dysbiosis^{27,70,71}.

BILE ACIDS AND THE GUT MICROBIOTA

The gut-to-liver axis plays a critical role in the regulation of metabolic homeostasis and in preventing metabolic

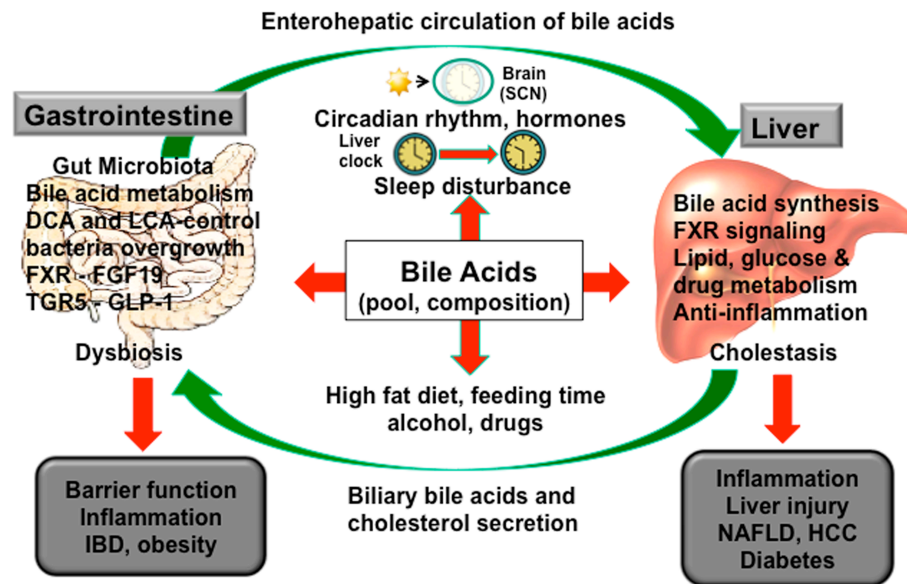


Figure 6. The gut-to-liver axis and circadian rhythms in bile acid metabolism. Bile acid synthesis in the liver is controlled in part by circadian expression of CYP7A1 expression. Bile acids control the gut microbiota population, and gut bacteria regulate bile acid metabolism, bile acid composition, and enterohepatic circulation of bile acids. Sleep disruption, high-fat diet (HFD), alcohol, and drugs alter the central clock in the hypothalamic suprachiasmatic nucleus (SCN) of the brain to desynchronize the peripheral clocks in the liver and intestine. Disruption of circadian rhythms alters bile acid homeostasis, causes liver and intestine inflammation and dysbiosis, and contributes to cholestatic liver injury, nonalcoholic fatty liver disease (NAFLD), diabetes, and inflammatory bowel diseases.

diseases⁷². Bile acids regulate the gut microbiome structure by controlling gut bacteria overgrowth and protecting the epithelial barrier via FXR signaling⁷³. FXR signaling shapes the gut microbiota and controls hepatic lipid metabolism^{74,75}. Gut bacteria metabolize bile acids to regulate bile acid composition and bile acid hydrophobicity. Intestinal bile acids are antimicrobial agents that control gut microbial overgrowth by altering membrane lipid composition, solubilizing membranes, and dissociating integral membrane proteins and damaging membrane integrity. Gut microbes deconjugate bile acids and convert primary bile acids to secondary bile acids in the colon. Gram-negative bacteria have a higher bile acid tolerance than Gram-positive bacteria. BSH activity is high in Gram-positive bacteria, such as *Lactobacillus*, *Enterococcus*, *Bacteroides*, and *Clostridium*. BSH deconjugates glycine- and taurine-conjugated bile acids to release glycine and taurine, which are metabolized to ammonia and CO₂, and also sulfite from taurine. Bile acids are carbon sources for energy metabolism in some Gram-negative anaerobic bacteria. Feeding a CA-containing diet increases the gut *Firmicutes-to-Bacteroidetes* ratio^{76,77} and causes dysbiosis and contributes to liver inflammation⁷⁸. Dietary saturated fats increase TCA and promote bile-tolerant and sulfur-producing *Bilophila wadsworthia* to increase proinflammatory cytokines and colitis in *Il10*^{-/-} mice⁷⁹. Bacteria capable of converting primary bile acids to secondary bile acids have been isolated from humans and rodents. The

bile acid inducible (Bai) operon in bile acid dehydroxylating gut bacteria have been mapped in *Clostridium scindens*, *Clostridium hylemonase*, *Clostridium hiranonis*, and *Clostridium sordellii*¹⁸. The *baiE* gene encodes the bile acid 7 α -dehydroxylase activities, whereas the *baiI* gene may encode the bile acid 7 β -dehydroxylase. Bacterial hydroxy-steroid dehydrogenases (HSD) epimerize the hydroxyl groups at 3-, 7-, and 12-C of bile acids. The α - and β -HSD activities in *Clostridium absonum* epimerize the 7 α -HO group of CDCA to the 7 β -HO group of UDCA, increasing the solubility and decreasing the toxicity of bile acids. However, the 7 β -dehydroxylase activities in *C. scindens* and *Clostridium hiranonase* and *Bacteroides* can convert UDCA back to LCA. Antibiotics alter the gut microbiota and impair the production of secondary bile acids, which are protective of gut barrier function. Bacteria with 7 α -dehydroxylase activity (i.e., *C. scindens*) may be used to increase secondary bile acids and alter gut bile acid composition to protect against *Clostridium difficile* infection in patients with antibiotic-associated diarrhea and colitis⁸⁰.

Recent studies have implicated Cyp8b1 in dyslipidemia, insulin resistance⁸¹, and atherosclerosis⁸². In diabetic patients, bile acid pool size and the serum ratio of 12 α -hydroxylated bile acids to non-12 α -hydroxylated bile acids are increased. *Cyp8b1*^{-/-} mice, germ-free mice and antibiotic-treated mice share similar metabolic phenotypes: they all have increased *Cyp7a1* expression and bile

acid synthesis, enlarged bile acid pool, and are resistant to HFD-induced hepatic steatosis due to antagonism of intestinal FXR activity by TMCA and increased *Cyp7a1* expression^{23,83}. Antibiotics modulate the gut microbiota and decrease *Cyp8b1* expression to alter bile acid profiles in mice and protect against diet-induced metabolic disorders⁸⁴. Germ-free mice have increased *Cyp7b1* expression and increased TMCA²³. Bile acids induce UCP-1 in brown adipose tissue to stimulate energy metabolism in mice housed at thermoneutrality⁸⁵. Reducing ambient temperature attenuated DIO in mice by increasing brown adipose tissue thermogenesis, reshaping gut microbiome, and changing bile acid profile similar to germ-free mice⁸⁶. Another study reports that *Cyp7b1* may play a role in cold-induced thermogenesis⁸⁷. Thus, cold-induced conversion of cholesterol to bile acids, by increasing the alternative pathway and altering bile acid composition, may shape the gut microbiome and promote adaptive thermogenesis in brown adipose tissues in mice.

Male *Fxr*^{-/-} mice are more susceptible to Western diet (WD)-induced hepatic steatosis, insulin resistance, and obesity compared to female mice. Bile acids and the microbiota may play a role in mediating sex differences in hepatic inflammation, dysbiosis, and NAFLD in mice⁸⁸⁻⁹⁰. The sex differences in WD-induced hepatic steatosis, insulin resistance, bile acids, and microbiota profiles are FXR dependent⁸⁹. Male *Fxr*^{-/-} mice have more severe hepatic inflammation and steatosis compared to female mice, and WD feeding aggravates hepatic steatosis more in these male mice⁸⁸. Normally, WD increases biliary secretion of bile acids and reshapes the gut microbiota in obesity by increasing *Firmicutes* and decreasing *Bacteroidetes*⁹¹. WD decreases *Firmicutes* and increases *Proteobacteria* in *Fxr*^{-/-} mice⁹². The incidence of streptozotocin (STZ)-HFD-induced HCC is significantly higher in male mice than in female mice. Metagenomic analysis showed differences in gut bacteria that are involved in bile acid metabolism between normal male and female mice. STZ-HFD treatment amplified the observed sex difference in gut microbiota. Female mice have a higher ratio of *Firmicutes* to *Bacteroidetes* compared to male mice. STZ-HFD treatment reduced *Firmicutes* and *Bacteroidetes* and markedly increased *Proteobacteria* in female mice, but much less so in male mice. It was found that STZ-HFD treatment caused more intrahepatic retention of hydrophobic bile acids (TCA, TCDC, and TLCA) in male mice compared to female mice. STZ-HFD treatment strongly reduced *Cyp7b1* mRNA expression, consistent with a much higher increase in TCA in male than female mice, and an altered gut microbiota between male and female mice.

Either activation or antagonizing intestinal FXR signaling resulted in reducing weight and increasing insulin sensitivity in DIO mice³⁴⁻³⁷. The underlying mechanisms

could be different. The intestine FXR antagonist Gly-MCA and the intestine-selective FXR agonist fexaramine may shape the gut microbiome differently, resulting in opposite effects on insulin resistance and obesity in mice. It seems clear that both intestinal FXR/FGF15 signaling and TGR5/GLP-1 signaling modulate hepatic metabolism. The gut-to-liver axis is critically involved in whole-body lipid, glucose, and energy metabolism.

BILE ACID SIGNALING IN LIVER PATHOBIOLOGY

FXR and TGR5 in Anti-Inflammation

Bile acids have different effects on cell proliferation and inflammation in different cell types and tissues⁵¹. CA feeding causes hepatocyte proliferation. More hydrophilic bile acids TCA and UDCA protect against apoptosis, while the hydrophobic bile acids TLCA and GCDCA cause hepatic apoptosis and liver injury. Activation of FXR and TGR5 signaling in mice by their respective agonists has been shown to protect against inflammation in the liver and intestine by inhibiting NF- κ B-induced inflammatory cytokine production and liver inflammation⁹³⁻¹⁰⁰. FXR agonists have been shown to protect against inflammation in cholestasis, cirrhosis, atherosclerosis, and IBD and are in clinical trials to treat NAFLD and primary biliary cirrhosis^{96,101,102}. FXR signaling also inhibits dextran sodium sulfate-induced IBDs^{103,104}. TGR5 agonist INT777 has been used to reduce body weight and stimulate GLP-1 secretion to improve insulin sensitivity²⁵, and attenuates atherosclerosis by reducing macrophage inflammation⁹⁹ and liver ischemia and reperfusion injury¹⁰⁵. The dual FXR and TGR5 agonist INT-767 has been shown to switch M1 proinflammatory responses to M2 anti-inflammatory responses and alleviate NAFLD in db/db mice¹⁰⁶ and atherosclerosis in *Apoe* and *Ldl* receptor double knock-out mice¹⁰⁷.

Cholestatic Liver Diseases

Cholestasis is a pathological condition where normal bile flow out of the liver is reduced or disrupted by tumors or inflammation, leading to intrahepatic accumulation of bile acids¹⁰⁸. Accumulation of cytotoxic bile acids activates NF- κ B-mediated proinflammatory cytokine production. High levels of toxic bile acids damage the bile duct epithelium and elevate biliary pressure to rupture the bile duct and expose hepatocytes to high concentrations of bile acids, inflammatory infiltration, and leads to hepatocyte cell death. Cholestasis could result from genetic defects in canalicular transporters (i.e., BSEP), mechanical obstruction of bile duct by gallstones or tumors, factors associated with pregnancy [intrahepatic cholestasis of pregnancy (ICP)], autoimmune destruction of the bile

ducts in and outside of the liver, or drug-induced liver toxicity^{109,110}. Mutations in the *FXR* gene have been shown to cause progressive familial intrahepatic cholestasis in human patients^{111,112}. Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are two common types of progressive chronic cholestasis diagnosed in humans. The etiology of PBC and PSC involves genetic, immune, and environmental factors. PBC is an autoimmune disease with high prevalence in females and is caused by inflammation in the small intrahepatic bile duct and impaired hepatic bile acid secretion and accumulation. PSC is an idiopathic liver disease characterized by chronic liver inflammation and progressive destruction of intrahepatic and extrahepatic bile ducts in men. Selective activation of intestinal FXR has been shown to increase FGF15, repress *Cyp7a1* and bile acid pool, and protect against intrahepatic cholestasis in bile duct-ligated mice and *Mdr2*^{-/-} mice³⁸.

BILE ACID THERAPY

UDCA (Ursodiol[™]) has been used as a bile acid replacement therapy to reduce bile acid toxicity for patients with bile acid synthesis deficiency, gallstone dissolution, and digestive diseases for several decades¹¹³. UDCA activates PKC and MAPK signaling to alleviate liver injury in cholestasis. UDCA increases hepatobiliary secretion and promotes biliary HCO₃⁻ secretion to protect hepatocytes and cholangiocytes from hydrophobic bile acid-induced liver injury¹¹⁴⁻¹¹⁶. However, UDCA is not effective in treating patients with PSC. Nor-ursodeoxycholic acid (norUDCA), a derivative of UDCA with shorter side-chain C₂₃, has been shown to increase HCO₃⁻ secretion and improve sclerosing cholangitis in the *Mdr2*^{-/-} model of cholangiopathy^{116,117}.

FXR Agonists

A synthetic CDCA derivative, 6-ethyl-CDCA (OCA, INT-747, OCALIVA[®]) is a selective FXR agonist (EC₅₀=0.099 μM) with therapeutic potential for treating cholestasis^{118,119}. OCA treatment showed effective protection in experimental cholestasis models¹²⁰. Recent clinical trials reported that OCA significantly improved liver tests in patients with PBC^{121,122} and reduced serum alkaline phosphatase, GGT, and alanine aminotransferase activities¹²¹. OCA improved nonalcoholic steatohepatitis (NASH) scores in clinical trials and is a promising therapy for NASH^{123,124}. The intestine-restricted FXR agonist fexaramine (EC₅₀=25 μM) has been shown to promote adipose tissue browning to reduce weight and diabetes in DIO mice³⁷.

TGR5 Agonists

Activation of TGR5 stimulates GLP-1 secretion from enteroendocrine L cells, which leads to stimulation

of insulin synthesis and secretion from pancreatic β-cells¹²⁵⁻¹²⁷. It also protects cholangiocytes from bile acid toxicity in cholestasis and is a potential therapy for cholestasis¹²⁵. However, TGR5 agonists may also promote proliferation, apoptosis, and progression of cholangiocarcinoma⁵¹. Several potent TGR5-selective agonists are in development for treating metabolic diseases^{128,129}. A synthetic bile acid derivative, INT-777 [6α-ethyl-23(S)-methyl-CA, EC₅₀=0.82 μM] is a TGR5-selective agonist and has been shown to protect intestinal barrier function and immune response to experimental colitis^{95,130}, reduce macrophage inflammation by activating cAMP signaling to inhibit NF-κB activity and inflammatory cytokine production, and reduce plaque formation and atherosclerosis in *Ldlr*^{-/-} mice⁹⁹. TGR5 agonists induced NO production and reduced monocyte adhesion in vascular endothelial cells¹³¹. A non-bile acid and potent TGR5 agonist has been shown to stimulate GLP-1 secretion and lower glucose^{132,133}. Other non-bile acid TGR5 agonists have been designed to treat type 2 diabetes^{133,134}.

FXR and TGR5 Dual Agonist

The dual FXR and TGR5 agonist INT-767 (EC₅₀=0.03 μM for FXR and EC₅₀=0.63 μM for TGR5) is a 6-ethyl-CDCA derivative with a C₂₃-sulfate group. INT-767 has been shown to improve diabetes and hepatic steatosis and decrease inflammation in *db/db* and DIO mice^{106,135,136}. INT-767 reduces liver injury in *Mdr2*^{-/-} mice¹³⁷. A recent study showed that INT-767 stimulates intracellular [Ca²⁺] and cAMP activity to stimulate GLP-1 secretion and improve glucose and lipid metabolism¹³⁸. Interestingly, INT-767 reduced expression of the genes in the classic bile acid synthesis pathway while inducing those in the alternate pathway to decrease TCA and increase TMCAs. TCA is highly efficacious in intestinal absorption of dietary cholesterol, and TMCA antagonizes intestinal FXR signaling. Surprisingly, OCA and INT-767 induce expression of intestinal *Tgr5* and pro-hormone convertase 1/3, which converts proglucagon to GLP-1 in the ileum. Consistently, *Fxr*^{-/-} and *Tgr5*^{-/-} mice have reduced GLP-1 secretion. This study uncovered a novel mechanism in which activation of FXR induces *Tgr5* gene expression and increases [Ca²⁺] and cAMP activity to stimulate GLP-1 secretion and improve hepatic glucose and lipid metabolism. Another FXR and TGR5 dual agonist has been designed to reduce weight and treat diabetes¹³⁹. Activation of both FXR and TGR5 may represent an effective therapy for treating hepatic steatosis, obesity, and diabetes¹³⁸.

CONCLUSIONS

Recent advances in the discovery of the role of bile acids in cell signaling and metabolism have contributed

enormously to the current understanding of the mechanism of regulation of bile acid homeostasis and hepatic metabolism. Bile acid signaling via FXR regulates hepatic metabolism, and intestinal FXR and TGR5 signaling cross-talk modulates the gut microbiome, bile acid homeostasis, and host metabolism. Gender differences in bile acids and gut microbiome in diet-induced steatosis, NASH, and hepatocellular carcinoma have been unveiled and need to be further studied to identify differentially regulated hormones, bacteria, bile acids, and metabolic pathways responsible for increased prevalence of these diseases in males^{89,90}. The emerging research of bile acid metabolism and homeostasis has been translated to drug therapy targeted to treating metabolic disorders. Bile acid receptors are signaling integrators, and bile acid derivatives are in clinical trials for treating dyslipidemia, NAFLD, diabetes, and cardiovascular diseases^{6,26}. Bariatric surgeries are effective in reducing weight and improving insulin resistance in obese patients, and FXR and TGR5 signaling have been implicated in increasing serum bile acids, GLP-1, and FGF19 after bariatric surgery¹⁴⁰⁻¹⁴². However, the underlying molecular mechanism of bile acid signaling in improving diabetes after bariatric surgery is not clear and needs to be elucidated to develop therapeutic strategies to cure diabetes and NAFLD.

ACKNOWLEDGMENTS: *This research was supported by NIH grants DK44442 and DK58379. The authors declare no conflicts of interest.*

REFERENCES

- Russell DW. Fifty years of advances in bile acid synthesis and metabolism. *J Lipid Res.* 2009;50(Suppl):S120-5.
- Chiang JY. Bile acids: Regulation of synthesis. *J Lipid Res.* 2009;50:1955-66.
- Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. *Physiol Rev.* 2009;89(1):147-91.
- Chiang JY. Bile acid metabolism and signaling. *Compr Physiol.* 2013;3:1191-212.
- Thomas C, Auwerx J, Schoonjans K. Bile acids and the membrane bile acid receptor TGR5—Connecting nutrition and metabolism. *Thyroid* 2008;18(2):167-74.
- Arab JP, Karpen SJ, Dawson PA, Arrese M, Trauner M. Bile acids and nonalcoholic fatty liver disease: Molecular insights and therapeutic perspectives. *Hepatology* 2017; 65(1):350-62.
- Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. *Nat Rev Gastroenterol Hepatol.* 2014;11(1):55-67.
- Chiang JY. Bile acid regulation of gene expression: Roles of nuclear hormone receptors. *Endocr Rev.* 2002; 23(4):443-63.
- Russell DW. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem.* 2003;72:1370174.
- Chiang JY. Regulation of bile acid synthesis: Pathways, nuclear receptors, and mechanisms. *J Hepatol.* 2004;40 (3):539-51.
- Axelsson M, Aly A, Sjovald J. Levels of 7 alpha-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. *FEBS Lett.* 1988;239(2):324-8.
- Honda A, Yamashita K, Numazawa M, Ikegami T, Doy M, Matsuzaki Y, Miyazaki H. Highly sensitive quantification of 7alpha-hydroxy-4-cholesten-3-one in human serum by LC-ESI-MS/MS. *J Lipid Res.* 2007;48(2):458-64.
- Takahashi S, Fukami T, Masuo Y, Brocker CN, Xie C, Krausz KW, Wolf CR, Henderson CJ, Gonzalez FJ. Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. *J Lipid Res.* 2016;57(12):2130-7.
- Pullinger CR, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, Verhagen A, Rivera CR, Mulvihill SJ, Malloy MJ, Kane JP. Human cholesterol 7alpha-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. *J Clin Invest.* 2002;110(1):109-17.
- Setchell KDR, Schwarz M, O'Connell NC, Lund EG, Davis DL, Lathe R, Thompson HR, Weslie Tyson R, Sokol RJ, Russell DW. Identification of a new inborn error in bile acid synthesis: Mutation of the oxysterol 7a-hydroxylase gene causes severe neonatal liver disease. *J Clin Invest.* 1998;102(9):1690-703.
- Feng L, Yuen YL, Xu J, Liu X, Chan MY, Wang K, Fong WP, Cheung WT, Lee SS. Identification and characterization of a novel PPARalpha-regulated and 7alpha-hydroxyl bile acid-preferring cytosolic sulfotransferase mL-STL (Sult2a8). *J Lipid Res.* 2017;58(6):1114-31.
- Ridlon JM, Kang DJ, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. *J Lipid Res.* 2006; 47(2):241-59.
- Ridlon JM, Harris SC, Bhowmik S, Kang DJ, Hylemon PB. Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes* 2016;7(1):22-39.
- Li T, Owsley E, Matozel M, Hsu P, Novak CM, Chiang JY. Transgenic expression of cholesterol 7alpha-hydroxylase in the liver prevents high-fat diet-induced obesity and insulin resistance in mice. *Hepatology* 2010;52(2): 678-90.
- Qi Y, Jiang C, Cheng J, Krausz KW, Li T, Ferrell JM, Gonzalez FJ, Chiang JY. Bile acid signaling in lipid metabolism: Metabolomic and lipidomic analysis of lipid and bile acid markers linked to anti-obesity and anti-diabetes in mice. *Biochim Biophys Acta* 2015;1851:19-29.
- Xie C, Jiang C, Shi J, Gao X, Sun D, Sun L, Wang T, Takahashi S, Anitha M, Krausz KW, and others. An intestinal farnesoid X receptor-ceramide signaling axis modulates hepatic gluconeogenesis in mice. *Diabetes* 2017;66(3):613-26.
- Ferrell JM, Boehme S, Li F, Chiang JY. Cholesterol 7{alpha}-hydroxylase-deficient mice are protected from high fat/high cholesterol diet-induced metabolic disorders. *J Lipid Res.* 2016;57:1144-54.
- Sayin SI, Wahlstrom A, Felin J, Jantti S, Marschall HU, Bamberg K, Angelin B, Hyotylainen T, Oresic M, Backhed F. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. *Cell Metab.* 2013; 17(2):225-35.
- Li-Hawkins J, Lund EG, Turley SD, Russell DW. Disruption of the oxysterol 7alpha-hydroxylase gene in mice. *J Biol Chem.* 2000;275(22):16536-42.
- Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, Macchiarulo A, Yamamoto H, Matakic C,

- Pruzanski M, and others. TGR5-mediated bile acid sensing controls glucose homeostasis. *Cell Metab.* 2009;10(3):167–77.
26. Porez G, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease: Thematic review series: New lipid and lipoprotein targets for the treatment of cardiometabolic diseases. *J Lipid Res.* 2012;53(9):1723–37.
27. Li T, Chiang JY. Bile acid signaling in metabolic disease and drug therapy. *Pharmacol Rev.* 2014;66(4):948–83.
28. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, Willson TM, Edwards PA. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci USA* 2006;103(4):1006–11.
29. Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, Moore DD, Auwerx J. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest.* 2004;113(10):1408–18.
30. Caron S, Huaman Samanez C, Dehondt H, Ploton M, Briand O, Lien F, Dorchie E, Dumont J, Postic C, Cariou B, and others. Farnesoid X receptor inhibits the transcriptional activity of carbohydrate response element binding protein in human hepatocytes. *Mol Cell Biol.* 2013;33(11):2202–11.
31. Matsukuma KE, Bennett MK, Huang J, Wang L, Gil G, Osborne TF. Coordinated control of bile acids and lipogenesis through FXR-dependent regulation of fatty acid synthase. *J Lipid Res.* 2006;47(12):2754–61.
32. Watanabe M, Horai Y, Houten SM, Morimoto K, Sugizaki T, Arita E, Matakai C, Sato H, Tanigawara Y, Schoonjans K, and others. Lowering bile acid pool size with a synthetic farnesoid X receptor (FXR) agonist induces obesity and diabetes through reduced energy expenditure. *J Biol Chem.* 2011;286(30):26913–20.
33. Prawitt J, Abdelkarim M, Stroeve JH, Popescu I, Duez H, Velagapudi VR, Dumont J, Bouchaert E, van Dijk TH, Lucas A, and others. Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. *Diabetes* 2011;60(7):1861–71.
34. Li F, Jiang C, Krausz KW, Li Y, Albert I, Hao H, Fabre KM, Mitchell JB, Patterson AD, Gonzalez FJ. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. *Nat Commun.* 2013;4:2384.
35. Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, Cai J, Qi Y, Fang ZZ, Takahashi S, and others. Intestinal farnesoid X receptor signaling promotes non-alcoholic fatty liver disease. *J Clin Invest.* 2015;125(1):386–402.
36. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, Brocker CN, Desai D, Amin SG, Bisson WH, and others. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. *Nat Commun.* 2015;6:10166.
37. Fang S, Suh JM, Reilly SM, Yu E, Osborn O, Lackey D, Yoshihara E, Perino A, Jacinto S, Lukasheva Y, and others. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. *Nat Med.* 2015;21(2):159–65.
38. Modica S, Petruzzelli M, Bellafante E, Murzilli S, Salvatore L, Celli N, Di Tullio G, Palasciano G, Moustafa T, Halilbasic E, and others. Selective activation of nuclear bile acid receptor FXR in the intestine protects mice against cholestasis. *Gastroenterology* 2012;142(2):355–65 e1–4.
39. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, Fukusumi S, Habata Y, Itoh T, Shintani Y, and others. A G protein-coupled receptor responsive to bile acids. *J Biol Chem.* 2003;278:9435–9440.
40. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, Itadani H, Tanaka K. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun.* 2002;298(5):714–9.
41. Keitel V, Cupisti K, Ullmer C, Knoefel WT, Kubitz R, Haussinger D. The membrane-bound bile acid receptor TGR5 is localized in the epithelium of human gallbladders. *Hepatology* 2009;50:861–70.
42. Keitel V, Ullmer C, Haussinger D. The membrane-bound bile acid receptor TGR5 (Gpbar-1) is localized in the primary cilium of cholangiocytes. *Biol Chem.* 2010;391(7):785–9.
43. Watanabe M, Houten SM, Matakai C, Christoffolete MA, Kim BW, Sato H, Messaddeq N, Harney JW, Ezaki O, Kodama T, and others. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006;439:484–9.
44. Li T, Holmstrom SR, Kir S, Umetani M, Schmidt DR, Kliewer SA, Mangelsdorf DJ. The G protein-coupled bile acid receptor, TGR5, stimulates gallbladder filling. *Mol Endocrinol.* 2011;25(6):1066–71.
45. Alemi F, Poole DP, Chiu J, Schoonjans K, Cattaruzza F, Grider JR, Bunnett NW, Corvera CU. The receptor TGR5 mediates the prokinetic actions of intestinal bile acids and is required for normal defecation in mice. *Gastroenterology* 2013;144(1):145–54.
46. Pean N, Doignon I, Garcin I, Besnard A, Julien B, Liu B, Branchereau S, Spraul A, Guettier C, Humbert L, and others. The receptor TGR5 protects the liver from bile acid overload during liver regeneration in mice. *Hepatology* 2013;58:1451–60.
47. Perino A, Pols TW, Nomura M, Stein S, Pellicciari R, Schoonjans K. TGR5 reduces macrophage migration through mTOR-induced C/EBPbeta differential translation. *J Clin Invest.* 2014;124(12):5424–36.
48. Hogenauer K, Arista L, Schmiedeberg N, Werner G, Jaksche H, Bouhelal R, Nguyen DG, Bhat BG, Raad L, Rauld C, and others. G-protein-coupled bile acid receptor 1 (GPBAR1, TGR5) agonists reduce the production of proinflammatory cytokines and stabilize the alternative macrophage phenotype. *J Med Chem.* 2014;57(24):10343–54.
49. Lou G, Ma X, Fu X, Meng Z, Zhang W, Wang YD, Van Ness C, Yu D, Xu R, Huang W. GPBAR1/TGR5 mediates bile acid-induced cytokine expression in murine Kupffer cells. *PLoS One* 2014;9(4):e93567.
50. Mobraten K, Haugbro T, Karlstrom E, Kleiveland CR, Lea T. Activation of the bile acid receptor TGR5 enhances LPS-induced inflammatory responses in a human monocyte cell line. *J Recept Signal Transduct Res.* 2015;35(5):402–9.
51. Reich M, Deutschmann K, Sommerfeld A, Klindt C, Kluge S, Kubitz R, Ullmer C, Knoefel WT, Herebian D, Mayatepek E, and others. TGR5 is essential for bile acid-dependent cholangiocyte proliferation in vivo and in vitro. *Gut* 2016;65(3):487–501.
52. Donepudi AC, Boehme S, Li F, Chiang JY. G-protein-coupled bile acid receptor plays a key role in bile acid

- metabolism and fasting-induced hepatic steatosis in mice. *Hepatology* 2017;65(3):813–27.
53. Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 2002;109(3):307–20.
 54. Kohsaka A, Laposky AD, Ramsey KM, Estrada C, Joshu C, Kobayashi Y, Turek FW, Bass J. High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab.* 2007;6(5):414–21.
 55. Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD, Panda S. Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc Natl Acad Sci USA* 2009;106(50):21453–8.
 56. Arble DM, Bass J, Laposky AD, Vitaterna MH, Turek FW. Circadian timing of food intake contributes to weight gain. *Obesity (Silver Spring)* 2009;17(11):2100–2.
 57. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. *Science* 2010;330(6009):1349–54.
 58. Hatori M, Vollmers C, Zarrinpar A, DiTacchio L, Bushong EA, Gill S, Leblanc M, Chaix A, Joens M, Fitzpatrick JA, and others. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab.* 2012;15(6):848–60.
 59. Zarrinpar A, Chaix A, Yooshep S, Panda S. Diet and feeding pattern affect the diurnal dynamics of the gut microbiome. *Cell Metab.* 2014;20(6):1006–1017.
 60. Pathak P, Li T, Chiang JY. Retinoic acid-related orphan receptor alpha regulates diurnal rhythm and fasting induction of sterol 12alpha-hydroxylase in bile acid synthesis. *J Biol Chem.* 2013;288(52):37154–65.
 61. Galman C, Angelin B, Rudling M. Bile acid synthesis in humans has a rapid diurnal variation that is asynchronous with cholesterol synthesis. *Gastroenterology* 2005;129(5):1445–53.
 62. Galman C, Angelin B, Rudling M. Pronounced variation in bile acid synthesis in humans is related to gender, hypertriglyceridaemia and circulating levels of fibroblast growth factor 19. *J Intern Med.* 2011;270(6):580–8.
 63. Lundasen T, Galman C, Angelin B, Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J Intern Med.* 2006;260(6):530–6.
 64. Kir S, Beddow SA, Samuel VT, Miller P, Previs SF, Suino-Powell K, Xu HE, Shulman GI, Kliewer SA, Mangelsdorf DJ. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. *Science* 2011;331(6024):1621–4.
 65. Morris CJ, Purvis TE, Hu K, Scheer FA. Circadian misalignment increases cardiovascular disease risk factors in humans. *Proc Natl Acad Sci USA* 2016;113(10):E1402–11.
 66. Konturek PC, Brzozowski T, Konturek SJ. Gut clock: Implication of circadian rhythms in the gastrointestinal tract. *J Physiol Pharmacol.* 2011;62(2):139–50.
 67. Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, and others. Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 2005;308(5724):1043–5.
 68. Maury E, Hong HK, Bass J. Circadian disruption in the pathogenesis of metabolic syndrome. *Diabetes Metab.* 2014;40(5):338–46.
 69. Ferrell JM, Chiang JY. Short-term circadian disruption impairs bile acid and lipid homeostasis in mice. *Cell Mol Gastroenterol Hepatol.* 2015;1(6):664–77.
 70. Voigt RM, Forsyth CB, Green SJ, Mutlu E, Engen P, Vitaterna MH, Turek FW, Keshavarzian A. Circadian disorganization alters intestinal microbiota. *PLoS One* 2014;9(5):e97500.
 71. Ferrell JM, Chiang JY. Circadian rhythms in liver metabolism and disease. *Acta Pharm Sin B* 2015;5(2):113–22.
 72. Wahlstrom A, Sayin SI, Marschall HU, Backhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metab.* 2016;24(1):41–50.
 73. Inagaki T, Moschetta A, Lee YK, Peng L, Zhao G, Downes M, Yu RT, Shelton JM, Richardson JA, Repa JJ, and others. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc Natl Acad Sci USA* 2006;103(10):3920–5.
 74. Zhang L, Xie C, Nichols RG, Chan SH, Jiang C, Hao R, Smith PB, Cai J, Simons MN, Hatzakis E, and others. Farnesoid X receptor signaling shapes the gut microbiota and controls hepatic lipid metabolism. *mSystems* 2016;1(5).
 75. Gonzalez FJ, Jiang C, Patterson AD. An intestinal microbiota-farnesoid X receptor axis modulates metabolic disease. *Gastroenterology* 2016;151(5):845–59.
 76. Islam KB, Fukiya S, Hagio M, Fujii N, Ishizuka S, Ooka T, Ogura Y, Hayashi T, Yokota A. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. *Gastroenterology* 2011;141(5):1773–81.
 77. Schnabl B, Brenner DA. Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 2014;146(6):1513–24.
 78. De Minicis S, Rychlicki C, Agostinelli L, Saccomanno S, Candelaresi C, Trozzi L, Mingarelli E, Facinelli B, Magi G, Palmieri C, and others. Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. *Hepatology* 2014;59(5):1738–49.
 79. Devkota S, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in *Il10^{-/-}* mice. *Nature* 2012;487(7405):104–8.
 80. Studer N, Desharnais L, Beutler M, Brugiroux S, Terrazos MA, Menin L, Schurch CM, McCoy KD, Kuehne SA, Minton NP, and others. Functional intestinal bile acid 7alpha-dehydroxylation by *Clostridium scindens* associated with protection from *Clostridium difficile* infection in a gnotobiotic mouse model. *Front Cell Infect Microbiol.* 2016;6:191.
 81. Li-Hawkins J, Gafvels M, Olin M, Lund EG, Andersson U, Schuster G, Bjorkhem I, Russell DW, Eggertsen G. Cholic acid mediates negative feedback regulation of bile acid synthesis in mice. *J Clin Invest.* 2002;110(8):1191–200.
 82. Slatk K, Gafvels M, Kannisto K, Ovchinnikova O, Paulsson-Berne G, Parini P, Jiang ZY, Eggertsen G. Abolished synthesis of cholic acid reduces atherosclerotic development in apolipoprotein E knockout mice. *J Lipid Res.* 2010;51(11):3289–98.
 83. Hu X, Bonde Y, Eggertsen G, Rudling M. Muricholic bile acids are potent regulators of bile acid synthesis via a positive feedback mechanism. *J Intern Med.* 2014;275(1):27–38.

84. Zhang Y, Limaye PB, Renaud HJ, Klaassen CD. Effect of various antibiotics on modulation of intestinal microbiota and bile acid profile in mice. *Toxicol Appl Pharmacol*. 2014;277(2):138–45.
85. Zietak M, Kozak LP. Bile acids induce uncoupling protein 1-dependent thermogenesis and stimulate energy expenditure at thermoneutrality in mice. *Am J Physiol Endocrinol Metab*. 2016;310(5):E346–54.
86. Zietak M, Kovatcheva-Datchary P, Markiewicz LH, Stahlman M, Kozak LP, Backhed F. Altered microbiota contributes to reduced diet-induced obesity upon cold exposure. *Cell Metab*. 2016;23(6):1216–23.
87. Worthmann A, John C, Ruhlemann MC, Baguhl M, Heinsen FA, Schaltenberg N, Heine M, Schlein C, Evangelakos I, Mineo C, and others. Cold-induced conversion of cholesterol to bile acids in mice shapes the gut microbiome and promotes adaptive thermogenesis. *Nat Med*. 2017;23(7):839–49.
88. Jena P, Sheng L, Liu H-X, Kalanetra KM, Mirsoian A, Murphy WJ, French SW, Krishnan VK, Mills DA, Wan Y-JY. Western diet-induced dysbiosis in FXR knockout mice causes persistent hepatic inflammation post antibiotic treatment. *Am J Pathol*. 2017;187(8):1800–13.
89. Sheng L, Jena PK, Liu H-X, Kalanetra KM, Gonzalez FJ, French SW, Krishnan VK, Miller DA, and Wan Y-JV. Gender differences in bile acids and microbiota in relationship with gender dissimilarity in steatosis induced by diet and FXR inactivation. *Sci Rep*. 2017;7(1):1748.
90. Xie G, Wang X, Zhao A, Yan J, Chen W, Jiang R, Ji J, Huang F, Zhang Y, Lei S, and others. Sex-dependent effects on gut microbiota regulate hepatic carcinogenic outcomes. *Sci Rep*. 2017;7:45232.
91. Clarke SF, Murphy EF, Nilaweera K, Ross PR, Shanahan F, O'Toole PW, Cotter PD. The gut microbiota and its relationship to diet and obesity: New insights. *Gut Microbes* 2012;3(3):186–202.
92. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe* 2008;3(4):213–23.
93. Fiorucci S, Cipriani S, Mencarelli A, Renga B, Distrutti E, Baldelli F. Counter-regulatory role of bile acid activated receptors in immunity and inflammation. *Curr Mol Med*. 2010;10(6):579–95.
94. Wang YD, Chen WD, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor kappa light-chain enhancer of activated B cells (NF-kappaB) in mice. *Hepatology* 2011;54(4):1421–32.
95. Cipriani S, Mencarelli A, Chini MG, Distrutti E, Renga B, Bifulco G, Baldelli F, Donini A, Fiorucci S. The bile acid receptor GPBAR-1 (TGR5) modulates integrity of intestinal barrier and immune response to experimental colitis. *PLoS One* 2011;6(10):e25637.
96. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. *J Immunol*. 2009;183(10):6251–61.
97. Renga B, Migliorati M, Mencarelli A, Fiorucci S. Reciprocal regulation of the bile acid-activated receptor FXR and the interferon-gamma-STAT-1 pathway in macrophages. *Biochim Biophys Acta* 2009;1792(6):564–73.
98. Guo C, Qi H, Yu Y, Zhang Q, Su J, Yu D, Huang W, Chen WD, Wang YD. The G-protein-coupled bile acid receptor Gpbar1 (TGR5) inhibits gastric inflammation through antagonizing NF-kappaB signaling pathway. *Front Pharmacol*. 2015;6:287.
99. Pols TW, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, Rizzo G, Gioiello A, Adorini L, Pellicciari R, and others. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metab*. 2011;14(6):747–57.
100. Perino A, Schoonjans K. TGR5 and Immunometabolism: Insights from physiology and pharmacology. *Trends Pharmacol Sci*. 2015;36(12):847–57.
101. Hageman J, Herrema H, Groen AK, Kuipers F. A role of the bile salt receptor FXR in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2010;30(8):1519–28.
102. Mencarelli A, Fiorucci S. FXR an emerging therapeutic target for the treatment of atherosclerosis. *J Cell Mol Med*. 2010;14(1–2):79–92.
103. Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen EC, Renooij W, Murzilli S, Klomp LW, Siersema PD, Schipper ME, Danese S, and others. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* 2011;60(4):463–72.
104. Wang YD, Chen WD, Wang M, Yu D, Forman BM, Huang W. Farnesoid X receptor antagonizes nuclear factor kappaB in hepatic inflammatory response. *Hepatology* 2008;48(5):1632–43.
105. Yang H, Zhou H, Zhuang L, Auwerx J, Schoonjans K, Wang X, Feng C, Lu L. Plasma membrane-bound G protein-coupled bile acid receptor attenuates liver ischemia/reperfusion injury via the inhibition of toll-like receptor 4 signaling in mice. *Liver Transpl*. 2017;23(1):63–74.
106. McMahan RH, Wang XX, Cheng LL, Krisko T, Smith M, El Kasmi K, Pruzanski M, Adorini L, Golden-Mason L, Levi M, and others. Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem*. 2013;288(17):11761–70.
107. Miyazaki-Anzai S, Masuda M, Levi M, Keenan AL, Miyazaki M. Dual activation of the bile acid nuclear receptor FXR and G-protein-coupled receptor TGR5 protects mice against atherosclerosis. *PLoS One* 2014; 9(9):e108270.
108. Ghonem NS, Assis DN, Boyer JL. Fibrates and cholestasis. *Hepatology* 2015;62(2):635–43.
109. Jacquemin E. Progressive familial intrahepatic cholestasis. *Clin Res Hepatol Gastroenterol*. 2012;36(Suppl 1): S26–35.
110. Zollner G, Trauner M. Mechanisms of cholestasis. *Clin Liver Dis*. 2008;12(1):1–26, vii.
111. Chen F, Ananthanarayanan M, Emre S, Neimark E, Bull LN, Knisely AS, Strautnieks SS, Thompson RJ, Magid MS, Gordon R, and others. Progressive familial intrahepatic cholestasis, type 1, is associated with decreased farnesoid X receptor activity. *Gastroenterology* 2004;126(3):756–64.
112. Gomez-Ospina N, Potter CJ, Xiao R, Manickam K, Kim MS, Kim KH, Shneider BL, Picarsic JL, Jacobson TA, Zhang J, and others. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. *Nat Commun*. 2016;7:10713.

113. Lioudaki E, Ganotakis ES, Mikhailidis DP. Lipid lowering drugs and gallstones: A therapeutic option? *Curr Pharm Des.* 2011;17(33):3622–31.
114. Hohenester S, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, Beuers U. A biliary HCO₃-umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. *Hepatology* 2012;55(1):173–83.
115. Prieto J, Garcia N, Marti-Climent JM, Penuelas I, Richter JA, Medina JF. Assessment of biliary bicarbonate secretion in humans by positron emission tomography. *Gastroenterology* 1999;117(1):167–72.
116. Beuers U. Drug insight: Mechanisms and sites of action of ursodeoxycholic acid in cholestasis. *Nat Clin Pract Gastroenterol Hepatol.* 2006;3(6):318–28.
117. Halilbasic E, Fiorotto R, Fickert P, Marschall HU, Moustafa T, Spirli C, Fuchsichler A, Gumhold J, Silbert D, Zatloukal K, and others. Side chain structure determines unique physiologic and therapeutic properties of norursodeoxycholic acid in Mdr2^{-/-} mice. *Hepatology* 2009;49(6):1972–81.
118. Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, Parks DJ, Willson TM. 6 α -ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem.* 2002;45(17):3569–72.
119. Pellicciari R, Costantino G, Camaioni E, Sadeghpour BM, Entrena A, Willson TM, Fiorucci S, Clerici C, Gioiello A. Bile acid derivatives as ligands of the farnesoid X receptor. Synthesis, evaluation, and structure-activity relationship of a series of body and side chain modified analogues of chenodeoxycholic acid. *J Med Chem.* 2004; 47(18):4559–69.
120. Fiorucci S, Clerici C, Antonelli E, Orlandi S, Goodwin B, Sadeghpour B, Sabatino G, Russo G, Castellani D, Willson TM, and others. Protective effects of 6-ethyl chenodeoxycholic acid, a farnesoid X receptor (FXR) ligand, in estrogen induced cholestasis. *J Pharmacol Exp Ther.* 2005;313:604–12.
121. Hirschfield GM, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M, Kowdley KV, Vincent C, Bodhenheimer HC Jr, Pares A, and others. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. *Gastroenterology* 2015; 148(4):751–61 e8.
122. Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, Drenth JP, Pockros PJ, Regula J, Beuers U, and others. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. *N Engl J Med.* 2016;375(7):631–43.
123. Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, Adorini L, Sciacca CI, Clopton P, Castelloe E, and others. Efficacy and safety of the farnesoid x receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 2013;145(3):574–82 e1.
124. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, and others. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): A multicentre, randomised, placebo-controlled trial. *Lancet* 2015;385(9972):956–65.
125. Pols TW, Noriega LG, Nomura M, Auwerx J, Schoonjans K. The bile acid membrane receptor TGR5: A valuable metabolic target. *Dig Dis.* 2011;29(1):37–44.
126. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. *Biochem Biophys Res Commun.* 2005;329(1):386–90.
127. Kumar DP, Asgharpour A, Mirshahi F, Park SH, Liu S, Imai Y, Nadler JL, Grider JR, Murthy KS, Sanyal AJ. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet alpha cells to promote glucose homeostasis. *J Biol Chem.* 2016;291(13):6626–40.
128. Sato H, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, Saladin R, Schoonjans K, Pellicciari R, Auwerx J. Novel potent and selective bile acid derivatives as TGR5 agonists: Biological screening, structure-activity relationships, and molecular modeling studies. *J Med Chem.* 2008;51(6):1831–41.
129. Tiwari A, Maiti P. TGR5: An emerging bile acid G-protein-coupled receptor target for the potential treatment of metabolic disorders. *Drug Discov Today* 2009; 14(9–10):523–30.
130. Pellicciari R, Sato H, Gioiello A, Costantino G, Macchiarulo A, Sadeghpour BM, Giorgi G, Schoonjans K, Auwerx J. Nongenomic actions of bile acids. Synthesis and preliminary characterization of 23- and 6,23-alkyl-substituted bile acid derivatives as selective modulators for the G-protein coupled receptor TGR5. *J Med Chem.* 2007;50(18):4265–8.
131. Kida T, Tsubosaka Y, Hori M, Ozaki H, Murata T. Bile acid receptor TGR5 agonism induces NO production and reduces monocyte adhesion in vascular endothelial cells. *Arterioscler Thromb Vasc Biol.* 2013;33(7):1663–9.
132. Duan H, Ning M, Chen X, Zou Q, Zhang L, Feng Y, Zhang L, Leng Y, Shen J. Design, synthesis, and antidiabetic activity of 4-phenoxy nicotinamide and 4-phenoxy pyrimidine-5-carboxamide derivatives as potent and orally efficacious TGR5 agonists. *J Med Chem.* 2012; 55(23):10475–89.
133. Duan H, Ning M, Zou Q, Ye Y, Feng Y, Zhang L, Leng Y, Shen J. Discovery of intestinal targeted TGR5 agonists for the treatment of type 2 diabetes. *J Med Chem.* 2015;58(8):3315–28.
134. Zambad SP, Tuli D, Mathur A, Ghalsasi SA, Chaudhary AR, Deshpande S, Gupta RC, Chauthaiwale V, Dutt C. TRC210258, a novel TGR5 agonist, reduces glycemic and dyslipidemic cardiovascular risk in animal models of diabetes. *Diabetes Metab Syndr Obes.* 2013;7:1–14.
135. Rizzo G, Passeri D, De Franco F, Ciaccioli G, Donadio L, Orlandi S, Sadeghpour B, Wang XX, Jiang T, Levi M, and others. Functional characterization of the semi-synthetic bile acid derivative INT-767, a dual farnesoid X receptor and TGR5 agonist. *Mol Pharmacol.* 2010; 78(4):617–30.
136. D'Amore C, Di Leva FS, Sepe V, Renga B, Del Gaudio C, D'Auria MV, Zampella A, Fiorucci S, Limongelli V. Design, synthesis, and biological evaluation of potent dual agonists of nuclear and membrane bile acid receptors. *J Med Chem.* 2014;57(3):937–54.
137. Baghdasaryan A, Claudel T, Gumhold J, Silbert D, Adorini L, Roda A, Vecchiotti S, Gonzalez FJ, Schoonjans K, Strazzabosco M, and others. Dual farnesoid X receptor/TGR5 agonist INT-767 reduces liver

- injury in the *Mdr2*^{-/-} (*Abcb4*^{-/-}) mouse cholangiopathy model by promoting biliary HCO output. *Hepatology* 2011;54(4):1303–12.
138. Pathak P, Liu H, Boehme S, Xie C, Krausz KW, Gonzalez F, Chiang JYL. Farnesoid X receptor induces Takeda G-protein receptor 5 crosstalk to regulate bile acid synthesis and hepatic metabolism. *J Biol Chem*. 2017;292(26):11055–69.
139. Carino A, Cipriani S, Marchiano S, Biagioli M, Santorelli C, Donini A, Zampella A, Monti MC, Fiorucci S. BAR502, a dual FXR and GPBAR1 agonist, promotes browning of white adipose tissue and reverses liver steatosis and fibrosis. *Sci Rep*. 2017;7:42801.
140. Steinert RE, Peterli R, Keller S, Meyer-Gerspach AC, Drewe J, Peters T, Beglinger C. Bile acids and gut peptide secretion after bariatric surgery: A 1-year prospective randomized pilot trial. *Obesity (Silver Spring)* 2013; 21(12):E660–8.
141. Ryan KK, Tremaroli V, Clemmensen C, Kovatcheva-Datchary P, Myronovych A, Karns R, Wilson-Perez HE, Sandoval DA, Kohli R, Backhed F, and others. FXR is a molecular target for the effects of vertical sleeve gastrectomy. *Nature* 2014;509:183–8.
142. McGavigan AK, Garibay D, Henseler ZM, Chen J, Bettaieb A, Haj FG, Ley RE, Chouinard ML, Cummings BP. TGR5 contributes to glucoregulatory improvements after vertical sleeve gastrectomy in mice. *Gut* 2015;66(2):226–34.