Bile Acid Signaling in Metabolic Disease and Drug Therapy

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Address correspondence to: John Y. L. Chiang, Department of Integrative Medical Sciences, Northeast Ohio Medical University, 4209 SR 44, Rootstown, OH 44272. E-mail: jchiang@neomed.edu dx.doi.org/10.1124/pr.113.008201.
Abstract—Bile acids are the end products of cholesterol catabolism. Hepatic bile acid synthesis accounts for a major fraction of daily cholesterol turnover in humans. Biliary secretion of bile acids generates bile flow and facilitates hepatobiliary secretion of lipids, lipophilic metabolites, and xenobiotics. In the intestine, bile acids are essential for the absorption, transport, and metabolism of dietary fats and lipid-soluble vitamins. Extensive research in the last 2 decades has unveiled new functions of bile acids as signaling molecules and metabolic integrators. The bile acid–activated nuclear receptors farnesoid X receptor, pregnane X receptor, constitutive androstane receptor, vitamin D receptor, and G protein–coupled bile acid receptor play critical roles in the regulation of lipid, glucose, and energy metabolism, inflammation, and drug metabolism and detoxification. Bile acid synthesis exhibits a strong diurnal rhythm, which is entrained by fasting and refeeding as well as nutrient status and plays an important role for maintaining metabolic homeostasis. Recent research revealed an interaction of liver bile acids and gut microbiota in the regulation of liver metabolism. Circadian disturbance and altered gut microbiota contribute to the pathogenesis of liver diseases, inflammatory bowel diseases, nonalcoholic fatty liver disease, diabetes, and obesity. Bile acids and their derivatives are potential therapeutic agents for treating metabolic diseases of the liver. 

I. Introduction

Bile acids are amphipathic molecules synthesized from cholesterol exclusively in the liver. Hepatic bile acid synthesis accounts for a major fraction of daily cholesterol turnover in humans. Biliary secretion of bile acids generates bile flow, and facilitates hepatobiliary secretion of lipids, endogenous metabolites, and xenobiotics. In bile, bile acids form mixed micelles with phospholipids and cholesterol, and bile acids are released into the small intestine after meal ingestion. Bile acids facilitate the intestinal digestion and absorption of dietary fat, steroids, drugs, and lipophilic vitamins. Most bile acids are efficiently reabsorbed in the ileum and transported back to the liver via portal circulation for bile acid recycling. The bile acid recycling pathway is critical for maintaining the pool size of both endogenous and exogenous bile acids, and is a major determinant of the overall bile acid concentration in the liver and body. The bile acid recycling pathway is highly regulated by a variety of factors, including diet, hormones, and gut microbiota. The regulation of bile acid recycling is crucial for maintaining bile acid homeostasis and modulating bile acid flux into the small intestine. 

ABBREVIATIONS: 3β-HSD, 3β-hydroxy-Δ5-Δ7-steroid dehydroxylase; ABC, ATP-binding cassette; ACC, acetyl-CoA carboxylase; AMPK, AMP-activated kinase; Apo, apolipoprotein; ASBT, apical sodium-dependent bile salt transporter; BDL, bile duct ligation; BML-1, brain and muscle ARNT-like protein-1; BSH, bile salt hydrolase; CA, cholic acid; CAR, constitutive androstane receptor; CCG, clock-controlled gene; CDCA, chenodeoxycholic acid; ChREBP, carbohydrate response element binding protein; CLOK, circadian locomotor output cycles kaput; Cry, cryptochrome; CYP7A1-tg, transgenic mice overexpressing CYP7A1 in the liver; C4, 7α-hydroxy-4-cholesten-3-one; D BoP, D-site binding protein; DCA, deoxycholic acid; E4BP4, E4 promoter binding protein 4; ER, endoplasmic reticulum; ERK, extracellular signal–regulated kinase; FAS, fatty acid synthase; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; FXR, farnesoid X receptor; G6Pase, glucose-6-phosphatase; GLP-1, glucagon-like peptide-1; GPAM, glycerol-3-phosphate acyltransferase 1; GPCR, G protein–coupled receptor; GW4064, 3-[2-[2-chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid; HDAC, histone deacetylase; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HNF, hepatic nuclear factor; IBD, inflammatory bowel disease; IEC, intraepithelial cholestasis of pregnancy; INT-774, 6a-ethyl-3a,7a,23-trihydroxy-24-nor-5β-cholan-23-sulfate; INT-777, 6a-ethyl-23(S)-methylcholic acid; LBD, ligand-binding domain; LCA, lathosterol; LDL, low-density lipoprotein; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; LPL, lipoprotein lipase; LPS, lipopolysaccharide; LRH-1, liver–related homolog-1; LXR, liver orphan receptor; MCA, muricholic acid; MDR, multidrug resistance protein; mEH, microsomal epoxide hydrolase; mRNA, microRNA; MRP, multidrug resistance-associated protein; NALFD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF–κB, nuclear factor–κB; NO, nitric oxide; Npas2, neuronal PAS domain protein 2; Ntcp, Na+-taurocholate cotransport peptide; OATP, organic anion transporter; OCA, obeticholic acid; OST, organic solute transporter; PCSK9, proprotein convertase subtilisin/kexin type 9; PEPCK, phosphoenolpyruvate carboxykinase; Per, period; PGC-1α, progressive familial intrahepatic cholestasis; PGC-1α, peroxisome proliferator–activated receptor γ coactivator-1α; PPAR, peroxisome proliferator–activated receptor; PXR, pregnane X receptor; Rev-erba, reverse-erythroid bason; ROS, retinoic acid–related orphan receptor α; RYGB, Roux-en-Y gastric bypass; S1P, sphingosine-1-phosphate; S1PR2, sphingosine-1-phosphate receptor 2; SHP, small heterodimer partner; SIRT, sirtuin; SphK, sphingosine kinase; SR-BI, scavenger receptor B1; SREBP, sterol response element binding protein; SULT, sulfotransferase; T2DM, type 2 diabetes mellitus; T-β-MCA, tauro-β-muricholic acid; TCA, taurocholic acid; TCPOBOP, 1,4-bis-[3,3-dichloropyridyloxy]benzene; TCA, taurodeoxycholic acid; TGR5, G protein–coupled bile acid receptor (Gpbar); TICE, transintestinal cholesterol excretion; TUDCA, tauroursodeoxycholic acid; UDCA, ursodeoxycholic acid; UTR, untranslated region; VDR, vitamin D receptor; VLDL, very low-density lipoprotein; VSG, vertical sleeve gastrectomy; VSMC, vascular smooth muscle cell; WAY-362450, 3-(3,4-difluorobenzoyl)-1,2,3,6-tetrahydro-1,1-dimethylazepino[4,5-b]indole-5-carboxylic acid 1-methylethyl ester.
 bile acid synthesis is a multistep reaction that involves enzymes localized in the endoplasmic reticulum (ER), mitochondria, cytosol, and peroxisomes (Russell, 2003).

1. The Classic Pathway. In humans, the classic pathway accounts for more than 90% of total bile acid production and thus is considered the major bile acid biosynthetic pathway. The classic pathway is initiated by a microsomal cholesterol 7α-hydroxylase (CYP7A1), which converts cholesterol to 7α-hydroxycholesterol and is the rate-limiting step in the classic pathway (Myant and Mitropoulos, 1977). Then 3β-hydroxy-Δ5-7C27-steroid dehydroxylase (3β-HSD) converts 7α-hydroxycholesterol to 7α-hydroxy-4-cholenoic acid-3-one (C4), which is the common precursor for CA and CDCA, and has been used as a serum marker for the rate of bile acid synthesis (Axelson et al., 1988; Honda et al., 2007). The microsomal sterol 12α-hydroxylase (CYP8B1) mediates the hydroxylation of C4 at the C-12 position, followed by several reactions including the steroid side chain cleavage by the mitochondrial sterol 27-hydroxylase (CYP27A1), leading to the synthesis of CA. CYP7A1 regulates the overall rate of bile acid production, whereas CYP8B1 regulates the CA/CDCA ratio in the bile acid pool. In mice, the majority of CDCA is converted to α-muricholic acid (MCA) and β-CDCA; therefore, CA and α- and β-MCAs are the major primary bile acids in the mouse bile acid pool.

2. The Alternative Pathway. In humans, the alternative pathway is thought to produce less than 10% of the total bile acids under normal physiologic conditions. In this pathway, the mitochondrial CYP27A1 catalyzes the first hydroxylation reaction of cholesterol to 27-hydroxycholesterol and 3β-hydroxy-5-cholestenolic acid, which is subsequently hydroxylated at the C-7 position by oxysterol 7α-hydroxylase (CYP7B1) to form 3β,7α-di hydroxy-5-cholestenolic acid. CYP7B1 catalyzes many hydroxylation reactions in steroid synthesis in steroidogenic tissues and oxysterol synthesis in peripheral tissues. The oxysterol intermediates formed in the peripheral tissues could be transported to the liver and mainly converted to CDCA. The alternative pathway may be responsible for the synthesis of about 50% of bile acids in rodents.

B. The Enterohepatic Circulation of Bile Acids

Conjugated bile acids synthesized in the hepatocytes are secreted across the canalicular (apical) membrane into the bile and stored in the gallbladder. After a meal, the duodenum secretes cholecystokinin, which stimulates gallbladder contraction and the release of bile acids into the intestinal tract. Along the small intestinal tract, micellar bile acids act as effective detergents to facilitate the solubilization of fatty acids and monoacylglycerols, digestion, and absorption of dietary lipids and fat-soluble vitamins. Bile acids are efficiently reabsorbed in the ileum, and transported back to the liver via portal blood.
for resecretion into the bile. This process is referred to as enterohepatic circulation of bile acids (Fig. 3). Only 5% of the total bile acid (approximately 0.5 g/day) is excreted into the feces, and this is replenished by the de novo synthesis in the liver. Bile acid transport across the plasma membrane is an active transport process.
that requires high-affinity bile acid transporters in the liver and the intestine (Trauner and Boyer, 2003). The bile acid pool size is defined as the total amount of bile acids in the enterohepatic circulation. It should be emphasized that bile acid compositions in humans and mice are very different. In humans, the highly hydrophobic bile acid pool consists of about 40% each of CA and CDCA, and 20% DCA. In mice, the highly hydrophilic bile acid pool consists of about 50% CA and 50% α- and β-MCs.

1. Hepatic Bile Acid Transport. Hepatocytes are polarized cells with basolateral (sinusoidal) and apical (canalicular) membrane domains (Fig. 4). Hepatocytes take up circulating bile acids through the basolateral membrane that is in direct contact with the portal plasma. This process is highly efficient with an approximately 80–90% first-pass extraction rate for conjugated bile acids. Bile acids cannot across the hepatocyte membrane, but require active transport systems (Meier and Stieger, 2002). The majority of circulating bile acids are taken up by hepatocytes via Na+-dependent cotransport systems. The Na+-dependent taurocholate transporter (NTCP; SLC10A1) has been identified as the major bile acid uptake transporter in the basolateral (sinusoidal) membrane of hepatocytes (Ananthanarayanan et al., 1988) (Fig. 4). Several earlier studies suggested a role of the microsomal epoxide hydrolase (mEH) in mediating the basolateral Na+-dependent bile acid uptake (von Dippe et al., 1993, 1996; Fretland and Omiecinski, 2000). Mice lacking mEH showed no apparent alteration in bile acid homeostasis (Miyata et al., 1999). By contrast, a point mutation that resulted in significantly decreased mEH expression in a human individual led to hypercholanemia, a condition of increased plasma bile acid levels in the absence of hepatocyte injury, suggesting impaired basolateral bile acid uptake rather than intrahepatic bile acid accumulation (Zhu et al., 2003). It is estimated that about 25% of bile acid uptake by hepatocytes is mediated by Na+-independent mechanisms (Trauner and Boyer, 2003). This pathway is mainly responsible for the uptake of unconjugated bile acids. Several organic anion transporters (OATPs), including OATP1A2, OATP1B1, and OATP1B3, have been identified as Na+-independent bile acid transporters.

Fig. 3. Enterohepatic circulation of bile acids. The human bile acid pool consists of approximately 3 g of bile acids. Food intake stimulates the gallbladder to release bile acids into the small intestine. An average man produces approximately 0.5 g bile acid per day by synthesis in the liver, and secretes approximately 0.5 g/day. Conjugated bile acids are efficiently reabsorbed in the ileum by active transport, whereas a small amount of unconjugated bile acids is reabsorbed by passive diffusion in the small and large intestines. The first-pass extraction of bile acids from the portal blood by the liver is very efficient. Small amounts of bile acids that spilled over into the systemic circulation are recovered in kidney. The bile acids in the pool are recycled 4–12 times a day.
The concentration of bile acids in the bile can be approximately 100- to 1000-fold higher than that in the hepatocytes. Canalicular bile acid transport against the concentration gradient thus represents the rate-limiting step in bile formation. The ATP-binding cassette (ABC) transporter bile salt export pump (BSEP; ABCB11), also known as the sister of P-glycoprotein, effluxes bile acids across the canalicular membrane (Childs et al., 1995). Patients with progressive familial intrahepatic cholestasis (PFIC) subtype 2 due to mutations in the BSEP gene show markedly elevated plasma bile acid levels with only less than 1% of normal biliary bile acid concentration, suggesting that BSEP is the major canalicular bile acid transport system in the hepatocytes (Strautnieks et al., 1997). The multidrug resistance–associated protein MRP2 (ABCC2), which mediates the transport of a wide range of organic substrates including bilirubin conjugates, glutathione, drugs, and so forth, also shows substrate specificity for divalent bile acids, such as sulfate-conjugated or glucuronidated N-acetylamidated bile acids. bile acids, phospholipids, and cholesterol are three major organic solutes of bile and once secreted, form mixed micelles to increase cholesterol solubility and lower the monomeric concentration of bile acids, thereby reducing their cytotoxicity to the bile duct. Cholesterol secretion into the bile is mediated by the ABCG5 and ABCG8 heterodimer at the canalicular membrane. The major phospholipid in the bile is phosphatidylcholine, which is excreted via the phospholipid flippase, multidrug-resistant protein MDR2 (ABCB4) (Smit et al., 1993).

2. Intestinal Bile Acid Transport. It is estimated that about 90–95% of bile acids are reabsorbed in the intestine with minimal daily loss in the feces. Therefore, the amount of bile acid synthesized by the liver to compensate the daily fecal loss and thus to maintain a constant bile acid pool size is also low under physiologic conditions. Intestinal bile acid reabsorption mainly occurs at the terminal ileum by the apical sodium-dependent bile salt transporter (ASBT; SLC10A2) (Wong et al., 1994; Schneider et al., 1995) (Fig. 4). Once absorbed into the enterocytes, bile acids bind to the intestinal bile acid binding protein and are transported to the basolateral membrane for secretion (Gong et al., 1994). The heterodimeric organic solute transporters OSTα and OSTβ appeared to be the major basolateral bile acid transport system in the intestine (Ballatori et al., 2005; Dawson et al., 2005). Overexpression of OSTα and OSTβ in mice enhanced basolateral efflux of taurocholate, whereas mice lacking osta showed significantly decreased intestinal bile acid absorption, plasma bile acids, and total bile acid pool (Rao et al., 2008). OSTα and OSTβ are also expressed in the human liver, and at relatively low levels in mouse liver (Ballatori et al., 2005). Both OSTα and OSTβ are localized at the basolateral membrane of the hepatocytes. OSTα and OSTβ are induced during cholestasis and thus mediate bile acid efflux into the circulation for renal excretion (Boyer et al., 2006; Cui et al., 2009). Interestingly, recent evidence suggests that OSTα/OSTβ also plays a role in mediating bile acid reuptake in the kidney (Soroka et al., 2010).

In the intestine, bile acids undergo multistep biotransformation usually catalyzed by enzyme activities in gut bacteria (Chiang, 1998; Ridlon et al., 2006). In the small and large intestine, some conjugated bile acids are deconjugated by bacterial bile salt hydrolases (BSHs) to become free bile acids. Unconjugated bile acids can cross the plasma membrane via passive diffusion, a process that accounts for a small fraction of bile acids recycled along the small and large intestines. In the large intestine, bacterial 7α-dehydroxylase removes a hydroxyl group from C-7 and converts CA to DCA and CDCA to LCA, respectively. These secondary bile acids are cytotoxic. DCA and, to a much less extent, LCA are reabsorbed in the large intestine via passive absorption. In humans, LCA is sulfated and N-acylamidated in the liver and secreted into bile. In mice, LCA is detoxified by hydroxylation at C-6 and/or C-7 in the intestine for fecal excretion. A small amount of LCA (approximately 1%) circulated to the liver is sulfated and efficiently secreted into the circulation for renal excretion. In the intestine, CYP3A4, CYP2B, CYP2C, and epimerases are involved in detoxification of LCA to more soluble hyocholic acid and ursodeoxycholic acid (UDCA) in humans. In mouse intestine LCA can be 7α-hydroxylated to CDCA, which is converted to α-MCA, β-MCA, ω-MCA, UDCA, hyodeoxycholic acid, and hyocholic acid (Fig. 2).

C. Regulation of Bile Acid Synthesis

It has been shown that feeding rats with bile acids resulted in a strong reduction of hepatic CYP7A1 enzyme activity and bile acid synthesis, whereas interruption of bile acid reabsorption in the intestine by bile acid binding resin, and subsequent reduction of bile acids returning to the liver, stimulated hepatic CYP7A1 enzyme activity and bile acid synthesis. This early evidence suggests that hepatic CYP7A1 and bile acid synthesis are under negative feedback regulation by bile acids. It is now clear that bile acid synthesis is mainly controlled by bile acids via the transcriptional repression of the CYP7A1 gene (Chiang, 2009). This bile acid feedback repression mechanism allows the liver to efficiently stimulate or inhibit bile acid synthesis in response to changes in bile acid levels and thus to maintain bile acid homeostasis. During cholestasis, repression of bile acid synthesis is a protective mechanism against hepatic bile acid accumulation and bile acid cytotoxicity.

The mechanisms by which bile acids inhibit hepatic CYP7A1 have been extensively investigated in the past decades (Chiang, 2009). These studies have shown that the nuclear receptor FXR plays a key role in mediating...
the bile acid feedback inhibition of CYP7A1 in the hepatocytes. It was first shown that upon activation by bile acids, hepatic FXR transcriptionally induced a nuclear receptor small heterodimer partner (SHP), which acted as a corepressor to inhibit the transcriptional activity of a nuclear receptor liver-related homolog-1 (LRH-1) or hepatocyte nuclear factor (HNF) 4α bound to the CYP7A1 gene promoter, and resulted in inhibition of CYP7A1 gene transcription (pathway 1, Fig. 5) (Chiang et al., 2000; Goodwin et al., 2000; Lu et al., 2000). However, the repression of CYP7A1 by bile acids and an FXR agonist was still observed in shp null mice, which implied that the FXR/SHP/LRH-1 cascade may not likely be the only pathway mediating bile acid feedback inhibition of CYP7A1 (Kerr et al., 2002; Wang et al., 2002). It was reported earlier that intraduodenal, but not intravenous, infusion of taurocholic acid (TCA) repressed CYP7A1 mRNA expression in rats (Pandak et al., 1995), suggesting that bile acids might induce an intestinal factor that mediates bile acid feedback inhibition of bile acid synthesis. In addition to the liver, FXR is also highly expressed in the intestine. Inagaki et al. (2005) reported that activation of FXR by bile acids or synthetic agonists in mice induced an intestinal fibroblast growth factor FGF15, which activates a liver fibroblast growth factor receptor FGFR4 to inhibit CYP7A1 and bile acid synthesis (pathway 2, Fig. 5). The FGF15/FGFR4 signaling requires a transmembrane protein called β-Klotho (Lin et al., 2007). Mice lacking either FGFR4 or β-Klotho had elevated hepatic CYP7A1 mRNA, enlarged bile acid pool size, and impaired bile acid feedback inhibition of CYP7A1 (Yu et al., 2000, 2005a). Thus, this intestine-to-liver bile acid sensing mechanism may play a critical role in maintaining overall bile acid homeostasis.

Human FGF19 shares approximately 51% amino acid sequence identity with mouse FGF15, and is considered to be the mouse FGF15 ortholog. FGF19 has been shown to repress CYP7A1 in human hepatocytes (Holt et al., 2003; Song et al., 2009). In contrast with FGF15 in mice, FGF19 mRNA is detectable in human livers and human hepatocytes, as well as gallbladder (Schaap et al., 2009; Song et al., 2009), and FGF19 protein is detectable in human blood circulation (Lundåsen et al., 2006). Cholestyramine treatment reduced plasma FGF19 levels in humans (Lundåsen et al., 2006). Circulating FGF19 levels were elevated in human patients with obstructive cholestasis, indicating that human hepatocytes produce FGF19 (Schaap et al., 2009). In human hepatocytes, FGF19 is highly inducible by bile acids or FXR agonists (Holt et al., 2003; Song et al., 2009). Such evidence suggests that in humans, both liver and intestine secrete FGF19 into the blood circulation, and that the FXR/FGF19 pathway is involved in bile acid sensing and regulation in both human liver and intestine, either via an autocrine or an endocrine manner.

The intracellular events after FGFR4 activation to repress CYP7A1 are incompletely understood (Fig. 5). In human hepatocytes, FGF19 repression of CYP7A1 was shown to be largely dependent on the activation of extracellular signal-regulated kinase (ERK) signaling, but the downstream target is still not clear (Song et al., 2009). In mice, the possible involvement of ERK, c-Jun N-terminal kinase, and SHP in mediating FGF15 repression of CYP7A1 has been suggested (Inagaki et al., 2005). It was shown that the repressive effect of CYP7A1 by adenovirus-mediated FGF15 overexpression in the liver was attenuated in shp knockout mice (Inagaki et al., 2005). On the other hand, the FGF15 repression of CYP7A1 was still very strong in shp knockout mice (Kong et al., 2012), suggesting that SHP is unlikely to be the major mediator of FGF15 repression of CYP7A1. Further studies are needed to delineate the intracellular mechanisms mediating the FGF15/FGF19 repression of CYP7A1 in hepatocytes.

FGF15 was also implicated in the regulation of gallbladder refilling in mice (Choi et al., 2006). Mice lacking FGF15 had almost empty gallbladders, and the gallbladder volume was restored by the administration of recombinant FGF15. By contrast, human gallbladders secrete high levels of FGF19 into the bile, and gallbladder FGF19 concentrations are about 100-fold higher than that in the blood circulation (Zweers et al., 2012). Both FGFR4 and β-Klotho were expressed
in the epithelial cells of the gallbladder, suggesting that the gallbladder should also respond to FGF19-activated intracellular signaling. The role of FGF19 signaling in the human gallbladder remains to be defined.

**II. Bile Acid Receptor Signaling in Liver Metabolism**

**A. Bile Acid–Activated Nuclear Receptors**

Nuclear receptors are a group of ligand-activated transcription factors that play important roles in embryogenesis, development, and metabolism (Mangelsdorf and Evans, 1995; Mangelsdorf et al., 1995; Chiang, 2002). Nuclear receptors play multiple roles in liver physiology and pathophysiology (Karpen and Trauner, 2010). The general structure of a nuclear receptor consists of an N-terminal DNA binding domain and a C-terminal ligand-binding domain (LBD) (Fig. 6). The N-terminal DNA binding domain is the most conserved region, containing two Zinc finger motifs that allow the nuclear receptor to bind to a consensus AGGTCA-like DNA sequence (termed the hormone response element) as a homodimer or heterodimer with the retinoid X receptor. A few nuclear receptors (e.g., HNF4 and LRH) bind DNA as monomers. The LBD is involved in ligand binding, nuclear receptor dimerization, and coregulator (coactivators or corepressors) interaction. Ligand binding causes a conformational change in the LBD, which leads to a shift of the helix 12 to allow the LBD to interact with the coactivators via the LXXLL motif—containing nuclear receptor box on the coactivators. Upon recruitment to the nuclear receptor, coactivators displace corepressors, and further recruit chromatin remodeling proteins to facilitate the assembly of general transcriptional complex that induces gene transcription. There are 48 nuclear receptor genes in the human genome and 49 in the mouse genome (Mangelsdorf et al., 1995).

Bile acids directly activate three nuclear receptors: FXR (Forman et al., 1995; Makishima et al., 1999; Parks et al., 1999), PXR (Xie et al., 2001), and VDR (Makishima et al., 2002). FXR and PXR are highly expressed in tissues that are exposed to bile acids, including the liver and the intestine (Forman et al., 1995; Kliewer et al., 1998), whereas VDR is widely expressed in most tissues. FXR can be activated by both free and conjugated bile acids; the hydrophobic bile acid CDCA is the most efficacious ligand of FXR (EC50 = approximately 10 μM), followed by LCA, DCA, and CA, whereas hydrophilic bile acids UDCA and MCA do not activate FXR. The secondary bile acid LCA and its metabolite 3-keto-LCA are the most potent activators of PXR and VDR, with an EC50 of approximately 100 nM. These nuclear receptors act as sensors of bile acid levels in the hepatocytes and the enterocytes, and mediate the pleiotropic effects of bile acids in the regulation of metabolic homeostasis.

1. **Farnesoid X Receptor Regulation of Bile Acid Homeostasis.** FXR regulates a network of genes in hepatic bile acid synthesis, biliary bile acid secretion, intestinal bile acid absorption, and hepatic bile acid uptake, and thus plays a key role in the regulation of bile acid homeostasis (Table 1). In addition to mediating the bile acid inhibition of bile acid synthesis, FXR activation by bile acids prevents hepatic bile acid accumulation via transcriptional induction of the apical bile acid efflux transporters BSEP (Ananthanarayanan et al., 2001) and MRP2 (Kast et al., 2002), the
phosphatidylcholine transporter MDR2 (Li et al., 2003), and the cholereter transporters ABCG5 and ABCG8 (Li et al., 2011b). It seems that FXR activation coordinates the biliary secretion of bile acids, cholesterol, and phospholipids, which form mixed micelles in the bile to prevent gallstone formation and bile acid damage to the bile duct epithelium (Li et al., 2003; Wittenburg et al., 2003; Moschetta et al., 2004). At the basolateral membrane of hepatocytes, the expression of bile acid uptake transporter NTCP is repressed by FXR, presumably via induction of SHP that represses the transactivation of NTCP by retinoic acid receptor and HNF4α (Denson et al., 2001). In conditions associated with hepatic bile acid accumulation, there is a compensatory induction of transporters at the basolateral membrane of the hepatocytes to efflux bile acids into the blood circulation, resulting in elevated plasma bile acid concentration and renal excretion of bile acids. FXR activation induces the OSTα and OSTβ expression at the sinusoidal membrane to efflux bile acids to the systemic circulation (Lee et al., 2006). Several basolateral drug transporters (MRP1, MRP3, and MRP4) are able to transport conjugated bile acids (Trauner and Boyer, 2003). These MRPs are induced during cholestasis in both mice and humans, but other studies suggest that induction of these basolateral MRPs may be due to activation of PXR rather than FXR (Zollner et al., 2007).

Similar to the effect of FXR activation in the hepatocytes, activation of intestine FXR by bile acids limits bile acid uptake and promotes basolateral bile acid secretion to decrease intracellular bile acid concentration. Bile acids inhibit mouse and human ASBT, but not rat ASBT (Arrese et al., 1998; Chen et al., 2003; Neimark et al., 2004). FXR induces the major intestinal bile acid efflux transporters OSTα and OSTβ located in the sinusoidal membrane (Lee et al., 2006).

Numerous studies have been carried out in fxr knockout mice to evaluate the role of FXR in regulating bile acid homeostasis and detoxification under bile duct ligation (BDL), bile acid feeding or drug-induced intrahepatic cholestasis (Wagner et al., 2003, 2011; Cui et al., 2009). Mice lacking FXR were phenotypically normal but showed elevated CYP7A1 mRNA expression and enlarged bile acid pool size (Sinal et al., 2000; Kok et al., 2003). When fed a CA-containing diet, fxr null mice showed more severe hepatotoxicity, which was accompanied by the lack of CYP7A1 repression and induction of FXR regulated transporters in the liver and the intestine. The fxr null mice were also more susceptible to bile acid–induced liver injury after BDL despite induction of the PXR-regulated bile acid detoxification network.

2. Farnesoid X Receptor Regulation of Triglyceride Metabolism. Bile acids are known to have lipid-lowering effects and control liver triglycerides by mechanisms that are not fully understood. It has long been known that treating human gallstone patients with CDCA decreases hepatic very low-density lipoprotein (VLDL) production and plasma triglyceride levels (Schoenfeld and Lachin, 1981), whereas treating hypercholesterolemia patients with bile acid–binding resins raised plasma triglyceride levels (Angelin et al., 1978). Studies conducted in fxr knockout mice further revealed that FXR deletion resulted in hepatic lipid accumulation and elevation of circulating total cholesterol and total triglycerides, as well as a proatherogenic lipoprotein profile. Conversely, activation of FXR by bile acids or FXR agonists decreased liver and plasma cholesterol and triglycerides in mice (Zhang et al., 2006a).

One mechanism by which FXR activation reduces hepatic fat accumulation and plasma triglyceride levels is the inhibition of hepatic de novo lipogenesis and VLDL overproduction. Obesity and diabetes are associated with hepatic fat accumulation, VLDL overproduction, and hypertriglyceridemia. It is generally thought that central obesity and insulin resistance lead to increased free fatty acid release from the adipose tissues and free fatty acid uptake by the liver. It is estimated that adipose-derived free fatty acid could account for up to 75% of hepatic triglycerides in nonalcoholic fatty liver associated with obesity and diabetes (Cohen et al., 2011). In addition, de novo lipogenesis from glycolysis is often induced in obesity and diabetes, which is accompanied by increased hepatic expression of steroid response element binding protein (SREBP)-1c. SREBP1c is basic helix-loop-helix
leucine-zipper transcription factors that are regulated by cholesterol/oxysterol and play a key role in the regulation of fatty acid and cholesterol synthesis (Brown and Goldstein, 1997; Horton et al., 2002; Shao and Espenshade, 2012). Two SREBP genes encode SREBP-1c and SREBP-2. SREBP-1c mainly regulates fatty acid synthesis, whereas SREBP-2 regulates cholesterol synthesis. SREBPs are synthesized as inactive precursors (125 kDa) in the ER. When intracellular oxysterol levels are high, SREBP/SREBP cleavage activation protein complex interacts with the insulin-induced gene (INSIG) and is retained in the ER. When intracellular oxysterol levels decrease, SREBP/SREBP cleavage activation protein escorted SREBP to the Golgi apparatus, where two sterol-sensitive proteases cleave an N terminus fragment (68 kDa). This processed or matured SREBP is translocated to the nucleus to bind to gene promoters containing the sterol response element and to activate target gene transcription (Wang et al., 1994). SREBP-1c induces the expression of a number of genes involved in de novo lipogenesis including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), acetyl-CoA synthetase, ATP-citrate lyase, malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and stearyl-CoA desaturase-1 (Brown and Goldstein, 1997; Horton et al., 2002). Insulin is known to induce both SREBP-1c mRNA expression by the liver orphan receptor (LXR) and proteolytic cleavage (Repa et al., 2000; Shao and Espenshade, 2012). It was suggested that the FXR/SHP cascade might repress LXRα induction of SREBP-1c and lipogenesis (Watanabe et al., 2004). More recently, it was reported that FXR activation inhibits another lipogenic transcriptional factor called the carbohydrate response element binding protein (ChREBP) (Caron et al., 2013). High levels of glucose initiate the nuclear translocation of ChREBP, which induces a set of genes that promote the conversion of glucose into fatty acids (Uyeda et al., 2002; Postic et al., 2007). It was shown that FXR directly interacted with ChREBP and inhibited ChREBP transactivation of its target gene L-pyruvate kinase. Numerous studies have shown that inhibition of ChREBP alleviated hepatic steatosis in mice (Dentin et al., 2004; Lizuka et al., 2004). ChREBP is also a transcriptional target of LXR, and thus may mediate the lipogenic effects of LXR activation (Cha and Repa, 2007). Taken together, these studies suggest that bile acids activate FXR to reduce hepatic lipogenesis, and thus reduce hepatic steatosis and VLDL overproduction.

Bile acid sequestrants decrease bile acid activation of FXR signaling to stimulate CYP7A1 gene expression, which reduces intracellular cholesterol to activate SREBP and induce the low-density lipoprotein receptor (LDLR) to reduce serum cholesterol. Transgenic overexpression of CYP7A1 in mice increased hepatic expression of SREBP and lipogenic genes (Miyake et al., 2001). Importantly, Li et al. (2010) reported that CYP7A1 transgenic mice fed a chow diet had increased hepatic VLDL secretion and elevated plasma VLDL triglyceride levels even in the presence of a significantly enlarged bile acid pool. Although the endogenous CYP7A1 mRNA was repressed more than 90% by an enlarged bile acid pool, none of the FXR target genes such as FAS, ACC, and stearyl-CoA desaturase-1 in fatty acid synthesis were repressed. Microarray analysis showed that hepatic CYP7A1 overexpression was associated with higher induction of SREBP-2 target genes in cholesterol synthesis than SREBP-1 target genes in lipogenesis (Li et al., 2013b). These studies show that increasing bile acid synthesis stimulates de novo cholesterol synthesis to provide cholesterol substrate for CYP7A1. This may shift acetyl-CoA from fatty acid synthesis to cholesterol synthesis and result in reducing lipogenesis.

FXR has been shown to induce peroxisome proliferator–activated receptor (PPAR)α, suggesting that FXR activation may promote hepatic fatty acid oxidation (Pineda Torra et al., 2003). FXR also induces hepatic expression and secretion of FGF21, a fasting-induced hormone involved in hepatic lipid oxidation and ketogenesis (Badman et al., 2007; Inagaki et al., 2007; Cyphert et al., 2012). This new evidence suggests that the triglyceride-lowering effect of FXR activation can also be attributed to increased hepatic fatty acid oxidation.

Plasma triglyceride is cleared after VLDL triglyceride is hydrolyzed by lipoprotein lipase (LPL) lining the endothelial cells of the peripheral tissues. Obesity and diabetes are reported to associate with impaired peripheral triglyceride clearance, contributing to diabetic hypertriglyceridemia (Pruneta-Deloche et al., 2004). Activation of FXR induces apolipoprotein (Apo) CII and ApoA5, which are LPL activators carried by VLDL, and represses ApoCIII, which is an LPL inhibitor (Kast et al., 2001; Claudel et al., 2003; Prieur et al., 2003). Another study showed that the VLDL receptor is induced upon FXR activation (Sivert et al., 2004). The VLDL receptor is expressed at low levels in the liver but is also expressed in peripheral tissues including heart, skeletal muscle, adipose, and blood vessels and mediates peripheral triglyceride clearance. In summary, these studies support that activation of FXR may lower plasma triglyceride by both decreasing hepatic triglyceride production and promoting plasma triglyceride clearance.

3. Farnesoid X Receptor Regulation of Glucose Metabolism. The first evidence linking FXR to glucose metabolism came from a study showing that hepatic FXR expression was decreased in streptozotocin-induced diabetic rats, which was corrected with insulin supplements (Duran-Sandoval et al., 2004). In fxr knockout mice, hepatic expressions of glucose metabolism genes showed altered response to refeeding (Duran-Sandoval et al., 2005). Bile acid synthesis was markedly increased during the postprandial period likely due to increased glucose influx and activation of insulin signaling (Li et al., 2012). A more recent study showed that FXR was
O-Glc-N-acylated under high glucose concentration to stimulate ligand-dependent FXR transactivating activity and increase cellular glucose flux and hexoseamine biosynthetic pathway (Berrabah et al., 2014). These studies indicate that FXR may be activated during the postprandial period to play a role in the regulation of glucose homeostasis (Kür et al., 2011; Potthoff et al., 2011; Li et al., 2012). Thus, activation of FXR by bile acid feeding or administration of the FXR agonist GW4064 (3-[2-[2-chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoazolyl]methoxy]phenyl]ethenyl]benzoic acid) lowered fasting plasma glucose and improved insulin sensitivity in obese and diabetic db/db mice (Ma et al., 2006; Zhang et al., 2006a), whereas FXR-deficient mice had insulin resistance and hyperglycemia (Ma et al., 2006).

Current antidiabetic drugs used to control fasting hyperglycemia act to either directly inhibit hepatic gluconeogenesis or to promote insulin secretion (Radziuk et al., 2003). The possibility that FXR activation inhibits hepatic gluconeogenesis to lower fasting plasma glucose has been extensively investigated in in vitro and in vivo models, but to date the role of FXR in the regulation of hepatic glucose production remains controversial. Bile acids and FXR repressed gluconeogenic genes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in primary hepatocytes and in liver cell lines by inducing SHP, which inhibits the transactivating activity of many transcription factors known to regulate these genes, including fasting-induced cAMP responsive element binding protein, forkhead box protein O1, CCAAT/element binding protein, glucocorticoid receptor, HNF3, and HNF4α (Yamagata et al., 2004). By contrast, one study showed that FXR bound to the promoter of PEPCK and FXR agonism induced PEPCK mRNA expression and glucose output in human and rat hepatocytes and in mouse liver (Stayrook et al., 2005). It should be noted that, despite such discrepancies, a majority of studies concluded that FXR activation and bile acid administration decreased fasting plasma glucose levels in diabetic mice. The effect of FXR in improving plasma glucose homeostasis may not be solely dependent on the direct inhibition of hepatic gluconeogenesis.

Several studies suggest that FXR regulates peripheral insulin sensitivity. Hyperinsulinemic euglycemic clamp studies demonstrated that FXR deficiency was associated with decreased whole body glucose disposal in mice (Cariou et al., 2006; Ma et al., 2006). Fxr knockout mice had elevated circulating and muscle free fatty acids, which explains the peripheral insulin-resistant phenotype observed in these mice (Ma et al., 2006). Consistently, FXR activation decreased free fatty acid levels and increased insulin sensitivity in mice (Zhang et al., 2006a). It is noticed that fxr knockout mice had smaller adipocytes, and FXR agonist GW4064 treatment increased adipose differentiation and insulin-dependent glucose uptake in 3T3-L1 cells in vitro (Cariou et al., 2006). Another study suggests that the FXR agonist INT-747 (6α-ethyl-3α,7α-dihydroxy-5β-cholan-24-oxic acid) induced adipose differentiation via induction of the expression of adipocyte-related genes including CCAAT/element binding protein-α and PPARγ (Rizzo et al., 2006). FXR is not expressed in skeletal muscle and is expressed in white adipose at a very low level, which raised the possibility that some insulin-sensitizing and glucose-reducing effects observed in FXR-deficient mice and in mice treated with FXR agonists or bile acids may be indirect or independent of FXR. Two recent studies showed that FXR deficiency in mice protected against genetic and diet-induced obesity and insulin resistance (Prawitt et al., 2011), whereas chronic administration of the FXR agonist GW4064 caused more weight gain when mice were fed a high-fat diet (Watanabe et al., 2011). Some of these effects may likely be due to an altered bile acid pool and signaling because fxr knockout may result in increased hepatic CYP7A1 and enlarged bile acid pool and tissue bile acid signaling, whereas the use of a nonbile acid–like FXR agonist such as GW4064 will likely diminish the bile acid pool.

FXR induces FGF15 in the intestine, which is secreted into the blood circulation and acts as a postprandial factor that promotes glycogen synthesis as a mechanism controlling postprandial glucose metabolism (Kür et al., 2011). It has been shown that plasma FGF19 increases during the postprandial period in humans, presumably due to increased bile acid signaling (Lundåsen et al., 2006). Interestingly, a few studies showed that FGF19 transgenic mice were resistant to diet-induced obesity and insulin resistance (Tomlinson et al., 2002; Fu et al., 2004). In addition, FGF19 has been shown to repress hepatic glucose production (Potthoff et al., 2011), promote glycogen synthesis (Kür et al., 2011), repress lipogenesis (Bhatnagar et al., 2009; Miyata et al., 2011a), and increase metabolic rate (Tomlinson et al., 2002; Fu et al., 2004). Fgf15 knockout mice showed glucose intolerance, increased hepatic gluconeogenesis, and reduced hepatic glycogen storage but unaltered overall insulin sensitivity. Decreased fasting FGF19 levels or impaired hepatic response to FGF19 have been reported in humans with fatty liver and insulin resistance (Schreuder et al., 2010; Wojcik et al., 2012). These studies suggest that bile acid regulation of hepatic glucose metabolism involves complex cross-talk between FXR-dependent pathways and FXR-independent signaling pathways. Some metabolic alteration observed in FXR loss-of-function or gain-of-function models may be due to indirect modulation of bile acid metabolism.

More recently, a few studies showed that FXR is also expressed in human and murine pancreatic β cells and may positively regulate glucose-dependent insulin secretion (Popescu et al., 2010; Renga et al., 2010; Düfer et al., 2012). The underlying mechanisms of this beneficial effect of FXR activation in the β cells are
not clear and both genomic and nongenomic actions have been suggested. For example, FXR activation was shown to stimulate insulin gene transcription (Renga et al., 2010). It was also shown that bile acids, such as CDCA, rapidly increased cytosolic Ca\(^{2+}\) concentrations and membrane depolarization in β cells (Düfer et al., 2012). Another study showed that FXR activation was associated with increased AKT phosphorylation and translocation of glucose transporter 2 to the cell membrane, and thus enhanced glucose uptake into pancreatic β cells (Renga et al., 2010). Whether these effects are mediated via nongenomic FXR action or via FXR-independent signaling pathways is still not clear.

4. Farnesoid X Receptor Regulation of Cholesterol Metabolism. The role of FXR in the development and progression of atherosclerosis has been extensively investigated (Hageman et al., 2010). It was shown that the synthetic FXR agonist INT-747 reduced aortic plaque formation in apoe knockout mice (Mencarelli et al., 2009). Similar antiatherogenic effects were also observed in ldlr knockout mice and in apoe knockout mice fed the synthetic FXR agonist WAY-362450 [3-(3,4-difluorobenzoyl)-1,2,3,6-tetrahydro-1,1-dimethylazepino[4,5-b]indole-5-carboxylic acid 1-methylethyl ester] (Hartman et al., 2009). Thus, current studies consistently support that FXR activation is antiatherogenic. By contrast, studies evaluating the role of FXR in the development of atherosclerosis in FXR loss-of-function models yielded inconsistent results. Hanniman et al. (2005) reported that FXR deletion in the apoe knockout mice fed a high-fat/high-cholesterol diet resulted in a more atherogenic lipoprotein profile with increased VLDL cholesterol, low-density lipoprotein-cholesterol (LDL-C), and decreased high-density lipoprotein (HDL)-cholesterol (HDL-C) and increased atherosclerotic lesion size. Opposing the findings from this study, two other studies found reduced atherosclerosis development when FXR was deleted in ldlr knockout mice and/or in apoe knockout mice (Guo et al., 2006; Zhang et al., 2006b). Reduced macrophage CD36 expression and low-density lipoprotein (LDL) uptake into macrophages have been reported in these mice, suggesting possible underlying mechanisms.

Mice lacking FXR had elevated LDL in the circulation when challenged with a high-cholesterol diet (Sinal et al., 2000). Activation of FXR by bile acids and synthetic FXR agonists mainly caused a reduction of HDL-C in wild-type mice, and decreased both LDL-C and HDL-C in hypercholesterolemia mice (Zhang et al., 2006a). How FXR activation reduced plasma LDL-C is still not fully understood. Plasma LDL-C is cleared via LDLR-mediated uptake. FXR activation was shown to repress proprotein convertase subtilisin/kexin type 9 (PCSK9), an LDLR inhibitor, in vitro (Langhi et al., 2008). Recent studies demonstrated that secreted PCSK9 promotes LDLR internalization and intracellular degradation and thus decreased LDLR recycling back to the plasma membrane (Maxwell et al., 2005). Gain-of-function mutation of PCSK9 was associated with premature atherosclerosis, whereas loss-of-function mutation of PCSK9 resulted in lower plasma LDL-C and reduced risk of coronary heart disease (Abifadel et al., 2003; Timms et al., 2004). However, activation of FXR significantly decreased plasma non-HDL cholesterol in apoe\(^{-/-}\) mice, suggesting that the cholesterol-lowering effects of FXR activation are not solely mediated by LDLR-mediated clearance (Hartman et al., 2009). FXR activation reduced hepatic lipogenesis and VLDL production, increased plasma triglyceride clearance, improved insulin sensitivity, and decreased circulating fatty acid levels, all of which could play a role in reducing plasma LDL-C upon FXR activation.

Plasma HDL transports cholesterol from peripheral tissues to the liver for catabolism to bile acids and biliary secretion. This process is called reverse cholesterol transport and plays a critical role in preventing the development of atherosclerosis (Brufau et al., 2011). The role of FXR in regulating HDL metabolism is still under debate. Mice lacking FXR showed either unaltered HDL-C (Sinal et al., 2000) or increased HDL-C (Caudel et al., 2002), whereas activation of FXR decreased HDL-C (Zhang et al., 2010). FXR inhibits the hepatic production of ApoAI, a key structural component of HDL (Caudel et al., 2002), suggesting that FXR may inhibit HDL biogenesis. It has been demonstrated that pharmacological activation of FXR reduced plasma HDL-C in a number of hyperlipidemia mouse models (Evans et al., 2009b; Hartman et al., 2009). It should be noted that although HDL is generally considered to be the major mediator of reverse cholesterol transport, the macrophage-derived cholesterol only accounts for a small portion of the total plasma HDL-C. Importantly, it was shown that activation of FXR promoted macrophage-to-feces reverse cholesterol transport in mice by inducing hepatic expression of the HDL receptor, scavenger receptor B1 (SR-B1) (Zhang et al., 2010). Fxr knockout mice showed a reduced rate of plasma HDL-C clearance (Lambert et al., 2003). SR-BI deficiency caused higher plasma HDL and increased atherosclerosis in mice (Rigotti et al., 1997; Trigatti et al., 2003). Human subjects with a heterozygous SR-BI mutation showed an approximately 50% increase in plasma HDL (Vergeer et al., 2006, 2011). Studies suggest that the antiatherogenic function of SR-BI may be mainly attributed to its role in mediating the cholesterol efflux from macrophages and hepatic HDL uptake (Ji et al., 1997; Covey et al., 2003; Zhang et al., 2005). Therefore, it is also possible that decreased plasma HDL upon FXR activation may partially result from increased hepatic HDL uptake (Mencarelli et al., 2009). The last step of reverse cholesterol transport is biliary secretion of cholesterol or bile acids for fecal excretion. FXR activation represses CYP7A1 and thus does not stimulate hepatic cholesterol catabolism. Mice lacking functional
ABCG5/ABCG8 showed reduced macrophage-to-feces reverse cholesterol transport when stimulated by an LXR agonist (Calpe-Berdiel et al., 2008). It is shown that cholate feeding induced hepatic ABCG5 and ABCG8 expression, which was lower in \textit{fxr} knockout mice (Repa et al., 2002; Yu et al., 2005b). Bile acid activation of FXR induced hepatic expression of ABCG5 and ABCG8 through a common FXR-responsive element located in the intergenic promoter shared by ABCG5 and ABCG8 (Li et al., 2011b). Thus, activation of FXR may simultaneously promote hepatic uptake of HDL-C and biliary secretion of free cholesterol via ABCG5/ABCG8 (Lambert et al., 2003). In addition to the classic peripheral-to-liver reverse cholesterol transport route, recent studies in mice revealed the existence of a transintestinal cholesterol excretion (TICE) pathway that is quantitatively significant in fecal cholesterol secretion (van der Velde et al., 2010; Brufau et al., 2011). TICE may also be present in humans, but the quantitative contribution to overall fecal neutral sterol excretion is not clear (van der Velde et al., 2010). The molecular mechanisms for TICE have not been fully defined. FXR is highly expressed in the intestine but its potential role in the regulation of TICE remains unclear.

5. Anti-Inflammatory Function of Farnesoid X Receptor. FXR is expressed in vascular smooth muscle cells (VSMCs) (Bishop-Bailey et al., 2004; Zhang et al., 2008). Recent evidence suggests that FXR activation may play a role in decreasing inflammation of the vasculature, which is critically involved in the progression of atherosclerosis (Bishop-Bailey et al., 2004; Li et al., 2007). In addition, FXR plays an anti-inflammatory role during liver injury (Wang et al., 2008; Zhang et al., 2009b) and in experimental models of inflammatory bowel disease (IBD) (Vavassori et al., 2009; Gadaleta et al., 2011b). The underlying molecular mechanism by which FXR reduces inflammation is not clear. It was reported that FXR induced \textit{SHP} to inhibit the expression of cyclooxygenase 2 and inducible nitric oxide (NO) synthase, which are involved in vascular inflammation and VSMC migration. FXR activation may also antagonize nuclear factor-\textit{κB} (NF-\textit{κB}) signaling to decrease proinflammatory cytokine production in the liver (Wang et al., 2008). Although some studies did not detect FXR expression in macrophages, others reported that FXR was expressed in macrophages and activation of FXR repressed lipopolysaccharide (LPS)-induced proinflammatory cytokine expression, an effect that was abolished in \textit{fxr}^{-/-} macrophages (Mencarelli et al., 2009). The potential anti-inflammatory role of FXR in macrophages remains to be further defined.

6. Bile Acid/Xenobiotic Receptors in Bile Acid and Drug Metabolism and Detoxification. PXR, constitutive androstane receptor (CAR), and VDR play important roles in the regulation of all three phases of drug metabolism and detoxification, as well as in bile acid metabolism and detoxification, including phase I drug/bile acid hydroxylation/oxidation, phase II drug/bile acid conjugation, and phase III drug/bile acid transport (Fig. 7) (Li and Chiang, 2013). In cholestasis, bile acids activate these nuclear receptors to induce expression of the genes involved in bile acid and drug metabolism,

![Fig. 7. Xenobiotic nuclear receptors in bile acid, drug, lipid, and glucose metabolism. The xenobiotic nuclear receptors PXR and CAR are highly expressed in the liver and intestine. VDR is highly expressed in the intestine, but is expressed at low levels in the liver. Activation of xenobiotic nuclear receptors by drugs, bile acids, and xenobiotics induces a network of genes involved in phase I, phase II, and phase III drug and bile acid metabolism and detoxification. CAR, PXR, and VDR inhibit CYP7A1 and thus bile acid synthesis via interaction with HNF4α and inhibition of HNF4α transactivation of CYP7A1. Similarly, CAR inhibits PEPCK and G6Pase involved in gluconeogenesis and inhibits SREBP-1c in lipogenesis. Activation of CAR decreases plasma glucose levels and improves hepatic steatosis in obesity and diabetes. By contrast, activation of PXR induces hepatic expression of PPARγ and CD36, leading to hepatic steatosis. FFA, free fatty acid; FoxO1, forkhead box protein O1; SCD1, stearoyl-CoA desaturase-1.](image-url)
conjugation, and transport in the liver and intestine as an adaptive response to protect against injury and inflammation.

a. **Pregnane X receptor.** Treating rodents with progrenolone-16α-carbonitrile, a mouse PXR agonist, repressed hepatic CYP7A1 activity (Ståhlberg, 1995), suggesting that PXR may regulate bile acid metabolism. This was subsequently confirmed by studies showing that progrenolone-16α-carbonitrile repressed CYP7A1 mRNA expression and biliary bile acid secretion, but failed to do so in PXR knockout mice (Staudinger et al., 2001). Two groups showed that rifampicin-activated PXR repressed human CYP7A1 gene transcription via inhibition of HNF4α and peroxisome proliferator–activated receptor γ coactivator-1α (PGC-1α) transactivation of CYP7A1 gene transcription (Bhalla et al., 2004; Li and Chiang, 2005). Activation of PXR in the intestine also induced FGF15 or FGF19 expression, and a PXR response element was identified in the promoter of the fgf19 gene (Wistuba et al., 2007; Wang et al., 2011a). PXR induces CYP3A enzymes, which play a major role in the hydroxylation of bile acids (Staudinger et al., 2001). PXR also induces the bile acid conjugation enzymes, sulfotransferase SULT2A1 and UDP-glucuronosyl N-transferase UGT1A1, the canalicular transporter Mrp2, and the basolateral transporter Oatp2 (Kliewer and Willson, 2002). Mice treated with LCA or subjected to BDL showed induction of PXR target genes involved in bile acid detoxification, whereas pxr knockout mice were more susceptible to hepatotoxicity caused by LCA treatment or BDL, supporting the significance of the adaptive activation of PXR in the prevention of bile acid toxicity during cholestasis (Staudinger et al., 2001; Stedman et al., 2005). In addition, numerous studies have demonstrated that pharmacological activation of PXR protected against bile acid–induced liver injury in experimental cholestasis models (Stedman et al., 2005). Rifampicin has been used to reduce pruritus (itch) associated with cholestasis in humans (Hofmann, 2002). The pathophysiological cause of pruritus is not completely known (Kremer et al., 2012), but may be associated with high tissue and plasma bile acid accumulation (Bunchorntavakul and Reddy, 2012). A recent study linked lysophospholipase D (autotaxin) and its product, lysophosphatidic acid, as mediators of cholestatic pruritus (Kremer et al., 2012). Rifampicin significantly reduced itch intensity and autotaxin activity in patients not responding to bile acid sequestrants. Rifampicin inhibited autotaxin expression in HepG2 cells overexpressed with PXR. This explains the anti-pruritic action of rifampicin by PXR-dependent transcriptional inhibition of autotaxin expression.

b. **Constitutive androstane receptor.** CAR and PXR can bind to the same xenobiotic response elements in the target gene promoters and thus it is not unexpected that PXR and CAR regulate an overlapping set of target genes in xenobiotic and bile acid metabolism. There is no evidence that bile acids bind or activate CAR, but CAR agonists have been shown to repress the CYP7A1 gene in hepatocytes (Miao et al., 2006). A number of studies showed that activation of CAR was beneficial for protecting against bile acid toxicity during cholestasis in mice (Guo et al., 2003; Saini et al., 2004; Stedman et al., 2005; Beilke et al., 2009). Mice lacking CAR had higher degree of liver injury than wild-type mice upon LCA treatment or in BDL-induced cholestasis, and such liver damage was aggravated in car/pxr double knockout mice, suggesting that PXR and CAR may play complementary roles in bile acid detoxification (Stedman et al., 2005). CAR may play an important role in inducing sulfation of LCA as demonstrated by the resistance of CAR transgenic mice to LCA toxicity and increased sulfated LCA without the induction of CYP3A (Saini et al., 2004). In a study by Guo et al. (2003), fxr/pxr double knockout mice treated with phenobarbital and synthetic analog TCPOBOP (1,4-bis[2-(3,5-dichloropyridyloxy)]benzene) were protected from CA-feeding-induced bile acid toxicity, which was accompanied by induction of CAR target genes carnitine palmitoyltransferase 2b, Cyp3a, Mrp2, Ugt1a1, and glutathione-S-transferase a.

c. **Vitamin D receptor.** The VDR acts as a bile acid sensor in the intestine and protects the gut from bile acid toxicity (Naggal et al., 2005). Activation of VDR by 1α,25-dihydroxyvitamin D3 induces CYP3A4, CYP2B, and CYP2C in intestinal cells, suggesting a role for VDR in drug and bile acid metabolism (Schmiedlin-Ren et al., 2001). Activation of VDR also induced SULT2A1 and thus simultaneously stimulated bile acid sulfation (Chatterjee et al., 2005). Furthermore, two bile acid transporters (MRP3 and ASBT) were shown to be VDR targets in the intestine (McCarthy et al., 2005; Chen et al., 2006). Accumulation of LCA in the gut may activate the VDR to convert LCA to less toxic intermediates for excretion. VDR mRNA and protein were detected at very low levels in primary human hepatocytes (Han et al., 2010). Treating primary human hepatocytes with 1α,25-dihydroxyvitamin D3 induced CYP3A, CYP2B, and CYP2C (Drocourt et al., 2002). During cholestasis, LCA levels may increase significantly in the liver. It was first shown that treating primary human hepatocytes with 1α,25-dihydroxyvitamin D3 repressed CYP7A1 mRNA expression (Han and Chiang, 2009). Consistently, a recent study showed that vdr−/– mice had higher hepatic cyp7a1 gene expression and enlarged bile acid pool size, whereas 1α,25-dihydroxyvitamin D3 treatment repressed hepatic cyp7a1 gene expression in mice (Schmidt et al., 2010). It was shown that vdr knockout mice had lower intestinal FGF15 expression, whereas 1α,25-dihydroxyvitamin D3 treatment increased intestinal FGF15 in mice. A VDR binding site was identified in the fgf15 gene promoter (Schmidt et al., 2010). In addition, 1α,
25-dihydroxyvitamin D₃ did not repress CYP7A1 in fgf15−/− mice, supporting that activation of intestinal VDR represses hepatic CYP7A1 via FGF15 signaling in mice. One study showed that 1α,25-dihydroxyvitamin D₃ treatments had no effect on hepatic or plasma bile acid levels in mice after BDL, suggesting a minimal role of VDR in modulating bile acid metabolism in cholestasis (Ogura et al., 2009). However, this study showed that VDR activation in BDL mice was associated with reduced proinflammatory cytokine expression, consistent with the known role of VDR in immunity. These results suggest that the anti-inflammatory properties of VDR may provide certain benefits during cholestasis. The role of VDR in regulating innate and adaptive immunity is reviewed in detail elsewhere (Nagpal et al., 2005). It was also shown that 1α,25-dihydroxyvitamin D₃ treatment was accompanied by increased renal MRP2, MRP3, and MRP4 mRNA expression and increased renal bile acid secretion (Nishida et al., 2009). However, this study also showed that 1α,25-dihydroxyvitamin D₃ treatment markedly increased hepatic CYP7A1 mRNA without altering total bile acid pool size or serum bile acid concentration in chow-fed mice. Further studies are needed to evaluate the beneficial effects of pharmacological activation of VDR in cholestasis.

7. Bile Acid/Xenobiotic Receptors in Glucose and Lipid Metabolism. Recent evidence suggests that the bile acid/xenobiotic receptors PXR and CAR also act as metabolic regulators (referred to as endobiotic receptors) under physiologic and pathologic conditions, and thus may be implicated in the development and treatment of metabolic diseases. It has long been known that phenobarbital reduces plasma fasting glucose and improves insulin sensitivity in diabetic patients (Lahtela et al., 1984). A few recent studies showed that activation of CAR by TCPOBOP improved hyperglycemia and insulin sensitivity in obese and diabetic ob/ob mice and high-fat diet–induced obese mice (Dong et al., 2009; Gao et al., 2009; Masuyama and Hiramatsu, 2012). It was reported that phenobarbital decreased the expression of PEPCK and G6Pase in both primary hepatocytes and mouse liver, suggesting that phenobarbital may inhibit hepatic gluconeogenesis (Kodama et al., 2004; Miao et al., 2006). On the other hand, the effect of CAR activation on plasma glucose levels may also be attributed to increased peripheral glucose clearance, as suggested by glucose tolerance testing and euglycemic clamp studies in both mice and humans (Lahtela et al., 1984).

A few recent studies have reported antiobesity and lipid-lowering effects of CAR agonists in mice (Gao et al., 2009; Sberna et al., 2011; Masuyama and Hiramatsu, 2012). The mechanisms by which CAR activation decreases weight gain is not clear, but reduced body weight gain in mice likely contributes to reduced hepatic steatosis and hepatic VLDL production and promotes the antiatherogenic lipid profile in mice treated with CAR agonists. In the liver, CAR activation decreased both SREBP-1c mRNA expression and the mature form of SREBP-1c, presumably via inducing Insig-1 involved in SREBP retention in the ER (Shao and Espenshade, 2012). CAR activation also increased reverse cholesterol transport, which may be due to increased hepatic bile acid excretion (Sberna et al., 2011).

Despite the fact that CAR and PXR can recognize common DNA response elements and regulate an overlapping set of target genes, activation of these two xenobiotic receptors surprisingly resulted in completely opposite outcomes in lipid metabolism. Liver-specific transgenic expression of a constitutively active PXR resulted in marked hepatic lipid accumulation, and treating “humanized” PXR transgenic mice with rifampicin also resulted in hepatic lipid accumulation (Zhou et al., 2006). Mechanistic studies suggest that PXR activation induces hepatic steatosis via a combined effect of enhanced lipogenesis and repressed fatty acid oxidation. It was shown that PXR increased the expression of the hepatic fatty acid uptake transporter fatty acid translocase FAT/CD36 (Zhou et al., 2008). This study also showed that PXR induced hepatic expression of PPARγ. Activation of PPARγ has been shown to cause hepatic fat accumulation. In addition, PXR activation was shown to promote an atherogenic lipoprotein profile in wild-type mice and increased the development of atherosclerosis in apoe−/− mice (Zhou et al., 2009). Consistently, PXR deficiency attenuated the development of atherosclerosis in apoe knockout mice (Sui et al., 2011). Studies so far suggest that the proatherogenic effect of PXR may be due to PXR induction of CD36 in the macrophages, which leads to increased uptake of oxidized LDL in atherogenic prone mouse models.

B. Bile Acid–Activated G Protein–Coupled Receptors

1. The G Protein–Coupled Bile Acid Receptor Is a Bile Acid–Activated Membrane Receptor. Bile acids are known to activate intracellular signaling pathways via nongenomic actions. Bile acid was shown to stimulate cAMP production in colonic cells, suggesting the existence of a bile acid–activated GPCR. Gas protein–coupled receptor TGR5 was recently identified as a bile acid–activated membrane receptor (Maruyama et al., 2002; Kawamata et al., 2003). Activation of TGR5 by bile acids stimulated adenylate cyclase, rapid intracellular cAMP production, and protein kinase A activation (Fig. 8). Both conjugated bile acids and free bile acids are known to bind and activate TGR5. Among all bile acids, TLCA is the most potent TGR5 agonist with an EC₅₀ value of 0.53 μM, followed by taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid, and TCA with EC₅₀ values of approximately 1.0, 4.4, and 7.7 μM, respectively. TGR5 is highly expressed along the intestinal tract, with the highest expression found in the ileum and colon (Kawamata et al., 2003). Despite the
liver being a major bile acid target organ, TGR5 expression in the liver is very low. TGR5 is expressed in liver sinusoidal endothelial cells, gallbladder epithelial cells, and Kupffer cells (Keitel et al., 2007, 2008). TGR5 is also expressed in nontraditional bile acid target organs including white and brown adipose, spleen, kidney, pancreas, lung, macrophages, and the central nervous system (Kawamata et al., 2003; Keitel et al., 2010). It is generally recognized that TGR5 signaling plays important roles in energy and glucose metabolism as well as anti-inflammation in the digestive system.

a. The G protein–coupled bile acid receptor in bile acid metabolism. Maruyama et al. (2006) previously reported that the total bile acid pool size is decreased in tgr5<sup>-/-</sup> mice, suggesting that TGR5 might regulate bile acid metabolism. Tgr5<sup>-/-</sup> mice had normal fecal bile acid secretion, suggesting that decreased bile acid pool may not be due to a role of TGR5 in regulating intestinal bile acid reabsorption. In addition, hepatic expression of CYP7A1 was unaltered in tgr5<sup>-/-</sup> mice. It has been reported that tgr5<sup>-/-</sup> mice are protected from lithogenic diet–induced cholesterol gallstone and bile acid feedback regulation may be altered (Vassileva et al., 2009; Li et al., 2011a). A recent study showed that tgr5<sup>-/-</sup> mice had a more hydrophobic bile acid composition with increased CYP8B1 and a decreased MCA/CA ratio, as well as more severe liver injury upon bile acid feeding or BDL (Péan et al., 2013). It was demonstrated that tgr5<sup>-/-</sup> mice had reduced liver regeneration capacity and TGR5 might protect the liver from bile acid overload during liver regeneration in mice. The mechanism by which tgr5 gene ablation alters bile acid metabolism is still largely unknown.

b. The G protein–coupled bile acid receptor in energy expenditure and glucose metabolism. Feeding mice a lithogenic diet (containing 0.5% CA and 0.2% cholesterol) prevented diet-induced weight gain (Watanabe et al., 2006). It seems that the antiobesity effect of bile acids is mediated through the activation of TGR5 in the brown adipose tissue in mice. Bile acid activation of TGR5, via cAMP signaling, induced type 2 deiodinase and the conversion of thyroxine to the active 3,5,3‘-triiodothyronine, which is known to stimulate mitochondrial oxidative phosphorylation and energy expenditure. A similar effect was also seen when mice were given a potent synthetic TGR5 agonist INT-777 [6a-ethyl-23(S)-methylcholic acid]. The effect of bile acids on body weight loss was independent of FXR activation because the FXR agonist GW4064 did not increase energy expenditure or reduce body weight in mice. Furthermore, it was later shown that chronic feeding of GW4064 to mice resulted in increased susceptibility to diet-induced weight gain, which was associated with a decreased bile acid pool and reduced energy expenditure (Watanabe et al., 2011). The role of TGR5 in energy metabolism in humans has not been studied.

TGR5 is highly expressed in the small and large intestines, which are exposed to high levels of bile acids. The colon is exposed to LCA, the most potent TGR5 agonist among all bile acids. Katsuma et al. (2005) first showed that bile acid–activated TGR5 stimulated glucagon-like peptide-1 (GLP-1) production in an enteroendocrine cell line. GLP-1 is known to promote insulin secretion and thus regulate glucose homeostasis. Thomas et al. (2009) further showed that administration of a potent TGR5 agonist INT-777 raised the intracellular ATP/ADP ratio and calcium influx, which leads to enhanced GLP-1 secretion. Both INT-777–treated mice and tgr5 transgenic mice showed improved glucose tolerance. Because GLP-1 mimetic and receptor agonists have shown promise in improving glucose homeostasis in diabetes, bile acid–based TGR5 agonists may be used to stimulate GLP-1 secretion in diabetic patients (Nauck, 2011). In contrast with these results obtained from TGR5 gain-of-function models, studies in tgr5<sup>-/-</sup> mice yielded inconsistent results. One study reported higher weight gain in female, but not male, tgr5<sup>-/-</sup> mice (Maruyama et al., 2006). Another study showed that neither under chow condition nor under...
a high-fat diet feeding condition did tgr5\(^{−/−}\) mice show higher body weight than wild-type controls (Vassileva et al., 2010). The high-fat diet feeding effect on insulin sensitivity showed sex-specific differences, with male tgr5\(^{−/−}\) mice showing impaired, but female tgr5\(^{−/−}\) mice showing improved, insulin sensitivity (Vassileva et al., 2010). Thus, it is possible that TGR5 is not highly activated by the physiologic concentration of circulating bile acids outside of the enterohepatic system, which may explain why loss of TGR5 did not have a major impact on weight gain and glucose metabolism. By contrast, pharmacological activation of TGR5 showed a clear beneficial effect on body weight reduction and glucose homeostasis. The physiologic role of TGR5 in the regulation of metabolic homeostasis needs further study.

c. G protein–coupled bile acid receptor modulation of immune response. TGR5 is highly expressed in monocytes and macrophages and in human spleen, and may play an anti-inflammatory role in the immune system. Thus far, TGR5 activation has shown protective effects in various inflammation-related diseases in experimental models (Kawamata et al., 2003; Keitel et al., 2008). In Kupffer cells, activation of TGR5 reduced LPS-stimulated proinflammatory cytokine production (Keitel et al., 2008). The specific TGR5 agonist INT-777 attenuated liver damage associated with high-fat diet–induced steatosis. Tgr5\(^{−/−}\) mice challenged with LPS showed higher plasma liver enzymes and elevated cytokine expression, whereas 23(S)-mCDCA, a highly selective TGR5 agonist, antagonized LPS-induced cytokine expression in mouse liver (Wang et al., 2011b). Since TGR5 is not expressed in hepatocytes, such effects of TGR5 activation may be attributed to its activation in Kupffer cells. The anti-inflammatory role of TGR5 in the liver is supported by recent studies demonstrating a protective role of TGR5 activation in cholestasis and nonalcoholic steatohepatitis (NASH) (McMahan et al., 2013; Péan et al., 2013). However, the underlying mechanism of TGR5 signaling in anti-inflammation in liver is completely unknown. In the vasculature, TGR5 activation by INT-777 attenuated atherosclerosis in ldlr\(^{−/−}\) mice, but not in ldlrtgr5 double knockout mice. Importantly, it was shown that INT-777 failed to attenuate atherosclerosis in ldlr\(^{−/−}\) mice transplanted with bone marrow of tgr5\(^{−/−}\) mice, demonstrating that the antiatherogenic effect of the TGR5 agonist was due to TGR5 activation in macrophages. The anti-inflammatory function of TGR5 in protection against IBD is mediated by inhibition of NF-κB–dependent proinflammatory cytokine production. It was demonstrated that a TGR5 selective agonist protected the integrity of intestinal barrier function, immune response, and proinflammatory cytokine production in experimental colitis models (Cipriani et al., 2011; Yoneno et al., 2013).

d. Novel functions of the G protein–coupled bile acid receptor. Two studies unveiled novel functions of TGR5 signaling in the brain. TGR5 is expressed in astrocytes and neurons, and is activated by neurosteroids, and functions as a neurosteroid receptor (Keitel et al., 2010). Another study showed that TGR5 may mediate bile acid–induced itch and analgesia associated with cholestasis (Alemi et al., 2013a). In this study, the investigators identified TGR5 in peptidergic neurons that transmit itch and pain in mouse dorsal root ganglia and in the spinal cord as well as in dermal macrophage–containing opioids. Alemi et al. (2013a) observed that scratching was reduced in tgr5\(^{−/−}\) mice but increased in TGR5 transgenic mice exhibiting spontaneous pruritus. Bile acids activate TGR5 on sensory nerves and stimulate the release of neuropeptides in the spinal cord that transmits itch and analgesia. TGR5 is highly expressed in the colon and has been linked to gut motor function. Tgr5 knockout mice have higher transit time and lower defecation, suggesting that TGR5 mediates the prokinetic action of bile acids in the intestine and is required for normal defecation (Alemi et al., 2013b). A single nucleotide polymorphism in the TGR5 gene has been associated with irritable bowel syndrome (Camilleri et al., 2011). TLCA increased endothelial NO synthase phosphorylation and NO production, and TGR5-mediated NO production significantly reduced tumor necrosis factor α–induced monocyte adhesion in vascular endothelial cells (Kida et al., 2013).

2. Sphingosine-1-Phosphate Receptor 2. A recent study identified the sphingosine-1-phosphate receptor 2 (S1PR2) as a bile acid–activated GPCR (Studer et al., 2012). S1PR2 is a Gαi class of GPCRs and is expressed in hepatocytes and many other tissues. It was previously reported that conjugated bile acids activated the ERK1/2 and AKT signaling pathways, which were sensitive to pertussis toxin inhibition in rat primary hepatocytes (Dent et al., 2005). Screening GPCRs in the lipid-activated family identified that TCA, TDCA, tauroursodeoxycholic acid (TUDCA), glycolytic acid, and glycodeoxycholic acid specifically activated S1PR2, but not other GPCRs in the family. Sphingosine-1-phosphate (S1P) is a potent pleiotropic lipid mediator that has been shown to activate at least five different S1PRs. S1P is the phosphorylated product of the membrane lipid sphingosine by sphingosine kinases SphK1 and SphK2. Extracellular signals activate SphK1, which is translocated from the cytosol to the plasma membrane to convert membrane sphingosine to S1P. SphK2 is located in the nucleus and is also activated by ERK1/2-mediated phosphorylation. S1P activates S1P receptors by an autocrine/paracrine manner. Molecular modeling shows that TCA is able to dock into the S1P binding site on S1PR2 (Studer et al., 2012). Sphingosine is also derived from ceramide or synthesized from serine and palmitoyl-CoA. It has been reported that DCA activates acidic sphingomyelinase to generate ceramide from sphingomyelin (Gupta et al., 2004). Both ceramide and S1P levels are increased in obese humans (Chavez...
and Summers, 2012). Ceramide production and signaling have been linked to the pathogenesis of insulin resistance and other obesity- and diabetes-associated metabolic disorders; in addition, promoting the conversion of ceramide to S1P may attenuate the deleterious effects of ceramide and high-fat diet–induced insulin resistance (Bruce et al., 2012). On the other hand, S1P has been implicated in the regulation of inflammation, cell death, and insulin sensitivity via activation of different S1P receptors in liver, muscle, and pancreas and immune cells (Adada et al., 2013). Interestingly, it was reported that SphK2 is associated with histone deacetylase histone deacetylase (HDAC)1/2, and S1P inhibits HDAC1/2 activity and stimulates histone acetylation and transcription of cyclin-dependent kinase inhibitor p21 and cFos (Hait et al., 2009).

III. Bile Acids and Gut Microbiota

Bile acids and gut microbiota are closely linked. Gut microbiota are involved in the biotransformation of bile acids through deconjugation, dehydroxylation, and re-conjugation of bile acids (Ridlon et al., 2006). Bile acids have antimicrobial activity by damaging the bacterial cell membrane and thus inhibiting bacteria outgrowth (Kurdi et al., 2006). Obstruction of bile flow by BDL caused bacterial proliferation and mucosal injury in the small intestine and bile acid treatment reduced bacteria outgrowth. It has been reported recently that bile acid activation of FXR induced genes involved in enter protection and inhibited bacterial overgrowth and mucosal injury (Inagaki et al., 2006).

Gut microbiota play important roles in pathogen defense, immunity, and nutrient harvest. Recent evidence suggests that there is a regulatory relationship between the development of obesity and altered gut microbiota (Aron-Wisnewsky et al., 2013). Obesity, caloric intake, and macronutrients of the diet affect gut microbial communities (Ley et al., 2005, 2006; Parks et al., 2013). It is suggested that intestinal microbes generate short chain fatty acids from dietary carbohydrates that otherwise cannot be used as energy (Topping and Clifton, 2001; Gill et al., 2006; Turnbaugh et al., 2006). Therefore, gut microbiota composition directly affects energy metabolism, leading to remarkable alterations of lipid, glucose and energy metabolism in the liver, muscle, and adipose. Most of such evidence has been obtained by studying the metabolism in germ-free mice. A number of studies showed that germ-free mice were protected against diet-induced obesity compared with the conventionally raised counterparts (Bäckhed et al., 2004; Turnbaugh et al., 2006). Interestingly, germ-free mice receiving caecal microbiota from ob/ob mice had higher energy absorption from food and more weight gain than germ-free mice harboring caecal microbiota from lean mice (Turnbaugh et al., 2006). Importantly, introducing gut microbiota from the cecum of conventionally raised mice to germ-free mice caused rapid weight gain and insulin resistance without increasing food intake (Bäckhed et al., 2004). The gut microbiota affected the release of gut hormones, such as peptide YY and GLP1 (Reimer and McBurney, 1996; Kok et al., 1998; Cani et al., 2004), and low-grade inflammation (Noverr and Huffnagle, 2004; Cani et al., 2007), which linked gut microbiota to the development of obesity and diabetes (Qin et al., 2012; Joyce and Gahan, 2014).

Figure 9 illustrates the interaction between bile acid metabolism and gut microbiota and the effects of high-fat diets, drugs, and circadian rhythm on liver and intestine inflammation and related metabolic diseases. Bile acids, through activation of FXR and TGR5 signaling, control microbiota overgrowth and composition and protect liver and intestine against inflammation. On the other hand, gut microbiota regulate bile acid biotransformation in the intestine, which alter bile acid composition and modulate FXR and TGR5 signaling. Bile acids appear to play a major role in regulation of the gut microbiome (Ridlon et al., 2014). Recent evidence suggests that increased biliary secretion of bile acids upon high-fat diet feeding may reshape the gut microbiota in obesity. Both Western diet–fed mice and genetically obese ob/ob mice showed a reduction in abundance of Bacteroidetes and an increase in abundance of Firmicutes (Clarke et al., 2012). Feeding rats with a CA-containing diet resulted in increased gut Firmicutes-to-Bacteroidetes ratio, which was also seen in obese mice (Islam et al., 2011). DCA, escaped from
reabsorption in the small intestine, enters the colon and exhibits the most potent antimicrobial activity and selective inhibition of gut bacteria growth, leading to altered composition of gut microbiota (Islam et al., 2011). Expansion of Firmicutes results in significant expansion of DCA-producing bacteria (Ridlon et al., 2014). Another recent study showed that dietary saturated fats induced TCA, which promotes expansion of low-abundance sulfur-reducing pathobiont, Bilophila wasdworthia, increases proinflammatory cytokines and incidence of colitis in il-10−/− mice (Devkota et al., 2012). In a recent human study, the animal-based diet rapidly altered the gut microbiome, increasing the abundance of bile-tolerant microorganisms (B. wasdworthia and Bacteroides) and decreasing Firmicutes (David et al., 2014). This study supports a link between dietary fat, bile acids, and the outgrowth of micro-organisms in IBDs.

Several early studies showed that the gut microbiota impacted bile acid metabolism and signaling. It was shown that germ-free rats had increased biliary bile acid concentration, increased intestinal fractional cholesterol absorption, and hypercholesterolemia (Wostmann, 1973). All bile acids are conjugated in germ-free rats, whereas β-MCA is converted to hyodeoxycholic acid and ω-MCA in conventionally raised rats (Madsen et al., 1976). In the intestine, bacterial BSHs deconjugate conjugated bile acids and are enriched in the human gut microbiome (Jones et al., 2008). More recent studies show that in germ-free mice and antibiotic-treated mice, tauro-conjugated bile acids are predominant in liver, kidney, plasma, and heart, especially tauro-β-muricholic acid (T-β-MCA) (Swann et al., 2011). Ampicillin increased hepatic primary bile acid synthesis and suppressed ileal FGF15 expression (Miyata et al., 2009). The marked decrease in intestine FGF15 expression reflects reduced intestine FXR activation by bile acids. In ampicillin-treated mice, fecal bile acid excretion rates were markedly reduced, portal bile acid concentrations increased, and ileal ASBT expression levels were increased (Miyata et al., 2011b). Taurodeoxycholic acid or CA administration to ampicillin-treated mice decreased ASBT expression. Analysis of the bile acid composition in the bile of germ-free mice showed an increased MCA/CA ratio. Because MCAs are hydrophilic bile acids and poor FXR agonists, the reduced FGF15 expression in the intestine resulted in increased CYP7A1. This is confirmed by a recent study of germ-free mice that T-α- and β-MCA were strong FXR antagonists (Şayın et al., 2013). Another study showed that an antioxidant, tempol, inhibited intestinal Lactobacilli and their BSH activity and resulted in increasing T-β-MCA, which inhibits FXR signaling and decreases obesity in mice (Li et al., 2013a). Tempol feeding reversed the Firmicutes/Bacteroides ratio and resulted in increasing T-β-MCA levels in the intestine. Interestingly, high-fat diet–fed intestine-specific fxr−/− mice had lower diet-induced obesity than wild-type mice. Another study shows that cyp8b1−/− mice, bile duct–ligated mice, antibiotic-treated mice, and germ-free mice all share similar bile acid phenotypes: increased bile acid synthesis, reduced bile acid reabsorption, and enlarged bile acid pool size (Hu et al., 2014). These data are consistent with the hypothesis that increasing T-α-MCA and T-β-MCA as well as TUDCA antagonize FXR activity. Increasing T-α-MCA and T-β-MCA may be a positive feedback mechanism to stimulate bile acid synthesis and increase bile acid pool size.

Bacterial dysbiosis has been associated with IBDs, obesity, type 2 diabetes mellitus (T2DM), nonalcoholic fatty liver disease (NAFLD), liver cirrhosis, and cancer (Kakiyama et al., 2013; Ridlon et al., 2013, 2014; Duseja and Chawla, 2014; Joyce and Gahan, 2014). Although strong evidence supports that gut microbiota can impact whole body metabolic homeostasis via the modulation of bile acid signaling, it is not clear how this could explain the observed metabolic changes and the development of metabolic disorders seen in germ-free mice. However, there seems to be a coordination between energy metabolism and bile acid signaling. Gut microbiota increased energy harvest from the diet, which may contribute to the development of obesity. At the same time, gut microbes may regulate the bile acid pool and composition, leading to increased hydrophobic bile acid content and activation of FXR and TGR5 signaling, which regulates lipid and glucose homeostasis in response to increased energy intake from the intestine. Similarly, it was shown that the total bile acid pool and the CA/MCA ratio were increased in obese mice (Li et al., 2012). Intestinal cholesterol absorption was also increased in obese mice. It was suggested that the increased bile acid pool may be a response to higher nutrient availability in obese mice (Li et al., 2012). Because altered gut microbiota in obese mice favor higher energy intake in the intestine, the altered bile acid metabolism and signaling in obese mice may be deemed as an adaptive response to handle such changes by increasing the bile acid–regulated network. Although a clear role of microbes in the regulation of bile acid metabolism has been demonstrated by comparing germ-free mice and conventionally raised mice, the contribution of altered microbiota to altered bile acid metabolism in obesity remains to be investigated. Dysbiosis is the major environmental factor that affects bile acid metabolism and contributes to diseases not only in the gastrointestinal system but also in chronic diseases, such as NAFLD, diabetes, and obesity (Jones et al., 2014). Treating dysbiosis by modulating bile acid metabolism by gut microbiota may be a therapeutic approach for improving health and preventing NAFLD and T2DM. Indeed, a recent study reported that probiotics modified microbiota to increase bile acid synthesis by deconjugation of bile acids for fecal excretion and antagonizing the FXR-FGF15 pathway.
axis by increased T-MCAs in mice (Degirolamo et al., 2014).

IV. Circadian Rhythm in Bile Acid and Drug Metabolism

The diurnal rhythm of bile acid synthesis in humans was recognized more than 30 years ago (Duane et al., 1979). Circadian rhythms play critical roles in many physiologic processes, including metabolism in most tissues and organ systems (Rutter et al., 2002; Laposky et al., 2008). Bile acid metabolism is among the first identified direct molecular output of the central clock (Bass and Takahashi, 2010). Circadian rhythms are disturbed by diets, eating patterns, shift work, chronic jet leg, and sleep deprivation and affect metabolism by contributing to the pathogenesis of metabolic diseases, diabetes, and obesity (Damiola et al., 2000; Laposky et al., 2008; Bass and Takahashi, 2010; Froy, 2010; Shi et al., 2013). Diurnal regulation of bile acid metabolism also affects drug metabolism as well as efficacy and pharmacokinetics of drug therapy.

A. Clock Genes and Nuclear Receptors

Circadian rhythms are regulated by the central clock located in the suprachiasmatic nucleus of the anterior hypothalamus in the brain (Fig. 10). External signals, most importantly the light and dark cycle, reset the brain central clock, which synchronizes with the peripheral clocks in nearly every tissue type that is influenced by environmental factors including fasting/feeding cycles and nutrient availability (Bass and Takahashi, 2010). The output of the brain clock, such as sleep/wake phases and eating behavior, affects peripheral clock outputs, such as energy metabolism (Froy, 2010; Asher and Schibler, 2011). The molecular circadian clock is driven by a transcriptional-translational feedback loop. In the brain and peripheral tissues, the primary clock genes circadian locomotor output cycles kaput (CLOCK) and brain and muscle ARNT-like protein-1 (BMAL1) regulate transcription of period 1 (Per1) and period 2 (Per2) and cryptochrome 1 (Cry1) and cryptochrome 2 (Cry2). Per/Cry complexes then inhibit BMAL1 and CLOCK expression in a negative feedback loop; this loop occurs with a period of approximately 24 hours (Fig. 10). In the...
clock output loop, the Bmal1/Clock or neuronal PAS domain protein 2 (Npas2) complex regulates clock-controlled genes (CCGs) by binding to the E-boxes (CANNTG) in the gene promoter. The connection of circadian rhythm to lipid metabolism was unveiled by the finding that reverse-erythroblastosis α (Rev-erbα) regulates the oscillation of SREBP (Le Martelot et al., 2009) and miRNA122 (Gatfield et al., 2009) in lipid metabolism. Rev-erbα and retinoic acid–related orphan receptor α (RORα) regulate Bmal1 and Clock by binding to a ROR response element in the gene promoters. In liver cells, the BMAL1/CLOCK complex binds to the E-box sequences in the Rev-erba and RORα gene promoters, and regulates the circadian rhythm of Rev-erbα and RORα expression. Rev-erba is activated by heme and plays a key role in connecting energy metabolism to lipid metabolism (Preitner et al., 2002; Le Martelot et al., 2009; Feng et al., 2011). Rev-erba and RORα regulate clock output genes containing ROREs, such as PEPCK, G6Pase in gluconeogenesis, CYP7A1 and CYP8B1 in bile acid synthesis, SREBP-1c, ACC, FAS, and microsomal triglyceride transfer protein in lipogenesis. Primary CCGs are D-site binding protein (DBP), E4 promoter binding protein 4 (E4BP4), transcription elongation factor (Tef), and hairy and enhancer of split 6 (Hes6). Disruption of primary clock genes, CCGs, and clock output genes exacerbates the development of metabolic diseases including fatty liver disease, diabetes, and obesity (Bass and Takahashi, 2010; Feng et al., 2011).

B. Diurnal Rhythm of Bile Acid Synthesis

Earlier studies in the 1990s observed that CYP7A1 activity, mRNA, and protein expression exhibited a strong diurnal rhythm, which peaked at the middle of the dark phase and decreased at the middle of the light phase in rats (Chiang et al., 1990; Noshiro et al., 1990; Falvey et al., 1995). More recent studies show that serum total bile acid levels were the lowest in the middle of the light phase and rapidly increased at the onset of the dark phase and also at the end of the dark cycle, indicating that mice have two episodes of eating during the dark phase (Zhang et al., 2011). Circadian profiles of bile acids show enhanced bacterial dehydroxylation during the fasting phase and reconjugation of bile acids in the fed phase. Cholestryramine feeding abolished the diurnal rhythm of serum bile acids, decreased α-MCA, β-MCA, ω-MCA, and UDCA, but increased DCA and CDCA. In free-fed mice, liver CYP7A1 mRNA expression rapidly increased to the highest level at the onset of the dark phase, and gradually decreased to the lowest level at 10:00 AM. SHP mRNA expression was the highest at 10:00 AM and the lowest at 10:00 PM, which is the inverse of the CYP7A1 expression pattern and is consistent with the negative regulation of CYP7A1 by the FXR/SHP pathway. Opposite to CYP7A1 diurnal rhythm, intestinal FGF15 mRNA showed a strong peak at 10:00 PM and a minor peak at 10:00 AM. Cholestryramine feeding markedly reduced intestinal FGF15 mRNA expression, but did not affect circadian rhythm of SHP and CYP7A1 mRNA. In intestine-specific FXR/−/− mice, the circadian rhythm of CYP7A1 expression is similar to wild-type mice but FGF15 mRNA levels are markedly reduced and show no diurnal rhythm (Stroeve et al., 2010). Thus, the diurnal rhythm of SHP, not FGF15, regulates the diurnal rhythm of bile acid synthesis.

Bile acid synthesis as indicated by serum C4 levels in humans shows two peaks during the day, the first at 1:00 PM and the second at 9:00 PM (Gälman et al., 2005). During the night, C4 levels decreased and returned to basal levels the next morning, completely opposite to that in rodents. In humans, the majority of bile acid synthesis occurs during the postprandial period (Duane et al., 1983). Circulating FGF19 levels also exhibit a pronounced diurnal rhythm with peaks 90–120 minutes after the postprandial rise in serum bile acids (Lundåsen et al., 2006). The FGF19 peaks preceded the decline of bile acid synthesis. Fasting abolished the diurnal rhythm of FGF19. Therefore, in humans, the diurnal rhythm of circulating FGF19 is controlled by the transintestinal bile acid flux. An inverse relationship between the fasting serum FGF19 and C4 levels is consistent with the hypothesis that FGF19 exerts feedback inhibition of CYP7A1 in humans (Gälman et al., 2011).

Circadian rhythm of CYP7A1 is regulated by a positive clock-controlled gene DBP (Lavery and Schibler, 1993) and negative clock genes DEC2 and Rev-erbα (Noshiro et al., 2004, 2007; Le Martelot et al., 2009). DBP expression follows a stringent circadian rhythm and is a key liver clock-controlled gene (Wuarin and Schibler, 1990). DBP stimulates CYP7A1 expression and regulates its diurnal rhythm (Lavery and Schibler, 1993; Preitner et al., 2002). Interestingly, Rev-erbα–deficient mice had a lower bile acid synthesis rate and reduced CYP7A1 expression, whereas SHP and E4BP4 expression were increased. It was suggested that Rev-erba induced CYP7A1 by inhibiting SHP and E4BP4 (Duez et al., 2008; Le Martelot et al., 2009). It is likely that Rev-erba expression levels determine the extent of SHP and E4BP4 in inhibition of CYP7A1. Rev-erba is an unstable protein with a very short half-life of less than 1 hour, and is activated by intracellular heme to recruit nuclear receptor corepressor 1 and HDAC3 to inhibit target gene transcription (Yin and Lazar, 2005; Yin et al., 2006).

Fasting and restricted feeding strongly affected the circadian rhythm of CYP7A1 and bile acid synthesis. Fasting strongly reduced CYP7A1 expression and blunted the circadian rhythm of CYP7A1, whereas restricted feeding rapidly stimulated CYP7A1 expression in mice (Li et al., 2012). Restricted feeding altered the circadian rhythm of bile acid synthesis and increased the
bile acid pool by approximately 20%. The circadian rhythm of CYP8B1 is opposite to that of CYP7A1. Fasting-induced RORα induced CYP8B1 expression in mice (Pathak et al., 2013). RORα and Rev-erbα bind to the same response element on the CYP8B1 gene promoter and regulate the diurnal rhythm of CYP8B1 expression. Disruption of per1/per2 increases serum bile acids and results in hepatic cholestasis (Ma et al., 2009). In per1/per2 knockout mice, circadian expression of clock genes, such as Rev-erbα, Clock, E4BP4, and DBP, were altered, which altered CYP7A1 circadian rhythm and bile acid synthesis.

C. Circadian Rhythm in Metabolic Diseases

The linking of clock genes to metabolic syndrome was first observed in clock mutant mice (Turek et al., 2005). Fasting and refeeding as well as the time of feeding have profound effects on circadian rhythms of hepatic lipid and glucose metabolism (Vollmers et al., 2009; Asher and Schibler, 2011; Hatori et al., 2012; Tong and Yin, 2013). During fasting, glucagon stimulates fatty acid mobilization from adipose tissues to the liver for generation of energy. In the fed state, insulin signaling stimulates lipogenesis and secretes VLDL for delivery of triglycerides to adipose tissue for storage. Serum triglyceride levels show a circadian rhythm (Rudic et al., 2004; Pan and Hussain, 2007). Clock mutant mice have hyper-triglyceridermia and altered diurnal rhythm of serum triglycerides (Pan et al., 2010). Expression of PPARα shows a circadian rhythm in liver and is abolished in clock mutant mice. PPARγ also exhibits a diurnal rhythm, which is regulated by E4BP4. Clock binds to the SHP promoter and induces SHP, which inhibits the microsomal triglyceride transfer protein involved in VLDL assembly. In Rev-erbα−/− mice, lipogenesis is stimulated (Feng et al., 2011). HDAC3 and Rev-erbα are colocalized in the promoter of genes in lipid metabolism, and deletion of either HDAC3 or Rev-erbα in mouse liver causes hepatic steatosis. Rev-erbα is also involved in the circadian rhythm of SREBP and modulates circadian rhythm of lipid, cholesterol, and bile acid synthesis (Le Martelot et al., 2009). Bmal1 and Clock are involved in glucose metabolism (Rudic et al., 2004). Gluconeogenesis is abolished in bmal1−/− mice and depressed in clock mutant mice. It was suggested that Bmal1 and Clock synchronize with peripheral clocks to control lipid and glucose metabolism. Clock mutant and per2−/− mice have increased food intake during the light cycle. A high-fat diet modulates glucose metabolism and amplifies circadian rhythm in glucose tolerance and insulin sensitivity. Interestingly, mice fed a high-fat diet only during the light phase (sleep period) gained significantly more weight than mice fed the same diet only during the dark phase (active period). Caloric intake and locomotor activity did not vary significantly between treatments, although day-fed mice trended toward being less active, which could contribute to decreased energy expenditure (Arble et al., 2009). Conversely, night-time–restricted feeding without reducing caloric intake has been shown to prevent obesity, insulin resistance, hepatic steatosis, and inflammation in mice fed a high-fat diet (Hatori et al., 2012). In this study, CYP7A1 expression shows a robust diurnal rhythm in time-restricted feeding but is blunted in free-fed mice. Serum cholesterol is lower and bile acids are higher in mice fed a time-restricted high-fat diet than in free-fed mice. Thus, a time-restricted feeding enhances diurnal rhythm of bile acid synthesis and may prevent high-fat diet–induced metabolic syndrome in mice.

In obese and diabetic mice, rhythmicity of the clock genes was attenuated (Ando et al., 2005a). Common genetic variations in the clock gene have been linked to NAFLD (Sookoian et al., 2007). Interestingly, a recent study shows that epigenetic regulation of insulin resistance was linked to NAFLD (Sookoian et al., 2010). The methylated/nonmethylated DNA ratios in CpG islands in the PGC-1α gene promoter in diabetic patients is correlated with serum insulin and a homeostasis model of assessment of insulin resistance. Chronic alcohol consumption disrupts clock genes and diurnal rhythms of liver metabolic genes (Filiano et al., 2013). However, the mechanism of ethanol effect on circadian clock expression is not clear. The ethanol diet used in chronic ethanol feeding is equivalent to a high-fat content of approximately 30% and ethanol feeding may alter eating behaviors and patterns as well as locomotor activity, thus possibly contributing to the observed disruption of circadian rhythm. Chronic alcohol consumption is known to alter lipid metabolism by stimulating lipogenesis and inhibiting fatty acid oxidation to contribute to alcoholic fatty liver disease (Gao and Bataller, 2011). Chronic alcohol feeding altered circadian rhythm in wild-type mice by delaying phase onset of activity and shortening the free-running period (Seggio et al., 2009). Alcohol treatment increased hepatic triglycerides in clock mutant mice more than in wild-type mice and altered lipogenic gene expression in clock mutant mouse liver (Kudo et al., 2009). The diurnal rhythm of many primary clock genes (Bmal1, Clock, Cry1/2, and Per1/2) and CCGs (Dbp, Npas2, and Rev-erbα) were altered in alcohol-fed mouse livers (Filiano et al., 2013) and caused phase shifting of diurnal rhythm of hepatic triglycerides, cholesterol, and bile acid levels (Zhou et al., 2014). Alcohol also alters the NAD/NADH ratio, which regulates sirtuin SIRT1 activity and downstream target genes Bmal1/Npas2/Clock (Nakahata et al., 2009). It was suggested that alcohol affects the diurnal rhythm through alteration of the redox state (NAD/NADH), reducing heme levels, and cellular energetics such as AMP/ATP and the AMP-activated kinase (AMPK).

D. Circadian Rhythm in Drug Metabolism

All three phases of drug metabolism exhibit circadian rhythm regulated by RORα and Rev-erbα, as well
as by several CCGs (Gachon and Firsov, 2011; Zmrzljak and Rozman, 2012). Circadian regulation of drug metabolism and detoxification is complicated by drug induction of drug-metabolizing enzymes. In general, mRNA expression of phase I drug oxidation cytochrome P450 enzymes is increased during the dark phase, whereas that of phase II drug conjugation enzymes and phase III drug transporters reached maximal levels during the light cycle. Circadian rhythms of the CYP genes are regulated by PAS (Per-Arnt-Sim) domain basic-leucine-zipper transcription factors, DBP, TEF, and hepatic leukemia factor (Hirao et al., 2006; Froy, 2009; Zhang et al., 2009c; Gachon and Firsov, 2011; Kośir et al., 2013). Rev-erba, RORs, PPARα, CAR, and PXR are involved in circadian expression of drug metabolism enzymes (Gachon and Firsov, 2011; Kośir et al., 2013). In RORα and RORγ double knockout mice, expression of several phase I and II drug metabolism enzymes are downregulated, suggesting that RORα and RORγ play a role in transcriptional regulation of circadian rhythm of drug-metabolizing enzyme expression (Kang et al., 2007). It appears that circadian rhythm of heme synthesis may link nuclear receptors to regulation of the circadian rhythms of drug metabolism. Heme is a ligand of Rev-erba and Rev-erβ3 that stimulates recruitment of corepressors (nuclear receptor corepressor 1/HDAC3) to inhibit transcription of Bmal1 and other target genes by decreasing histone acetylation (Yin et al., 2006, 2007; Burris, 2008). Heme binds to Npas2, a PAS domain protein, by inhibiting DNA binding activity of Bmal1/Npas2 complex (Crumbley et al., 2010). Heme also regulates Per2 by stimulating the Bmal1/Npas2 complex and regulates the circadian rhythm of δ-aminolevulinic acid synthase 1 (Alas1), the rate-limiting enzyme in heme synthesis (Kaasik and Lee, 2004). PXR and CAR induce alas1 gene expression (Podvinec et al., 2004), which affects PXR and CAR regulation of drug metabolism and circadian rhythm. Alas1 is also regulated by PGC-1α, which is induced by fasting (Handschin et al., 2005), thus linking nutritional status to heme synthesis and circadian rhythm of drug metabolism.

Diurnal variations in drug absorption, distribution, metabolism, and excretion are important for designing therapeutic drugs for optimal efficacy and dosing, as well as for minimizing side effects and injury to the tissues and organ systems. Absorption of many drugs is sensitive to the time of day of drug administration (Musiek and Fitzgerald, 2013). Most drugs are absorbed more efficiently in the morning and after a meal when bile acid synthesis reaches its peaks. Bile acids facilitate intestinal drug absorption and transport. Circadian rhythms and fasting and feeding cycles affect drug transporter MDR1 (ABCB1) in the intestinal brush border membrane, and efflux transporters (MRP3, ABCC3) in the sinusoidal membrane (Ando et al., 2005b). MDR1 exhibits a circadian rhythm regulated by hepatic leukemia factor and E4BP4 (Ando et al., 2005b; Murakami et al., 2008). Other transporters such as MRP2 and breast cancer–related protein also show circadian rhythms (Stearns et al., 2008). The pharmacodynamics of drugs is also regulated by the circadian rhythm of drug transporters, receptors, and enzymes and affects the efficacy of drugs, especially for chemotherapies (Musiek and Fitzgerald, 2013), as well as the detoxification of drugs (Zmrzljak and Rozman, 2012).

V. MicroRNA in Lipid and Glucose Metabolism

miRNAs are small noncoding RNAs that, after base paring with complementary sequences in the target mRNAs, promote mRNA degradation or inhibit protein synthesis. Recent studies identified several miRNAs involved in hepatic lipid metabolism (Krützfeldt and Stoffel, 2006; Moore et al., 2010, 2011; Najafi-Shoushtari et al., 2010; Fernández-Hernando et al., 2011; Rottiers and Nääär, 2012; Sacco and Adeli, 2012; Ramírez et al., 2013b), metabolic diseases (Fernández-Hernando et al., 2013), and atherosclerosis (Aryal et al., 2014). The most abundant miRNA in liver, miR-122a, plays a key role in regulating cholesterol biosynthesis and lipid metabolism in mice (Krützfeldt et al., 2005; Esau et al., 2006; Elmén et al., 2008). Inhibition of miR-122a expression in mice using antagonirs or antisense oligonucleotides have uncovered the phenotypes of reduced serum cholesterol and triglyceride levels, increased fatty acid oxidation and decreased hepatic fatty acid and cholesterol synthesis rate (Krützfeldt et al., 2005; Esau et al., 2006; Elmén et al., 2008). Inhibition of miR-122a resulted in reducing plasma cholesterol and improving steatosis in a diet-induced obese mouse model (Esau et al., 2006). In NASH patients, miR-122a expression was reduced 63%, implicating a role for miR-122a in inhibiting fatty acid synthesis and preventing inflammation, proliferation, and steatosis (Cheung et al., 2008). The mechanism of miR-122a in regulation of cholesterol metabolism is unknown. miR-122a antagonirs do not affect miR-122 expression, and the miR-122a binding site is not present in the 3′-untranslated region (UTR) of mRNAs encoding 3-hydroxy-3-methylglutaryl-CoA reductase and the LDLR in mouse livers. It was speculated that miR-122a might indirectly induce genes by inhibiting the expression of a repressor. A putative miR-122a binding sequence has been identified in the 3′-UTR of CYP7A1 mRNA (Song et al., 2010). Results from this study provide a plausible mechanism that miR-122a antagonirs may induce CYP7A1 and result in reducing serum cholesterol and triglyceride levels.

MiR-33a, which is encoded by intron 16 of the SREBP-2 gene, was recently shown to regulate cellular cholesterol homeostasis (Najafi-Shoushtari et al.,
2010), biliary bile acid secretion (Allen et al., 2012), fatty acid oxidation, and cell proliferation (Cirera-Salinas et al., 2012). It was shown that when cellular cholesterol levels decrease, miR-33a expression is coinduced with SREBP-2 mRNA. miR-33a inhibits ABCA1 and ABCG1 to reduce cellular cholesterol efflux, thus retaining cellular cholesterol. Studies in mice treated with an anti–miR-33a or in genetic miR-33a-deficient mice showed that antagonism of miR-33 induced ABCA1 in macrophages and in the liver, increased serum HDL levels, and promoted macrophage-to-feces reverse cholesterol transport. In addition, antagonism of miR-33a was shown to promote regression of atherosclerosis in ldlr−/− mice and nonhuman primates (Rayner et al., 2011). These studies suggest that miR-33a acts in a synergistic manner with SREBP-2 to regulate cellular cholesterol homeostasis. Study of transgenic mice overexpressing CYP7A1 in the liver (CYP7A1-tg mice) showed a parallel increase of SREBP-2 and miR-33a expression levels in liver. Overexpression of miR-33a in the liver decreased bile acid pool, increased hepatic cholesterol, and decreased serum cholesterol in these mice. This study suggests that coinduction of miR-33a with SREBP-2 may provide a negative feedback loop to maintain cholesterol and bile acid homeostasis (Li et al., 2013b). The cardioprotective effects of miR-33a antagonism can be attributed to not only HDL biogenesis in the liver, but also to cholesterol efflux from the macrophages, which is the first step in reverse cholesterol transport (Rayner et al., 2011). In addition, miR-33a and miR-33b inhibit the genes in fatty acid β-oxidation, such as carnitine O-octanoyltransferase, hydroxyacyl-coenzyme A dehydrogenase, and carnitine palmitoyl transferase 1A, and the energy sensors AMPKα1 and NAD+−dependent SIRT6. Antagonism of miR-33 may be a therapeutic strategy to induce CYP7A1 activity and bile acid synthesis to treat fatty liver diseases, diabetes, and obesity. Interestingly, miR-33b, encoded by intron 17 of the SREBP-1a gene, regulates glucose metabolism (Ramírez et al., 2013a). It is interesting to note that miR-33b is expressed in human liver, but not in mouse liver. Overexpression of miR-33b inhibits PEPCK and G6PaseC3 expression and reduces glucose production in hepatocytes. miR-33b also inhibits glucose induction of cAMP responsive element binding protein 1, SRC1, RORα, and PGC-1α expression in human hepatocytes. SIRT6 and AMPKα are also miR-33b target genes. In glycolysis, miR-33b induces glucokinase but not other glycolytic genes. In glycogenolysis, miR-33b reduces glycogen phosphorylase and phosphoglucomutase mRNA expression in HepG2 cells. However, miR-33b binding sites are not present in mouse SIRT6, PEPCK1, or G6PaseC3. In rhesus monkeys, glucose infusion, but not insulin, increases SREBP-1c and miR-33b levels and decreases PEPCK and G6PC3 mRNA and protein levels in the liver. SREBP-1c and miR-33b may coordinate regulate hepatic glucose production.

miR-34a and miR-24 have emerged as key regulators of hepatic lipid metabolism (Rottiers and Näär, 2012). MiR-34a binding sites have been identified in the 3′-UTR and miR-24 binding sites in the coding region of HNF4a mRNA (Takagi et al., 2010). Overexpression of these two miRNAs downregulated HNF4a mRNA levels and decreased CYP7A1 and CYPR1B1 expression in HepG2 cells. miR-24a expression is increased in high-fat diet–fed mice and in human hepatocytes incubated with fatty acids (Ng et al., 2014). Insig1 was identified as a target of miR-24. Inhibition of miR-24 increased Insig1 and subsequently decreased hepatic lipid accumulation. Antagonizing miR-24 and miR-34a may be used as a therapy to stimulate conversion of cholesterol to bile acids and to reduce high-fat diet–induced hyperlipidemia for NAFLD and atherosclerosis.

A high-throughput small mRNA sequencing detected 150 miRNAs in mouse liver. High-fat diet treatment revealed that 50 miRNAs were increased more than 2-fold. Unbiased in silico analysis identified miR-27b as a regulatory hub in lipid metabolism in humans and mice (Vickers et al., 2013). miR-27b downregulated mRNA expression of PPARγ, angiopoietin-like protein 3 (ANGPTL3), and glycerol-3-phosphate acyltransferase 1 (GPAM), all involved in lipid metabolism. In apero−/− mice on a high-fat diet, miR-27a is upregulated in the liver. ANGPTL3 is highly expressed in the liver and is an inhibitor of LPL, which hydrolyzes triglycerides in VLDL and chylomicrons. Plasma levels of ANGPTL3 correlate with high serum lipids. GPAM is highly expressed in the liver and catalyzes the first and rate-limiting step in de novo triglyceride synthesis. ANGPTL3 and GPAM genetic polymorphisms are significantly associated with plasma lipid levels (Musunuru et al., 2010; Teslovich et al., 2010). miR-27b oligomers may be used to inhibit ANGPTL3 to stimulate LPL as a therapy to promote VLDL metabolism and prevent NAFLD. In CYP7A1-tg mouse livers, ANGPTL3 mRNA levels were significantly reduced, suggesting that bile acids may be used to inhibit ANGPTL3 and stimulate LPL.

VI. Bile Acid Signaling in Metabolic Diseases and Therapeutics

Bile acids are known to increase cell proliferation and apoptosis in the liver and intestine (Solá et al., 2006; Amaral et al., 2009). Accumulation of toxic endobiotics and xenobiotics causes damage to cells and organs in the digestive tract. Inborn errors in bile acid metabolism in pediatric human patients have been identified in almost every gene in bile acid synthesis and conjugation and have been extensively reviewed (Heubi et al., 2007; Ng et al., 2014). This section reviews bile acid signaling in inflammatory diseases of the digestive system including cholestatic liver diseases, IBDs, and fatty liver diseases as well as bile acid–based therapies.
A. Cholestatic Liver Diseases

1. Cholestasis. Cholestasis is caused by a disruption of bile flow, which results in a lack of bile in the intestine, accumulation of toxic bile acids and other metabolites in the liver, and increased bile acids in the systemic circulation (Trauner et al., 1998). Congenital or acquired defects in bile flow can cause obstructive cholestasis. Obstruction of the bile ducts by tumors or stones, genetic mutations of bile acid transporter genes, and acquired dysregulation of bile transport system by drugs, pregnancy, and pathophysiological conditions causes intrahepatic and extrahepatic cholestasis (Wagner et al., 2009). Congenital or acquired defects in canalicular membrane transporters are the major cause of intrahepatic cholestasis. Congenital cholestasis occurs early in life and patients have jaundice, pruritus, low absorption of fat-soluble vitamins leading to slow growth, and progressive liver damage by increased hepatic and serum levels of bile acids. PFIC and benign recurrent intrahepatic cholestasis are autosomal recessive diseases. PFIC1 (also known as Byler disease) is linked to mutations in the ATP8B1 gene, which codes a P-type ATPase, functioning as an aminophospholipid flippase that maintains membrane asymmetry by inward flipping of phosphatidylserine from the outer leaflet of the plasma membrane. PFIC2 is linked to BSEP mutations and polymorphisms that have been linked to intrahepatic cholestasis of pregnancy (ICP) (Pauli-Magnus et al., 2004; Noe et al., 2005; Lang et al., 2006) and drug-induced liver injury (Lang et al., 2007). ICP is a reversible form of intrahepatic cholestasis associated with adverse pregnancy outcomes (Eloranta and Kullak-Ublick, 2008). PFIC3 is linked to MDR3 mutations and patients have high levels of γ-glutamyl transpeptidase activity, progressive cholestasis, and bile duct damage, and may require liver transplant. Phospholipids in bile are required for mixed micelle formation with bile acids and cholesterol (Jacquemin, 2001; Pauli-Magnus et al., 2004). Without forming mixed micelles, bile acids damage the canalicular membrane and cholangiocytes. MDR3 mutations may cause cholesterol gallstone diseases and have been linked to ICP (Jacquemin, 2001; Wasmuth et al., 2007). Genetic polymorphisms and heterozygote mutations of the PFIC1, PFIC2, and PFIC3 genes may increase susceptibility to acquired cholestasis in adults including ICP, drug-induced liver injury, primary biliary cirrhosis, and primary sclerosing cholangitis. FXR variants have been identified in ICP patients (Van Mil et al., 2007) and affect FXR target genes ABCB11 and ABCB4 as well as ATP8B1 expression in ICP (Müllenbach et al., 2005; Painter et al., 2005).

Mutations in the MRP2 gene have been linked to Dubin-Johnson syndrome, a disease characterized by chronic hyperbilirubinemia (Keitel et al., 2003). MRP2 excretes conjugated bile acids, bilirubin, and other organic anions. Patients have elevated bile acids and cholestasis. MRP2 mutations have also been linked to ICP. The FXR gene has been identified as a candidate gene for the cholesterol gallstone susceptibility locus Lith7 in mice (Wittenburg et al., 2003). A study of obstructive cholestasis in human patients shows that bile acid synthesis is suppressed but CYP7A1 expression is not altered (Bertolotti et al., 2001). By contrast, it was reported that CYP7A1 mRNA expression is repressed in human patients with obstructive extrahepatic cholestasis, likely due to increased FGF19 expression in hepatocytes (Schaap et al., 2009).

2. Bile Acids as Therapeutic Agents. CDCA and UDCA have been used for gallstone dissolution and inborn errors of bile acid synthesis for many decades (Chiang, 2009). UDCA (ursodiol) is an approved drug for treating primary biliary cirrhosis. Recently, therapies targeted to FXR, TGR5, and bile acid metabolism have been developed for treatment of metabolic diseases and cholestatic liver diseases (reviewed in Claudel et al., 2004; Trauner and Halilbasic, 2011; Fiorucci et al., 2012; Porez et al., 2012; Chiang, 2013; Li and Chiang, 2013; Swanson et al., 2013; Schaap et al., 2014).

Activation of FXR inhibits CYP7A1 to reduce bile acid synthesis, and inhibits NTCP and OATPs to reduce sinusoidal uptake of bile acids. FXR also may upregulate MRP3/MPR4 and OSTα/β in the sinusoidal membrane as an adaptive response to efflux bile acids into systemic circulation in obstructive cholestasis. Bile acids also play a protective role in controlling bacterial overgrowth in the intestine (Inagaki et al., 2006). Thus, obstruction of bile flow or knockout of the fxr gene in mice increases bacterial growth and mucosal injury in the intestine, and bile acid administration reduces bacterial growth in obstructive cholestasis. CDCA, CA, and UDCA have been used for effective gallstone dissolution for many years. CDCA and CA are not toxic to humans. UDCA has been used to treat bile acid-deficient patients as a replacement of bile acids in the pool. CA is converted to DCA, which is highly toxic and is a colon cancer promoter. CA is more efficient in intestinal absorption of cholesterol than other bile acids and may cause gallstones in human patients (Wang et al., 2006). UDCA has been used in traditional Chinese medicine for treating digestive disease for several centuries. UDCA is a highly soluble and nontoxic bile acid. UDCA reduces the cytotoxicity of the circulating bile acid pool, and thus protects cholangiocytes, stimulates hepatobiliary secretion, and inhibits liver cell apoptosis (Solá et al., 2006). UDCA may also activate PXR and induce PXR target genes, including CYP3A4, SULTs, UDP-glucuronosyl N-transferases, BSEP, MDR3, and MRP4, for detoxification of bile acids. norUDCA is a side chain-shortened C23 homolog of UDCA that cannot be
increased susceptibility to colitis in il-10 knockout mice. This bile acid derivative reverses sclerosing cholangitis in the Mdr2<sup>−/−</sup> (Acbh4) model of cholangiopathy by reducing bile acid hydrophobicity, increasing bile flow, and inducing bile acid detoxification, and reducing inflammation and fibrosis progression (Moustafa et al., 2012).

**B. Inflammatory Gastrointestinal Diseases**

IBDs are chronic condition of inflammation in intestinal mucosa. IBDs include Crohn’s disease of the gastrointestinal tract and ulcerative colitis in the colon. Intestinal microbiota, epithelial dysfunction, and aberrant mucosal immune response all contribute to IBD (Vavassori et al., 2009; Cipriani et al., 2011; Gadaleta et al., 2011b). In an early study of dextrin sodium sulfate– or trinitrobenzene sulfonic acid–induced colitis, CYP7A1 expression, bile acid secretion, bile acid content in gut and liver, and bile acid pool size were increased; by contrast, in indomethacin-induced small bowel inflammation, CYP7A1 expression, bile acid secretion, and bile acid pool were reduced but the hepatic acute phase and cytokine response were increased (Dikopoulos et al., 2007). A recent study of TGR5 knockout mice showed abnormal morphology of colonic mucous and altered epithelial tight junctions as well as increased intestinal permeability and susceptibility to develop dextrin sodium sulfate–induced colitis. As described in section III, a diet high in saturated fat induces TCA and organic sulfur to promote sulfite-reducing pathobiont *B. wadsworthia* and results in increased susceptibility to colitis in *il-10<sup>−/−</sup>* mice (Devkota et al., 2012). Interestingly, feeding a low-fat diet supplemented with TCA, but not glycocholic acid, increased *B. wadsworthia* abundance and colitis in *il-10<sup>−/−</sup>* mice. This study demonstrated a high-fat diet/ bile acid/microbiota axis in pathogenesis of IBD. Several studies show that activation of FXR and TGR5 signaling protects against IBD by antagonizing Toll-like receptor 4/tumor necrosis factor α and NF-κB signaling and intestinal barrier structures and functions (Cipriani et al., 2011; Gadaleta et al., 2011a). A recent study show that bile acid activation of TGR5 signaling can induce macrophage differentiation toward interleukin-12–producing dendritic cells (Ichikawa et al., 2012) and inhibit the production of proinflammatory cytokines by intestinal macrophages in Crohn’s disease (Yoneno et al., 2013). An IBD patient study showed a link of genetic variation in FXR to IBD and ileal SHP mRNA levels were reduced in Crohn’s colitis, but not in ulcerative colitis (Nijmeijer et al., 2011). It was concluded that FXR activation in the ileum is reduced in Crohn’s patients, secondary to altered enterohepatic circulation of bile acids. TGR5 and FXR agonists may have therapeutic benefit to Crohn’s disease patients (Lian et al., 2011; Pols et al., 2011b; Stojanovic et al., 2012; Stepanov et al., 2013).

**C. Fatty Liver Disease, Diabetes, and Obesity**

Diabetes and fatty liver diseases are inflammatory diseases associated with dyslipidemia and hepatic insulin resistance. NAFLD is the most common chronic liver disease affecting approximately 20–40% of the adult population in the United States, and has a high prevalence of T2DM and obesity (Farrell and Larter, 2006; Brunt, 2010; Cohen et al., 2011). Accumulation of cytotoxic bile acids causes inflammation and injury to the liver. In diabetic patients, serum 12α-hydroxylated bile acids and their conjugates are increased and correlated with insulin resistance (Thomas et al., 2008; Haessler et al., 2013). Increasing CA facilitates intestinal absorption of dietary cholesterol and fats and contributes to cholesterol gallstone diseases, NAFLD, and diabetes.

Metabolic syndrome is a collection of five clinical symptoms including hypertension, hyperglycemia, hypertriglyceridemia, insulin resistance, and central obesity (Reaven, 1988). Metabolic syndrome contributes to chronic heart disease, atherosclerosis, T2DM, and NAFLD. Dyslipidemia causes insulin resistance and inflammation, and pathogenesis of NAFLD (Ginsberg et al., 2006; Cohen et al., 2011). NAFLD is a spectrum of chronic liver abnormalities from simple steatosis to NASH to liver cirrhosis (Yeh and Brunt, 2007). In hepatic steatosis, more than 5% of hepatocytes accumulate lipid droplets. Hepatic steatosis is caused by alcohol, drugs, hepatitis, insulin resistance, high-fat diet, and other factors, and is reversible. About 30% of steatotic patients develop NASH, which involves hepatic ballooning, inflammatory infiltrates, and cell death. About 10–29% of NASH patients will progress to cirrhosis and hepatocellular carcinoma, the end stage of liver disease. NAFLD is closely associated with obesity, insulin resistance, and hepatic steatosis (Farrell and Larter, 2006; Tiniakos et al., 2010). The progression of hepatic steatosis to NASH requires at least “two hits”: hepatic steatosis is the first hit, followed by inflammation as the second hit (Day and James, 1998). Other factors, such as insulin resistance and oxidative stress, accelerate the progression from hepatic steatosis to NASH. Excessive accumulation of free fatty acids and triglycerides in hepatocytes may cause lipotoxicity and hepatocyte apoptosis (Trauner et al., 2010). Free fatty acids cause liver injury by increasing inflammation, the unfolded protein response or ER stress, and damaging mitochondria and increasing reactive oxidizing species. Autophagy is a critical lysosomal mechanism for degradation of cell organelles and maintenance of normal cell function and prevention of diseases in NASH (Czaja et al., 2013). Lipophagy (autophagy of lipids) degrades cellular fats to maintain lipid homeostasis (Singh et al.,...
Based on the phenotypes of CYP7A1-tg mouse model (Li et al., 2010, 2011b), increasing bile acid synthesis with reduced CA in the pool may be a strategy for preventing high-fat diet–induced NAFLD and improving insulin resistance. A recent lipidomic analysis identified seven lipid markers, including lysophosphatidylcholine, phosphatidylcholine, sphingomyelin, and ceramide, that were significantly decreased in serum of CYP7A1-tg mice fed a high-fat diet. Metabolomics analysis identified 13 metabolites in bile acid synthesis, including taurochenodeoxycholic acid, TDCA, TUDCA, TCA, and T-β-MCA, that differed between CYP7A1-tg and wild-type mice. Interestingly, T-β-MCA was significantly increased only in intestine of CYP7A1-tg mice. This study suggest that reducing 12α-hydroxylated bile acids and increasing T-β-MCA, an FXR antagonist, may reduce the high-fat diet–induced increase of phospholipid, sphingomyelin, and ceramide and may ameliorate diabetes and obesity (Qi et al., 2014). CA facilitates dietary cholesterol absorption and contributes to cholesterol gallstone disease, and may also cause insulin resistance and NAFLD.

The therapeutic potential of bile acids and derivatives for treating hepatic and biliary diseases, NAFLD, and metabolic syndrome has been extensively reviewed (Thomas et al., 2008; Fiorucci and Baldelli, 2009; Zollner and Trauner, 2009; Lian et al., 2011; Pols et al., 2011a,b; Adorini et al., 2012; Hollman et al., 2012; Stojanecvic et al., 2012; McMahan et al., 2013; Mudalair et al., 2013; Stepanov et al., 2013; Duboc et al., 2014) and will be briefly summarized. Bile acid sequestrants have long been used for treating gallstone disease. The second-generation bile acid sequestrants are currently in clinical trials for metabolic diseases (Davidson et al., 1999; Aldridge and Ito, 2001; Bays et al., 2008; Brufau et al., 2010; Shang et al., 2010; Nwose and Jones, 2013). Gastric bypass surgeries are effective in reducing weight and improving insulin resistance and are increasingly popular for treatment of T2DM (Patti et al., 2009; Simonen et al., 2012; Gerhard et al., 2013).

1. Farnesoid X Receptor Agonists. Many studies have shown that activation of FXR inhibits inflammation in liver and intestine and FXR agonists are potential therapeutic drugs for metabolic and inflammatory diseases. A synthetic bile acid derivative, obeticholic acid (OCA, 6-ethyl-CDCA or INT-747) is a potent and selective FXR agonist that has anticholestatic effects (Pellicciari et al., 2004). In animal studies, OCA increases insulin sensitivity, inhibits gluconeogenesis, inhibits lipogenesis, and has anti-inflammatory and antifibrotic properties (Adorini et al., 2012). OCA ameliorates high-fat diet–induced obesity and insulin resistance in mice, as well as insulin resistance and fatty liver in Zucker rats (fa/fa) (Cipriani et al., 2010). OCA antagonizes NF-κB–stimulated inflammation in liver (Wang et al., 2008), modulates innate immunity in animal models of colitis (Vavassori et al., 2009), inhibits and preserves the intestinal barrier in IBD (Nijmeijer et al., 2011), and inhibits VSMC inflammation and migration. A phase II clinical trial of OCA for NAFLD and T2DM patients showed improved insulin sensitivity, reduced γ-glutamyl-transpeptidase levels (a marker of NASH), and weight loss (Mudalair et al., 2013).

2. G Protein–Coupled Bile Acid Receptor Agonists. TGR5 signaling inhibits the production of proinflammatory cytokines in Crohn’s disease (Yonenoo et al., 2013). Drugs targeting to TGR5 have the potential to treat metabolic and inflammatory diseases. Oleandrin acid extracted from olive leaves has a structure similar to bile acids and has been shown to be a specific TGR5 agonist (Sato et al., 2008). A bile acid derivative INT-777 is a selective and potent TGR5 agonist (Pellicciari et al., 2007, 2009). In animal studies, INT-777 improves glucose tolerance, stimulates GLP-1 secretion from enteroendocrine L cells and improves insulin sensitivity, and increases intracellular ATP/ADP ratio and calcium mobilization in obese mice (Thomas et al., 2009). Activation of TGR5 also reduces atherosclerotic lesions by reducing macrophage inflammation and lipid loading (Pols et al., 2011a). TGR5 agonists also reduce inflammation in liver and intestine to prevent liver inflammation and IBD (Pols et al., 2011b). The FXR and TGR5 dual agonist INT-767 (6α-ethyl-3α,7α,23-trihydroxy-24-nor-5β-cholan-23-sulphate) has been shown to improve NAFLD by modulating hepatocyte monocyte activity (McMahan et al., 2013). Other non-bile acid compounds have been reported as specific TGR5 agonists (Evans et al., 2009a; Herbert et al., 2010).

3. Bile Acid Sequestrants. Many studies in animals and human diabetic patients showed that bile acids improved glycemic control (Claudel et al., 2005; Prawitt et al., 2014). In diabetes, bile acid pool size is enlarged, likely due to the altered circadian rhythm of CYP7A1 and the fasting to refeeding response (Li et al., 2012). Disruption of enterohepatic circulation of bile acids by bile fistula or feeding of bile acid sequestrants reduces bile acid pool size and stimulates hepatic bile acid synthesis. Increasing hepatic bile acid synthesis may inhibit gluconeogenesis and lipogenesis but stimulate glycolysis, fatty acid oxidation, and glycogenesis. Bile acid sequestrants are known to reduce serum cholesterol by stimulating bile acid synthesis, increasing LDLR-mediated uptake of serum LDL cholesterol for treating hypercholesterolemia. Cholestyramine and colestipol are classic bile acid sequestrants that have been used for gallstone dissolution and lipid lowering in humans. Colesevelam, a second-generation bile acid sequestrant, has been used in combination with statins or antidiabetic drugs for increasing glycemic control and improving insulin resistance (Davidson et al., 1999; Aldridge and Ito, 2001; Bays et al., 2008; Brufau et al., 2010; Shang et al., 2010). The underlying
mechanism of the glucose-lowering effect of bile acid sequestrants is not completely known. Recent reports show that colesvelam may mediate its action through induction of GLP-1 secretion in rats (Shang et al., 2010). Colesvelam may reduce hepatic glucose production and improve hyperglycemia and hyperinsulinemia in diet-induced obese mice (Potthoff et al., 2013). This effect may be mediated through activation of TGR5 and GLP-1 release (Nwose and Jones, 2013). A recent study shows that colesvelam increases GLP-1 levels and improves $\beta$-cell function, but has no effect on insulin sensitivity (Beysen et al., 2012). Colesvelam improves glycemic control in T2DM patients, but is not correlated with changes in bile acid metabolism (Brufau et al., 2010). In these patients, CA synthesis is increased and colesvelam treatment preferentially increases CA but decreases CDCA and DCA, thus decreasing the hydrophobicity of the bile acid pool without changing the total bile acid pool size. There is no correlation between bile acid kinetics and glucose metabolism in these patients.

4. Gastric Bypass Surgery. Gastric bypass is currently being used as a treatment for reducing weight and T2DM. Laparoscopic adjustable gastric banding, laparoscopic sleeve gastrectomy, and the Roux-en-Y gastric bypass (RYGB) are the most common bariatric surgeries for reducing weight of obese and diabetic patients (Bradley et al., 2012). These procedures decrease energy intake and improve energy balance. RYGB surgery creates a small pouch in the stomach and connects it to the proximal jejunum to form the Roux limb, which is anastomosed to the duodenal limb to form a Y configuration (Bradley et al., 2012; Mells and Anania, 2013). After RYGB surgery, bile acids are secreted to the empty duodenum and directly into the ileum, bypassing a large portion of the stomach and duodenum and 150 cm of the jejunum. Several recent studies report that serum bile acids are increased after RYGB surgery in obese and diabetic patients and are correlated with improved glycemic control, lipid oxidation, and weight loss (Patti et al., 2009; Simonen et al., 2012; Gerhard et al., 2013). RYGB surgery significantly increases bile flow, fasting serum bile acids, and FGF19 levels (Pournaras et al., 2012). Before gastric bypass, diabetic RYGB patients have lower levels of FGF19 and higher bile acids than nondiabetic patients (Patti et al., 2009; Gerhard et al., 2013). After RYGB surgery, serum FGF19 levels are correlated with increased CYP7A1 and bile acid synthesis in diabetic patients, and serum FGF19 and bile acid levels are increased more in diabetic patients with remission than nondiabetic patients and diabetic patients with no remission (Gerhard et al., 2013). It was concluded that the FGF19/CYP7A1 bile acid synthesis pathway might be involved in remission of T2DM after RYGB surgery. The mechanism for improving insulin resistance and glycemic control is not clear. A recent study of vertical sleeve gastrectomy (VSG) in mice showed that increased circulating bile acids after VSG might change gut microbial communities (Ryan et al., 2014). Gastric bypass changes gut microbiota and is associated with weight loss and inflammation (Zhang et al., 2009a; Furet et al., 2010; Graessler et al., 2013). In FXR knockout mice, the ability of VSG to reduce body weight and improve glucose tolerance was significantly reduced, suggesting that FXR signaling may be involved in weight loss and improving glycemic control in bariatric surgery (Zhang et al., 2009a; Furet et al., 2010; Graessler et al., 2013). After gastric bypass surgery, partially digested food and bile acids are mixed at the distal small intestine and bile acids may spill over to the large intestine, where they activate FXR to induce FGF19 and TGR5 in the large intestine to induce GLP-1 release (Kir et al., 2011). GLP-1 stimulates insulin secretion from the pancreas and improves insulin sensitivity, whereas FGF19 stimulates glycogen synthesis in hepatocytes and reduces serum glucose (Kir et al., 2011; Schaap et al., 2014). Further study using TGR5 knockout and FXR and TGR5 double knockout mice may provide more information about the underlying molecular mechanisms.

VII. Future Perspectives

Basic research in bile acid metabolism and signaling in the last 20 years has unveiled an important role for bile acids in integration of hepatic lipid, glucose, and energy metabolism. Maintaining bile acid homeostasis is essential for protection against bile acid toxicity and inflammation in the gastrointestinal tract. Circadian rhythms of bile acids are critical in maintaining metabolic homeostasis and preventing metabolic disorders. Much is to be learned about the liver-gut microbiota axis and host metabolism. Understanding bile acid–microbiome interaction is critical for treatment and prevention of inflammatory gastrointestinal diseases, NAFLD, diabetes, and obesity. Progress in translation of basic research in bile acid metabolism to clinical applications for patient treatment and drug therapies for liver diseases and diabetes has been made in recent years. Bile acids and their derivatives have the unwanted side effect of pruritus, which may cause low patient compliance. Nonbile acid–based agonists specific for FXR and TGR5 may be developed for treating NAFLD and IBD. miRNA therapy has great potential for treating liver-related diseases. Current research in bile acid metabolism relies on genetically modified mouse models. Future research using proteomics, metabolomics, lipidomics, and megagenomic sequencing will identify biomarkers for diagnosis of these diseases. It is anticipated that new treatment strategies for inflammatory metabolic diseases of the digestive system will be developed.


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