Bile acid receptors link nutrient sensing to metabolic regulation

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a common liver disease in Western populations. Non-alcoholic steatohepatitis (NASH) is a more debilitating form of NAFLD characterized by hepatocellular injury and inflammation, which significantly increase the risk of end-stage liver and cardiovascular diseases. Unfortunately, there are no available drug therapies for NASH. Bile acids are physiological detergent molecules that are synthesized from cholesterol exclusively in the hepatocytes. Bile acids circulate between the liver and intestine, where they are required for cholesterol solubilization in the bile and dietary fat emulsification in the gut. Bile acids also act as signaling molecules that regulate metabolic homeostasis and inflammatory processes. Many of these effects are mediated by the bile acid-activated nuclear receptor farnesoid X receptor (FXR) and the G protein-coupled receptor TGR5. Nutrient signaling regulates hepatic bile acid synthesis and circulating plasma bile acid concentrations, which in turn control metabolic homeostasis. The FXR agonist obeticholic acid has had beneficial effects on NASH in recent clinical trials. Preclinical studies have suggested that the TGR5 agonist and the FXR/TGR5 dual agonist are also potential therapies for metabolic liver diseases. Extensive studies in the past few decades have significantly improved our understanding of the metabolic regulatory function of bile acids, which has provided the molecular basis for developing promising bile acid-based therapeutic agents for NASH treatment.

Keywords

Nuclear receptor; TGR5; Metabolic syndromes; Fatty liver disease

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive hepatic fat accumulation (steatosis) that is not caused by alcohol consumption (1,2). NAFLD is a common liver disease and affects ~30% of the Western population (1,2). Currently, simple hepatic steatosis does not require clinical treatment. However, in some patients it may progress to nonalcoholic steatohepatitis (NASH), which is a more debilitating form of

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NAFLD characterized by the presence of hepatocellular injury and inflammation. Patients with NASH have a significantly increased risk of developing fibrosis, cirrhosis, liver cancer and liver failure. In addition, patients with NASH have a significantly higher risk of cardiovascular diseases (CVD), which are the leading cause of morbidity and mortality among these patients (3,4). NASH pathogenesis is incompletely understood and is considered a result of complex interactions between genetic and environmental factors. Patients with NASH are often obese and diabetic and commonly possess other features of metabolic syndrome, such as insulin resistance, dyslipidemia and hypertension. It is thought that over-nutrition and obesity cause adipocyte stress and dysfunction, leading to inflammatory infiltration and adipose insulin resistance. As a result, uncontrolled lipolysis causes increased free fatty acid release and fatty acid lipotoxicity in non-conventional fat storage tissues, such as the skeletal muscle, pancreas and liver (2,5). Adipose-derived fatty acids serve as a major source of hepatic fat in NAFLD (2,5). Increased hepatic fat accumulation in the presence of insulin resistance further promotes hepatic triglyceride overproduction, which is a key contributing factor to dyslipidemia and higher CVD risk. Unfortunately, there are no approved therapies for NASH. Patients who develop end-stage liver disease require a liver transplantation. New therapeutic interventions that treat both liver-related and cardiovascular-related complications in patients with NASH are still needed.

Bile acids are cholesterol derivatives produced only in the hepatocytes of the liver (6). Hepatic bile acid synthesis represents the only quantitatively significant route for cholesterol elimination. Bile acids are amphipathic physiological detergents that facilitate dietary cholesterol, lipid and fat-soluble vitamin absorption in the small intestine (6,7). Studies over the past couple decades have demonstrated that bile acids act as signaling molecules that regulate intracellular signaling pathways. Many of these pathways are critically involved in the regulation of lipid, glucose and energy metabolism. In addition, bile acid synthesis and plasma bile acid concentrations are sensitive to circadian and nutrient regulation, which suggests that bile acid signaling integrates nutrient sensing to the maintenance of metabolic homeostasis. Drugs targeting bile acid metabolism and signaling have been used clinically to treat patients with hypercholesterolemia and hyperglycemia (6). New therapies targeting bile acid signaling pathways are currently being developed to treat fatty liver diseases. In this review, we will summarize the current knowledge of bile acid synthesis regulation and the mechanisms underlying bile acid signaling regulation of metabolic homeostasis. These comprise the molecular basis for the development of bile acid-based therapies for fatty liver disease.

2. A brief introduction on bile acid metabolism

Cholesterol conversion into bile acids involves several enzymatic and non-enzymatic reactions (Fig. 1A). Hepatocytes are the only cell type that expresses all the required bile acid synthetic enzymes, which are located in different intracellular compartments, including the endothelium reticulum (ER), cytosol, mitochondria and peroxisomes. Cholesterol 7α-hydroxylase (CYP7A1), which is a cytochrome P450 (CYP) enzyme residing in the ER, catalyzes the first and rate-limiting step in the classic bile acid synthesis pathway to convert cholesterol to 7α-hydroxycholesterol. 7α-hydroxycholesterol is subsequently converted to

two primary bile acids, chenodeoxycholic acid (CDCA) and cholic acid (CA) (Fig. 1A). CA synthesis involves the C-12 hydroxylation of 7α-hydroxy-4-choesten-3-one to 7α, 12α-dihydroxy-4-cholesten-3-one, which is catalyzed by another cytochrome p450 enzyme sterol 12α-hydroxylase (CYP8B1) that is located in the ER (Fig. 1B). As displayed in Fig 1A, CDCA can also be produced via the alternative bile acid biosynthesis pathway, in which cholesterol is first converted to 27-hydroxycholesterol by the mitochondrial sterol 27-hydroxylase (CYP27A1). Newly synthesized bile acids are conjugated to the amino acids glycine or taurine on the side chain to form N-acyl amidates, a process that is catalyzed sequentially by two enzymes, bile acid-CoA ligase and bile acid-CoA:amino acid N-acyltransferase (8,9). Conjugation of bile acids increases bile acid water-solubility under physiological pH and decreases bile acid toxicity. The bile of human patients with defective bile acid-conjugating enzymes contains high levels of unconjugated bile acids, which causes fat-soluble vitamin malabsorption, growth retardation and liver injury (10,11). Human bile contains glycine- and taurine-conjugated bile acids in a roughly 3:1 ratio, while mouse bile contains predominantly taurine-conjugated bile acids. In this review, the terms CDCA, CA and other bile acid species mentioned later will be used to refer to both the conjugated and unconjugated forms unless specified.

Bile acids circulate between the liver and the intestine in a process called enterohepatic circulation of the bile, which is stimulated by nutrient intake and occurs a few times a day in humans (Fig. 2). Bile acids are secreted across the apical membrane of hepatocytes and stored in the gallbladder. Bile acid secretion into the bile against the concentration gradient is mediated by ATP binding cassette transporter B11 (also called the bile salt export pump [BSEP]) (12). Cholesterol and phospholipids are two other major constituents in the bile, and their efflux is mediated by ATP-binding cassette (ABC) transporters, the ABCG5/ABCG8 heterodimer (13) and canalicular phospholipid floppase multi-drug resistance 3, respectively (14). Some bile acids can be absorbed by cholangiocytes after they are secreted by hepatocytes. Cholangiocytes take up unconjugated bile acids via passive diffusion and conjugated bile acids via the apical sodium-dependent bile salt transporter (ASBT) (15). Bile acids in cholangiocytes are then secreted into the peribiliary plexus via organic solute transporter (OST) α and OSTβ, which form a heterodimer, and subsequently taken up by hepatocytes (15,16). Meal intake stimulates the release of bile acids into the intestinal tract where bile acids help emulsify dietary lipids and thus facilitate dietary lipid and fat-soluble vitamin absorption. In the small intestine, some colonized bacteria express bile salt hydrolases, which convert some of the conjugated bile acids to unconjugated bile acids. These unconjugated bile acids can then serve as substrates for bacterial 7α-dehydroxylase, which mediates the C-7 dehydroxylation reaction of primary bile acids to produce secondary bile acids deoxycholic acid (DCA) from CA and lithocholic acid (LCA) from CDCA. The majority of bile acids are efficiently reabsorbed in the ileum and transported back to the liver via portal circulation. The daily fecal loss of bile acids is approximately 5%, which is replenished by *de novo* bile acid biosynthesis in the hepatocytes to maintain a relatively constant bile acid pool. The ileum has high expression of bile acid transporters. ASBT mediates bile acid uptake into enterocytes, and OSTα/OSTβ heterodimers at the basolateral membrane of the enterocyte mediate bile acid efflux into the portal circulation. Conjugated bile acids in the portal circulation are taken up primarily by the sodium-dependent

taurocholate transporter (17). Because the first pass extraction rate of portal bile acids by the liver is approximately 90% (18), bile acid concentrations in the systemic circulation are significantly lower than those of the portal blood under normal physiology. Under normal conditions, bile acid excretion through the kidney is minimal. However, in cholestasis, where bile acid excretion via the biliary route is impaired, more bile acids are secreted across the basolateral side of hepatocytes into the systemic circulation (19–21). Basolateral bile acid efflux from the hepatocyte can be mediated by multidrug resistance-related protein (MRP) 3, MRP4 and OSTα/OSTβ heterodimer (16,21,22). Increased systemic bile acid concentrations lead to renal bile acid excretion.

In humans, the two primary bile acids CA and CDCA make up approximately 70%–80% of the total bile acid pool. DCA is the major secondary bile acid, which may account for approximately 20% of the total bile acid pool. Among all bile acid species, the secondary bile acid LCA is highly toxic. LCA is efficiently detoxified, and only trace amounts of LCA are found in the bile. In mice and rats, CDCA and UDCA are converted to α-muricholic acids (MCA) and βMCA, respectively. MCAs have a hydroxyl group at the C-6 position and are more hydrophilic than CDCA. In C57BL6J mice, the bile acid pool contains roughly 50% MCA, ~40% CA and ~10% CDCA (23). A recent study revealed that murine Cyp2C70 was involved in MCA formation in mice (24). Humans do not appear to have a homolog of murine Cyp2c70, which may explain the different bile acid composition between mice and humans.

3. Bile acid receptors in the regulation of metabolism and inflammation

Bile acid exerts its regulatory function by activating a number of nuclear receptors and signal transduction pathways in hepatic and extrahepatic tissues. Nuclear receptors are intracellular ligand-activated transcription factors (25). A typical nuclear receptor consists of a DNA-binding domain and a ligand-binding domain. Most nuclear receptors bind to the consensus DNA sequences in their target gene promoters and regulate gene transcription in a ligand-dependent manner. Bile acids are endogenous ligands of three nuclear receptors: the farnesoid X receptor (FXR) (26–28), the pregnane X receptor (29) and the vitamin D receptor (30). These bile acid receptors sense bile acid concentrations in the enterohepatic system and in turn regulate bile acid homeostasis and detoxification mechanisms. Pregnane X and vitamin D receptor activation induces many phase-I cytochrome p450s, phase II conjugating enzymes and phase-III transporters that are involved in bile acid and drug detoxification in the hepatocyte and the intestine (reviewed in (6)). In this review, we will primarily focus our discussion on the role of FXR in the regulation of bile acid, lipid and glucose metabolism. Bile acids also activate cell surface receptors, such as the G protein-coupled receptor TGR5 and the sphingosine-1-phosphate receptor 2. TGR5 is expressed in the intestine and colon as well as metabolically active tissues, including skeletal muscle and brown adipose tissue, and is involved in the regulation of glucose and energy metabolism. Finally, bile acid-activated FXR induces the growth hormone fibroblast growth factor (FGF) 15 in mice and its ortholog FGF19 in humans. Recent studies revealed a novel role of FGF15/19 in the regulation of postprandial glucose metabolism in the liver.

3.1. FXR regulation of bile acid synthesis and transport

In the enterohepatic system, FXR is expressed in hepatocytes and enterocytes that are routinely exposed to high bile acid concentrations. Both unconjugated and conjugated bile acids, including CDCA, CA, DCA and LCA, can activate FXR (26,27). The most potent FXR ligand among all endogenous bile acid species is CDCA, which activates human FXR with an EC_{50} of ~10 μmol/L and murine FXR with an EC_{50} of ~50 μmol/L. In mice, the hydrophilic bile acid MCA does not activate FXR.

The role of FXR in the regulation of bile acid homeostasis has been extensively investigated. *De novo* bile acid biosynthesis is subject to bile acid feedback inhibition, which helps maintain bile acid homeostasis. Extensive studies over the past two decades support the conclusion that FXR plays a central role in mediating bile acid feedback inhibition of bile acid synthesis (6). Bile acids and FXR repress several bile acid biosynthetic genes, including *CYP7A1*, *CYP8B1* and *CYP27A1*. However, major research focus has been placed on understanding the mechanisms underlying the transcriptional regulation of the rate-limiting gene *CYP7A1* in the classic bile acid biosynthetic pathway. FXR can sense elevated bile acid concentrations in either the hepatocyte or the enterocyte and in turn decreases hepatic bile acid synthesis. In hepatocytes, FXR transcriptionally induces an atypical nuclear receptor called small heterodimer partner (SHP). Unlike other nuclear receptors, SHP does not have a DNA-binding domain, and it primarily interacts with other transcriptional factors as a transcriptional co-repressor. Two nuclear receptors, hepatocyte nuclear factor 4α and liver receptor homolog 1, bind to the *CYP7A1* gene promoter and play a key role in maintaining basal *CYP7A1* gene transcription. SHP interacts with both hepatocyte nuclear factor 4α and liver receptor homolog 1 as a co-repressor, and FXR-induction of SHP results in *CYP7A1* inhibition (31,32). When bile acid levels increase in the intestine, FXR induces FGF15, which acts as an endocrine hormone to inhibit hepatic *CYP7A1* gene transcription after binding to FGF receptor 4 (FGFR4) on hepatocytes (33,34). In mice, *Fgfr4* deletion resulted in significantly higher hepatic *CYP7A1* expression, increased bile acid synthesis and enlarged bile acid pool size, while active FGFR4 over-expression reduced hepatic *CYP7A1* expression and bile acid pool size (35). Furthermore, studies have demonstrated that FGFR4 forms a plasma membrane signaling complex with β-Klotho (36), cytoplasmic tyrosine phosphatase SHP-2 (37) and FGF receptor substrate 2 (38). Additionally, ligand binding to FGFR4 activates extracellular signal-regulated kinase 1/2 signaling to cause transcriptional repression of the *CYP7A1* gene (33,39,40). Humans do not express FGF15. In humans, FGF19 shares ~51% amino acid sequence homology with mouse FGF15. In contrast to mouse FGF15, which is expressed in enterocytes but not hepatocytes, human FGF19 is expressed in both hepatocytes and enterocytes and is transcriptionally induced by FXR and bile acids. Bile acids and FXR agonists have been shown to directly induce *FGF19* mRNA expression in human hepatocytes (39,41). In addition, plasma FGF19 levels were elevated in patients with obstructive cholestasis, which was presumably a result of hepatic FGF19 induction (42). Studies have revealed that human FGF19 also inhibits human *CYP7A1* gene transcription via the FGFR4/extracellular signal-regulated kinase signaling cascade in human hepatocytes, suggesting that FXR/FGF15/19 regulation of *CYP7A1* is a conserved mechanism (39,41). Although FGF15 is not expressed in mouse hepatocytes or gallbladder, it has been reported to regulate gallbladder refilling in mice (43). Mice lacking

Fgf15 had almost empty gallbladders, which was restored by recombinant FGF15 administration. In contrast, *FGF19* mRNA expression could be detected in human gallbladders (39,42). Interestingly, human gallbladders contain high FGF19 levels that are approximately one hundred-fold higher than those in the blood circulation (44). Gallbladder epithelial cells express the FGF19 receptor FGFR4 and β-Klotho (44). However, FGF19 function in the human gallbladder has not been well defined.

In addition to the inhibition of *de novo* bile acid biosynthesis, FXR activation also reduced intracellular bile acid accumulation by modulating bile acid transporters in hepatocytes and enterocytes. When bile acid levels increase in hepatocytes, bile acids activate FXR to induce BSEP to promote biliary bile acid secretion (45) and simultaneously inhibit the sodium-dependent taurocholate transporter and basolateral bile acid uptake (46). Similarly, intestinal bile acid accumulation activated FXR to induce basolateral bile acid efflux transporters OSTα and OSTβ (47) and repressed the apical bile acid uptake transporter ASBT (48). This thereby inhibited intestinal bile acid re-absorption and promoted bile acid secretion into the portal blood. In the kidney, bile acids are excreted via MRP2 and MRP4 into the urine. In addition, ASBT and OSTα/β are involved in re-absorption of bile acids in the renal proximal tubules. FXR repression of ASBT in the kidney may presumably increase renal bile acid excretion. A recent report revealed that FXR was highly expressed in the kidney, and its activation by bile acids induced renal aquaporin 2 and decreased urine volume in mice (49).

3.2. Bile acid signaling links nutrient sensing and metabolic homeostasis

Almost all major metabolic pathways respond to changes in nutrient availability. The metabolic switch from catabolism to anabolism during the fasting-to-fed transition is subjected to complex regulation by pancreatic hormones, incretins, adipokines and growth factors. After food intake, insulin promotes glucose uptake into the muscle and adipose to prevent prolonged postprandial hyperglycemia. Insulin also induces hepatic lipogenesis and thus couples postprandial glycolysis to fatty acid synthesis in the liver. Furthermore, insulin inhibits hepatic glycogenolysis, gluconeogenesis and hepatic very low density lipoprotein(VLDL) secretion. During fasting, attenuated insulin signaling and elevated glucagon action in the liver and adipose tissue stimulate hepatic glucose production and adipose lipolysis. Fatty acids are transported to the liver to be used for fatty acid oxidation, ketogenesis and VLDL assembly. The fasting-to-fed transition is often impaired in individuals with obesity and peripheral insulin resistance. In obesity and diabetes, uncontrolled adipose fatty acid release is considered a major contributor to hepatic steatosis and hyperlipidemia. Free fatty acid deposition in the skeletal muscle is also an initial trigger of muscle insulin resistance. In the liver, increased fatty acid influx into the hepatocytes causes lipotoxicity, which is thought to be an underlying cause of NASH pathogenesis.

Both hepatic bile acid synthesis and plasma bile acid concentrations increase in response to food intake (50,51). Postprandial metabolism is highly active. Studies suggest that bile acid signaling plays an important role in the regulation of postprandial lipid, glucose and energy metabolism. After a meal, cholecystokinin stimulates gallbladder contraction and bile acid release into the small intestine, which thus increases the trans-flux of bile acids across enterocytes, resulting in postprandial elevation of plasma bile acid concentrations in humans

(50,51). In mice, plasma bile acid concentrations were lower during the light cycle and increased at onset of the dark cycle as well as after refeeding in mice (23). Hepatic CYP7A1 activity and bile acid synthesis also exhibited a strong diurnal rhythm (50). In humans, plasma 7α-hydroxy-4-cholesten-3-one, which is a surrogate marker for hepatic CYP7A1 activity, increased during postprandial periods (50). In mice, refeeding after fasting also caused marked induction of hepatic *Cyp7a1* mRNA expression and hepatic CYP7A1 activity (23). The mechanism underlying the diurnal control of hepatic CYP7A1 expression is a result of complex regulation by clock genes and insulin and glucagon signaling, which is reviewed elsewhere (6). The following sections will summarize current knowledge regarding how bile acids act as metabolic regulators that integrate nutrient signaling with the regulation of metabolic homeostasis.

3.3. FXR regulation of fatty acid metabolism

Early studies demonstrated that using CDCA for gallstone dissolution in humans also decreased plasma triglyceride levels (52). Bile acid sequestrants are a class of cholesterol-lowering drugs that bind bile acids in the intestine and prevent bile acid reabsorption and return to the liver. Removal of bile acids by bile acid sequestrants induces hepatic *CYP7A1* expression and cholesterol catabolism, which achieves cholesterol-lowering effects. It was found that treating hypercholesterolemia with bile acid sequestrants often raised plasma triglyceride levels in many human patients (53,54). These observations collectively suggest that bile acids may have triglyceride-lowering effects. Later studies in mice revealed that when challenged with a high fat diet, *Fxr* knockout mice displayed worsened hepatic lipid accumulation and hyperlipidemia compared with wild type control mice. In contrast, pharmacological activation of FXR by a semisynthetic FXR agonist attenuated diet-induced hepatic steatosis and hyperlipidemia in mice (55). These studies suggest that FXR plays a role in mediating the triglyceride-lowering effects of bile acids. Studies to date have provided extensive molecular insights on the mechanisms through which FXR regulates fatty acid and triglyceride metabolism. These bile acid- and FXR-regulated pathways are summarized below.

3.3.1. De novo *lipogenesis*—As mentioned earlier, obesity and insulin resistance cause increased *de novo* lipogenesis in the liver, which is a major contributor to fat accumulation and VLDL overproduction (56). Adipose-derived free fatty acids are the primary source of hepatic triglyceride synthesis in NAFLD (5). In addition, excess carbohydrates are also converted to fatty acids in the liver. Hepatic *de novo* lipogenesis is regulated by two transcriptional factors: sterol-regulatory element-binding protein-1 (SREBP-1) and carbohydrate-responsive element-binding protein (ChREBP). Studies have demonstrated that FXR activation represses both SREBP-1 and ChREBP and their target genes in *de novo* lipogenesis in the liver (57,58). SREBPs are a group of basic helix-loop-helix leucine zipper transcription factors involved in the regulation of fatty acid and cholesterol metabolism primarily in hepatocytes but also in some extrahepatic tissues (59). SREBPs are synthesized as precursors that are sequestered in the ER by forming a protein complex with SREBP cleavage activation protein and insulin-induced genes (Insig1 and Insig2). SREBP activation involves a sterol-sensing mechanism, whereby decreased oxysterol levels in the ER promote SREBP/SREBP cleavage activation protein complex translocation to the Golgi apparatus. In

the Golgi apparatus, SREBPs are cleaved by two serine proteases S1P and S2P, releasing an N-terminal transcriptionally active form of SREBP that enters the nucleus to activate target gene transcription. There are two SREBP isoforms: SREBP-2, which primarily induces genes involved in cholesterol metabolism, and SREBP-1, which is a master inducer of lipogenic genes. In addition to proteolytic cleavage activation, hepatic *SREBP-1* gene transcription is also highly induced in fatty livers (60). *Srebp-1* transgenic mice displayed elevated hepatic lipogenic gene expression and developed hepatic steatosis on a chow diet (61). Currently identified SREBP-1-induced lipogenic genes include acetyl-CoA carboxylase, fatty acid synthase, acetyl-CoA synthase, ATP-citrate lyase, malic enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and stearoyl-CoA desaturase-1 (59,62). FXR activation by either bile acids or a synthetic FXR agonist decreased hepatic *SREBP-1* expression. Mechanistically, FXR-induced SHP repressed LXRα activation of the *SREBP*-1 gene promoter (58). Both high dietary sugar intake and postprandial hyperglycemia cause increased carbohydrate influx into the glycolysis pathway in hepatocytes. Pyruvate, the end product of glycolysis generated by liver pyruvate kinase, is converted to acetyl-CoA, which enters the TCA cycle. In the presence of high levels of NADH and ATP during the postprandial state, acetyl-CoA-derived citrate is preferentially shuttled back to the cytosol and used for fatty acid synthesis and triglyceride production. In response to carbohydrate influx, glucose induces ChREBP nuclear translocation and transcriptional activation of a set of lipogenic genes, including liver pyruvate kinase, fatty acid synthase and acetyl-CoA carboxylase, to facilitate fatty acid synthesis (63,64). Studies have demonstrated that ChREBP inhibition alleviated hepatic fat accumulation in mice (65,66).

3.3.2. Fatty acid oxidation—Recently, several genes involved in hepatic fatty acid oxidation have been identified as novel FXR targets, suggesting that bile acids also promote hepatic fatty acid oxidation via FXR activation. It was first shown that bile acids induced peroxisome proliferator-activated receptor α (PPARα) expression in human hepatocytes but not in mouse hepatocytes because of the lack of a conserved FXR binding site in the mouse *Ppara* gene promoter (67). PPARα is a nuclear receptor that can be activated by endogenous free fatty acids as well as fibrates, a class of lipid-lowering drugs. Activation of PPARα transcriptionally induces hepatic fatty acid oxidation genes, including the rate-limiting gene carnitine palmitoyltransferase 1A. Therefore, increased cellular free fatty acid levels can feedforward to promote fatty acid oxidation via PPARα activation. More recently, it was shown that FXR also transcriptionally induced liver carboxylesterase 1 (CES1), and over-expression of CES1 in mouse liver decreased hepatic triglyceride accumulation (68). CES1 is a neutral cholesterol ester and triglyceride hydrolase that is highly expressed in hepatocytes and macrophages. Previous studies have established a protective role of CES1 in both NAFLD and atherosclerosis (68,69). Hepatocyte CES1 mobilized intracellularly-stored cholesterol ester in the lipid droplets and simultaneously promoted cholesterol conversion into bile acids in mice (70). In macrophages, CES1 stimulated cholesterol efflux and prevented foam cell formation and inflammatory macrophage activation (69). Xu *et al.* demonstrated that CES1 also converted triglycerides to free fatty acids, which led to the feedforward activation of PPARα (68). As a result, FXR induction of CES1 simultaneously promoted hepatic fatty acid oxidation driven by both increased substrate availability and

induction of fatty acid oxidation genes. FGF21 is another catabolic hormone induced by PPARα in the hepatocytes. In addition, a previous study demonstrated that FXR also induced hepatic expression and secretion of FGF21 (71). In the liver, FGF21 has broad metabolic actions, including stimulation of hepatic lipid oxidation, ketogenesis and gluconeogenesis, and as a result, reduces triglyceride levels and improves insulin sensitivity (72,73). The mechanism of action of FGF21 in various metabolic tissues is reviewed in detail elsewhere (74).

3.4. FXR regulation of glucose metabolism

In type 2 diabetes, peripheral insulin resistance, especially in the skeletal muscle, impairs glucose clearance resulting in postprandial hyperglycemia. In addition, hepatic gluconeogenesis is increased because of the attenuated suppressive effects of insulin, which leads to fasting hyperglycemia. Feeding mice a CA-containing diet or administration of an FXR agonist reduced fasting plasma glucose levels and improved insulin sensitivity in obese and diabetic mice (55,75). The glucose-lowering effects of bile acids and FXR may be primarily attributed to the reduction in hepatic glucose production via transcriptional repression of key gluconeogenic genes, including the genes encoding phosphoenolpyruvate carboxykinase, *PEPCK,* and glucose 6-phosphatase, *G6Pase.* To date, FXR-induced SHP has been shown to interact with and inhibit *PEPCK* and *G6Pase* transactivation through cAMP response element-binding protein (76), forkhead transcription factor O1 (77) and glucocorticoid receptor (78). Elevation of intracellular glucose concentrations can induce FXR expression and enhance FXR activity via *O*-Glc-*N*-acylation (79,80), suggesting that FXR can also sense intracellular glucose levels and consequently control glucose metabolism. In humans, circulating plasma FGF19 concentrations exhibited a pronounced diurnal rhythm and peaked during the postprandial period (51). Recently, bile acid/FXR-induced gut FGF15 has been suggested to act as a postprandial hormone, in addition to insulin, that regulates hepatic glucose metabolism (81,82). FGF15/19 has also been shown to inhibit hepatic lipogenesis (83) and increase metabolic rate (84). *Fgf19* transgenic mice were resistant to diet-induced obesity and insulin resistance (84). Human patients with fatty liver disease and insulin resistance had decreased fasting FGF19 levels or an impaired hepatic response to FGF19 (85,86).

3.5. Bile acid regulation of autophagy

Autophagy is a well-conserved cellular self-degradation process that helps eliminate protein aggregates and damaged organelles to maintain cellular integrity (87). Autophagy is also a catabolic process that produces nutrients and energy by degrading macromolecules in response to nutrient deprivation (88). Cellular autophagy is under the reciprocal control of nutrient-sensing mechanistic target of rapamycin and AMP-activated protein kinase signaling in response to changes in nutrient availability and growth hormone signaling (88). As a result, autophagy is normally induced after fasting/starvation and repressed during the postprandial period. Recent studies revealed that autophagy transported intracellularly stored lipids to the lysosomes for mobilization, a process termed "lipophagy" (89). Several recent studies have linked bile acid signaling to the regulation of hepatic autophagic activity. Whole genome chromatin binding assays revealed that FXR bound to many autophagy genes, and FXR activation caused transcriptional repression of autophagy gene expression and

decreased hepatic autophagic activity (90,91). In addition, Manley *et al.* reported that hepatic autophagic flux was inhibited in cultured primary hepatocytes treated with bile acids and in the liver of *Fxr* knockout mice because of increased bile acid concentrations (92). This study suggested that bile acids impaired hepatic autophagic flux by inhibiting autophagosome-lysosome fusion. Consistent with the repressive effect of bile acids on autophagic activity, administration of the bile acid sequestrant cholestyramine induced hepatic autophagy in mice, which was likely a combined result of reduced hepatic mechanistic target of rapamycin signaling and bile acid concentrations (93). The novel role of bile acid signaling regulation of hepatic autophagy further supports the general notion that bile acids act as nutrient sensors to regulate hepatic metabolism during the fasting-to-fed transition.

3.6. Bile acid-activated G protein-coupled receptors

The Gαs protein-coupled receptor TGR5 is a bile acid-activated membrane receptor (94,95). TGR5 signals through the adenylate cyclase/cAMP cascade, resulting in protein kinase A activation. TGR5 can be activated by both conjugated bile acids and free bile acids, including CDCA, CA, DCA and LCA. LCA is the most potent TGR5 agonist with an EC_{50} of ~0.5 μM, while DCA, CDCA and CA activate TGR5 with EC_{50} values ranging between 1 and 10 μM. In the enterohepatic system, TGR5 is expressed in the intestine, with relatively higher expression found in the terminal ileum and colon (94). In the liver, TGR5 is not expressed in hepatocytes but is expressed in sinusoidal endothelial cells (96), gallbladder epithelial cells and Kupffer cells (97). In other extrahepatic tissues, TGR5 is expressed in the white and brown adipose tissue, spleen, kidney, pancreas, lung, macrophages and central nervous system (94).

3.6.1. TGR5 in energy expenditure and glucose metabolism—TGR5 was first reported to mediate the anti-obesity effect of bile acids (98). Feeding mice a CA-containing diet activated TGR5 in the brown adipose tissue and skeletal muscle. TGR5 subsequently induced type 2 deiodinase and the conversion of thyroxine to the active 3,5,3′-tri-iodothyronine and thus increased energy expenditure. A similar anti-obesity effect was also observed when mice were administered a potent synthetic TGR5 agonist INT-777 (99). In contrast to the clear anti-obesity effects of pharmacological TGR5 activation, TGR5 deficiency did not appear to have a profound impact on obesity development in mice. One study revealed higher weight gain in female but not male *TGR5* knockout mice (100). However, another study demonstrated that *TGR5* knockout mice did not gain more weight than wild type controls under chow-fed conditions or after a high fat diet challenge (101). As mentioned earlier, systemic bile acid concentrations are usually very low because of the efficient first pass extraction rate of bile acids from the portal blood by the liver. Therefore, TGR5 in the muscle and adipose may not be highly activated by physiological concentrations of systemic bile acids.

TGR5 has also been shown to exhibit hypoglycemic effects by stimulating glucagon-like peptide-1 (GLP-1) production. GLP-1 is a gut incretin secreted by the enteroendocrine L cells in the ileum and colon. During the postprandial period, dietary carbohydrates and fats stimulate GLP-1 secretion, which enhances insulin secretion from the pancreatic β cells, inhibits glucagon production from the α cells (102), slows gastric emptying and promotes

satiety (103,104). These metabolic effects are thought to collectively contribute to the glucose-lowering effect of GLP-1. It was first reported that bile acids can induce GLP-1 production in the enteroendocrine cell line STC-1 via TGR5 activation (105). It was later confirmed in mice that administration of the TGR5 agonist INT-777 enhanced GLP-1 secretion and improved glucose homeostasis (99). Currently, GLP-1 mimetics and inhibitors of dipeptidyl peptidase-4, the enzyme that rapidly inactivates GLP-1 in the circulation, are clinically used as novel type 2 diabetes therapies. Recently, the bile acid sequestrant colesevelam, which has been used as a cholesterol-lowering drug, was found to also exhibit glucose-lowering effects when given to patients with type 2 diabetes mellitus (106). This effect has been attributed, at least in part, to GLP-1 induction in the gut (107). It is thought that colesevelam, by preventing bile acid re-absorption in the ileum, may increase the amount of bile acids that reach the colon, where TGR5 is highly expressed. Furthermore, bile acid sequestrants may also delay dietary fat solubilization and absorption, which allows a higher concentration of dietary fatty acids to reach the distal ileum and induce GLP-1 secretion (108). As such, bile acid sequestrants may synergistically enhance the stimulatory effect of dietary nutrients and bile acids on gut GLP-1 production after a meal. More recently, studies have suggested the involvement of TGR5 signaling in mediating the beneficial effects of gastric bypass surgery (109,110). Many of the metabolic improvements, particularly in glucose homeostasis, were attenuated in *TGR5* knockout mice after vertical sleeve gastrectomy. Such effects may be partially attributed to altered bile acid composition and action in TGR5 target tissues, which is consistent with altered bile acid metabolism in *TGR5* knockout mice (100,111).

3.6.2 The anti-inflammatory role of TGR5 in macrophages—TGR5 is functionally expressed in macrophages and plays an anti-inflammatory role. To date, TGR5 activation has been shown to attenuate inflammation in various experimental models, including diabetes, NAFLD, atherosclerosis, cholestasis and inflammatory bowel disease (94,97,112–116). Recently, several new studies have revealed novel mechanistic insights on the anti-inflammatory effects of TGR5 signaling in macrophages. TGR5 activation was first shown to antagonize nuclear factor kappa(NF)-κB signaling in macrophages, leading to reduced cytokine expression upon lipopolysaccharide challenge (112). TGR5 activation has also been shown to decrease chemokine expression, which, under obese conditions, reduced adipose macrophage infiltration and improved insulin sensitivity (117). In addition to antagonizing cellular pathways that induce cytokine synthesis at the transcriptional level, a new study reported that TGR5 activation could reduce cytokine maturation and secretion by inhibiting Nod-like receptor pyrin domain-containing protein 3 (NLRP3) inflammasome activation (118). The inflammasome is a pattern-recognition receptor-containing multi-protein oligomer that recognizes pathogen-derived pathogen-associated molecular patterns and endogenously produced danger-associated molecular patterns (119). Inflammasome activation can in turn activate caspase-1, which cleaves the pro-inflammatory cytokines Interleukin **(IL)-1β** and IL-18 to their bioactive forms. High fatty acid and glucose levels and intracellular accumulation of cholesterol crystals have all been shown to activate the NLRP3 inflammasome, which thus links metabolic stress and intracellular organelle damage to tissue inflammation (120–123). The study by Guo *et al.* demonstrated that TGR5

activation caused protein kinase A-dependent phosphorylation and ubiquitination of NLRP3, resulting in decreased NLRP3 inflammasome activation (118).

4. Conclusions

Extensive studies over the past two decades have significantly expanded our knowledge of the physiological and pathophysiological functions of bile acid metabolism and signaling. Bile acids are no longer considered merely physiological detergent molecules that facilitate nutrient absorption, but are also important regulators of various cellular processes in lipid, glucose and energy metabolism and immune responses. Under normal physiology, bile acid signaling plays a role in integrating nutrient sensing to the regulation of metabolic homeostasis. Dysregulation of bile acid homeostasis may underlie the pathogenesis of many human diseases. Importantly, new mechanistic understanding of bile acid signaling action in the liver and extrahepatic tissues to date has laid the groundwork for the development of promising bile acid-based drug therapies for the treatment of liver and metabolic diseases. Indeed, bile acid sequestrants have been used for a long time as a cholesterol-lowering therapy in addition to statins. More recently, the bile acid sequestrant colesevelam has been approved to be used in combination therapies to improve glycemic control in type 2 diabetes because of its effects on inducing gut GLP-1 production (53,106,124). Additionally, the FXR agonist obeticholic acid has been approved for primary biliary cholangitis treatment (125,126). Obeticholic acid treatment has also been shown to significantly improve fibrosis and NAFLD Activity Score in a completed Phase 2b FLINT trial (127). Other bile acid-based therapies, such as the selective TGR5 agonist and the FXR and TGR5 dual agonist, are being tested in preclinical studies and phase 1 trials as potential therapies for metabolic and inflammatory diseases (113,128–131).

Acknowledgments

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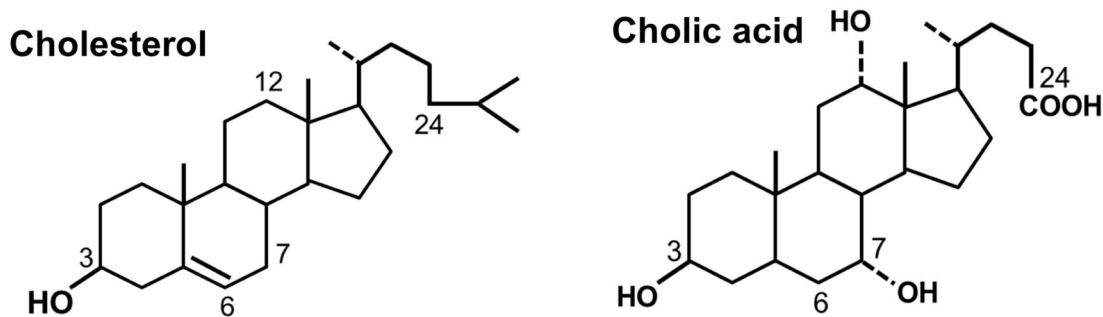
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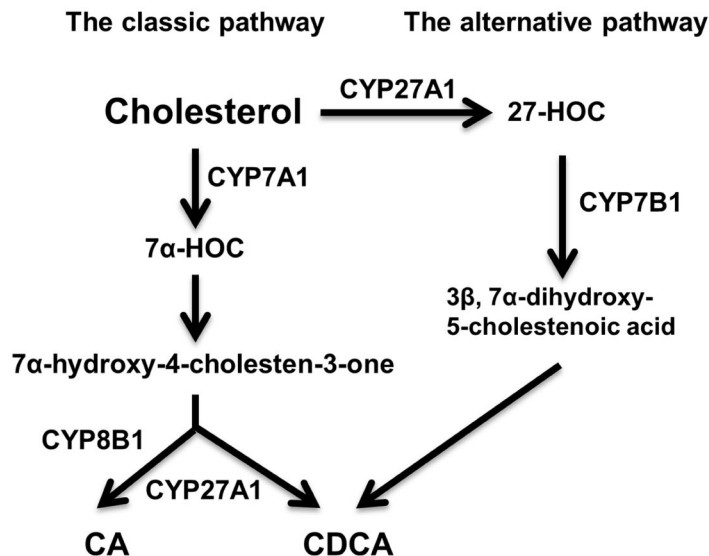
B

Figure 1. Bile acid synthesis
A. Primary bile acids can be synthesized by two pathways in hepatocytes. The classic bile acid synthesis pathway is considered the primary pathway in humans. In this pathway, cholesterol is first converted to 7α-hydroxycholesterol (7α-HOC) by the rate-limiting enzyme cholesterol 7α-hydroxylase (CYP7A1) in the endoplasmic reticulum. 7α-hydroxy-4 cholesten-3-one (C4) is a common precursor for chenodeoxycholic acid(CDCA) and cholic acid (CA). Sterol 12α-hydroxylase (CYP8B1) catalyzes the C-12 hydroxylation of C4, which leads to the synthesis of CA. In the alternative pathway, cholesterol is first converted to 27-hydroxycholesterol (27-HOC) by mitochondrial sterol 27-hydroxylase (CYP27A1). Oxysterol 7α-hydroxylase (CYP7B1) then catalyzes C-7 hydroxylation. The alternative pathway only produces CDCA. **B**. Cholesterol and cholic acid molecular structures.
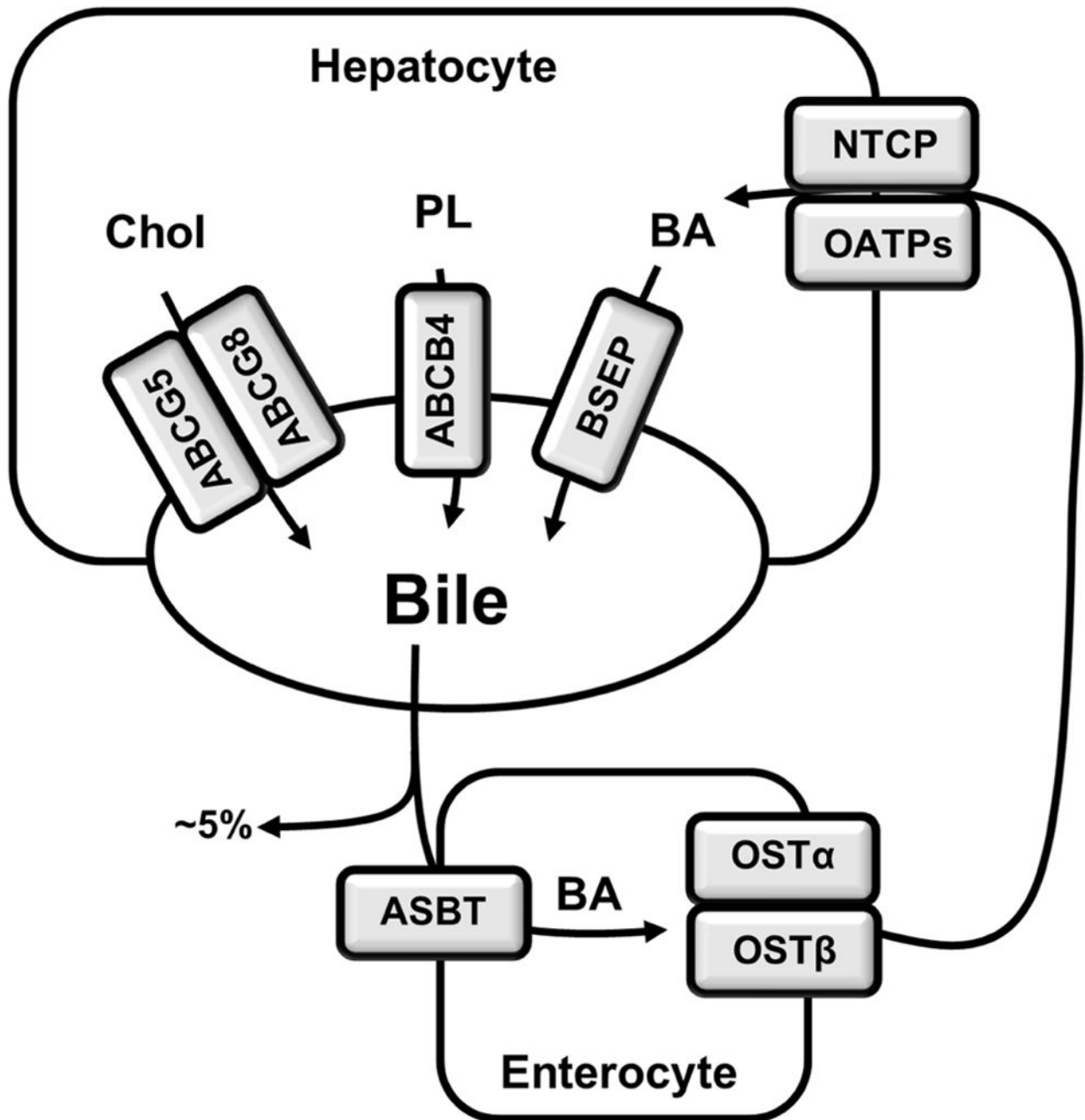
Figure 2. Bile acid transport in the enterohepatic circulation

In the enterohepatic circulation, bile acids are secreted from the canalicular side of hepatocytes into the bile and stored in the gallbladder. Meal intake stimulates gallbladder bile release into the small intestine, where ~95% of bile acids can be re-absorbed in the ileum and secreted into the portal circulation. Approximately 5% of the total bile acids are lost in feces. Bile acids are taken up by the hepatocytes from the basolateral side and re-secreted into the bile. Bile acid uptake and secretion are mediated by transporters. Bile salt export pump (BSEP) is the primary canalicular bile acid efflux transporter in hepatocytes.

Cholesterol is secreted into the bile by the ATP binding cassette (ABC)G5/ABCG8 heterodimer, and phospholipids are secreted by ABCB4 into the bile. Cholesterol, bile acids and phospholipids are the major constituents in the bile. They form mixed micelles for cholesterol solubilization and to prevent bile acid damage to the biliary system. In the small intestine, bile acids are taken up into the enterocytes by apical sodium-dependent bile salt transporter (ASBT) and are secreted into the portal circulation by the organic solute transporter (OST)α/OSTβ heterodimer. Sodium–dependent taurocholate transporter(NTCP) is the major basolateral conjugated bile acid uptake transporter on hepatocytes, while OATP isoforms can also mediate uptake of primarily unconjugated bile acids.