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## Bile Acid Metabolism and Signaling

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### Abstract

Bile acids are important physiological agents for intestinal nutrient absorption and biliary secretion of lipids, toxic metabolites, and xenobiotics. Bile acids also are signaling molecules and metabolic regulators that activate nuclear receptors and G protein-coupled receptor (GPCR) signaling to regulate hepatic lipid, glucose, and energy homeostasis and maintain metabolic homeostasis. Conversion of cholesterol to bile acids is critical for maintaining cholesterol homeostasis and preventing accumulation of cholesterol, triglycerides, and toxic metabolites, and injury in the liver and other organs. Enterohepatic circulation of bile acids from the liver to intestine and back to the liver plays a central role in nutrient absorption and distribution, and metabolic regulation and homeostasis. This physiological process is regulated by a complex membrane transport system in the liver and intestine regulated by nuclear receptors. Toxic bile acids may cause inflammation, apoptosis, and cell death. On the other hand, bile acid-activated nuclear and GPCR signaling protects against inflammation in liver, intestine, and macrophages. Disorders in bile acid metabolism cause cholestatic liver diseases, dyslipidemia, fatty liver diseases, cardiovascular diseases, and diabetes. Bile acids, bile acid derivatives, and bile acid sequestrants are therapeutic agents for treating chronic liver diseases, obesity, and diabetes in humans.

### Introduction

Bile acids are the end products of cholesterol catabolism (32,34,159). Conversion of cholesterol to bile acids accounts for the daily turnover of a major fraction of cholesterol in humans. Bile acid synthesis generates bile flow and biliary secretion of bile acids, phospholipids, cholesterol, drugs, and toxic metabolites. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are the major primary bile acids synthesized in human livers, and are conjugated with taurine or glycine for secretion into bile. Bile salts form mixed micelles with phospholipids and cholesterol and stored in the gallbladder, secreted into the intestinal tract to facilitate digestion and absorption of nutrients. Most bile acids are reabsorbed in the ileum and are transported back to the liver via portal blood circulation to inhibit bile acid synthesis. Enterohepatic circulation of bile acids is highly efficient in humans and is an important physiological system not only for nutrient absorption and xenobiotic disposal, but also for maintaining metabolic homeostasis. The mechanism of bile acid feedback inhibition of its own synthesis has been studied for more than 50 years, but

the underlying molecular mechanism is still not clear. The recent discovery that bile acids are endogenous ligands of a nuclear receptor farnesoid X receptor (FXR) has provided some mechanistic insight into the role of bile acids in the regulation of gene transcription (28, 31, 65), but the physiological relevance of the FXR-dependent pathways in regulation of bile acid metabolism remains elusive. Many recent studies have provided strong evidence that bile acid-activate FXR plays a critical role in maintaining metabolic homeostasis (5, 33, 86, 87, 106, 189). Bile acid-activated membrane G protein-coupled receptors, TGR5 (aka Gpbar-1, G-protein-coupled bile acid receptor) appear to play a role in stimulating energy metabolism, protecting liver and intestine from inflammation and steatosis, and improving insulin sensitivity (189). Another recently identified bile acid-activated GPCR, sphingosine-1-phosphate receptor 2 (S1P2) may also play a role in lipid metabolism (183). The following sections will cover bile acid synthesis and metabolism, its regulation by nuclear receptor, the recently uncovered role of bile acids in integrated regulation of lipid, glucose, and energy metabolism. Diseases in bile acid synthesis and transport, cholestasis, and therapeutic potential of bile acids and derivatives for treating metabolic diseases are briefly reviewed. See Table 1 for a list of abbreviations.

## Bile Acid Metabolism

Bile acids (or bile salts) are derived from cholesterol. In mammals, all bile acids are C<sub>24</sub>-5 $\beta$ -bile acids (cholanoic acid). The steroid nucleus has four fused carbon rings consisting of three 6-carbon rings and one 5-carbon ring. Conversion of cholesterol to bile acids involves hydroxylation, saturation of the double bond at C<sub>5</sub>-C<sub>6</sub>, epimerization of the 3-hydroxyl group, and oxidative cleavage of a 3-carbon unit from the side chain. The 3-hydroxyl group in all bile acids has a  $\alpha$ -configuration. All bile acids have a 5 $\beta$ -hydrogen group and a *cis*-configuration along the plane of the fused A and B ring. In CA all three hydroxyl groups and the carboxyl group are faced to one side of the carbon skeleton forming a hydrophilic face opposing the highly hydrophobic face. Thus, bile acids are amphipathic molecules with powerful detergent properties. Most bile acids are conjugated to the amino acids glycine or taurine and form sodium salts in physiological pH to increase their solubility.

Human liver synthesizes about 200 to 600 mg bile acids per day and excretes the same amount in feces. The net daily turnover of bile acids is about 5% of a total bile acid pool of about 3 g. Conversion of cholesterol to bile acids involves 17 distinct enzymes located in the cytosol, endoplasmic reticulum, mitochondria, and peroxisomes (Fig. 1). These enzymes catalyze modifications of the steroid ring and oxidative cleavage of three carbons from the side chain of cholesterol to form C<sub>24</sub> bile acids. There are two major bile acid biosynthetic pathways (34). In the neutral bile acid pathway (or classic pathway), steroid ring modification precedes side-chain cleavage, whereas in the acidic pathway side-chain cleavage preceded steroid ring modifications. These are five hydroxylases involved in bile acid synthesis. The classic pathway is initiated by cholesterol 7 $\alpha$ -hydroxylase (CYP7A1), the only rate-limiting enzyme in bile acid synthesis, and synthesizes two primary bile acids, CA and CDCA in human liver. A microsomal sterol 12 $\alpha$ -hydroxylase (CYP8B1) is required for synthesis of CA, without 12 $\alpha$ -hydroxylase the product is CDCA. The acidic pathway (or alternative pathway) is initiated by sterol 27-hydroxylase (CYP27A1), a mitochondria cytochrome P450 enzyme, which is widely distributed in most tissues and macrophages. It

has been reported that the acidic pathway may contribute about 9% of total bile acid synthesis in human hepatocytes (52). The acidic pathway may be quantitatively important in bile acid synthesis in patients with liver diseases and in the neonates.

In humans, most bile acids are amino conjugated at the carboxyl group (amidation) with the ratio of glycine to taurine conjugates of about 3 to 1. In mice, most bile acids (>95%) are taurine conjugated. Conjugation of bile acids increases ionization and solubility at physiological pH, prevents  $\text{Ca}^{2+}$  precipitation, minimizes passive absorption, and resistant to cleavage by pancreatic carboxypeptidases. In the distal intestine, conjugated-CA and CDCA are first deconjugated, and then bacterial 7 $\alpha$ -dehydroxylase converts CA and CDCA to DCA and lithocholic acid (LCA), respectively. DCA and LCA are the secondary bile acids (damaged bile acids). Most LCA is excreted into feces and small amounts of LCA is circulated to the liver and rapidly conjugated by sulfation and excreted into bile. Sulfation is the major pathway for detoxification of hydrophobic bile acids in humans (82). In mice, CDCA is hydroxylated to  $\alpha$ -muricholic acid and 6 $\beta$ -muricholic acids, two major primary bile acids in mice. The 7 $\alpha$ -hydroxyl groups in CDCA also can be epimerized to 7 $\beta$  to form ursodeoxycholic acid (UDCA). Hydroxylation at the 6 $\alpha$ / $\beta$  or 7 $\beta$ -position increases bile acid solubility and reduces bile acid toxicity.

## Enterohepatic Circulation of Bile Acids

Bile acids synthesized in the liver are immediately secreted into bile, reabsorbed in the intestine and transported back to the liver. The enterohepatic circulation of bile acids (Fig. 2) is very efficient in humans. Small amounts of bile acids may spill over into the systemic circulation, reabsorbed when passing through the renal tubules in the kidney, and are then circulated back to the liver through systemic circulation. Some bile acids secreted in the bile duct are reabsorbed in the cholangiocytes (bile duct epithelial cells) and recycled back to hepatocytes (the cholangiohepatic shunt). Bile acids are stored in the gallbladder. After each meal, cholecystokinin secreted from the intestine stimulates gallbladder contraction to empty bile acids into the intestinal tract. When passing down the intestinal tract, small amounts of unconjugated bile acids are reabsorbed in the upper intestine by passive diffusion. Most bile acids (95%) are reabsorbed in the brush border membrane of the terminal ileum, transdiffused across the enterocyte to the basolateral membrane, and secreted into portal blood circulation to liver sinusoids and are taken up into hepatocytes. DCA is reabsorbed in the colon and recycled with CA and CDCA to the liver. A bile acid pool of ~3 g consisting of ~40% CA, 40% CDCA, 20% DCA, and trace amount of LCA, is recycled 4 to 12 times a day. Bile acids lost in the feces (~0.5 g/day) are replenished by *de novo* synthesis in the liver to maintain a constant bile acid pool.

## Regulation of Bile Acid Synthesis

### Bile acid feedback regulation

Studies of bile acid metabolism in the 1960s showed that bile acids, cholesterol, thyroid hormone, glucocorticoid, insulin, and circadian rhythms regulated CYP7A1 activity and the rate of bile acid synthesis [reviewed in refs. (34,136)]. Interruption of enterohepatic circulation of bile acids by bile acid binding resins such as cholestyramine or biliary

diversion by bile fistula strongly stimulates CYP7A1 enzyme activity and bile acid synthesis in rats. Feeding rats with bile acids strongly reduced CYP7A1 enzyme activity and bile acid synthesis. These results imply that bile acid synthesis is regulated by a negative feedback mechanism, and bile acids returning to the liver via enterohepatic circulation may directly or indirectly inhibit bile acid synthesis by inhibiting CYP7A1 activity. Many studies show that bile acids inhibit whereas cholesterol stimulates CYP7A1 mRNA, protein and enzyme activity and bile acid synthesis in rodents. It was concluded that CYP7A1 activity is mainly regulated by transcriptional mechanism.

It has been noted that multiple CYP7A1 transcripts exist in rat hepatocytes and the 3'-untranslated regions (3'-UTRs) of CYP7A1 mRNAs are unusually long (118). The half-life of CYP7A1 mRNA has been estimated to be very short, about 30 min (8, 139). It is possible that bile acids may reduce CYP7A1 mRNA stability via the bile acid response elements located in the 3'-UTR (2,8). However, posttranscriptional regulation of CYP7A1 has not been studied in detail. One recent study shows that apoB editing complex 1 (Apobec-1) regulates the stability of CYP7A1 by binding to the conserved AU-rich sequences in the 3'-UTR (213). The *Apobec-1* locus (*Lith6*) encodes the RNA-specific cytidine deaminase involved in production of Apob48 in small intestine and rodent liver. In *Apobec-1*<sup>-/-</sup> mice, expression of *Cyp7a1* mRNA and protein are significantly reduced and these mice are susceptible to lithogenic diet-induced gallstone disease. Apobec-1 mediates posttranscriptional regulation of mouse *Cyp7a1* expression by stabilizing *Cyp7a1* mRNAs.

### Nutrient regulation of bile acid synthesis

Since liver metabolism is highly active during the postprandial period and humans undergo fasting-to-refeeding cycles several times a day, there is a physiological link between induction of bile acid synthesis and regulation of postprandial nutrient metabolism.

Nutrients may play a key role in regulating bile acid synthesis, which in turn regulates nutrient absorption and metabolic homeostasis.

CYP7a1 is a highly specific hydroxylase that only uses cholesterol as the substrate and insert a hydroxyl group at the 7 $\alpha$ -position. This enzyme is located in the cholesterol-poor endoplasmic reticulum. Thus the availability of cholesterol as substrate ( $K_m$  effect) regulates the specific activity of CYP7A1. It has been suggested that newly synthesized cholesterol is the preferred substrate. Therefore, there is a direct link of *de novo* cholesterol synthesis to bile acid biosynthesis in hepatocytes. Stimulation of bile acid synthesis reduces hepatic cholesterol/oxysterol levels and results in stimulating *de novo* cholesterol synthesis to provide substrate for CYP7A1 (Fig. 3). When intracellular oxysterol levels decrease, a basic helix-loop-helix-leucine zipper protein called steroid response element binding protein-2 (SREBP-2) precursor (125 kDa) is dissociated from insulin induced gene-1 (Insig-1) and Insig-2 in the endoplasmic reticulum membrane and is escorted by a steroid sensitive SREBP cleavage-activating protein to the Golgi apparatus, where two oxysterol-sensitive proteases S1P and S2P cleave and release the N-terminal 68 kDa transcription factor, which enters the nucleus, binds to the SRE on the promoters of the genes encoding all enzymes in cholesterol synthesis (HMG-CoA reductase, as an example) and the LDL receptor (20, 54, 56, 85, 214) (Fig. 3). When intracellular oxysterol levels are high,

SREBP-2 is retained in the endoplasmic reticulum and cholesterol synthesis is inhibited. In mice, feeding a high cholesterol diet stimulates bile acid synthesis by activating an oxysterol-activated nuclear receptor LXR $\alpha$ , which induces *Cyp7a1* gene transcription. SREBP-1a and SREBP-1c are transcribed by the *SREBP-1* gene and induce all genes in lipogenesis (64). LXR $\alpha$  induces the *SREBP-1c* gene, but not SREBP-2 gene transcription (47). Insulin and LXR $\alpha$  induce all genes in lipogenesis by inducing SREBP-1c gene transcription (30, 168, 169).

Early studies in rats showed that the bile acid pool size increased in diabetic rats and insulin treatment reduced bile acid pool size, inhibited CYP7A1 and CYP8B1 activities, and altered bile acid composition (184). In streptozocin-treated diabetic rats CYP7A1 activity is increased suggesting that insulin represses CYP7A1 while lack of insulin induces CYP7A1 (184). It has been reported that glucagon/cAMP and fasting induce *Cyp7a1* expression, which parallels the induction of Peroxisome proliferator-activated receptor  $\gamma$  coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) and phosphoenolpyruvate carboxykinase (PEPCK) in gluconeogenesis (46, 170), suggesting that *Cyp7a1* expression and bile acid synthesis are up regulated during fasting as a feed forward signal for intestinal nutrient absorption. In contrast, in human patients serum 7 $\alpha$ -hydroxy-4-cholesten-3-one (C4)-levels, reflecting the rate of bile acid synthesis, are reduced during the fasting and increased during the postprandial state (123), suggesting that bile acid synthesis is induced during the postprandial period and is inhibited during fasting. It was thought that species differences might explain the differential regulation of bile acid synthesis in the fasting-to-refeeding cycle. More recent studies show that insulin and glucose induce, while glucagon represses *CYP7A1* gene expression in primary human hepatocytes (109,115,174). *In vivo* studies also show that glucose and insulin rapidly induce *CYP7A1* gene expression and bile acid synthesis leading to an enlarged bile acid pool and elevated circulating bile acids (112). Insulin may have dual functions, stimulating CYP7A1 at physiological concentrations but inhibiting at high concentrations found in an insulin resistant state (109). Insulin signaling activates AKT/protein kinase B (PKB), and possibly also the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase 1/2 (ERK1/2) pathway to inhibit *CYP7A1* gene transcription. Insulin is known to induce SREBP-1c, which may inhibit *CYP7A1* expression by interacting with hepatocyte nuclear factor 4 $\alpha$  (HNF4 $\alpha$ ). Glucose induces CYP7A1 via an epigenetic mechanism by increasing histone acetylation status of the *CYP7A1* gene promoter (112). On the other hand, glucagon and cAMP strongly inhibit CYP7A1 expression via activation of PKA, which phosphorylates HNF4 $\alpha$  to abolish its DNA-binding activity, and result in inhibition of *CYP7A1* expression in human hepatocytes (174). These recent studies clearly established that nutrients play a key role in regulation of bile acid synthesis during fasting to refeeding cycles.

Conjugated bile acids activate both AKT and the MAPK/ERK1/2 pathway. Taurocholic acid activation of tyrosine phosphorylation of epidermal growth factor receptors (EGFRs) is sensitive to pertussis toxin and G $\alpha$ i in rodent hepatocytes (50, 87). Unconjugated bile acids (DCA) can activate ERK1/2 and AKT pathways by stimulating the synthesis of mitochondria reactive oxidizing species, which activates EGFRs (57). AKT phosphorylates FoxO1 and inhibits PEPCK and glucose-6-phosphatase (G-6-Pase) in gluconeogenesis.

AKT phosphorylates and inhibits glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) activity to activate glycogen synthesis in rat primary hepatocytes (58). This implies that bile acids may mimic the insulin action in regulating glucose metabolism by stimulating glycogen synthesis and inhibiting gluconeogenesis. Hydrophobic bile acids are known to induce apoptosis in hepatocytes, and hydrophilic bile acids increase intracellular cAMP and activate MAPK and PI3K pathways to protect hepatocytes from apoptosis (5, 153, 157).

In diabetic patients, bile acid pool and fecal bile acids are elevated, and are decreased upon insulin treatment (12). In both mouse models of type I and type II diabetes, bile acid pool sizes increase, but the fasting-to-refeeding regulation of the *cyp7a1* gene is lost (112). In diabetic mice, the *CYP7A1* gene promoter is hyperacetylated, thus, these mice have higher basal CYP7A1 activity, rate of bile acid synthesis, and larger bile acid pool. The implication of bile acids in obesity and diabetes is further supported by a recent clinical study demonstrated that serum bile acids were higher in patients with prior gastric bypass than in overweight and severely obese patients without gastric bypass, and serum bile acids were positively correlated with serum glucagon-like peptide-1 (GLP-1) (141). It is likely that in gastric bypass patients, bile acid synthesis may be increased due to reduced bile acid feedback, resulting in increased bile acid synthesis and improved glucose tolerance in obese patients. This is consistent with a recent study that lowering circulating bile acids worsened diet-induced obesity and diabetes, while increasing bile acid pool size improved glucose homeostasis (206). Interestingly, a recent metabolomics study identified bile acids as the most elevated metabolites in human sera after an oral glucose challenge in patients with normal glucose tolerance, but this response was blunted in patients with impaired glucose tolerance (166). It should be clarified that increased bile acid pool is not the cause of diabetes but the consequence of dysregulation of bile acid metabolism and altered metabolic homeostasis. Serum bile acid levels have become biomarkers for diagnosis of liver diseases, diabetes, and obesity.

## Bile Acid-Activated Nuclear Receptors

Nuclear receptors are ligand-activated transcription factors that play important roles in embryogenesis, development, and metabolism (31). Figure 4 shows a general structure of nuclear receptors. The N-terminal A and B domains contain the activation function-1 domain, which is the most variable region in nuclear receptors. The C domain is a highly conserved region containing two Zn<sup>2+</sup> fingers responsible for binding to a hormone response element (HRE) with two tandem AGGTCA repeating sequences spaced by 1–5 nucleotides arranged in direct repeat, everted repeat, or inverted repeat. Most nuclear receptors bind to a HRE as homodimers, or heterodimers with retinoid X receptor. In general, in the absence of a ligand, nuclear receptors bind corepressors and are inactive. Upon ligand binding, coactivators are recruited to displace corepressors. Nuclear receptor/coactivators interact with other mediators to stimulate RNA polymerase II and induce gene transcription. The D or hinge domain has a nuclear localization sequence and is also involved in DNA binding. The E domain is the ligand-binding domain (LBD) conserved in nuclear receptors within the same subfamily. The LBD contains coactivator interaction motifs LXXLs and also is important in transactivation (AF-2). There are 48 nuclear receptor genes in the human genome. Nuclear receptors are classified as type I endocrine receptors, type II adopted



receptors, and type III orphan receptors (the ligands of which have not been firmly established).

### Bile acid-activated nuclear receptors

Bile acids directly activate three nuclear receptors, FXR (127, 140, 199), pregnane X receptor (PXR) (212), and vitamin D receptor (VDR) (126). FXR is activated by free and conjugated-bile acids; the hydrophobic bile acid CDCA is the most efficacious bile acid ligand of FXR ( $EC_{50} = \sim 10 \mu\text{mol/L}$ ), followed by LCA, DCA, and CA, while hydrophilic bile acids UDCA and MCA do not activate FXR. LCA and its metabolite 3-keto-LCA are the most efficacious bile acid ligands for both VDR and PXR ( $EC_{50} = \sim 100 \text{ nmol/L}$ ). PXR is highly expressed in the liver and intestine, and plays more important roles in detoxification of bile acids, drugs, and toxic compounds by activating phase I drug metabolizing P450 enzymes, phase II drug conjugation enzymes, and phase III drug transporters (178,194,212). PXR is known to inhibit *CYP7A1* gene transcription by inhibiting HNF4 $\alpha$  and PGC-1 $\alpha$  transactivation of the *CYP7A1* gene (15, 110). Interestingly, PXR null mice are more sensitive to lithogenic diet-induced gallstone disease and have reduced bile acid pool (81). This is because the lithogenic diet (containing 1% cholesterol and 0.5% CA) reduces *Cyp7a1* expression through activation of FXR and induction of fibroblast growth factor 15 (FGF15). VDR also inhibits *CYP7A1* gene transcription by interacting with HNF4 $\alpha$ , competing for coactivators and recruiting corepressors to inhibit *CYP7A1* gene transcription (78). FXR, PXR, and VDR may coordinately regulate bile acids, lipoproteins, drugs, glucose, and energy metabolism (65, 189). Bile acids also have been shown to activate several cell signaling pathways involved in regulation of bile acid metabolism (87). Thus, bile acid activation of nuclear receptors and cell signaling pathways converge to regulate a complex cellular metabolism network [see recent reviews in Refs. (38, 86, 106, 189)].

### Roles of FXR in regulation of bile acid synthesis and transport

Many studies have implicated FXR in the regulation of bile acid synthesis, biliary bile acid secretion, intestinal bile acid absorption, and hepatic uptake of bile acids (24, 55, 106, 133, 172, 221). FXR activates target gene transcription mainly by binding to an inverted repeat with one-base spacing (IR1) in the gene promoter. *Fxr* knockout mice have increased bile acid synthesis and *Cyp7a1* expression suggesting FXR-mediated bile acid inhibition of *Cyp7a1* (172). The negative effect of FXR may be through an indirect mechanism by induction of a negative nuclear receptor small heterodimer partner (SHP) that inhibits transactivators of *CYP7A1* and *CYP8B1* by HNF4 $\alpha$  and liver-related homolog-1 (LRH-1). FXR also regulates bile acid conjugation by inducing *BACS* and *BAAT* gene transcription. FXR induces a bile salt export pump (BSEP, a ATP binding cassette transporter, ABCB11) located in the canalicular membrane of hepatocytes (Fig. 5). BSEP is the principal bile acid efflux pump, which utilizes ATP hydrolysis to secrete conjugated-bile acids against a biliary bile acid concentration about 1000-fold higher than in hepatocytes. FXR induces a multidrug resistant protein 2/3 (MDR2/3, ABCB4), which effluxes phosphatidylcholine (Fig. 5). FXR also induces multidrug resistance-related protein 2 (MRP2, ABCC2), which effluxes glucuronidated and sulfated bile acids, organic anions and drugs (224). ABC-half transporters, ABCG5 and ABCG8 heterodimer are responsible for biliary excretion of

cholesterol. FXR induces ABCG5 expression. In the gallbladder, bile acids form the mixed micelles with phosphatidylcholine and cholesterol. This allows for storage of bile acids in high concentrations (mmol/L) and prevents cholesterol from precipitating in the gallbladder. In the terminal ileum, conjugated bile acids are reabsorbed by apical sodium-dependent bile salt transporter (ASBT) located in the brush border membrane of the enterocytes (Fig. 5). Once inside the enterocytes, bile acids bind to the ileum bile acid binding protein, which is highly induced by FXR (193). Bile acids are excreted into portal circulation by the organic solute transporter  $\alpha$  and  $\beta$  dimer (OST $\alpha/\beta$ ) located in the basolateral membrane of enterocytes (9,44). OST $\alpha/\beta$  appears to be the major bile acid efflux transporter in the intestine (10). OST $\alpha/\beta$  also acts as the secondary bile acid efflux transporter in sinusoidal membrane. FXR induces OST $\alpha/\beta$  gene transcription (66). Bile acids are circulated via portal blood to hepatocytes, where a sinusoidal Na<sup>+</sup>-dependent taurocholate cotransport peptide (NTCP) uptakes bile acids into hepatocytes. FXR inhibits NTCP gene transcription (49). Thus, FXR plays a critical role in enterohepatic circulation of bile acids by regulating bile acid synthesis, biliary bile acid secretion, intestinal bile acid reabsorption and secretion, and bile acid uptake into hepatocytes. Defective regulation of these FXR target genes impairs enterohepatic circulation of bile acids, and contributes to cholestatic liver diseases (93, 103, 192, 223). FXR, PXR and constitutive androstane receptor (CAR) may play a complementary role in detoxification of bile acids and protection against cholestasis (76).

#### The FXR/SHP/LRH-1 pathway

FXR inhibits *CYP7A1* gene transcription by indirect mechanisms. It is thought that bile acid-activated FXR induces a negative nuclear receptor SHP to inhibit *CYP7A1* and *CYP8B1* gene transcription (Fig. 5) (74,121). SHP is an atypical receptor without a DNA-binding domain. SHP inhibits the trans-activating activity of liver related homologue-1 (LRH-1), and results in inhibiting *CYP7A1* gene transcription (74,121). SHP also interacts with HNF4 $\alpha$  to block HNF4 $\alpha$  interaction with peroxisome PGC-1 $\alpha$  and results in inhibition of *CYP7A1* and *CYP8B1* transcription (48, 219). The FXR/SHP mechanism is supported by the finding that SHP and *CYP7A1* mRNA expression levels show an inversed correlation, and *CYP7A1* expression and bile acid synthesis is induced in *Shp* knockout mice. Paradoxically, bile acid feeding to *Shp* knockout mice inhibits *CYP7A1* expression and bile acid synthesis suggesting that redundant pathway may exist for bile acid inhibition of *CYP7A1* (101, 201). The physiological relevance of the FXR/SHP pathway in bile acid feedback inhibition remains unclear.

#### The FXR/FGF19/FGFR4 pathway

Another FXR-dependent mechanism is based on the observation that GW4064 induces an intestine hormone, FGF15, which activates a hepatic FGF receptor 4 (FGFR4) signaling to inhibit *CYP7A1* mRNA expression (Fig. 5) (83). A subsequent study shows that FXR induces FGF15, a mouse orthologous of human FGF19 in mouse intestine, and the expression of FGF15 mRNA is inversely correlated to the *CYP7A1* mRNA expression levels in mouse liver (88). Both *Fgfr4*<sup>-/-</sup> and *Fgf15*<sup>-/-</sup> mice have increased bile acid pool, fecal bile acid secretion, and *CYP7A1* expression (218). Overexpression of a constitutively active FGFR4 represses *CYP7A1* expression and decreases bile acid pool size in wild type mice (217). These results are consistent with the proposed role of FGF15/FGFR4 signaling



in mediating bile acid inhibition of bile acid synthesis. It has been suggested that intestine-derived FGF15 functions as an enterohepatic signal to activate FGFR4 signaling, which inhibits CYP7A1 in hepatocytes (88). Furthermore, GW4064 represses CYP7A1 expression in liver *Fxr* knockout mice but not in intestinal *Fxr* knockout mice (102). Interestingly, FXR-mediated repression of CYP8B1 expression is more dependent on liver FXR than intestinal FXR, and FGF15 represses CYP7A1 but not CYP8B1 expression. These results provide strong evidences that the intestinal FXR but not liver FXR is required for bile acid inhibition of *CYP7A1* gene transcription in mice. FGF19 activation of FGFR4 signaling requires a membrane-bound glycosidase  $\beta$ -Klotho (120). In  $\beta$ -*Klotho* knockout mice, bile acid synthesis, and secretion and CYP7A1 expression was increased, but CYP8B1 expression was not altered (90). FGF15 has not been identified in mouse sera and liver, and bile acids do not induce FGF15 expression in mouse liver. Thus, the physiological relevance of the FGF15/FGFR4 pathway in bile acid feedback regulation of bile acid synthesis in mice remains unclear.

In human patients serum FGF19 levels exhibit a diurnal rhythm with two major peaks at 3 and 9 pm, which are ~90 to 120 min following the peaks of serum bile acids and C4 (122). These observations are consistent with the concept that FGF19 is secreted from the intestine to blood circulation in response to postprandial efflux of bile acids to inhibit bile acid synthesis in the liver. In contrast to mice, GW4064 and bile acids are able to induce FGF19 synthesis and secretion in human hepatocytes (176). This study shows that CDCA and GW4064 rapidly induce FGF19 mRNA expression, FGF19 protein secretion, and tyrosine phosphorylation of FGFR4, but inhibit CYP7A1 mRNA expression in primary human hepatocytes suggesting that liver-produced FGF19 is secreted from hepatocytes to activate FGFR4 signaling in hepatocytes by an autocrine or paracrine mechanism. Furthermore, knockdown of SHP expression by siRNA does not affect FGF19 inhibition of CYP7A1 mRNA expression in primary human hepatocytes, suggesting that SHP may not be required in FGF19 signaling (176). It is also noted that induction of FGF19 is sustained for at least 24 h but induction of SHP mRNA by CDCA and GW4064 is transient, reaching the maximum in 1 to 3 h and reducing to the basal levels after 6 h of treatment (176). All these data show a lack of correlation between SHP and CYP7A1 expression levels in FGF19 signaling. The study by Song et al. (176) demonstrates that FGF19/FGFR4 signaling activates and phosphorylates mainly the MAPK/ERK1/2 signaling pathway in human primary hepatocytes (Fig. 5). However, the down stream factor(s) of the FGF19 pathway that inhibits *CYP7A1* gene transcription remain unknown.

Expression of FGF19 in human sera and hepatocytes has been reported recently in a patient patients with extrahepatic cholestasis (160) Serum FGF19 levels were higher in cholestatic patients than in noncholestatic patients and postcholestatic patients who received a biliary stent to drain bile acids. FGF19 mRNA could be detected in the majority of liver specimens with a wide range of expression levels, which were much higher in the cholestatic group than in the drained and noncholestatic group. CYP7A1 mRNA expression levels were lower in the cholestatic group than in the control and drained groups. These data suggest that bile acids accumulated in cholestatic liver could induce FGF19 expression as an adaptive response to cholestatic liver injury.

## Bile Acid-Activated G protein-Coupled Receptors

### G protein bile acid receptor-1 (Gpbar-1, aka TGR5)

Recently, a G $\alpha$ s protein-coupled receptor TGR5 has been identified as a bile acid-activated membrane receptor (96, 128). TGR5 is expressed in many tissues including gall-bladder, spleen, liver, intestine, kidney, skeletal muscle, pancreas, adipocytes, and macrophages (Fig. 6). TGR5 is not expressed in hepatocytes, but has been detected in Kupffer cells (hepatic resident macrophages), liver sinusoidal endothelial cells, and gallbladder epithelial cells (97, 98, 100). Conjugated and free bile acids bind and activate TGR5 in the order of TLCA>LCA>DCA>CDCA>CA. TGR5 signaling stimulates cAMP, which activates PKA and target gene expression. In brown adipose tissue TGR5 signaling stimulates the conversion of ATP to cAMP, which activates a type 2 iodothyronine deiodinase (DIO2). DIO2 converts thyroxine T<sub>4</sub> to the biologically activate hormone T<sub>3</sub>, which is known to stimulate mitochondrial oxygen consumption and energy metabolism (207). TGR5 knockout mice have reduced bile acid pool and accumulate fats when fed a high fat diet (129). Interestingly, *Tgr5*<sup>-/-</sup> mice are protected from lithogenic diet-induced gallstone disease (196). In liver and intestine, TGR5/cAMP signaling has anti-inflammatory function by inhibiting nuclear factor NF- $\kappa$ B-mediated inflammatory cytokine production (204), and protects intestinal barrier integrity and prevents colitis (36). TGR5 stimulates gallbladder refilling (113). A study of human gallstone patients reports that TGR5 mRNA and protein are expressed in all patients, and TGR5 mRNA, but not protein expression levels, are increased in gallstone patients (97). This study shows localization of TGR5 in the apical membrane and recycling of endosomes in gallbladder epithelial cells. TGR5 is colocalized with cystic fibrosis transducer regulator and ASBT, suggesting the coupling of TGR5 in bile acid uptake and chloride secretion.

Activation of TGR5 inhibits proinflammatory cytokine production by macrophages and inhibits atherosclerosis (146). Bile acid-activated TGR5 signaling may play a critical role in protection against inflammatory diseases including fatty liver disease, inflammatory bowel diseases, atherosclerosis, and diabetes. It has been reported that bile acids and TGR5 stimulate GLP-1 secretion in an enteroendocrine cell line STC-1. Knockdown of TGR5 mRNA expression by siRNA reduced GLP-1 secretion suggesting that bile acids induce GLP-1 secretion by TGR5-dependent cAMP production (95). GLP-1 plays a critical role in regulating glucose homeostasis, appetite, insulin and glucagon secretion in pancreas, and diabetes. Activation of TGR5 by a specific TGR5 agonist 6 $\alpha$ -ethyl-23(S)-methyl-CA (6-EMCA, INT-777) stimulates GLP-1 secretion from enteroendocrine L cells, increases intracellular calcium mobilization, and improves insulin sensitivity (188). Interestingly, *Tgr5* null mice did not develop gallstones when fed a lithogenic diet (196). These mice have impaired bile acid feedback, and upregulation of CYP7A1 may prevent gallstone formation in *Tgr5* null mice.

### Sphingosin-1-phosphate receptor 2

A recent study identified S1P2 as a bile acid-activated GPCR (183). S1P2 is expressed in hepatocytes. Conjugated bile acids, TCA, TDCA, TUDCA, GCA, and GDCA are able to activate S1P2 signaling, which has been shown to activate ERK1/2 and AKT signaling in rat

hepatocytes (50) (Fig. 7). S1P receptor 2(S1P2) is a G $\alpha$ i class of GPCR. Molecular modeling shows TCA is able to dock into the S1P binding site in S1P2 (183). TCA appears to have a low affinity to S1P2. Sphingosine 1-phosphate is a phosphorylated product of a membrane lipid sphingosine by sphingosine kinase 1 (SphK1) and sphingosine kinase 2 (SphK2). Extracellular signal activates SphK1, which is translocated from cytosol to the plasma membrane to convert membrane-derived sphingosine to S1P, which activates S1P receptors by an autocrine/paracrine manner. SphK2 is located in the nucleus and shuttle between the cytosol and nucleus. Phosphorylation of SphK2 by p-ERK1/2 activates SphK2. S1P is a potent pleiotropic lipid mediator that has been shown to activate at least five different GPCR. Bile acids only activate S1P2 in hepatocytes. S1P2 activates the insulin receptor/AKT pathway through activation of a tyrosine kinase, Src, and EGFRs. It has been suggested that the S1P2/ERK1/2/AKT pathway may phosphorylate and stabilize SHP, which is known to be unstable and rapidly degraded via the ubiquitin-proteasome degradation pathway (130). If this is the case then S1P may inhibit CYP7A1 and bile acid synthesis by stabilizing and activating SHP to inhibit CYP7A1 gene transcription. Similarly, the S1P/SHP pathway may inhibit peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ )-mediated activation of fatty acid oxidation, and SREBP-1c-mediated fatty acid synthesis. However, the mechanism of the S1P/SHP pathway in inhibition of fatty acid synthesis and PPAR $\alpha$ -regulated fatty acid oxidation remain unclear. Bile acid-activated S1P2 signaling may activate and phosphorylate ERK1/2, which is known to inhibit Cyp7a1 resulting in inhibiting bile acid synthesis (177) (Fig. 7). S1P has been shown to bind to and inhibit histone deacetylase 1 (HDAC1) and histone deacetylase 2 (HDAC2) involved in epigenetic regulation of gene transcription, thus stimulates histone acetylation and the rate of gene transcription. It has been suggested that the S1P2/AKT pathway may regulate hepatic glucose and lipid metabolism via inhibition of GSK3 $\beta$  and resulting in activation of glycogen synthase. Thus S1P2 signaling may stimulate glycogenesis and reduce serum glucose. AKT is known to phosphorylate FoxO1, which is excluded from the nucleus resulting in inhibiting gluconeogenesis (151). Further *in vivo* study is needed to confirm that bile acids are endogenous ligands, which bind to S1P2 with high affinity and specificity, and to unveil the physiological function of this bile acid-activated S1P2/ERK1/2/AKT signaling pathway.

## Bile Acids in Inflammation

Bile acid synthesis is under tight control to prevent excessive intracellular accumulation of bile acids. Hydrophobic bile acids are potent inflammatory agents that cause injury to liver, intestine, and other tissues, whereas hydrophilic bile acids are anti-inflammatory. Bile acid-activated FXR and TGR5 signaling suppress inflammation in macrophages, intestine, and hepatocytes by inhibiting NF- $\kappa$ B nuclear translocation and antagonizing NF- $\kappa$ B-dependent induction of proinflammatory cytokines (36, 63, 146, 197). Conversely, proinflammatory cytokines could ameliorate FXR signaling and inhibit FXR target gene expression (68, 69). This inhibition may be through activation of TNF $\alpha$  and IL-1 $\beta$  signaling. The NF- $\kappa$ B subunits p65 and p50 may physically interact with FXR and block FXR activity. The role of FXR and TGR5 in atherosclerosis and metabolic syndrome has recently been suggested (77, 146, 147).

Bile acids are known to activate proinflammatory cytokine (TNF $\alpha$  and IL-1 $\beta$ ) release from Kupffer cells (132). These cytokines may cross from sinusoid to hepatocytes to activate cytokine receptors Toll-like receptors signaling and activate the Ras/MEK4/7/JNK1/2 signal cascade, which phosphorylates HNF4 $\alpha$  and reduces its binding to the BAREs and results in inhibiting *CYP7A1* and *CYP8B1* gene transcription (92, 114). On the other hand, physiological concentrations of bile acids activate FXR and TGR5 signaling to reduce NF- $\kappa$ B-mediated proinflammatory cytokine production. TGF $\beta$ 1 secreted from Kupffer cells activates the Toll-like receptor 4 in hepatic stellate cells (HSCs), which also secrete TGF $\beta$ 1 to activate the TGF $\beta$ 1 receptor and the Smad signaling pathway in hepatocytes. Bile acids stimulate TGF $\beta$ 1 expression in hepatocytes and activate the latent TGF $\beta$ 1 in HSC and activate HSC. Smad3 recruits HDAC and a repressor mSin3A to inhibit HNF4 $\alpha$  activation of *CYP7A1* gene transcription (111). TGF $\beta$ 1 and bile acids activate the Ras/MAPK/JNK pathway to phosphorylate a tumor suppressor p53 (42, 210), which interacts with HNF4 $\alpha$  (125) and inhibits HNF4 $\alpha$  transactivation of *CYP7A1*. Another cytokine, hepatocyte growth factor (HGF) has been shown to inhibit bile acid synthesis in primary human hepatocytes (175). HGF secreted from HSCs activates a tyrosine receptor kinase cMet and rapidly inhibits *CYP7A1* expression. HGF induces cJun and SHP, and the ERK1/2 and JNK pathways. This leads to recruitment of cJun and SHP, but inhibits coactivators PGC-1 $\alpha$  and CBP binding to *CYP7A1* chromatin and results in inhibited *CYP7A1* gene transcription.

## Integration of Bile Acid Signaling and Liver Metabolism

Bile acid synthesis rate is correlated to serum triglyceride levels in hyperlipidemia patients (6). Bile acid sequestrants increase bile acid and triglyceride synthesis, while CDCA treatment reduces serum triglycerides in hyperlipoproteinemic patients (7). There early studies in human patients suggest an integrated regulation of bile acid synthesis and serum triglycerides. More recent studies show that bile acid signaling through FXR and TGR5 not only regulates triglycerides, but also glucose and energy metabolisms (106,147,148,187,189). Figure 8 illustrates the role of FXR and TGR5 in regulation of lipid and energy metabolism in the liver, adipocytes, and intestine. Figure 9 shows the role of FXR in regulation of glucose metabolism in the liver.

### Lipid metabolisms

The finding that FXR null mice have increased hepatic bile acids, cholesterol, triglycerides, and proatherogenic serum lipoprotein profiles provides the first experimental evidence that bile acid-activated FXR plays a role in regulation of lipid metabolism (172). It has been shown that FXR induces expression of human apolipoprotein C-II (94), which is an activator of lipoprotein lipase involved in lipolysis of triglycerides carried by triglyceride-rich lipoproteins, VLDL and chylomicron (Fig. 8). FXR also regulates several other genes involved in lipoprotein and triglyceride metabolism, including ApoA-V, ApoC-III, apoE, PPAR $\alpha$ , and syndecan-1 (38,86). The role of FXR in triglyceride metabolism is also supported by the finding that overexpression of FXR by adenovirus-mediated transduction or treatment with GW4064 reduces serum triglyceride levels in wild-type and diabetic mice (222), and the FXR/SHP pathway inhibits SREBP-1c and other lipogenic genes and results in reducing serum triglycerides and VLDL production in mice (208).

The liver and biliary system are an integral part of the reverse cholesterol transport system (21). The liver contributes to more than 50% of reverse cholesterol transport system that transports cholesterol from liver and peripheral tissues to the liver for conversion to bile acids. Reverse cholesterol transport plays a critical role in preventing accumulation of cholesterol and oxysterols in macrophages and protecting against atherosclerosis (75, 84, 108, 186, 209, 220). Transgenic over-expression of CYP7A1 in mice prevents high fat diet-induced atherosclerosis in wild-type and LDL receptor-deficient mice (131, 154), and prevents high fat diet-induced obesity and insulin resistance (117). Increasing bile acid synthesis stimulates *de novo* cholesterol synthesis, but increases biliary cholesterol secretion, without increasing intestinal cholesterol absorption, thus maintains whole body cholesterol homeostasis (116).

### Glucose metabolism

Many recent studies have implicated FXR in regulation of hepatic glucose metabolism (Fig. 9). An early study reports that bile acids inhibit PEPCK suggesting that bile acid synthesis and gluconeogenesis are coordinately regulated and linked to the fasting-to-refeeding cycle in mice (46). It has been reported that activation of FXR inhibits PEPCK by SHP-dependent inhibition of HNF4 $\alpha$  and FoxO1 (215). In contrast, another report shows that activation of FXR by a FXR agonist GW4064 stimulates PEPCK in hepatocytes (181). Furthermore, bile acid activation of FXR stimulates the insulin/AKT pathway, which phosphorylates and inactivates GSK3 $\beta$ , and results in stimulating glycogen synthesis (117). Activation of FXR by GW4064 or a specific FXR agonist 6-ethyl-CDCA in mouse models of diabetes inhibits gluconeogenesis (37, 124, 222). Thus, FXR may inhibit gluconeogenesis but stimulates glycolysis and glycogenesis to improve glucose tolerance and insulin sensitivity. It has been reported that high concentration of insulin inhibits while glucose increases FXR expression (53). In pancreatic  $\beta$  cell line, FXR stimulates glucose-induced insulin transcription and secretion (158). At high glucose concentrations, FXR stimulates insulin secretion via AKT phosphorylation and translocation of glucose transporter-2 (GLUT2) at the plasma membrane of pancreatic  $\beta$  cells and GLUT4 in hepatocytes.

Several animal studies report that FXR plays a role in regulation of serum glucose and insulin resistance in mouse models (124,222). FXR null mice developed severe fatty liver, increased circulating free fatty acids associated with elevated serum glucose and impaired glucose and insulin tolerance (124). FXR-deficiency also reduces adipose tissue mass, lowers serum leptin concentrations and impairs glucose tolerance and insulin resistance, and treatment with GW4064 improved insulin resistance in obese *ob/ob* mice (26). In diabetic *db/db* mice, activation of FXR by GW4064 or overexpressing FXR lowers serum glucose (222). In contrast, a recent study reports that loss of FXR in obese and diabetic mice reduces body weight and improves peripheral insulin sensitivity (150). Another report shows that feeding GW4064 to high fat diet-fed mice caused increased susceptibility to diet-induced obesity, fatty liver and hypertriglyceridemia (206). In principal, activation of FXR should reduce bile acid synthesis, bile acid pool size, and energy expenditure, whereas administration of bile acids restores bile acid pool and should reverse these abnormalities. This apparent paradox of FXR and bile acid actions in glucose metabolism may be explained by TGR5 signaling. Activation of TGR5 signaling has been shown to stimulate

intestinal GLP-1 secretion and improve liver and pancreatic function and enhance glucose tolerance in obese mice (Fig. 6) (188). Activation of TGR5 signaling by a TGR5 specific agonist 6 $\alpha$ -ethyl-23(*S*)-methyl-CA (INT-777) stimulates GLP-1 release in enteroendocrine cells. This may be due to an increase of intracellular ATP/ADP ration and calcium mobilization.

### Energy metabolism

Fatty acids and glucose are two major fuel molecules in the body. By controlling lipid and glucose metabolism, bile acids play a central role in energy metabolism. The role of FXR in energy metabolism is implicated by the finding that FGF19 increases metabolic rate, reduces adiposity and reverses diet-induced and leptin-deficient diabetic mice (67, 191). FGF19 induces hepatic leptin receptor, reduces acetyl-CoA carboxylase 2 expression, and increases fatty acid oxidation. FXR deficiency in mice increases susceptibility to torpor, supporting the role FXR plays in energy metabolism (25). It has been reported recently that FGF19 suppresses insulin-induced fatty acid synthesis in hepatocytes by inhibiting lipogenic gene expression and PGC-1 $\alpha$  by inducing SHP (16). *Shp* null mice show increased energy expenditure, PGC-1 $\alpha$  expression, and diet-induced obesity, suggesting that SHP may be involved in energy production in brown adipocyte tissue by inhibiting PGC-1 $\alpha$  expression (202).

Bile acids increase energy expenditure in brown adipose tissue and TGR5-dependent cAMP activation of a DIO2 is required for the bile acid effect on energy metabolism (Fig. 6) (207). In *DIO2*<sup>-/-</sup> mice, bile acids are unable to increase energy production. Knockout of the *Tgr5* gene reduces bile acid pool size by 25%, and female *Tgr5* null mice show significant weight gain and fat accumulation when fed a high fat diet (129). These phenotypes are consistent with the role of TGR5 in energy metabolism. However, adult humans have very little brown adipose tissue and the role of TGR5 in energy metabolism in man is not clear. An *in vitro* study shows that TGR5 regulates energy metabolism in human muscle cells. However, TGR5 levels in human skeletal muscle, adipose tissue and intestine are very low (96). The role of TGR5 in muscle energy metabolism is not known.

## Bile Acids in Human Diseases

### Bile acid metabolism defects

Bile acids increase cell proliferation and apoptosis in the liver and intestine (5, 173). Accumulation of toxic endobiotics and xenobiotics causes damage to cells and organs in the digestive tract. Inborn errors in bile acid metabolism have been identified by analysis of abnormal bile acid metabolites in human patients (163) and identification of mutations or enzyme deficiencies in bile acid metabolism. Decrease in bile acid synthesis can be caused by a primary defect in the enzymes involved in the bile acid biosynthetic pathways. Inborn error of bile acid synthesis can produce abnormal bile acid metabolites with altered steroid side chains or steroid nucleus structures. These metabolites are toxic and can cause cholestatic liver disease early in infancy and progressive neurological disease later in childhood and into adulthood. The diseases are characterized by various phenotypes depending on specific deficiency of enzymes in the pathways, such as jaundice,



hyperbilirubinemia, giant cell hepatitis, neurologic dysfunctions, cholesterol gallstones, premature heart disease, cholestatic liver diseases, malabsorption of fat and fat-soluble vitamins, etc. These diseases can be treated effectively with bile acid replacement therapy if diagnosed early. Nine inborn errors of bile acid synthesis have been reported, including CYP7A1 deficiency (152), CYP7B1 deficiency (39, 165),  $3\beta$ - $5$ - $C_{27}$  steroid oxidoreductase (HSD3B7) mutations (40,161,185),  $4$ - $3$ -oxysteroid  $5\beta$ -reductase deficiency (51, 73, 107, 164, 171), CYP27A1 mutations (cerebrotendinous xanthomatosis (17,18), bile acid conjugation deficiency (27, 35), and two or three defect in peroxisomal enzymes or biogenesis (19, 60, 61, 72, 80, 162).

### Cholestatic liver diseases

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 (192). 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 intra- and extrahepatic cholestasis (198).

### Transporter defects

Congenital or acquired defects in canalicular membrane transporters can cause accumulation of bile acids in hepatocytes and in systemic circulation. 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. Familial progressive intrahepatic cholestasis (FPIC) and benign recurrent intrahepatic cholestasis are autosomal recessive diseases linked to mutations in ATP8B1 (Type 1, PFIC1), BSEP (Type 2, PFIC2), and MDR3 (Type 3, PFIC3). 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. The mechanism of ATP8B1 mutations in pathogenesis of PFIC1 is not clear. More recent data suggest that ATP8B1 counterbalances the effect of ABCB4 (PFIC3), a phospholipid floppase, and that ATP8B1 may be an anchor of the actin cytoskeleton for microvilli formation in the brush border membrane. Several studies have shown reduced hepatic FXR expression levels in PFIC1 patients (4, 29). A recent study reports that hepatic FXR mRNA expression is not altered and ATP8B1 deficiency may disrupt the bile canalicular membrane structure and cause cholestasis (23).

PFIC2 is linked to BSEP mutations (182). Bile acids accumulated in the liver and in systemic circulation cause hepatitis and liver damage requiring liver transplant in pediatric patients. BSEP mutations and polymorphisms have been linked to intrahepatic cholestasis of pregnancy (ICP) (105,137,142) and drug-induced liver injury (104). ICP is a reversible form of intrahepatic cholestasis associated with adverse pregnancy outcomes (55).

PFIC3 is linked to mutation of MDR3 (ABCB4), a phospholipid floppase that flops phosphatidylcholine to the outer leaflet of the canalicular membrane. PFIC3 patients have

high levels of  $\gamma$ -glutamyl transpeptidase activity, progressive cholestasis, bile duct damage, and may require liver transplant. Patients have low phospholipids in bile, which are required for mixed micelle formation with bile acids and cholesterol (91, 142). Without forming mixed micelles, bile acids damage the canalicular membrane and cholangiocytes. MDR3 mutations may cause cholesterol gallstone diseases and has been linked to ICP (91, 205).

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 (PBC), and primary sclerosing cholangitis (PSC). FXR variants have been identified in ICP patients (195), and affect FXR target genes *ABCB11*, and *ABCB4*, and *ATP8B1* expression in ICP (135, 138).

Mutations in the *MRP2* gene have been linked to Dubin-Johnson syndrome, a disease characterized by chronic hyperbilirubinemia (99). MRP2 excretes conjugated bile acids, bilirubin, and other organic anions. Patients have elevated bile acids and cholestasis and MRP2 mutations have been linked to ICP.

### Flow defects

Congenital or acquired defects in bile flow can cause obstructive cholestasis. Blockage of the bile duct by gallstones or tumors causes intrahepatic cholestasis due to accumulation of bile acids in the liver and absent in the intestine. The *FXR* gene has been identified as a candidate gene for the cholesterol gallstone susceptibility locus *Lith7* in mice (211). A study of obstructive cholestasis in human patients shows that bile acid synthesis is suppressed but *CYP7A1* expression is not altered (13). In contrast, another recent study reports that *CYP7A1* mRNA expression is repressed in human patients with obstructive extrahepatic cholestasis, likely due to increased *FGF19* expression in hepatocytes (160).

### Bile Acids as Therapeutic Agents

Bile acids are therapeutic agents for cholestatic liver diseases. In principal, activation of FXR inhibits *CYP7A1* and reduces bile acid synthesis, and inhibits NTCP and OATPs to reduce sinusoidal uptake of bile acids. FXR also may upregulate *MRP3*, *MRP4*, and *OST $\alpha/\beta$*  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 (89). 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.

The therapeutic potential of bile acids and derivatives for treating hepatic and biliary diseases, nonalcoholic fatty liver diseases (NAFLDs), and metabolic syndrome are now well recognized (62, 189, 224). NAFLD is the most prevalent chronic liver disease affecting ~30% of the population in developed countries. Metabolic syndrome is a collection of five clinical symptoms including hypertension, hyperglycemia, hypertriglyceridemia, insulin resistance, and central obesity (155, 156) (Fig. 10). Metabolic syndrome contributes to chronic heart disease atherosclerosis, type II diabetes, and NAFLD. Dyslipidemia causes insulin resistance and inflammation, and pathogenesis of NAFLD (41,70). NAFLD is a

spectrum of chronic liver abnormalities from simple steatosis to nonalcoholic steatohepatitis (NASH) to liver cirrhosis (216). 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 to NASH, which involves hepatic ballooning, inflammatory infiltrates, and cell death. About 10% to 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 (59, 190). The progression of NAFLD to NASH requires at least “two-hits,” hepatic steatosis is the first hit, followed by inflammation as the second hit (45). Other factors such as insulin resistance and oxidative stress accelerate the progression from NAFLD to NASH.

Based on the results from CYP7A1 overexpressing mouse model (116, 117), 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 through activating FXR and TGR5 signaling to stimulate energy metabolism and increasing glucose tolerance and insulin sensitivity.

Many studies in animals and human diabetic patients report that bile acids may improve glycemic control (38). Disruption of enterohepatic circulation of bile acids by bile fistula and feeding bile acid sequestrants may reduce bile acid pool, which is enlarged in diabetes. Increasing hepatic bile acid synthesis may inhibit gluconeogenesis and lipogenesis, and stimulate glycolysis, fatty acid oxidation, and glycogenesis. Gastric bypass is currently being used as a treatment for reducing weight and type 2 diabetes mellitus (T2DM). However, the mechanism for improving insulin resistance and glycemic control is not known. Increases of serum bile acids after Roux-en-Y gastric bypass surgery may be linked to improved glucose and lipid metabolism (141). It is likely that increased serum bile acids after gastric bypass may play a role in improving glycemic control. Another recent study shows that Roux-en-Y gastric bypass significantly increases bile flow and fasting serum bile acids and FGF19 in patients (149).

### **Bile acid displacement and replacement**

CDCA, CA, and UDCA have been used for effective gallstone dissolution for many years. CDCA and CA are not toxic to humans. CDCA 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 (200). UDCA has been used in traditional Chinese medicine for treating digestive disease for several centuries. UDCA (Ursodiol™) is a highly soluble and nontoxic bile acid, and has been approved by FDA for gallstone dissolution and primary biliary cirrhosis. UDCA reduces the cytotoxicity of the circulating bile acid pool, and thus, protects cholangiocytes, stimulates hepatobiliary secretion, and inhibits liver cell apoptosis (173). UDCA may also activate PXR and induce PXR target genes, CYP3A4, SULTs, UGTs, BSEP, MDR3, and MRP4 for detoxification of bile acids. Norursodeoxycholic acid (norUDCA) is a side chain-shortened C<sub>23</sub> homolog of UDCA that cannot be conjugated and is secreted into bile, reabsorbed by

cholangiocytes, and returned to liver. Cholehepatic shunt of norUDCA leads to increase in bicarbonate in bile and hyperchloresis. This bile acid derivative reverses sclerosing cholangitis in the *Mdr2*<sup>-/-</sup>(*Abcb4*) model of cholangiopathy by ameliorating of bile acid hydrophobicity, stimulating bile flow, inducing bile acid detoxification and anti-inflammation and antifibrosis (134). Clinical trials of norUDCA for PBC and PSC are underway in Europe.

### Bile acid sequestrants

Bile acid sequestrants, like cholestyramine, colestipol, and colesevelam, have been used for gallstone dissolution and lipid lowering in humans. Colesevelam is the second-generation bile acid sequestrants that have been used for lipid lowering in combination with statins, and with antidiabetic drugs for increasing glycemic control and insulin resistance (3, 11, 22, 43, 71, 167). These drugs bind bile acids in the intestine and interrupt enterohepatic circulation of bile acids, and result in stimulating bile acid synthesis, increasing LDL receptors and reducing serum cholesterol levels. Cholestyramine and colesevelam also have glucose-lowering effect and improve glycemic control for T2DM in some clinical studies (11, 14, 71, 179, 180). The underlying mechanism of glucose-lowering effect of bile acid sequestrants is not clear. Recent reports show that colesevelam may mediate its action through regulation of FXR/FGF19 and TGR5/GLP-1 signaling pathways (167). Colesevelam reduces bile acids in the intestine and should reduce FGF15 synthesis and consequently induces hepatic CYP7A1 expression and bile acid synthesis. However, effect of colesevelam on improving glucose tolerance and insulin sensitivity are not conclusive in human studies. A recent study shows that colesevelam increases GLP-1 levels and improves  $\beta$  cell function, but has no effect on insulin sensitivity (14). Colesevelam improves glycemic control in T2DM patients, but is not correlated with changes in bile acid metabolism (22). In T2DM patients, CA synthesis increase and colesevelam treatment preferentially increases CA but decreased CDCA and DCA, thus decreasing the hydrophobicity of bile acid pool without changing total bile acid pool size. There is no correlation between bile acid kinetics and glucose metabolism in these patients. Several clinical trials of colestipol, cholestyramine and colesevelam for type II diabetes have been approved by US FDA (79).

### Bile acids and derivatives for liver and metabolic 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 (143). In animal studies, OCA increases insulin sensitivity, inhibits gluconeogenesis, inhibits lipogenesis, and also has anti-inflammatory and anti-fibrotic properties (1). OCA ameliorates high fat diet-induced obesity and insulin resistance in mice, and insulin resistance and fatty liver in Zucker rats (*fa/fa*) (37). OCA antagonizes NF- $\kappa$ B-stimulated inflammation in liver (203), modulates innate immunity in animal models of colitis (197), inhibits and preserves the intestinal barrier in inflammatory bowel disease (69), and inhibits vascular smooth muscle cell inflammation and migration (119). OCA has been in phase II clinical trials for type 2 diabetic patients with NAFLD. Patients have improved insulin sensitivity, liver  $\gamma$ -glutamyltransferase levels (a marker of NASH) and weight loss. In phase II clinical studies of PBC patients, OCA significantly lowered alkaline phosphatase after 12 weeks of treatment. However, the majority of patients have pruritus, a common symptom of

cholestasis and side effect of bile acid therapies. A larger National Institute of Diabetes and Digestive and Kidney Diseases-sponsored phase IIb trial OCA for NASH network will start soon.

Another bile acid derivative 6 $\alpha$ -ethyl-23(S)-methyl-CA (EMCA or INT-777) is a selective and potent TGR5 agonist (144, 145). In animal studies, EMCA 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 (188). Activation of TGR5 also reduces atherosclerotic lesions by reducing macrophage inflammation and lipid loading (146). TGR5 agonists also reduce inflammation in liver and intestine to prevent liver inflammation and IBD (147, 204).

## Conclusion

Basic research in bile acid metabolism and signaling in last 20 years has greatly improved our current knowledge and understanding of bile acid-mediated integration of liver metabolism and diseases. Bile acid-based therapies have great potential for treatment of liver diseases and diabetes. Current research in bile acid metabolism relies on genetically modified mouse models. Future research using proteomic, metabolomic, and lipidomic approaches will help in identifying bile acid biomarkers for diagnosis and treatment of human metabolic diseases.

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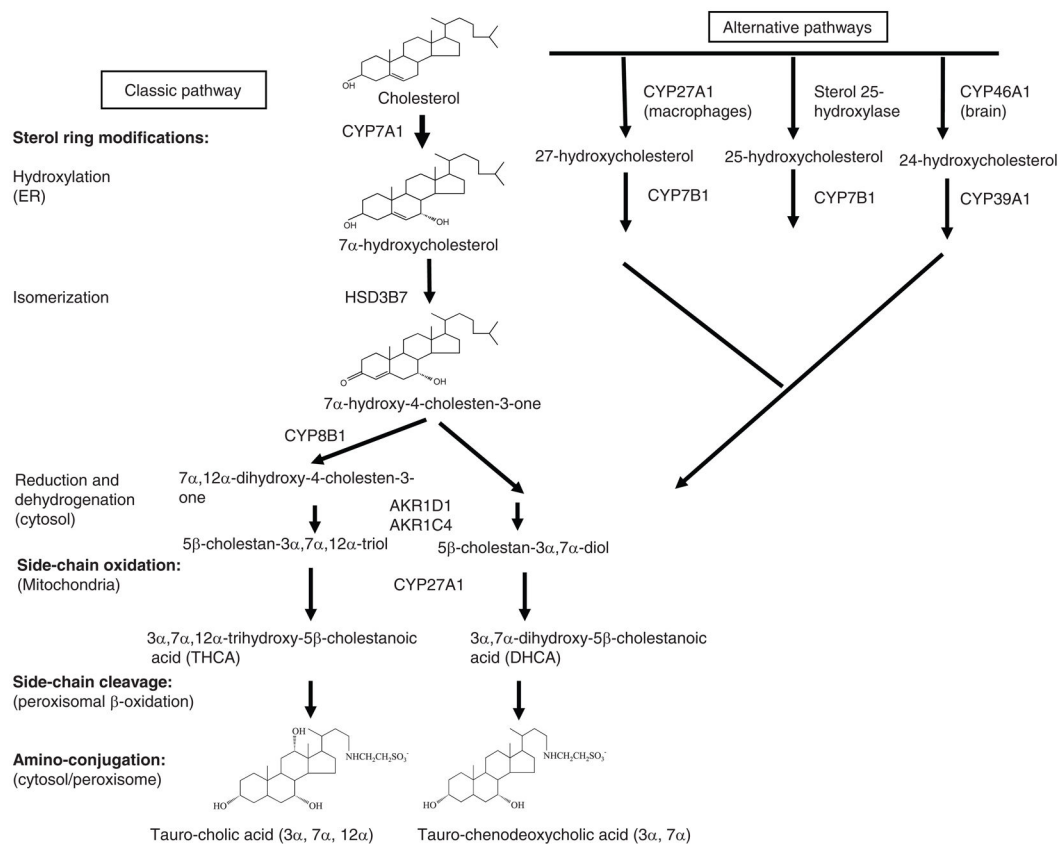
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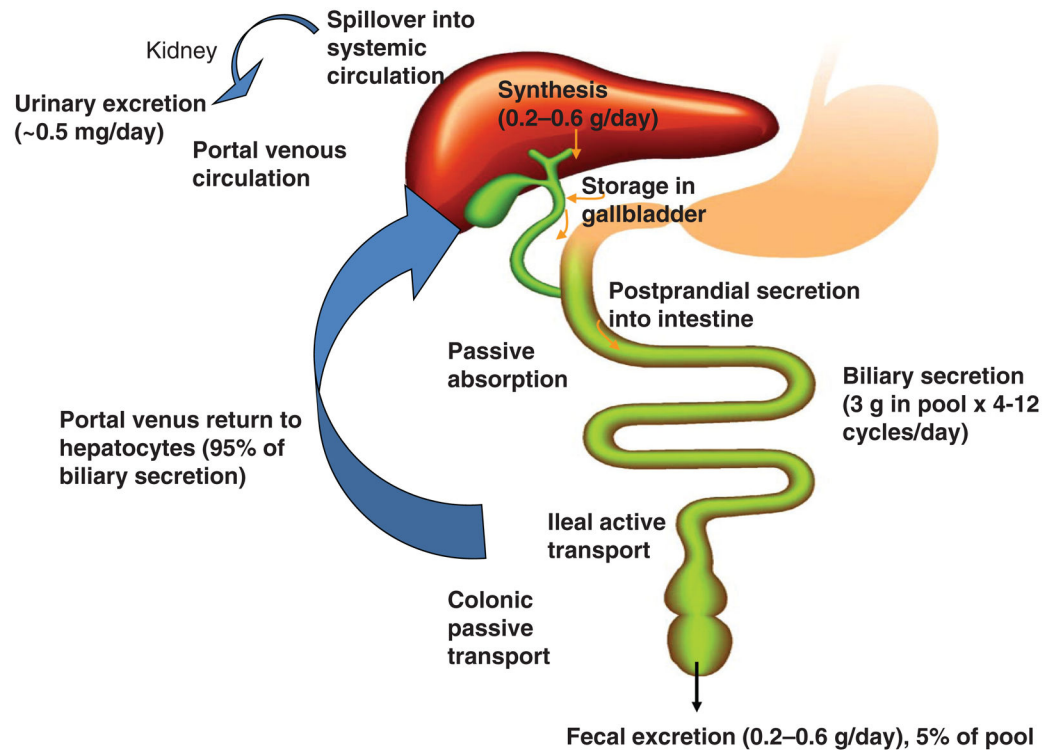


**Figure 1.**

Bile acid biosynthetic pathways. Two major bile acid biosynthetic pathways are shown. The neutral (or classic) pathway is initiated by cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) located in the endoplasmic reticulum of the liver, whereas the acidic (or alternative) pathway is initiated by mitochondrial sterol 27-hydroxylase (CYP27A1). There are three sterol hydroxylases that convert cholesterol to oxysterols: CYP27A1 in macrophages and other tissues, microsomal sterol 25-hydroxylase in liver microsomes, and sterol 24-hydroxylase (CYP46A1) in the brain. Oxysterol 7 $\alpha$ -hydroxylase (CYP7B1) is nonspecific and catalyzes hydroxylation of 27- and 25-hydroxycholesterol to 3 $\beta$ , 7 $\alpha$ -dihydroxy-5-cholestenoic acid and 5-cholesten-3 $\beta$ , 7 $\alpha$ , 25-triol, respectively. A brain-specific oxysterol 7 $\alpha$ -hydroxylase (CYP39A1) catalyzes hydroxylation of 24-hydroxycholesterol to 5-cholesten-3 $\beta$ , 7 $\alpha$ , 24(*S*)-triol. These oxysterols could be converted to CDCA if transported to the liver. In the liver, 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD, HSD3B7) convert 7 $\alpha$ -hydroxycholesterol to 7 $\alpha$ -hydroxy-4-cholesten-3-one (C4), which is converted to 7 $\alpha$ , 12 $\alpha$ -dihydroxy-4-cholesten-3-one by a sterol 12 $\alpha$ -hydroxylase (CYP8B1), leading to synthesis of cholic acid (CA). Without 12 $\alpha$ -hydroxylation, the pathway produces CDCA. Aldos-keto reductase 1D1 (AKR1D1) and AKR1C1 catalyze isomerization and saturation of the steroid ring. Then CYP27A1 catalyzes steroid side-chain oxidation to form cholestanolic acids, THCA, and DHCA. Bile acid-Co-A synthase (BACS) or very long-chain Co-A synthase (VLCS) in the endoplasmic reticulum (ER) ligates Co-A to the carboxyl groups. Bile acid thioesters are transported into peroxisomes, where an  $\alpha$ -methylacyl-CoA racemase (AMACR) converts

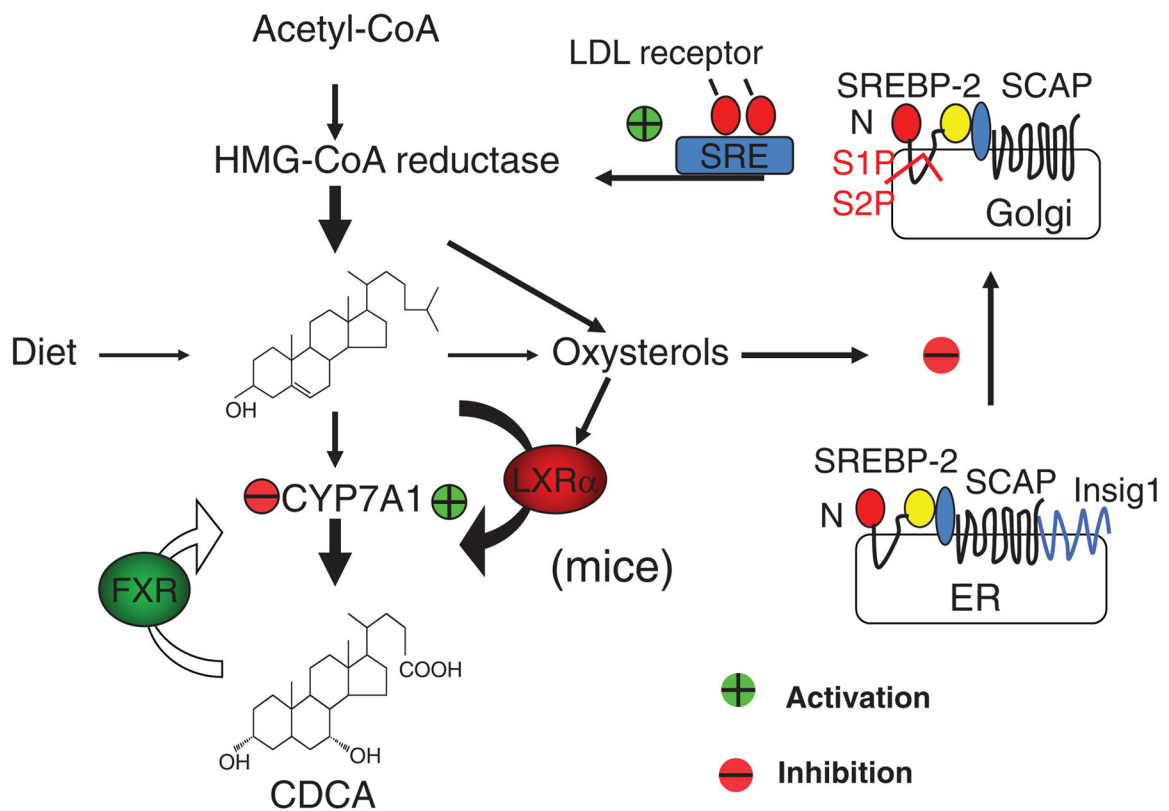


the methyl group from 25(R) to 25(S) conformation, and three peroxisomal  $\beta$ -oxidation enzymes, branched-chain acyl-CoA oxidase, D-bifunctional enzyme, and thiolase (or sterol carrier protein x) catalyze oxidative cleavage of a propionyl group from the steroid side-chain to form choly-CoA and chenodeoxycholy-CoA. Cytosolic or peroxisomal bile acid: amino-acid transferase (BAAT) catalyzes conjugation of amino acids, glycine or taurine to the carboxyl group of choly-CoA and chenodeoxycholy-CoA to form tauro- or glycol-conjugated CA or CDCA. ER: endoplasmic reticulum.



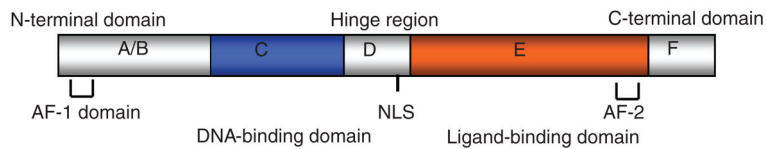
**Figure 2.**

Enterohepatic circulation of bile acids. An average man produces ~0.5g bile acid per day by synthesis in the liver, and secretes ~0.5g/day. This daily turnover of bile acids accounts for about 5% of total bile acid pool. The remaining 95% of bile acids in the pool are recycled 4 to 12 times a day. Most bile acids are reabsorbed in the ileum by active transport, while a small amount is reabsorbed by passive diffusion in the upper intestine to portal blood for circulation to the liver. Small amounts of bile acids spilled over into the systemic circulation are recovered in kidney.



**Figure 3.**

Bile acid synthesis regulates cholesterol homeostasis in hepatocytes. Cholesterol homeostasis is maintained by dietary uptake of cholesterol, *de novo* cholesterol synthesis from acetyl-CoA, and conversion of cholesterol to bile acids. Oxysterols are derived from cholesterol and bile acids. When intracellular cholesterol/oxysterol levels are high, steroid response element binding protein 2 (SREBP-2) precursor (125 kDa) interacts with insulin induced gene 1/2 (Insig1/2) and is retained in endoplasmic reticulum (ER) membrane. When intracellular oxysterol levels are low, SREBP cleavage and activating protein (SCAP) escorts SREBP-2 precursor to the Golgi apparatus, where sterol sensitive proteases S1P and S2P are activated to cleave a N-terminal fragment (65 kDa), which is translocated to the nucleus to bind to the steroid response elements in the gene promoters of all cholesterologenic genes and stimulates *de novo* cholesterol synthesis. Oxysterols activate LXR $\alpha$ , which induces *CYP7A1* gene transcription to stimulate bile acid synthesis in mice, but not humans. Bile acids (CDCA) activate farnesoid X receptor (FXR) to inhibit *CYP7A1* gene transcription and bile acid synthesis. This may lead to increased cholesterol levels and inhibited *de novo* cholesterol synthesis and absorption of dietary cholesterol.



#### 48 nuclear receptors in human:

##### Type I: Endocrine receptors

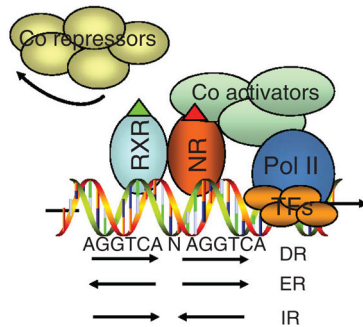
ER $\alpha$ , $\beta$ ; PR; AR; GR; MR; RAR $\alpha$ , $\beta$ , $\gamma$ ; TR $\alpha$ , $\beta$ ; VDR; EcR

##### Type II: Adopted orphan receptors

RXR $\alpha$ , $\beta$  ( $\delta$ ); PPAR $\alpha$ , $\beta$ , $\gamma$ ; LXR $\alpha$ , $\beta$ ; FXR, PXR, CAR

##### Type III: Orphan receptors

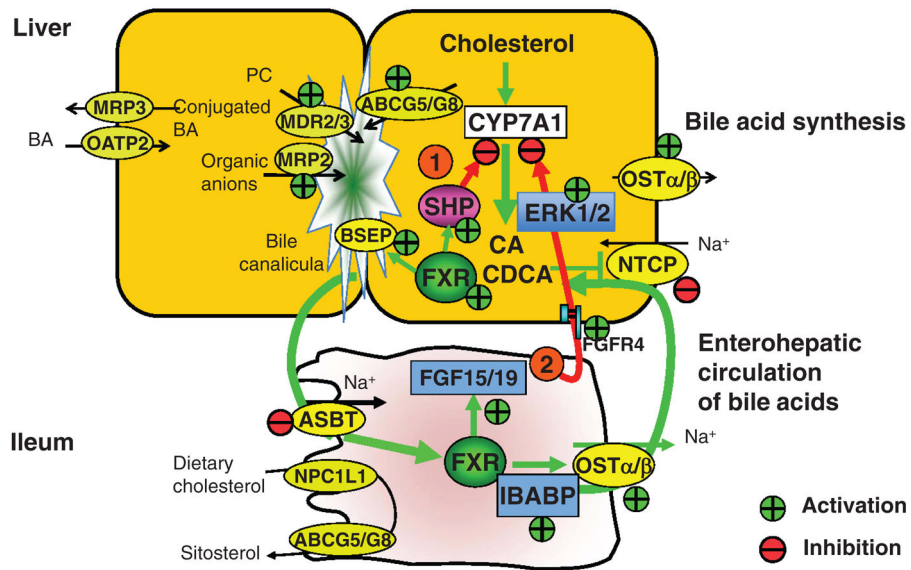
SF-1, LRH-1, DAX-1, SHP, NGFI-B $\alpha$ , $\beta$ , $\gamma$ ; ROR $\alpha$ , $\beta$ , $\gamma$ ; ERR $\alpha$ , $\beta$ , $\gamma$ ; RVR $\alpha$ , $\beta$ , $\gamma$ ; GCNF, TR2,4; HNF-4 $\alpha$ , COUP-TF $\alpha$ , $\beta$ , $\gamma$



#### Bile acid-activated nuclear receptors: FXR, PXR, CAR, VDR

**Figure 4.**

Nuclear receptors. The general structure of nuclear receptors is shown on the top. The NR1 family of genes involved in metabolic regulation, and their respective endogenous ligands are shown. The putative nuclear receptor response element binding sequence, arranged in direct repeat (DR), everted repeat (ER), and inverted repeat (IR), is shown. Ligand-activated receptors recruit coactivators to replace corepressors and results in transactivation of target gene expression. AF-1-2, activation function-1 and -2; CAR, constitutive androstane receptor; FXR, farnesoid X receptor; LXR, liver orphan receptor; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; NLS, nuclear localization sequence.



**Figure 5.**

Farnesoid X receptor (FXR) regulates enterohepatic circulation of bile acids. Major bile acid transporters in human hepatocytes and enterocytes are shown. Enzymes and transporters regulated by FXR are indicated. In hepatocytes, bile acids activate FXR to inhibit *CYP7A1* gene transcription by two pathways: (i) FXR induces small heterodimer partner (SHP), which inhibits *CYP7A1* by inhibiting nuclear receptors liver related homologue-1 (LRH-1) or hepatocyte nuclear factor (HNF4), which bind to the *CYP7A1* promoter. (ii) In enterocytes, FXR induces intestinal hormone fibroblast growth factor 19 (FGF19), which is circulated to hepatocytes to activate FGF receptor 4 (FGFR4) signaling to inhibit *CYP7A1* via activation of the extracellular stress-activated receptor kinase 1/2 (ERK1/2) pathway. FXR induces bile salt export pump (BSEP) to efflux bile acids into bile; multidrug resistance protein 2/3 (MDR2/3) to efflux phosphatidylcholine (PC) to bile; and MDR related protein 2 (MRP2) to efflux organic anions including glucuronidated- and sulfated-bile acids, organic anions, and drugs into bile. Bile acids also facilitate efflux of cholesterol to bile by ATP binding cassette G5/G8 (ABCG5/G8). In the bile, bile acids, PC, and cholesterol form mixed micelles, which are stored in the gallbladder. In the brush border membrane of the ileum, bile acids are reabsorbed by the apical sodium bile salt transporter (ASBT). In enterocytes, bile acids activate FXR, which induces ileum bile acid binding protein (IBABP) to bind bile salts and may facilitate intracellular transport of bile acids to organic solute transporter  $\alpha/\beta$  ( $OST\alpha/\beta$ ) located in the basolateral membrane for efflux of bile acids into portal circulation. Bile acids in portal blood are reabsorbed into hepatocytes by  $Na^+$ -dependent taurocholate cotransport peptide (NTCP). FXR inhibits NTCP transcription as a feedback inhibition of bile acid uptake to prevent liver injury. In the sinusoidal membrane of enterocytes and hepatocytes, FXR also induces MRP3/4 to efflux bile acids as an adaptive response to cholestasis. In hepatocytes, FXR also induces  $OST\alpha/\beta$  to efflux bile acids into sinusoidal blood to prevent bile acid accumulation in hepatocytes. MRP3 may be induced by FXR as an adaptive response to cholestasis. Bile acids returned to hepatic sinusoid are also taken up by  $Na^+$ -independent organic anion transport proteins (OATP2). Many of these membrane transporters (ASBT,  $OST\alpha/\beta$ , and MRP2/3) also are present in cholangiocytes for

reabsorption of bile acids, and in renal proximal tubule cells for reabsorption of bile acids from blood circulation and excretion of hydrophilic bile acids.

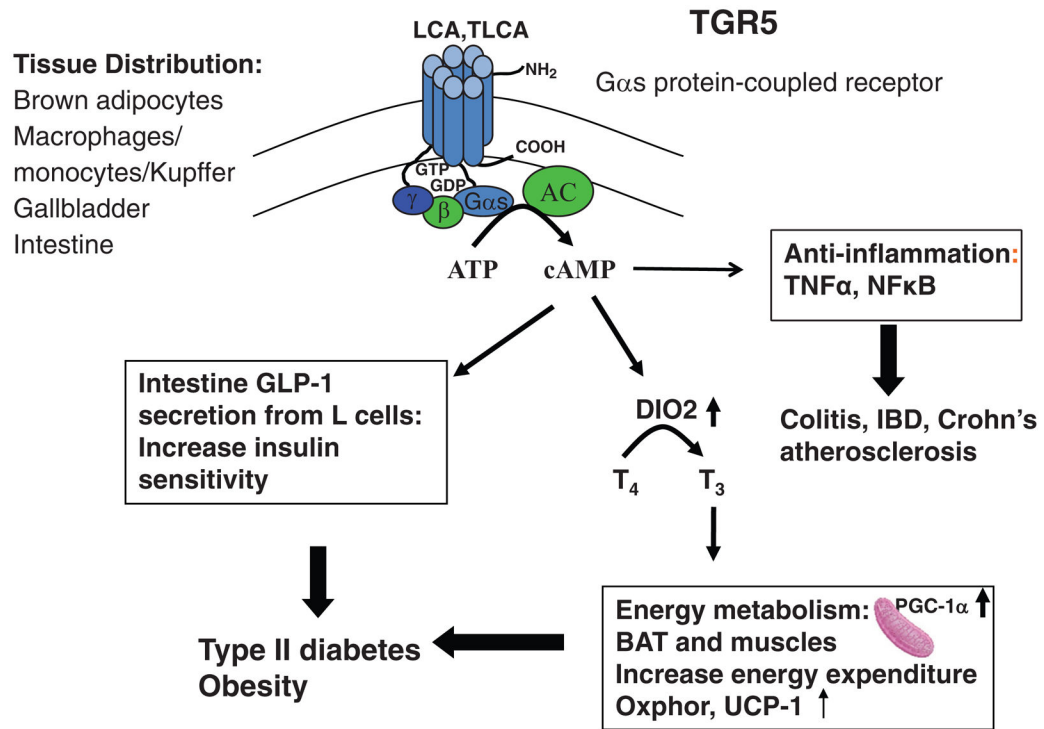
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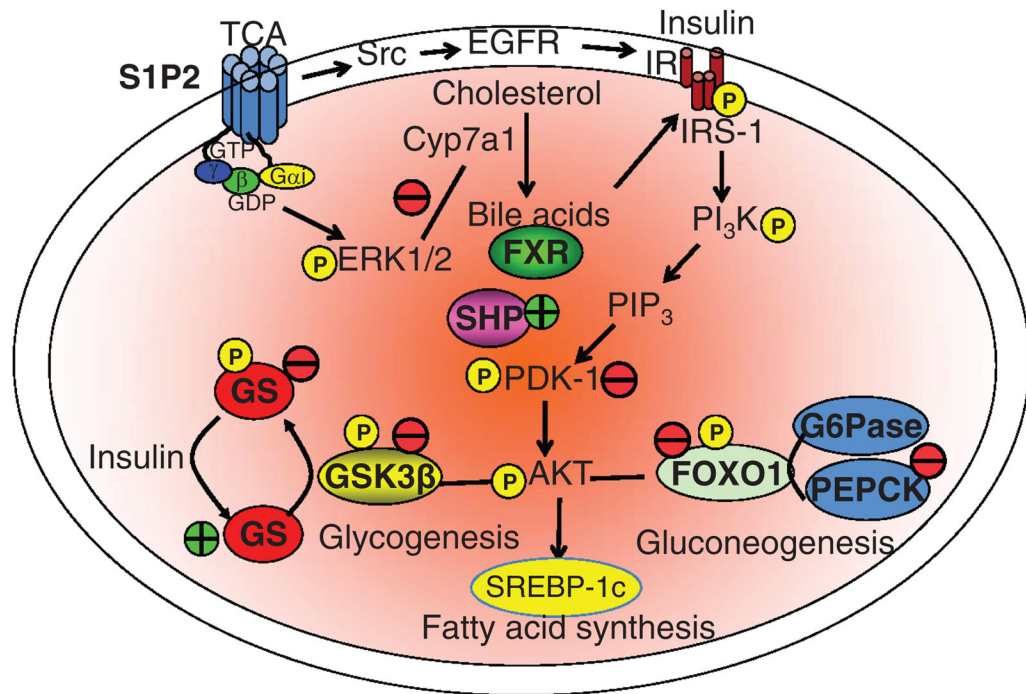




**Figure 6.**

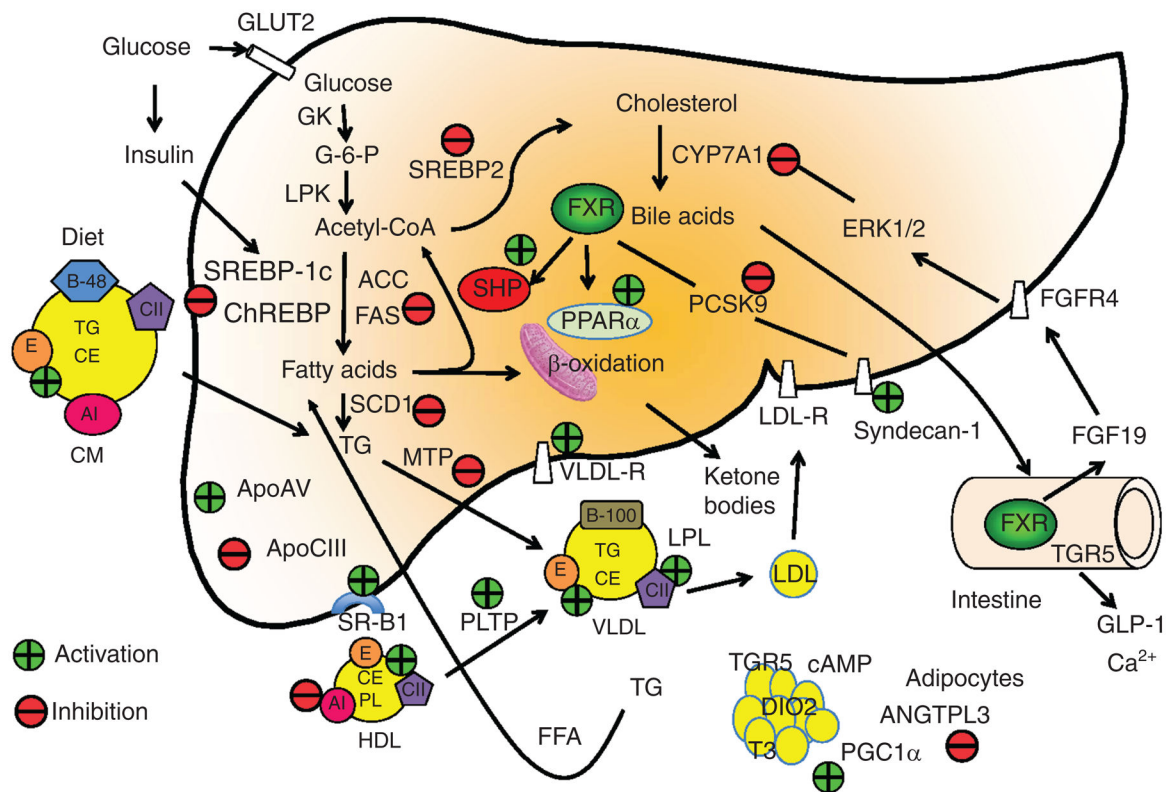
Bile acid-activated TGR5 signaling. TGR5 is expressed in brown adipocytes, macrophages/monocytes and hepatic Kupffer cells, gallbladder epithelium, and intestine, with especially high levels in the colon. TGR5 is the first G protein-coupled receptor (GPCR) identified as a bile acid-activated membrane receptor. TGR5 is a Gαs GPCR activated by secondary bile acids, lithocholic acid (LCA) and TLCA to induce cAMP signaling through activation of adenylyl cyclase (AC). TGR5 signaling may increase insulin sensitivity through two mechanisms. (i) In brown adipose tissue, cAMP induces type 2 deiodinase (DIO2), which converts and activates thyroid hormone T<sub>4</sub> to T<sub>3</sub> to stimulate energy metabolism in mitochondria by activating oxidative phosphorylation (OXphor) and uncoupling protein-1 (UCP-1). (ii) In the intestine, cAMP stimulates glucagon like peptide-1 (GLP-1) in L cells, which stimulates insulin secretion in the pancreas. TGR5 also has anti-inflammatory functions by antagonizing of TNFα and NF-κB-dependent induction of proinflammatory cytokines in intestine and macrophages, thus, protecting against colitis, inflammatory bowel disease and Crohn's disease, and also atherosclerosis.

### S1P2: G $\alpha$ i protein-coupled receptor



**Figure 7.**

Bile acid-activated S1P2 signaling. Tauro-conjugated bile acids stimulates sphingosine-1-phosphate receptor 2 (S1P2), a G $\alpha$ i protein-coupled receptor, which activates the extracellular signal-regulated kinase 1/2 (ERK1/2) and AKT pathways. S1P2 signaling activates the insulin receptor/AKT pathway through activation of Src and epidermal growth factor receptors (EGFRs) leading to activation of the insulin receptor (IR), which phosphorylates and activates insulin receptor substrate-1 (IRS-1). IRS-1 phosphorylates phosphatidylinositol 3-kinase (PI3K), which phosphorylates phosphatidylinositol 4', 5' bis-phosphate to phosphatidylinositol 3', 4', 5'-trisphosphate (PIP<sub>3</sub>). PIP<sub>3</sub> then phosphorylates pyruvate dehydrogenase kinase-1 (PDK-1) to phosphorylate and activate AKT (also known as PKB). Phosphorylated AKT phosphorylates and inactivates glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) and resulting in dephosphorylation and activation of glycogen synthase (GS), a key enzyme in glycogenesis. AKT also phosphorylates and inactivates a transcription factor, FoxO1 and resulted in inhibiting phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in gluconeogenesis. S1P2 signaling also may activate FXR/SHP pathway to inhibit peroxisome proliferator-activated receptor  $\alpha/\gamma$  (PPAR $\alpha/\gamma$ )/PGC-1 $\alpha$ -mediated fatty acid oxidation and steroid response element binding protein 1c (SREBP-1c)-mediated fatty acid synthesis. Therefore, S1P2 signaling may reduce serum glucose and triglycerides, and improve insulin sensitivity by stimulating glycogenesis and inhibiting gluconeogenesis and lipogenesis.



**Figure 8.**

Farnesoid X receptor (FXR) regulation of hepatic glucose and lipid metabolism in liver, adipocytes, and intestine. Glucose and insulin stimulate glycolysis to form acetyl-CoA, which is a precursor of cholesterol and fatty acids. The FXR/small heterodimer partner (SHP) pathway may inhibit steroid response element binding protein 1c (SREBP-1c), which induces all genes involved in lipogenesis, acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl CoA desaturase (SCD). The FXR/SHP pathway also inhibits SREBP-2, which induces all genes in *de novo* cholesterol synthesis. FXR activates mitochondria fatty acid  $\beta$ -oxidation by inducing peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ ). FXR inhibits PCSK9, which is an inhibitor of LDL receptor. Thus FXR induces LDL-R and also syndecan-1 involved in cholesterol uptake. FXR inhibits mitochondria triglyceride transport protein (MTP), which is required for assembly of VLDL particles with ApoB100. FXR also induces ApoE, which is a high affinity ligand of LDL receptor and scavenger receptor B1 (SR-B1), and a component of VLDL and chylomicron remnants, which are taken up by ApoE receptors. FXR also induces phospholipid transport protein (PLTP) involved in reverse cholesterol transport of cholesterol from peripheral tissues to liver by HDL/SR-B1 receptor-mediated mechanisms. On the other hand, FXR inhibits ApoA1, a component of HDL, and ANGPTL3, which is involved in hydrolysis of triglycerides in liver and adipocytes. FXR induces FGF19 synthesis in the intestine, which activates FGFR4 receptor in hepatocytes to activate ERK1/2 signaling to inhibit CYP7A1 and bile acid synthesis. In colon, bile acids activate TGR5 signaling to stimulate GLP-1 release. GLP-1 increases insulin sensitivity. TGR5 in brown adipocytes stimulates cAMP

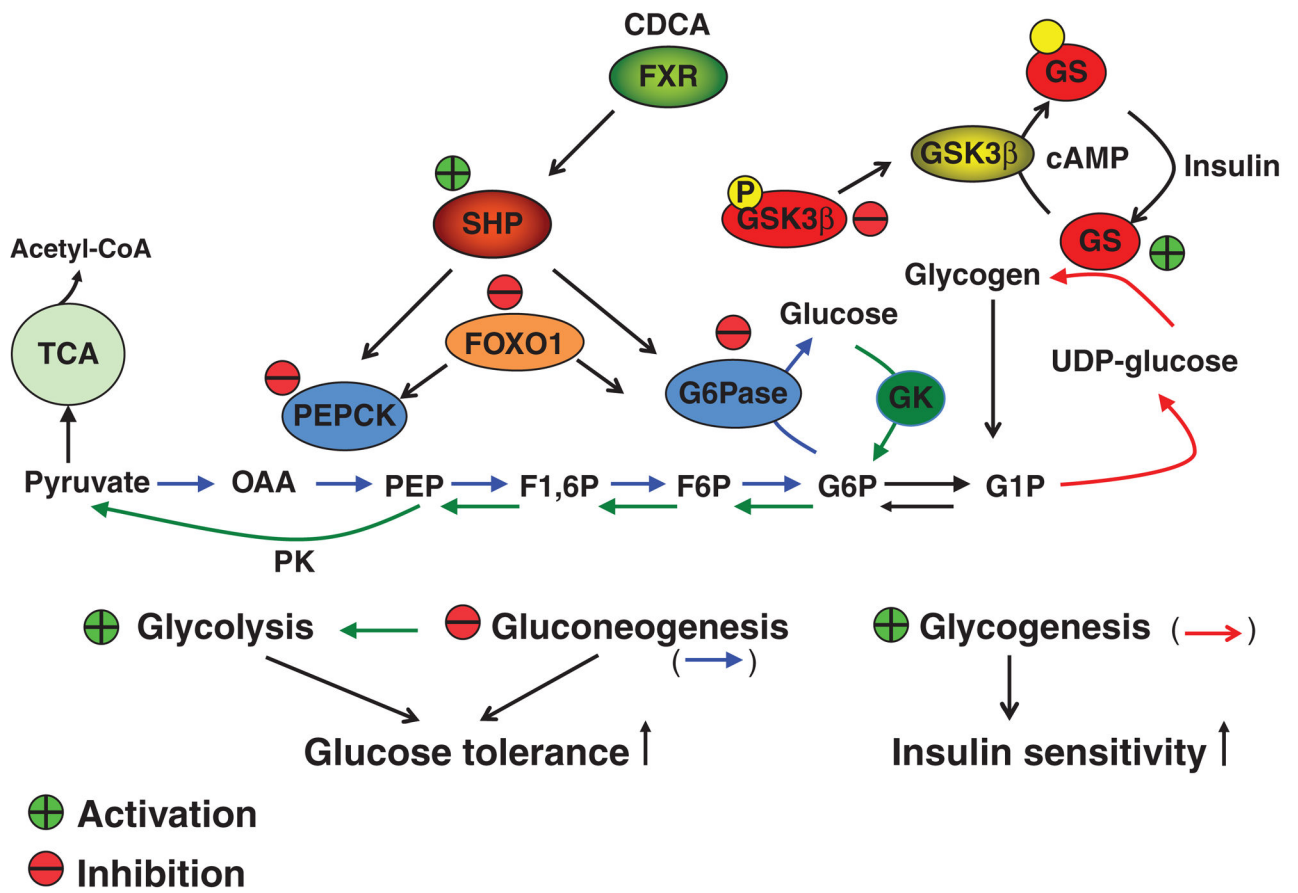
production, which induce deiodinase 2 (DIO2) to convert  $T_4$  to  $T_3$ , which stimulates mitochondrial energy metabolism via activation of PGC-1 $\alpha$ .

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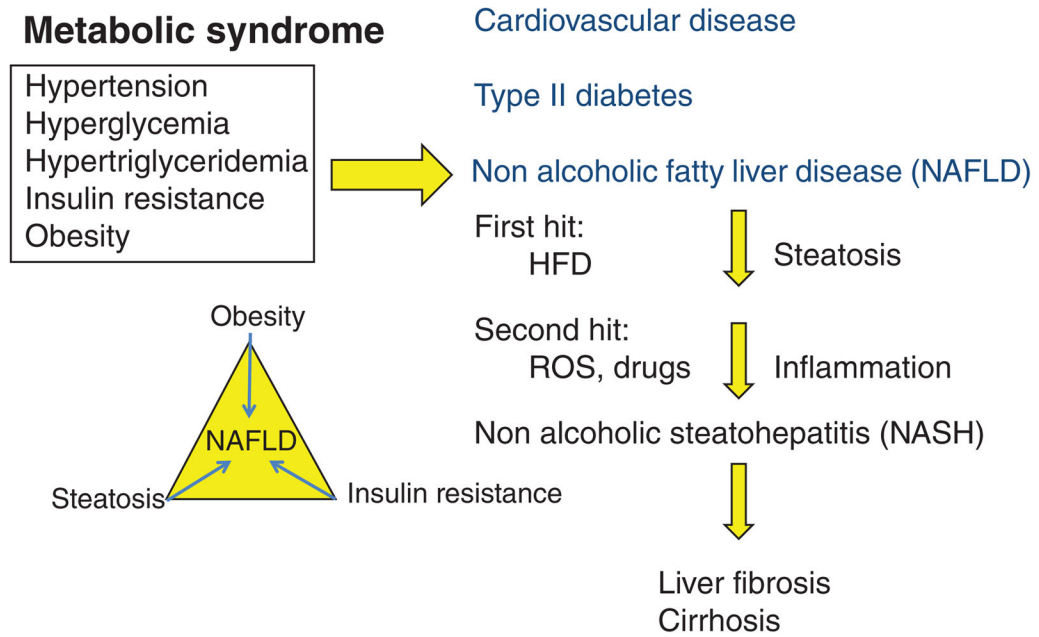
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**Figure 9.**

FXR regulation of hepatic glucose metabolism. FXR signaling phosphorylates and inhibits glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ), which is an inhibitor of glycogen synthase activity. This results in stimulating glycogenesis. The FXR/SHP pathway inhibits PEPCK and glucose 6-phosphatase (G6Pase) to inhibit gluconeogenesis. This results in increasing glucose tolerance and insulin sensitivity.



**Figure 10.**

Mechanism of nonalcoholic fatty liver disease (NAFLD). Metabolic syndrome is a constellation of five clinical symptoms, hypertension, hyperglycemia, hypertriglyceridemia, insulin resistance, and obesity. Metabolic syndrome is linked to cardiovascular disease, type II diabetes, and NAFLD. NAFLD is progressed to nonalcoholic steatohepatitis (NASH) by many factors. The first hit is high fat diet (HFD)-induced hepatic steatosis, followed by inflammation involving reactive oxidizing species, drugs, and endoplasmid reticulum stress to NASH. NASH patients have prevalence for liver fibrosis and cirrhosis, while NAFLD is linked to obesity, steatosis, and insulin resistance. HFD, high fat diet; ROS, reactive oxidizing species.



Table 1

## Abbreviations list

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ABC	ATP binding cassette
ASBP	Apical sodium-dependent bile salt transporter
AKR	Aldo-ketose reductase
AMACR	$\alpha$ -methyl-acyl-CoA racemase
BACS	Bile acid-coenzyme A synthase
BSEP	Bile salt export pump
CA	Cholic acid
CAR	Constitutive androstane receptor A
CDCA	Chenodeoxycholic acid
CYP7A1	Cholesterol 7 $\alpha$ -hydroxylase
CYP8B1	Sterol 12 $\alpha$ -hydroxylase
CYP27A1	Sterol 27-hydroxylase
CTX	Cerebrotendinous xanthomatosis
DCA	Deoxycholic acid
EGFR	Epidermal growth factor receptor
ERK	Extracellular signal-regulated kinase
FGF	Fibroblast growth factor
FGFR4	FGF receptor
FXR	Farnesoid X receptor
GPCR	G protein-coupled receptor
G6Pase	Glucose-6-phosphatase
HNF4 $\alpha$	Hepatic nuclear factor 4 $\alpha$
HSD	Hydroxysteroid dehydrogenase
ICP	Intrahepatic cholestasis of pregnancy
Insig	Insulin induced gene
JNK	Jun-N-terminal kinase
LCA	Lithocholic acid
LRH-1	Liver related homologue
LXR	Liver orphan receptor
MAPK	Mitogen-activated protein kinase
MCA	Muricholic acid
MDR	Multidrug resistant protein
MRP	Multidrug resistance related protein
NAFLD	Nonalcoholic fatty liver disease
NASH	Nonalcoholic steatohepatitis
NTCP	Na <sup>+</sup> -taurocholate cotransport peptide
OST $\alpha/\beta$	Organic solute transporter $\alpha/\beta$
PBC	Primary biliary cirrhosis
PEPCK	Phosphoenolpyruvate carboxykinase
PFIC	Progressive familial intrahepatic cholestasis

PGC-1 $\alpha$	Peroxisome proliferator activated receptor $\gamma$ co-activator 1 $\alpha$
PSC	Primary sclerosing cholangitis
PXR	Pregnane X receptor
RXR	Retinoid X receptor
SCAP	SREBP cleavage-activating protein
SHP	Small heterodimer partner
S1P	Sphingosine 1-phosphate
S1P2	S1P receptor 2
SREBP	Steroid response element binding protein
SULT	Sulfotransferas
UGT	UDP-glucuronosyl N-transferase
UDCA	Ursodeoxycholic acid
VDR	Vitamin D receptor

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