Bile Acids are Nutrient Signaling Hormones

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Abstract

Bile salts play crucial roles in allowing the gastrointestinal system to digest, transport and metabolize nutrients. They function as nutrient signaling hormones by activating specific nuclear receptors (FXR, PXR, Vitamin D) and G-protein coupled receptors [TGR5, sphingosine-1 phosphate receptor 2 (S1PR2), muscarinic receptors]. Bile acids and insulin appear to collaborate in regulating the metabolism of nutrients in the liver. They both activate the AKT and ERK1/2 signaling pathways. Bile acid induction of the FXR-α target gene, small heterodimer partner (SHP), is highly dependent on the activation PKCζ, a branch of the insulin signaling pathway. SHP is an important regulator of glucose and lipid metabolism in the liver. One might hypothesize that chronic low grade inflammation which is associated with insulin resistance, may inhibit bile acid signaling and disrupt lipid metabolism. The disruption of these signaling pathways may increase the risk of fatty liver and non-alcoholic fatty liver disease (NAFLD). Finally, conjugated bile acids appear to promote cholangiocarcinoma growth via the activation of S1PR2.

Keywords

1. Introduction

In the past, bile salts were considered to be just detergent molecules that were required for the solubilization of cholesterol in the gall bladder, promoting digestion of dietary lipids and stimulating absorption of lipids, cholesterol and fat-soluble vitamins in the intestines (1). Bile salts were also known to stimulate bile flow, promote cholesterol secretion from the liver, and to have antibacterial properties. However, in 1999, three independent laboratories reported that bile acids were natural ligands for the farnesoid X receptor (FXR-α)(2–4). The

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recognition that bile acids activated specific nuclear receptors started a renaissance in the field of bile acid research. Since 1999, bile acids have been reported to activate other nuclear receptors (preganane X receptor, vitamin D receptor), G protein coupled receptors [TGR5, sphingosine-1-phosphate receptor 2 (S1PR2), muscarinic receptor 2 (M$_2$)] and cell signaling pathways (JNK 1/2, AKT, and ERK 1/2)(5, 6). Deoxycholic acid (DCA), a secondary bile acid, has also been reported to activate the epidermal growth factor receptor (EGFR) (7). It is now clear that bile acid function as hormones or nutrient signaling molecules that help to regulate glucose, lipid, lipoprotein, energy metabolism and inflammatory responses (5, 6). The role of bile acid-mediated signaling pathways in nonalcoholic fatty liver diseases has been discussed in several excellent reviews (8–12). In this brief review, we will focus on how the insulin signaling pathway and FXR-α cross-talk to regulate hepatic nutrient metabolism.

2. Enterohepatic Circulation of Bile Acids

Bile acids are synthesized from cholesterol in liver hepatocytes, conjugated to either glycine or taurine and actively secreted *via* ABC transporters on the canalicular membrane into biliary bile. Conjugated bile acids are often referred to as bile salts. Bile acid synthesis represents a major out-put pathway of cholesterol from the body. Bile acids are actively secreted from hepatocytes *via* the bile salt export protein (BSEP, ABCB11) along with phospholipids by ABCB4 and with cholesterol by ABCG5/ABCG8 in a fairly constant ratio under normal conditions(13, 14). Bile acids are detergent molecules and form mixed micelles with cholesterol and phospholipids which help keep cholesterol in solution in the gall bladder. Eating stimulates the gall bladder to contract emptying its contents into the small intestines. Bile salts are crucial for the solubilization and absorption of cholesterol and lipids as well as lipid soluble vitamins (A, D, E, and K). They activate pancreatic enzymes and form mixed micelles with lipids in the small intestines promoting their absorption. Bile acids are efficiently recovered from the intestines, primarily the ileum, by the apical sodium dependent transporter (ASBT). Bile acids are secreted from ileocytes, on the basolateral side, by the organic solute OSTα/OSTβ transporter(15). Secondary bile acids, formed by 7α-dehydroxylation of primary bile acids by anaerobic gut bacteria, can be passively absorbed from the large bowel or secreted in the feces. Absorbed bile acids return to the liver via the portal blood where they are actively transported into hepatocytes primarily via the sodium taurocholate cotransporting polypeptide (NTCP, SLC10A1)(16). Bile acids are again actively secreted from the hepatocyte into bile which stimulates bile flow and the secretion of cholesterol and phospholipids. Bile acids undergo enterohepatic circulation several times each day (Figure 1). During their enterohepatic circulation approximately 500–600 mg/day are lost via fecal excretion and must be replaced by new bile acid synthesis in the liver. The bile acid pool size is tightly regulated as excess bile acids can be highly toxic to mammalian cells.

3. Synthesis of Primary and Secondary Bile Acids

There are two pathways of bile acid synthesis in the liver, the neutral pathway and the acidic pathway (Figure 2). The neutral pathway is believed to be the major pathway of bile acid synthesis in man under normal physiological conditions. The neutral pathway is initiated by

cholesterol 7α-hydroxylase (CYP7A1) which is the rate-limiting step in this biochemical pathway. CYP7A1 is a cytochrome P450 monooxygenase and the gene encoding this enzyme is highly regulated by a feed-back repressive mechanism involving the FXR-dependent induction of fibroblast growth factor 15/19 (FGF15/19) by bile acids in the intestines. FGF15/19 binds to the fibroblast growth factor receptor 4 (FGFR4)/β-Klotho complex in hepatocytes activating both the JNK1/2 and ERK1/2 signaling cascades (17–19). Activation of the JNK1/2 pathway has been reported to down-regulate CYP7A1 mRNA in hepatocytes(20). FGFR4 and β-Klotho null mice have increased levels of CYP7A1 and upregulated bile acid synthesis (21, 22). Moreover, treatment of FXR null mice with a specific FXR agonist failed to repress CYP7A1 in the liver(23). These results support an important role of FGF15, synthesized in the intestines by activation of FXR, in the regulation of CYP7A1 and bile acid synthesis in the liver. CYP7A1 has also been reported to be down-regulated by glucagon (24, 25) and pro-inflammatory cytokines (15) and up-regulated by glucose and insulin during the postprandial period (26).

The neutral pathway of bile acid synthesis produces both cholic acid (CA) and chenodeoxycholic acid (CDCA) (Fig. 2). The ratio of CA and CDCA is primarily determined by the activity of sterol 12α-hydroxylase (CYP8B1). The gene encoding CYP8B1 is also highly regulated by bile acids. Bile acids induce the gene encoding small heterodimer partner (SHP) in the liver via activation of the farnesoid X receptor (FXR-α). SHP is an orphan nuclear receptor without a DNA binding domain. It interacts with several transcription factors including hepatocyte nuclear factor 4 (HNF4α) and liver-related homolog-1 (LRH-1) and acts as a dominant negative protein to inhibit transcription. In this regard, a liver specific knockout of LRH-1 completely abolished the expression of CYP8B1, but had little effect on CYP7A1 (27, 28). These results suggest that the interaction of SHP with LRH-1, caused by bile acids, may be the key regulator of hepatic CYP8B1 and the ratio of CA/CDCA.

The acidic or alternative pathway of bile acid synthesis is initiated in the inner membrane of mitochondria by sterol 27-hydroxylase (CYP27A1). This enzyme also has low sterol 25-hydroxylase activity (29, 30). CYP27A1 is capable of further oxidizing the 27-hydroxy group to a carboxylic acid (31). Unlike, CYP7A1, CYP27A1 is widely expressed in various tissues in the body where it may produce regulatory oxysterols (32). Even though CYP27A1 is the initial enzyme in the acidic pathway of bile acid synthesis, it may not be the rate limiting step. The inner mitochondrial membrane is very low in cholesterol content. Hence, cholesterol transport into the mitochondria appears to be a rate limiting step in this pathway (33). In this regard, it has been reported that the expression of the gene encoding steroidogenic acute regulatory (StAR D1) protein, markedly increases (5-fold) the rates of bile acid synthesis in primary hepatocytes via the acidic pathway (32, 34). Over expression of the gene encoding CYP27A1 only minimally increases (<2-fold) bile acid synthesis. The acidic pathway is believed to become more dominant in an individual with cirrhotic liver disease as the neutral pathway is repressed by inflammation (20, 35).

The acidic pathway of bile acid synthesis is now being viewed as an important pathway for generating regulatory oxysterols. For example, 25-hydroxycholesterol and 27-hydroxycholesterol are natural ligands for the liver X receptor (LXR) which is involved in

regulating cholesterol and lipid metabolism (35, 36). Moreover, recent studies report that 25-hydroxycholesterol, formed by CYP27A1, can be converted into 5-cholsten-3β-25-diol-3-sulfate in the liver (37). The sulfated 25-hydroxycholesterol is a regulator of inflammatory responses, lipid metabolism and cell proliferation and is located in the liver (36, 38, 39). Recent evidence suggests that sulfated 25-hydroxycholesterol is a ligand for peroxisome proliferator-activated receptor gamma (PPAR), which is a major regulator of inflammation and lipid metabolism (39). The 7α-hydroxylation of oxysterols is catalyzed by oxysterol 7α-hydroxylase (CYP7B1)(40). This biotransformation allows some of these oxysterols to be converted to bile acids. Finally, oxysterols generated in extrahepatic tissues can be transported to the liver and metabolized into bile acids.

The primary bile acids CA and CDCA are converted into deoxycholic acid (DCA) and lithocholic acid (LCA), respectively, by a small population of intestinal anaerobic bacteria (41, 42). Bile acid 7α-dehydroxylation occurs via a multistep biochemical pathway found in a few species of the genus *Clostridium(41)*. However, bile acids must be deconjugated by bile salt hydrolase before 7α-dehydroxylation occurs. Bile salt hydrolase is found in a large number of different intestinal bacteria. The levels of DCA in the bile acid pool of humans can vary from 1% to over 50% as the human liver is unable to convert DCA back to CA (41). As the amount of DCA is increased in the bile acid pool there is an increase in the hydrophobicity and toxicity to mammalian cells. The amount of DCA in the bile acid pool in man is determined primarily by levels and activity of bile acid 7α-dehydroxylating gut bacteria and intestinal transit time (43). A Western diet appears to increase the levels of bile acid 7α-dehydroxylating bacteria in the intestines and is associated with an increase in DCA in bile (44). The composition of the human bile acid pool is important as bile acids are now known to be regulatory molecules which vary in their ability to activate different nuclear receptors and G-protein coupled receptors (GPCRs)(6). Hence, the bile acid pool composition can regulate the physiology of cells in the gastrointestinal system by differentially regulating different nuclear receptor and GPCRs and by direct toxicity to mammalian cells.

4. The secondary bile acid 7-oxolithocholic acid is reduced by host 11β-hydroxysteroid dehydrogenase 1

A number of gut bacterial have bile acid 7α-hydroxysteroid dehydrogenase activity which can generate 7-oxolithocholic acid (7-oxo-LCA) from chenodeoxycholic acid (45). 7-Oxo-LCA that is absorbed from the gut is reduced to chenodeoxycholic acid and small amounts of ursodeoxycholic acid by 11β-hydroxysteroid dehydrogenase 1 (11β-HSDH-1) in the liver (46, 47). There are two known isoforms of 11β-HSDH in humans and rodents, 11β-HSDH-1 and 11β-HSDH2. 11β-HSDH-2 has been reported to primary oxidize the 11β-hydroxy group of cortisol converting it to cortisone, essentially inactivating this glucocorticoid. In contrast, 11β-HSDH-1 appears to primarily function to reduce cortisone to cortisol producing an active glucocorticoid(48). Because 11β-HSDH-1 specifically reduces 7-oxo-LCA and this secondary bile acid accumulates in 11β-HSDH null mice, it has been proposed that 7-oxo-LCA may be a useful serum marker for 11β-HSDH-1 deficiency. Moreover, 7-oxo-LCA, or

analogs, might function as competitive inhibitors of 11β-HSDH-1 which could be useful in treating various metabolic diseases.

5. Bile Acids Vary in their Ability to Activate Nuclear Receptors and GPCRs

Bile acids can activate several different nuclear receptors (FXR, PXR and Vitamin D) and GPCRs (TGR5, S1PR2, and [M_2] Muscarinic receptor). The ability of different bile acids to activate FXR-α occurs in the following order CDCA>LCA = DCA>CA; for the pregnane X receptor (PXR) LCA>DCA>CA and the vitamin D receptor, 3-oxo-LCA>LCA>DCA>CA (49). LCA is the best activator of PXR and the vitamin D receptor which correlates with the hydrophobicity and toxicity of this bile acid toward mammalian cells. Activation of PXR and the vitamin D receptor induces genes encoding enzymes which metabolize LCA into a more hydrophilic and less toxic metabolite (49). These nuclear receptors appear to function in the protection of cells from hydrophobic bile acids. In contrast, FXR-α appears to play a much more extensive role in the body by regulating bile acid synthesis, transport, and enterohepatic circulation. Moreover, FXR-α also participates in the regulation of glucose, lipoprotein and lipid metabolism in the liver as well as a suppressor of inflammation in the liver and intestines (6). FXR null mice rapidly develop liver cancer suggesting FXR is a tumor suppressor (50, 51). Bile acids and FXR participate in the regulation of the level and composition of the intestinal microbiome by regulating antibacterial defenses in the small intestines and by direct effects on gut bacteria (52, 53).

TGR5, also referred to as membrane-type bile acid receptor (M-BAR), was the first GPCR to be reported to be activated by bile acids in the order LCA>DCA>CDCA>CA(54). TGR5 is a $G\alpha_s$ type receptor which activates adenyl cyclase activity increasing the rate of the synthesis of c-AMP (55). TGR5 is widely expressed in human tissues including: intestinal neuroendocrine cells, gall bladder, spleen, brown adipose tissue, macrophages and cholangiocytes, but not hepatocytes (55). TGR5 may play a role on various physiological processes in the body. TGR5 appears to be important in regulating energy metabolism. It has been postulated that bile acids may activate TGR5 in brown adipose tissue, activating type 2 iodothyroxine deiodinase, leading to increased levels of thyroid hormone and stimulation of energy metabolism (56). Moreover, TGR5 has been reported to promote the release of glucagon-like peptide-1 release from neuroendocrine cells which increases insulin release in the pancreas (57). These results suggest that TGR5 may play a role in glucose homeostasis in the body. TGR5 is a potential target for drug development for treating type 2 diabetes and other metabolic disorders (55, 58).

Taurine conjugated bile acids have been reported to activate specific muscarinic receptors (59). There are five different muscarinic receptors (M_1 to M_5) which are differentially expressed in various tissues in the body. Lithocholytaurine has been reported to activate the M_3 muscarinic receptor on gastric chief cells stimulating the secretion of pepsinogen (60). Muscarinic receptors may play an important role in colon cancer. Muscarinic receptors are over expressed in colon cancer cells and activation stimulates proliferation, migration and invasion (61). Moreover, activation of muscarinic receptors stimulates matrix metalloproteinase 1-dependent invasion of colon cancer cells (62). However, the role of

taurine conjugated bile acids in activating muscarinic receptors to promote colon cancer is unclear as most bile acids found in feces are unconjugated.

6. Interplay of Sphingosine 1-phosphate Receptor 2, Insulin and FXR in Regulating Hepatic Metabolism

Both unconjugated and conjugated bile acids activate the insulin signaling (AKT) and ERK1/2 pathways in hepatocytes (63). Interesting, insulin and bile acids both activated glycogen synthase activity to a similar extent in primary rat hepatocytes. Moreover, the addition of both insulin and bile acids to the culture medium resulted in an additive effect on activation of glycogen synthase activity in primary hepatocytes. Infusion of taurocholate (TCA) into the chronic bile fistula rat rapidly activated the AKT and ERK1/2 signaling pathway and glycogen synthase activity (63). In addition, there was a rapid down-regulation of the gluconeogenic genes, PEP carboxykinase (PEPCK) and glucose-6-phophatase (G-6-Pase) and a marked up-regulation of SHP mRNA in these sample livers (64). These results suggest that TCA functions much like insulin to regulate hepatic glucose metabolism both *in vitro* and *in vivo*.

Conjugated bile acids activate the insulin signaling (AKT) and ERK 1/2 pathways via a pertussis toxin sensitive mechanism in primary hepatocytes and *in vivo* (63, 64). These results suggest that conjugated bile acids activate a $G\alpha_i$ coupled GPCR in hepatocytes. The addition of pertussis toxin or expression dominant negative $G\alpha_i$ in primary rat hepatocytes blocked the ability of TCA to activate the AKT signaling pathway and to down-regulate PEPCK and G-6-Pase. Surprisingly, inhibiting PI_3 kinase with Wortmannin, but not an AKT or ERK1/2 chemical inhibitor, markedly decreased the ability of TCA to induce SHP mRNA. Moreover, inhibiting PKCζ with a chemical inhibitor or shRNA markedly inhibited TCA induction of SHP and the bile salt exporter (ABCB11), both FXR target genes (64). PKCζ is activated by phosphoinositide-dependent protein kinase-1 (PDK-1), a branch pathway of the insulin signaling pathway. Chemical inhibitors of other isoforms of PKC had no effect on the induction of SHP by TCA in primary hepatocytes. It has been reported that PKCζ phosphorylates FXR-α (65) and may allow for its activation of target gene expression. In contrast, phosphorylation of FXR-α by AMPK inhibits the ability of FXR to induce target genes (66). PKCζ has been reported to be important for the translocation of the bile acid transporters NTCP (SLC10A1) and BSEP (ABC B11) to the basolateral and canalicular membranes, respectively (65, 67). Finally, it has been recently reported that PKCζ phosphorylates SHP allowing both to translocate to the nucleus and down-regulate genes via epigenetic mechanisms (68). In total, these results all suggest that the insulin signaling pathway is an important regulator of FXR-α activation and bile acid signaling in the liver (Figure 3).

The identity of the major $G\alpha_i$ GPCR activating the AKT and ERK1/2 signaling pathway in primary hepatocytes was recently reported to be the S1PR2 by screening lipid-activated GPCRs expressed in HEK-293 cells. All conjugated bile acids tested activated the S1PR2. Moreover, JTE-013, a S1PR2 chemical antagonist or a recombinant lentivirus encoding S1PR2 shRNA markedly inhibited the activation of ERK1/2 and AKT in primary hepatocytes and in the chronic bile fistula rat (69).

The activation of the insulin signaling pathway and FXR-α appear to collaborate in the coordinate regulation of glucose, bile acid and lipid metabolism in the liver. SHP, an FXR target gene, is an important pleotropic regulator of multiple metabolic pathways in the liver (Figure 3). The S1PR2 appears to be an important regulator of hepatic lipid metabolism as S1PR2 null mice rapidly (2 weeks) develop overt fatty livers on a high fat diet as compared to wild-type mice (unpublished data). It is well established that inflammation and the synthesis of inflammatory cytokines i.e. TNFα inhibit insulin signaling by activation of the JNK 1/2 signaling pathway which phosphorylates insulin receptor substrate 1 (70). Inflammation is believed to be an important factor in the development of type 2 diabetes and fatty liver disease. A Western diet is correlated with low grade chronic inflammation and insulin resistance. Inhibition of the insulin signaling pathway may decrease the ability of bile acids to activate FXR-α, induce SHP and other FXR target genes leading to an increased risk of fatty liver and non-alcoholic fatty liver disease (NAFLD) (55).

6.1 Conjugated bile acids stimulate cholangiocarcinoma growth via the S1PR2

Cholangiocarcinoma (CCA) is an often fatal cancer of the biliary tract and its occurrence is associated with chronic cholestasis and elevation of conjugated primary bile acids in the liver and serum. Bile duct obstruction has been reported to promote the growth of CCA. *In vitro*, conjugated but not unconjugated bile acids promote the growth of CCA (71, 72). In recent studies, Lui R *et al*. reported that the S1PR2 is highly expressed in both rat and human CCA lines and in human CCA tissues (73). TCA activated the AKT and ERK1/2 signaling pathways in both human and rat CCA cells in culture. Moreover, TCA stimulated CCA cell proliferation, migration and invasion was strongly inhibited by JTE-013, a chemical antagonist of the S1PR2, and by a lentiviral shRNA directed against S1PR2. Finally, FXR-α and ASBT were significantly down-regulated in CCA as compared to normal cholangiocytes. These data suggest that conjugated bile acids promote the invasive growth of CCA via the S1PR2. S1PR2 and FXR may represent new targets for treating CCA.

7. Summary and Future Directions

There appears to be extensive interplay between bile salts and insulin signaling in the regulation of nutrient metabolism in both the intestines and liver. Bile salts play a key role in the solubilization and absorption of nutrients from the intestines. The absorption of nutrients stimulates the secretion of insulin from the pancreas. Moreover, bile acids may also stimulate the secretion of insulin by activating TGR5 in intestinal neuroendocrine cells resulting in the secretion of glucagon-like peptide-1. In the liver, bile salts and insulin both activate the AKT and ERK1/2 signaling pathways which yields a stronger signal than either alone. The activation of PKCζ, a branch of the insulin signaling pathway, is required for the optimal induction of FXR target genes and the regulation of the cellular location of bile acid transporters (Figure 3).

Perhaps an important future direction of bile acid signaling research would be to determine to what extent inflammation affects bile acid signaling and hepatic metabolism. Moreover, it is currently unclear what physiological role activation of the ERK1/2 by bile acids and insulin has on hepatic nutrient metabolism. The elucidation of epigenetic mechanisms of

gene regulation in the liver by bile acids may be important for regulating nutrient metabolism during the feed/fast cycle. Finally, the role of the S1PR2 and conjugated bile acids in the growth of cholangiocytes and CCA is another important direction for research in this area. The study of bile acids as hormones continues to be a growing and expanding field of biomedical research.

Acknowledgments

List of Abbreviations

ASBT	apical sodium dependent transporter
AKT	protein kinase B
BSEP	bile salt export protein (ABCB11)
CA	cholic acid
CCA	cholangiocarcinoma
CDCA	chenodeoxycholic acid
CYP7A1	cholesterol 7α-hydroxylase
CYP7B1	oxysterol 7α-hydroxylase
CYP27A1	sterol 27-hydroxylase
CYP8B1	12α-hydroxylase
DCA	deoxycholic acid
EGFR	epidermal growth factor receptor
ERK1/2	extracellular signal-regulated kinase
FGF15/19	fibroblast growth factor 15/19
FXR	farnesoid x receptor
G-6-Pase	glucose-6-phosphatase
GCA	glycocholic acid
GDCA	glycodeoxycholic acid
GPCR	G-protein coupled receptor
HNF4a	hepatocyte nuclear factor 4
LCA	lithocholic acid
LRH-1	liver-related homolog-1
LXR	liver X receptor
M1–5	muscarinic receptor 1–5

NAFLD	non-alcoholic fatty liver disease
NTCP	sodium taurocholate cotransporting polypeptide
P13K	phosphatidylinositol-3-kinase
PEPCK	PEP carboxykinse
PXR	pregnane X receptor
S1P	sphingosine 1-phosphate
S1PR2	sphingosine 1-phosphate receptor 2
SHP	small heterodimer partner
TCA	taurocholate

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Highlights

- Bile acids are important signaling molecules.

- Bile acids can activate nuclear receptors and GPCRs.

- Bile acid-mediated signaling pathways play important roles in lipid and glucose metabolism.

- Dysregulation of bile acid-mediated signaling pathways contributes to various metabolic diseases.
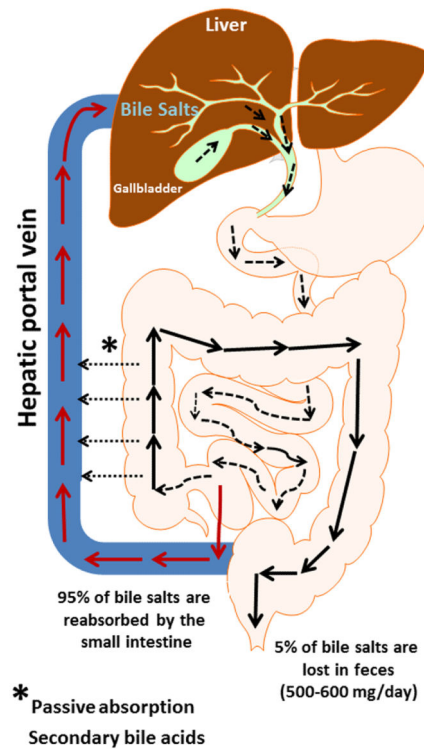
Figure 1. Enterohepatic circulation of bile acids
Bile acids are synthesized and conjugated mainly to glycine or taurine in hepatocytes. Bile acids travel to the gall bladder for storage during the fasting state. During digestion, bile acids travel to the duodenum *via* the common bile duct. 95% of the bile acids delivered to the duodenum are absorbed back into blood within the ileum and circulate back to the liver through the portal vein. 5% of bile acids are lost in feces.
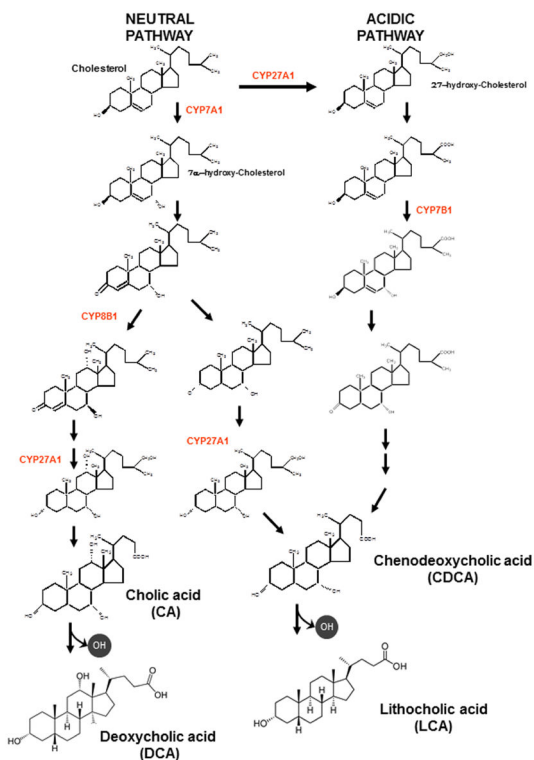
Figure 2. Biosynthetic pathways of bile acids

Two major pathways are involved in bile acid synthesis. The neutral (or classic) pathway is controlled by cholesterol 7α-hydroxylase (CYP7A1) in the endoplasmic reticulum. The acidic (or alternative) pathway is controlled by sterol 27-hydroxylase (CYP27A1) in mitochondria. The sterol 12α-hydroxylase (CYP8B1) is required to synthesis of cholic acid (CA). The oxysterol 7α-hydroxylase (CYP7B1) is involved in the formation of chenodeoxycholic acid (CDCA) in acidic pathway. The neutral pathway is also able to form CDCA by CYP27A1.
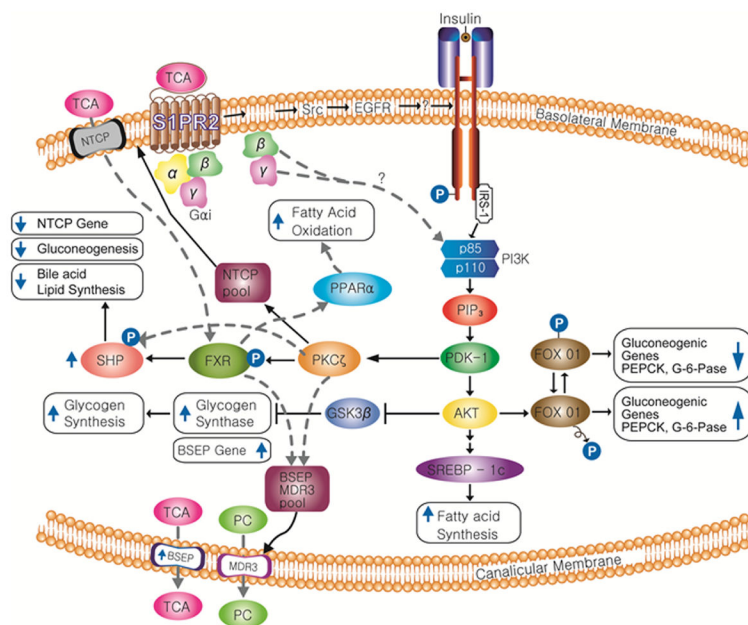
Figure 3. Interrelationship between sphingosine 1-phosphate receptor 2 and the insulin signaling pathway in regulating hepatic nutrient metabolism

S1PR2, sphingosine 1-phosphate receptor 2; Src, Src Kinase; EGFR, epidermal growth factor receptor; PPARα, peroxisome proliferator-activated receptor alpha; NTCP, Na+/taurocholate cotransporting polypeptide; BSEP, bile salt export pump; PC, phosphotidylcholine; PECK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; PDK1, phosphoinositide-dependent protein kinase 1; AKT, protein kinase B; SREBP, sterol regulatory element-binding protein; PKCζ, protein kinase C zeta; FXR, farnesoid x receptor; SHP, small heterodimeric partner; MDR3, phospholipid transporter (ABCB4); GSK3β, glycogen synthase kinase 3 beta.