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Bile Acids, Gut Microbiome and the Road to Fatty Liver Disease

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Abstract

This article describes the complex interactions occurring between diet, the gut microbiome, and bile acids in the etiology of fatty liver disease. Perhaps 25% of the world's population may have nonalcoholic fatty liver disease (NAFLD) and a significant percentage (~20%) of these individuals will progress to nonalcoholic steatohepatitis (NASH). Currently, the only recommended treatment for NAFLD and NASH is a change in diet and exercise. A Western-type diet containing high fructose corn syrup, fats, and cholesterol creates gut dysbiosis, increases intestinal permeability and uptake of LPS causing low-grade chronic inflammation in the body. Fructose is a "lipogenic" sugar that induces long-chain fatty acid (LCFA) synthesis in the liver. Inflammation decreases the oxidation of LCFA, allowing fat accumulation in hepatocytes. Hepatic bile acid transporters are downregulated by inflammation slowing their enterohepatic circulation and allowing conjugated bile acids (CBA) to increase in the serum and liver of NASH patients. High levels of CBA in the liver are hypothesized to activate sphingosine-1-phosphate receptor 2 (S1PR2), activating pro-inflammatory and fibrosis pathways enhancing NASH progression. Because inflammation appears to be a major physiological driving force in NAFLD/NASH, new drugs and treatment protocols may require the use of anti-inflammatory compounds, such as berberine, in combination with bile acid receptor agonists or antagonists. Emerging new molecular technologies may provide guidance in unraveling the complex physiological pathways driving fatty liver disease and better approaches to prevention and treatment.

Introduction

Nonalcoholic fatty liver disease (NAFLD) is becoming a global epidemiological problem, which affects about 25% of the adult population (58). Progression of NAFLD to nonalcoholic steatohepatitis (NASH) is among the top etiologies for cirrhosis and hepatocellular carcinoma (HCC) (89). The imbalance between lipid uptake or *de novo* synthesis and lipid secretion results in excessive lipid accumulation in hepatocytes. It has been well recognized that inflammation is the key driving force of NAFLD to NASH

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progression (4). NAFLD/NASH is often associated with obesity, insulin resistance, type 2 diabetes (T2DM), and metabolic syndrome (90). However, its pathogenesis remains incompletely understood.

In the last few decades, scientists have recognized the human body as a complex ecosystem of interacting prokaryotic and eukaryotic cells. The adult human body consists of approximately 10^{13} mammalian cells and 2 to 5×10^{13} prokaryotic cells that colonize the human body at different sites (77). Moreover, the microbial metagenome is much greater than the human genome as the microbiome represents approximately 99% of the functional genes in the body (68). Elucidating the host-microbe interactions is very important in developing strategies to prevent and treat very common diseases of the liver and gastrointestinal tract (GI). Quantitatively, most of the bacteria associated with the human body are found in the GI tract, especially the colon that contains one of the most densely populated natural ecosystems known. In recent years, using the 16S rRNA gene, the most invariant bacterial gene, and high-throughput sequencing, scientists have a much better understanding of the diversity of the human gut microbiome. It has been estimated that the human colon contains at least 150 to 200 “phylotypes” at any given time. Bacterial 16S rRNA genes sharing >97% to 99% identity are generally referred to as a “phylotype” or operational taxonomic unit (OTU) (44). There have been identified six divisions/phyla of bacteria in the human colon. Two major divisions, the *Bacteroidetes* (Gram-negative anaerobes) and *Firmicutes* (Gram-positive anaerobes) represent greater than 90% of the total species of gut microbiota. Moreover, bacterial diversity in the colon ecosystem is almost entirely due to changes at lower taxonomic levels, that is, genus, species, and strains (24, 68).

The Role of Diet in Regulating Gut Microbiota and Inflammation

The composition of the human gut microbiota can be altered by diet, bile acids, liver diseases, antibiotics, gender, age, intestinal transit time, and numerous other factors. Dysbiosis of the gut microbiota can enhance the pathophysiology of several chronic diseases, including liver and GI diseases (71). The gut microbiota can utilize both endogenous and exogenous substrates for growth. Endogenous substrates include greater than 100g wet weight of sloughed intestinal cells per day as well as bile components. Host antigens may select for gut bacteria that are capable of degrading these as a source of carbon and energy. In this regard, Hoskins and Boulding (34) reported that fecal samples from blood group B individuals had 5×10^{4} higher levels of bacteria capable of degrading B antigens compared to individuals with A or O blood groups. Exogenous or dietary substrates can be quite variable depending upon the type of diet consumed, which include Western, Mediterranean, Paleolithic, Vegetarian, Ketogenic as well as hybrid forms of each diet (27). Alteration of the structure of the human gut microbiome by changes to either plant-based or animal-based diets can occur within 24 to 48h postconsumption (17). A study by O’Keefe et al. (64) showed that switching the diets of African Americans (Western diet) with rural South Africans (plant-based diet high in fiber and resistant starch) for 2 weeks resulted in a rapid change in the gut microbiota for each group. African Americans on a Western diet are at high risk for colon cancer, while rural South Africans have a very low risk for colon cancer. The results showed that Ki67, a mucosal cell proliferative

marker, and the fecal metabolome markedly changed in each group. African Americans showed a decrease in Ki67 in intestinal cells, secondary bile acids, deoxycholic acid (DCA), and lithocholic acid (LCA), and an increase in fecal butyric acid on the rural South African diet. The rural South Africans showed the opposite effects on the Western diet. Western-type diets containing high fructose corn syrup (HFCS), saturated fats, and cholesterol and low in complex carbohydrates also rapidly altered the gut microbiota composition, intestinal barrier function, and immune system (22, 74). Western-type diets have been reported to increase intestinal permeability with increased absorption of bacterial components, such as lipopolysaccharides (LPS) and lipoteichoic acids, which enhance the synthesis of pro-inflammatory cytokines and cyclooxygenase-2 (COX2) induction through toll-like receptor-4 (TLR4) and toll-like receptor-2 (TLR2), respectively, in the intestines and liver (51). Western diets create a low-grade chronic inflammation in the intestine and liver and systemically drive the pathophysiology of numerous chronic diseases (22, 74).

Western Diets and Hepatic Sugar Metabolism

During the last 40 years, there have been major changes in the dietary habits of individuals in the United States and worldwide, with associated increases in the numbers of overweight and obese individuals. One of the significant dietary changes was the wide-scale introduction of HFCS into diets in the early 1980s and beyond as well as increased long-chain saturated fatty acids (LCSF). Farmers have known for centuries that feeding corn to animals resulted in weight gain. HFCS is a liquid mixture of fructose (50%–65%) and the remainder glucose (65). Fructose is a “lipogenic” sugar in humans and other animals, and its transport and metabolism in the liver are mediated by different pathways compared to glucose (32). Fructose metabolism in the liver stimulates the synthesis of long-chain fatty acids (LCFA) *via* induction of sterol regulatory element-binding protein 1C (SREBP-1c), which induces genes encoding enzymes in the fatty acid biosynthesis pathway. The pathway of fructose metabolism in the human liver is mostly unregulated as compared to glucose metabolism. Once in the liver, fructose is phosphorylated by fructokinase C at carbon 1 producing fructose-1-phosphate, which is then cleaved to dihydroxyacetone phosphate and glyceraldehyde by aldolase B. Because fructose is rapidly metabolized with consumption of ATP, excess uric acid is formed through activation of AMP deaminase and is further catabolized to uric acid (19). The generation of uric acid may further enhance inflammation and fat accumulation in the liver. The combination of increased LCSF synthesis in the liver and decreased oxidation due to increased inflammation contributes to fatty liver disease.

LCSF, such as palmitate, has been reported to activate cellular stress pathways and the induction of JNK-dependent hepatocyte apoptosis (55). Moreover, LCSF stimulates the release of extracellular vesicles from hepatocytes containing tumor necrosis factor-related apoptosis-inducing ligand that induces the expression of pro-inflammatory cytokines IL1-β and IL-6 in macrophages (33). Inflammatory cytokines, such as TNF-α, can quench insulin signaling by activating the JNK signaling cascade by phosphorylating insulin receptor substrate 1 (2). The accumulation of fat in the liver, with enhanced inflammation, over time can lead to NAFLD and up to 20% of these individuals go on to develop NASH (39).

Enterohepatic Circulation of Bile Acids

Bile acids are synthesized from free cholesterol in liver hepatocytes. The major bile acids biosynthetic pathway, termed the classical pathway, starts with the 7α-hydroxylation of cholesterol catalyzed by cholesterol 7α-hydroxylase (CYP7A1), located in the smooth ER (36, 93). In humans, this pathway leads to the synthesis of cholic acid (CA, 3α,7α,12α-trihydroxy-5β-cholan-24-oic acid) and chenodeoxycholic acid (CDCA, 3α,7α-dihydroxy-5β-cholan-24-oic acid) *via* a multistep pathway (36). The alternative pathway of bile acid synthesis begins in the mitochondria with the 27-hydroxylation of cholesterol catalyzed by cholesterol 27-hydroxylase (CYP27A1). This pathway is believed to form mostly CDCA and may generate important regulatory oxysterols (70). Bile acids are conjugated at the 24-carboxyl group to either glycine or taurine before active transport from the hepatocyte primarily by the canalicular bile salt export protein [BSEP, ATP-binding cassette subfamily B, member 11 (ABCB11)] along with cholesterol (ABCG5/G8, ATP-binding cassette subfamily G, member 5/8), phosphatidylcholine (ABCB4, ATP-binding cassette subfamily B, member 4), conjugated bilirubin (multidrug resistance protein 2, MRP2), and other metabolites (93). Biliary bile components are stored in the gallbladder and released into the small bowel following a meal. Conjugated bile acids (CBA) function in the small bowel to promote the absorption of cholesterol, LCFA, as well as fat-soluble vitamins A, D, E, and K by forming mixed micelles that promote uptake by enterocytes. Bile acids move down the GI *via* gut peristalsis and are actively transported by ileal enterocytes by the apical sodium-dependent bile acid transporter (ASBT) (18, 93). Once inside ileal enterocytes, bile acids activate the farnesoid X receptor (FXR), upregulating the gene encoding fibroblast growth factor 15 (FGF-15) in mice, fibroblast growth factor 19 (FGF-19) in humans (76). Intracellular bile acids are transported into the portal blood by the heterodimeric organic solute transporter (OST), OSTα-OSTβ, a facilitated diffusion transporter on the basolateral membranes of ileal enterocytes. Bile acids and FGF-15/19 are transported to the liver *via* the portal vein. Bile acids are taken up by hepatocytes, primarily by the Na^+-taurocholate co-transporting polypeptide (NTCP). In addition, the basolateral multidrug resistance-associated proteins (MRP3 or ABCC3 and MRP4 or ABCC4) and OSTα-OSTβ are involved in ATP-dependent bile acid export from hepatocytes to systemic circulation (42). FGF-15/19 binding to fibroblast growth factor receptor 4 (FGFR4) on hepatocytes activates the extracellular signal-regulated kinases (ERK) signaling cascade and downregulates CYP7A1, the rate-limiting enzyme in the classical bile acid synthesis pathway (Figure 1). In this manner, the enterohepatic circulation of bile acids helps to maintain homeostatic bile acid synthesis rates, glucose, lipid, energy metabolism as well as the immune system (13).

During the enterohepatic circulation of bile acids, several hundred milligrams of bile acid enter the colon, where gut bacteria can biotransform bile acids into a variety of metabolites (72, 73). However, the two most important “gateway” biotransformations are catalyzed by bile salt hydrolases (BSH) and the multistep bile acid 7α-dehydroxylation pathway (7α-DeOH) (Figure 2) (72). Hydrophobic secondary bile acids can be absorbed from the large bowel *via* passive diffusion and transported to the liver *via* the portal vein. The levels of DCA in biliary bile can be quite high (>50%) in some individuals as the human liver is not capable of the 7α-hydroxylation of DCA reforming CA, as is the case with rodents (72).

Bile acids are major regulators of the structure of the gut microbiome. Resistance to bile acids is a major selective pressure regulating the gut microbiome structure. Bile acids may alter the structure of the human gut microbiome in a variety of ways: (i) deconjugation of bile salts increases the hydrophobicity and alters the chemical properties of individual bile acids. Unconjugated bile acids are generally more toxic to gut bacteria than CBA (72). A small population of gut bacteria belonging to the genus *Clostridium* is able to convert the unconjugated primary bile acids, CA and CDCA, into DCA and LCA, respectively. DCA is reported to be up to 10-time more toxic to some gut bacteria than CA, probably because it disrupts bacterial cytoplasmic membrane structure and function (15). The epimerization of the 3α>3β-hydroxyl group of DCA by gut bacteria decreases the toxicity of this bile acid by decreasing hydrophobicity (21) (Figure 2). (ii) Bile acids have been reported to inhibit the growth and translocation of bacteria in the small bowel *via* an FXR-dependent mechanism involving the secretion of antibacterial peptides (37). This probably has a stronger effect on the composition of mucosal-associated gut microbiota than those found in the lumen of the intestine. (iii) Certain gut bacteria including, *Clostridium scindens* and other species of 7α-DeOH gut bacteria, secrete antibiotics or antibacterial compounds (40). These antibacterial compounds may be important regulators of the gut microbiome structure along with secondary bile acids. In this regard, feeding CA to rats shifted the gut microbiome from a approximately 1:1 ratio of *Firmicutes/Bacteroidetes* to >98% *Firmicutes* (38), and feeding CA to mice increased levels of bile acid 7α-DeOH bacteria approximately 1000-fold (72). Moreover, feeding a high-fat diet (HFD) containing fructose and cholesterol increases the *Firmicutes/Bacteriodetes* ratio, possibly due to increased bile acid loss into the colon.

Effects of Fatty Liver Disease on Hepatic Bile Acid Synthesis, Transporters, and Serum Bile Acid Levels

Changes in rates of primary bile acid synthesis in the liver, formation of secondary bile acids by gut bacteria, induction of FGF-15/19 in the intestines, and increase of the serum bile acid levels, may be important indicators of pathophysiological processes occurring in the liver-gut-microbiome axis due to fatty liver disease. The downregulation of hepatic NTPC by LPS was reported more than 20 years ago (81). Moreover, in a rat liver model of obstructive cholestasis, there was a downregulation of BSEP and NTCP in periportal hepatocytes due to induction of TNFα and IL-1β (23). The downregulation of BSEP is hypothesized to be primarily due to the negative effects of TNF-α and IL-1β on the interaction of FXR:RXR (retinoid x receptor) heterodimer that activates the BSEP promoter (26). The OSTα/β is a heterodimeric solute transport protein located in the basolateral membranes of liver and intestinal epithelial cells (8). OSTα/β was found to be significantly upregulated in liver tissues from NASH and primary biliary cholangitis (PBC) patients. Upregulation of OSTα/β is considered a physiological marker for cholestatic liver disease (56). However, levels of OSTα/β mRNA or protein have not been measured in NAFLD patients. Downregulation of NTCP and other hepatocyte uptake transporters by inflammation may increase the level of serum bile acids. In this regard, Puri et al. (67) identified and quantitated serum bile acids in biopsy-proven NAFLD and NASH patients and compared them to healthy controls. The results showed a significant increase in total conjugated primary bile acids and decreased secondary bile acids in NASH patients compared to NAFLD patients and controls. There

was a stepwise increase in total serum bile acids from controls to NAFLD to NASH patients. The increase in serum CBA was associated with higher grades of steatosis, lobular and portal inflammation, and hepatocyte ballooning, correlating a role of increased serum bile acids with pathophysiological effects in the liver. Additional studies by Lake et al. (43) suggest that in NASH patients, there is a shift to more bile acid synthesis *via* the alternative pathway with upregulation of oxysterol 7α-hydroxylase (CYP7B1) and increased taurine conjugated primary bile acid. Studies by Mouzaki et al. (60) showed that NASH patients had significantly higher levels of total fecal unconjugated primary bile acids as compared to controls. Moreover, levels of the bile acid biosynthesis serum marker, 7α-hydroxy-4-cholesten-3-one (C4) was significantly upregulated in NASH patients; however, FGF-19 levels were not significantly different. Mouse models of NASH also show increases in hepatic inflammation markers, downregulation of bile acid transporters, except OSTβ, key genes involved in bile acid synthesis, and an increase in serum total conjugated primary bile acids, especially taurocholate (TCA) (80, 83) (Figure 3).

Possible Role of Sphingosine1-phosphate Receptor 2 in NASH Progression

Studer et al. (79) first reported that CBA activate ERK1/2 and protein kinase B (AKT) signaling pathways through sphingosine-1-phosphate receptor 2 (S1PR2) in rodent hepatocytes. The activation of ERK1/2 and AKT by TCA in primary rat hepatocytes or in the chronic bile fistula rat was inhibited by JET-013, a chemical S1PR2 antagonist. Moreover, S1PR2 shRNA markedly inhibited ERK1/2 and AKT activation by TCA in primary rat hepatocytes. Additional studies discovered that S1PR2 activated nuclear sphingosine kinase 2 (SphK2) in mouse hepatocytes and the chronic bile fistula rat (61). Sphingosine-1-phosphate (S1P) has been shown to be an endogenous inhibitor of histone deacetylases 1 and 2 that regulate levels of histone acetylation and gene expression (30). SphK2 has been reported to be a key regulator of genes involved in LCFA metabolism, and S1PR2$^{-/-}$ and SphK2$^{-/-}$ mice rapidly developed fatty livers on (61, 62). SphK2 is significantly downregulated in a mouse model of NASH on an HFD (83). S1PR2 appears to be an important bile acid sensor during the feeding and fasting cycle and is activated by CBA returning from the intestines following a meal. CBAs secreted from the liver are stored in the gallbladder during fasting. The decreased levels of hepatic CBA cause the inactivation of S1PR2. In contrast, there is evidence that constant activation of S1PR2 results in the induction of pro-inflammatory and proliferative signaling pathways in cells in the liver that may occur during cholestasis. In this regard, TCA promoted cholangiocarcinoma cell growth and cyclooxygenase 2 expression *via* S1PR2 [Figure 3, (35, 49, 50)]. Our recent studies also showed that the long noncoding RNA H19 activates pro-inflammatory and fibrotic markers in cirrhotic liver and bile duct ligated mice. In the multidrug resistance 2 knockout (Mdr2$^{-/-}$) mouse that serves as a model of cholestatic cholangiopathies, TCA induced expression of H19 and fibrotic genes *via* S1PR2 (46). The increased serum levels of CBA, especially TCA, have been reported in NASH patients and may activate S1PR2 in cholangiocytes and other liver cells to activate pro-inflammatory pathways and fibrotic gene expression, accelerating fatty liver pathogenesis. Finally, other studies reported that CBAs promote the growth of esophageal adenocarcinoma cells, activation of YAP and β-catenin signaling pathways *via* S1PR2 (48). It is unknown if S1PR2 and CBAs might play a role in HCC development.

Liver Disease and Gut Dysbiosis

A comparison of the gut microbiota in individuals with a healthy liver, NAFLD, NASH, and cirrhosis shows an increase in gut dysbiosis with advancing disease (7). Most studies show a decrease in gut microbiota diversity, an increase in bacteria containing LPS, and a decrease in bacteria producing short-chain fatty acids (SCFA), especially butyrate (57). Bile acids, SCFA, and LPS activate different biosynthetic, metabolic, pro-, and anti-inflammatory signaling pathways in host cells. In this regard, bile acids activate specific nuclear receptors (FXR, Vitamin D, PXR) and G-protein coupled receptors (GPR) (TGR5, S1PR2, $M_{2,3}$-muscarinic) while SCFA activates GPR (41 and 43) and propionate and butyrate activate the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ) (3, 75). LPS can bind to TLR-4 to activate pro-inflammatory signaling pathways (14). Western diets are associated with an increase in the risk of obesity, T2DM, NAFLD, and NASH and chronic low-grade inflammation (12). It has been observed that enhanced inflammation associated with HFD is due to the absorption of LPS and stimulation of the synthesis of pro-inflammatory cytokines through activating TLR-4. Higher levels of LPS have been reported in obese individuals compared to healthy controls, possibly due to higher levels of Gram-negative gut bacteria, especially members of the family *Enterobacteriaceae*, and increased intestinal barrier permeability (12, 59). Humans are one of the most sensitive animal species to the pro-inflammatory effects of LPS. Cani et al. (10) have reported that HFD can alter the composition of the gut microbiota, decreasing tight junction proteins, zonula occludens (ZO1) and occludin, allowing increase absorption of LPS. HFD may also increase absorption of LPS *via* the lymphatics by incorporation into chylomicrons in the small bowel. Germ-free animals and those treated with antibiotics are highly resistant to developing fatty liver disease implicating the gut microbiota as an important factor in inducing fatty liver disease (9).

Slowing of the enterohepatic circulation brought on by increased systemic inflammation, downregulating hepatic bile acid transporters may decrease levels of bile acids in the intestine. This may have the effect of altering the gut microbiota as intestinal bile acids are important regulators of the structure of the gut microbiome. Moreover, bile acids are signaling molecules activating anti-inflammatory pathways through activation of FXR and TGR5, as well as increasing the secretion of gut antibacterial peptides (25). Moreover, intestinal bile acids regulate bile acid synthesis in the liver by stimulating the synthesis of FGF-19 in ileal enterocytes *via* activation of FXR (Figure 4) (41).

Studies by Bajaj et al. (7) shows major changes in the stool microbiota composition comparing healthy controls to decompensated cirrhotic patients where there were significant decreases in potentially beneficial autochthonous bacteria including members of families *Lachnospiraceae, Ruminococcaceae,* and *Clostridiales* XIV and increases in potential pathogenic families including *Staphyloccaeae, Enterobacteriaceae,* and *Enterococcaceae*. Qin et al. (68) also observed major changes in cirrhotic patients compared to controls, and gene analysis suggested that many of the gut bacteria were from the oral cavity. Moreover, changes in both oral and stool microbiome in cirrhotic patients were also reported by Bajaj et al. (1, 6). It was discovered that in the salvia of cirrhotic patients, the levels of autochthonous bacterial families decreased and potential pathogenic families

Enterobacteriaceae and *Enterococcaceae* increased. These results might represent a system-wide change in immunity to host microbiota in cirrhotic patients. In patients with cirrhosis, which have a smaller bile acid pool than control patients, there is a loss of bile acid 7α-DeOH gut bacteria and a shift to a more "toxic" Gram-negative gut microbiota (55). When patients with cirrhosis are transplanted with a new liver, there is an increase in bile acid secretion, an increase in fecal secondary bile acid synthesis, and a return to a more "normal" and diverse gut microbiota with less systemic inflammation (33). These results show the intimate connection between bile acids and the liver-gut axis (Figure 5).

Possible Role of Berberine and Other Anti-inflammatory Compounds in Treating Fatty Liver Disease

Berberine is a natural pentacyclic isoquinoline alkaloid present in many plants used in ancient medicine, such as Berberis vulgaris (barberry fruit), goldenseal, Orgon grapes, Coptis chinensis, and has been used in Asia for thousands of years as a folk remedy for various digestive disorders, especially for diarrhea and infectious diseases (86). During the last two decades, berberine has been extensively studied for its beneficial effects on metabolic diseases, including NAFLD and diabetes (6). Numerous studies have reported that berberine has various biological activities, such as anti-inflammatory, lipid-lowering, and an antidiabetic effect (63). Our previous studies in rodent NAFLD models showed the beneficial effects of berberine on preventing NAFLD disease progression is mainly through modulating bile acid metabolism in the gut-liver axis (28). Although the bioavailability of intragastrically administered berberine was much lower than that of intraperitoneally administered berberine, it had a stronger lipid-lowing effect, indicating that the GI is the major functional site of berberine (28). A number of mechanisms have been identified underlying berberine's beneficial effects, such as activating AMP-activated protein kinase (AMPK), inhibiting Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome activation, promoting glucagon-like peptide-1 (GLP-1) secretion, attenuating ER stress and oxidative stress, regulating microRNAs (miRs) (20, 45, 52, 54, 69, 84). Our recent study using the best available diet-induced NASH mouse model showed that berberine significantly prevented NASH disease progression by targeting multiple pathways (5, 83). Bile acid analysis further showed that berberine had a significant impact on bile acid composition and fatty acid metabolism. Since berberine is mainly accumulated in the intestinal tract, our studies suggest that gut microbiota may be the primary target of berberine in modulating metabolic processes. A growing number of studies showed that berberine modulates not only the structure but also the number of gut microbiota (16, 29, 91, 92). A recent study reported that the combination of berberine, tocotrienols and coffee extracts improved metabolic profile and hepatic lipid accumulation in the HFD-feeding mouse NAFLD model via modulating gut microbiota and hepatic miR-122 and miR-34a (16).

Licorice is another ancient medicinal plant with a long history as a remedy for inflammatory diseases and metabolic disorders (31, 53, 87). Our previous study showed that 18β-glycyrrhetinic acid, the major component of licorice root extract, prevented free fatty acid-induced hepatic lipotoxicity *via* modulating lysosomal and mitochondrial functions (85). It also has been reported that glycyrrhetinic acid alleviated hepatic inflammation injury in

viral hepatitis disease *via* the high mobility group box protein 1 (HMGB1)-TLR4 signaling pathway (78). A recent study showed that diammonium glycyrrhizinate exerted its protective effect against HFD-induced NAFLD *via* modulating gut microbiota and preventing HFD-induced disruption of intestinal barrier functions. Diammonium glycyrrhizinate reduced the ratio of Firmicutes-to-Bacteroidetes and increased the levels of SCFA-producing bacteria (47). There is increasing evidence indicating the beneficial effects of polyphenols, alkaloids, and terpenoids from herbal medicine, vegetables, and fruits in regulating lipid, glucose, and energy metabolism *via* different signaling pathways in the liver and modulating gut microbiota (66, 82, 88, 95).

Future Directions

The physiological interactions between diet, gut microbiome, and bile acids in regulating normal physiological and biochemical pathways in the body are just beginning to be elucidated. Dysregulation of these pathways increases the risk of chronic diseases, including fatty liver disease. These are important medical issues as there are no currently accepted medical treatments for fatty liver disease, such as NASH, other than dietary changes and exercise. In this regard, it is currently believed that 25% of the world's population may have NAFLD, of which up to 20% may progress to NASH and perhaps 5% of these individuals may end up with cirrhosis and/or HCC (89). Because dietary habits affect so many physiological and biochemical pathways in the body, treatment protocols for NASH may require multiple approaches and drug combinations (94). For example, the FXR agonist (obeticholic acid) has been used to treat NASH patients but with limited success. However, in the background of enhanced inflammation, cellular levels of FXR may be downregulated. Moreover, phosphorylation of FXR by protein kinase C-zeta (PKC-zeta) is required for optimal activation but may be inhibited by inflammation (11). Therefore, chronic inflammation may alter drug effectiveness requiring an anti-inflammatory compound, such as berberine and other complementary medicines, for optimal treatment results. Fatty liver diseases appear to have complex etiologies, but with the emerging new molecular technologies, there is an opportunity to gain a better understanding of these and improved treatment protocols in the future.

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List of Abbreviations and Acronyms

ABCB11	ATP-binding cassette subfamily B, member 11
ABCG5/G8	ATP-binding cassette subfamily G, member 5/8
AKT	protein kinase B
AMPK	AMP-activated protein kinase

ASBT	apical sodium-dependent bile acid transporter
BSEP	bile salt export protein
BSH	bile salt hydrolases
CA	cholic acid
CBA	conjugated bile acids
CDCA	chenodeoxycholic acid
COX2	cyclooxygenase-2
CYP27A1	cholesterol 27-hydroxylase
CYP7A1	cholesterol 7α-hydroxylase
CYP7B1	oxysterol 7α-hydroxylase
DCA	deoxycholic acid
ERK	extracellular signal-regulated kinases
FGF-15	fibroblast growth factor 15
FGF-19	fibroblast growth factor 19
FGFR4	fibroblast growth factor receptor 4
FXR	farnesoid X receptor
GI	gastrointestinal tract
GLP-1	glucagon-like peptide-1
GPR	G-protein coupled receptors
HCC	hepatocellular carcinoma
HFCS	high fructose corn syrup
HFD	high-fat diet
LCA	lithocholic acid
LCFA	long-chain fatty acids
LCSF	long-chain saturated fatty acids
LPS	lipopolysaccharides
miRs	microRNAs
MPR2	multidrug resistance protein 2
NAFLD	nonalcoholic fatty liver disease

NASH	nonalcoholic steatohepatitis
NLRP3	nod-like receptor family pyrin domain containing 3
NTCP	Na^+-taurocholate co-transporting polypeptide
OST	organic solute transporter
OTU	operational taxonomic unit
PBC	primary biliary cholangitis
PKC-zeta	protein kinase C-zeta
PPARγ	peroxisome proliferator-activated receptor γ
RXR	retinoid x receptor
S1P	sphingosine-1-phosphate
S1PR2	sphingosine-1-phosphate receptor 2
SCFA	short-chain fatty acids
SphK2	sphingosine kinase 2
SREBP-1c	sterol regulatory element-binding protein 1c
T2DM	type 2 diabetes
TCA	taurocholate
TLR2	toll-like receptor-2
TLR4	toll-like receptor-4
ZO1	zonula occludens

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Didactic Synopsis

Major teaching points

- Western-type diets containing high fructose corn syrup, lipids, and cholesterol create chronic inflammation in the body.
- Bile acids are important signaling molecules regulating glucose, lipid, energy metabolism as well as the structure of the gut microbiome.
- Inflammation appears to slow the enterohepatic circulation of bile acids allowing them to increase in serum, enhancing inflammation and fibrotic pathways in the liver.
- Naturally occurring compounds, such as berberine, may be useful in treating fatty liver disease by inhibiting pro-inflammatory pathways arising in the gut.

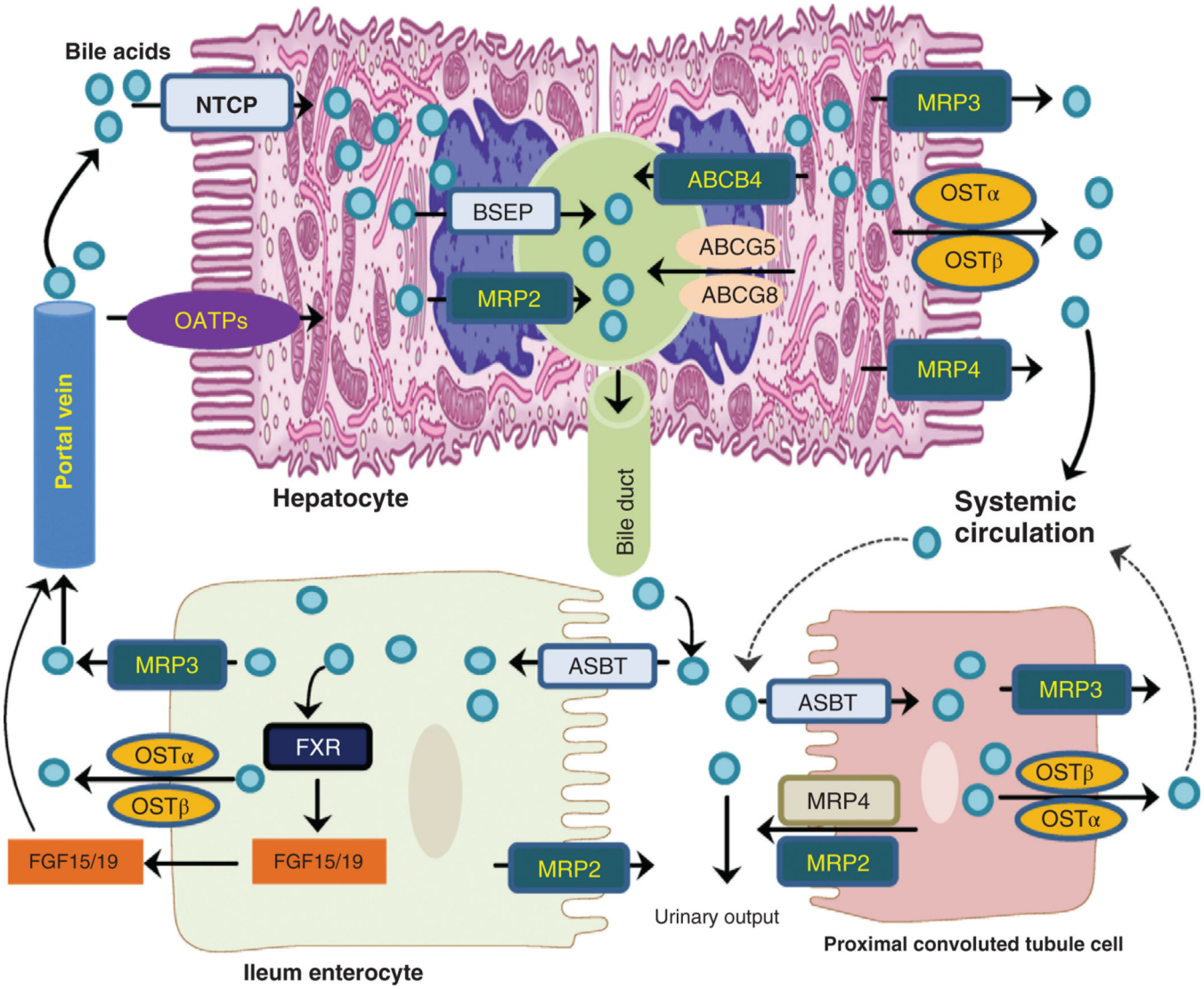

Figure 1. Bile acid transporters in the liver hepatocyte, ileal enterocyte, and proximal convoluted tubule in the kidney.

Bile acids are actively transported from the hepatocytes by the bile salt export protein (BSEP) or multidrug resistance protein 2 (MPR2) into bile duct. After secretion into the intestine, they are efficiently recovered by leal enterocytes using the apical sodium-dependent bile acid transporter (ASBT) and into the portal vein *via* the heterodimeric organic solute transporter (OSTα/β) on the basolateral membrane. Bile acids may also undergo hepatic-renal cycling, especially during cholestasis. Bile acids secreted by the kidney are usually modified by the sulfation of hydroxyl groups. The multidrug resistance-associated proteins (MRP3 or ABCC3 and MRP4 or ABCC4) and OSTα-OSTβ on the basolateral membrane are involved in ATP-dependent bile acid export from hepatocytes to systemic circulation.

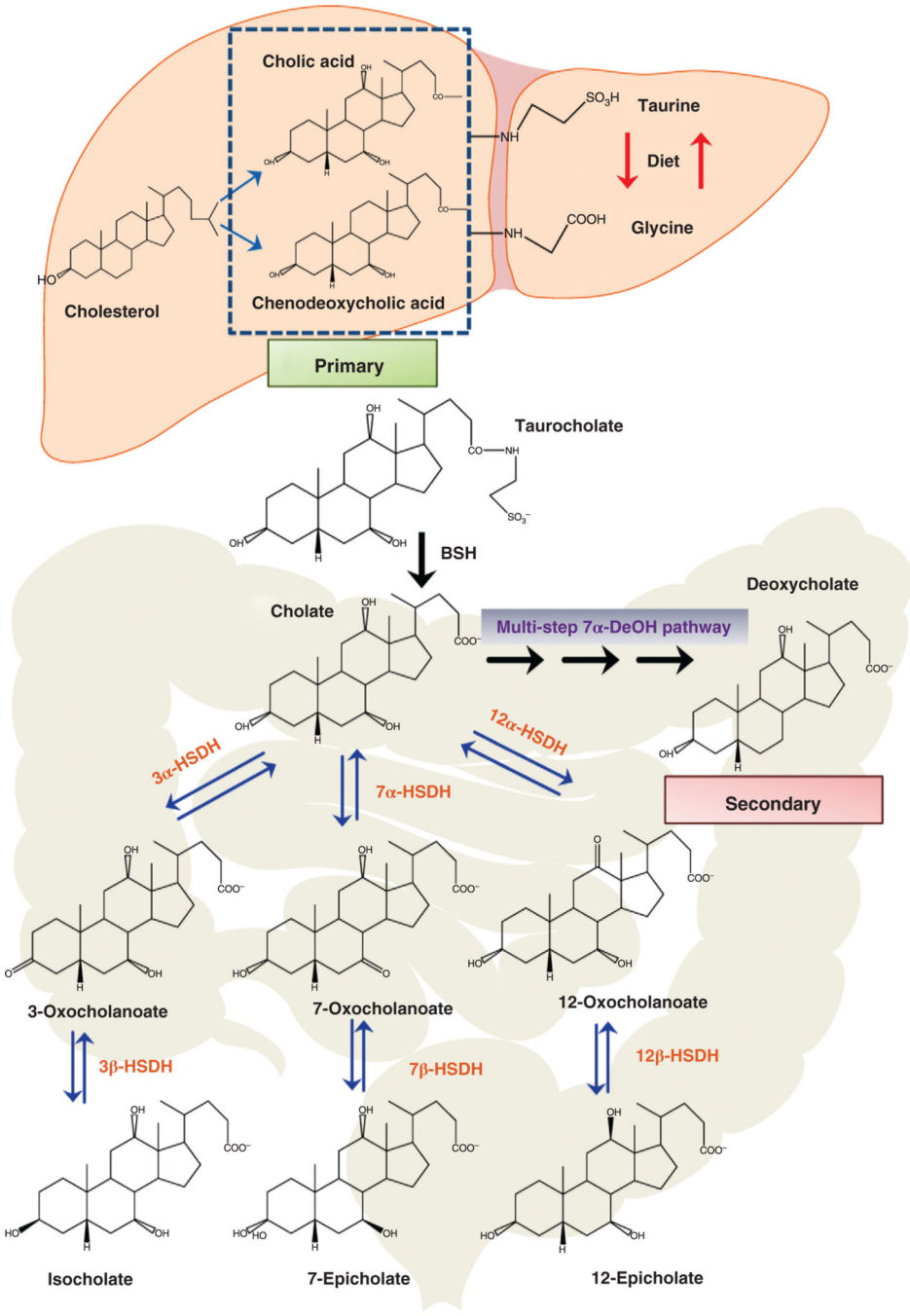

Figure 2. Synthesis of the primary bile acids cholic acid and chenodeoxycholic acid from cholesterol in liver hepatocytes and metabolism by gut bacteria.

The primary bile acids, cholic acid, and chenodeoxycholic acid are synthesized in the hepatocytes from cholesterol and conjugated with taurine or glycine. Taurocholate is biotransformed by gut bacteria expressing bile salt hydrolases (BSH) to cholic acid and taurine. Gut bacteria can oxidize hydroxyl groups at the 3α, 7α, and 12α position on the steroid ring by 3α-hydroxysteroid dehydrogenases (3α-HSDH), 7α-hydroxysteroid dehydrogenases (7α-HSDH), and 12α-hydroxysteroid dehydrogenases (12α-HSDH), respectively. Oxo-bile acids may be further metabolized at the 3β, 7β, or 12β position by 3β-

hydroxysteroid dehydrogenase (3β-HSDH), 7β-hydroxysteroid dehydrogenase (7β-HSDH) and 12β-hydroxysteroid dehydrogenase (12β-HSDH), respectively, producing iso and epi bile acids. Primary bile acids can be biotransformed to secondary bile acids by removing the 7α-hydroxyl group via a multistep 7α-dehydroxylation (7α-DeOH) biochemical pathway found in some species of the genus *Clostridium*.

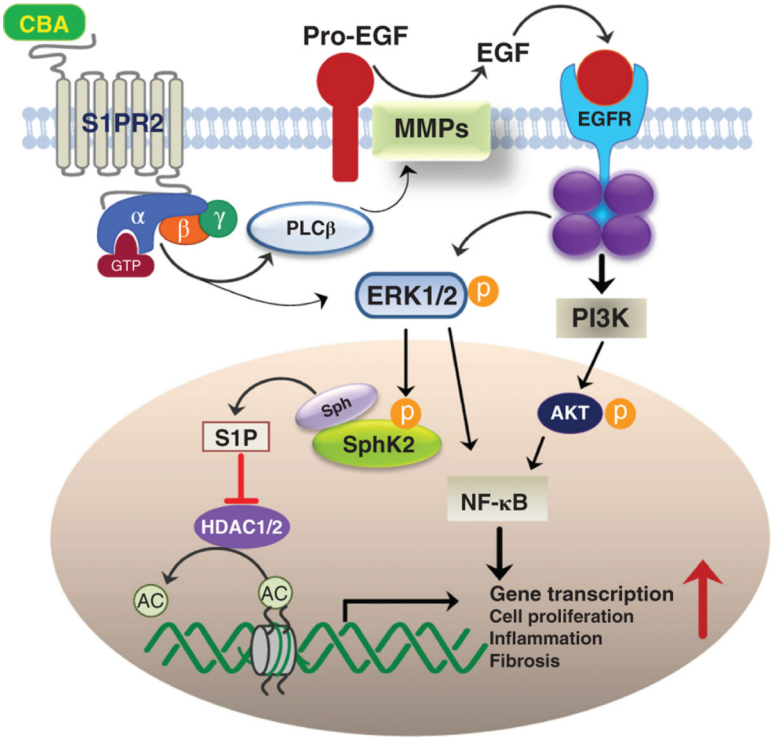

Figure 3. Activation of the ERK1/2 and AKT signaling pathways by conjugated bile acids (CBA) *via* S1PR2.

High levels of CBA activate S1PR2 in liver cells, enhancing the upregulation of genes encoding pro-inflammatory and fibrosis mediators. Phosphorylated ERK1/2 is translocated into the nucleus, where it activates sphingosine kinase 2 (SphK2) *via* phosphorylation. SphK2 produces sphingosine-1-phosphate (S1P) that is a potent inhibitor of histone deacetylase 1 and 2 (HDAC1/2), allowing the epigenetic upregulation of gene transcription.

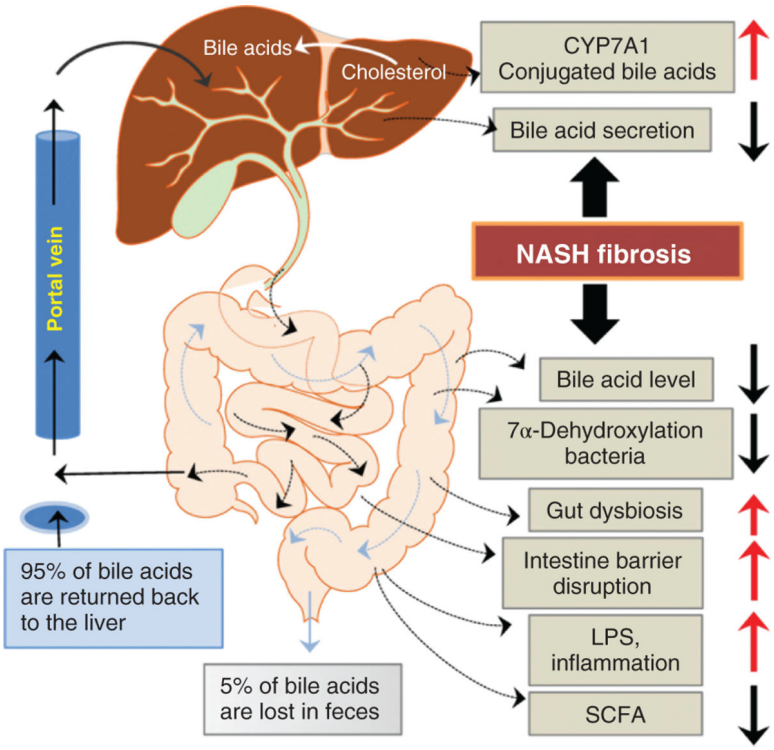

Figure 4. Alteration of the enterohepatic circulation of bile acids by NASH.

Liver diseases interrupt the enterohepatic circulation of bile acids, enhancing gut dysbiosis. This has the downstream effect of increasing gut permeability and absorption of pro-inflammatory mediators such as LPS. In NASH, there is an increase in the serum CBA that may activate the S1PR2 in hepatic cells, enhancing inflammation and activating pro-fibrotic gene expression in stellate cells.

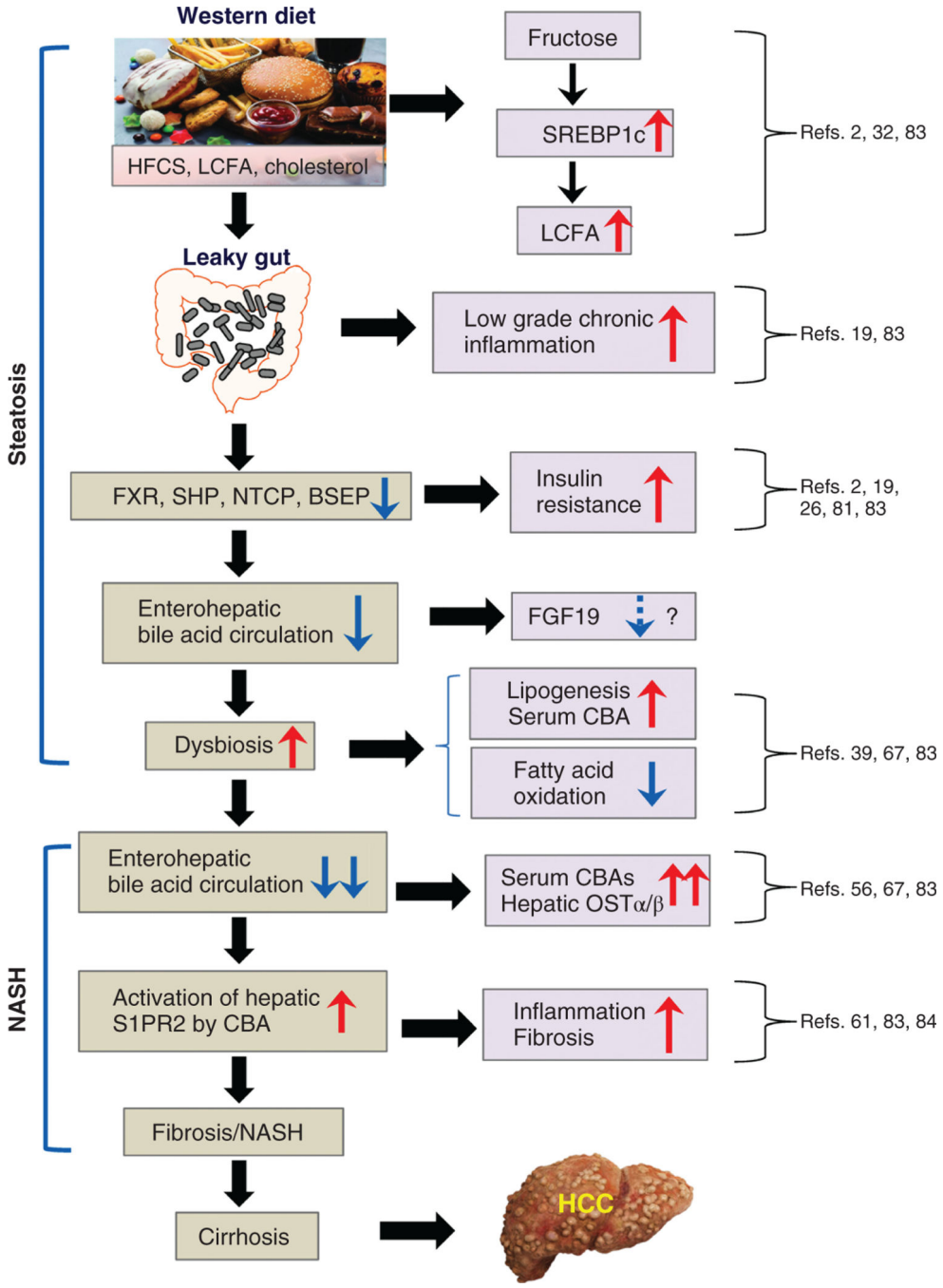

Figure 5. The possible role of diet, gut dysbiosis and bile acids in the development of steatosis and NASH.

The road to Steatosis and NASH begins by consuming a Western-type diet containing large amounts of HFCS and fats, resulting in constant low-grade systemic inflammation due to gut dysbiosis and absorption of pro-inflammatory bacterial molecules. Under these dietary conditions, there is an upregulation of long-chain fatty acid synthesis in the liver and decreased oxidation and secretion of fats. Inflammation also downregulates bile acid transporters in the liver, slowing their enterohepatic circulation and allowing an increase

in CBA in the liver, which activates S1PR2 stimulating hepatic inflammation and fibrosis pathways and promoting the development of cirrhosis and hepatocellular carcinoma (HCC).