Review

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Dysregulated bile acid homeostasis: unveiling its role in metabolic diseases

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Abstract: Maintaining bile acid homeostasis is essential for metabolic health. Bile acid homeostasis encompasses a complex interplay between biosynthesis, conjugation, secretion, and reabsorption. Beyond their vital role in digestion and absorption of lipid-soluble nutrients, bile acids are pivotal in systemic metabolic regulation. Recent studies have linked bile acid dysregulation to the pathogenesis of metabolic diseases, including obesity, type 2 diabetes mellitus (T2DM), and metabolic dysfunction-associated steatotic liver disease (MASLD). Bile acids are essential signaling molecules that regulate many critical biological processes, including lipid metabolism, energy expenditure, insulin sensitivity, and glucose metabolism. Disruption in bile acid homeostasis contributes to metabolic disease *via* altered bile acid feedback mechanisms, hormonal dysregulation, interactions with the gut microbiota, and changes in the expression and function of bile acid transporters and receptors. This review summarized the essential molecular pathways and regulatory mechanisms through which bile acid dysregulation contributes to the pathogenesis and progression of obesity, T2DM, and MASLD. We aim to underscore the significance of bile acids as potential diagnostic markers and therapeutic agents in the context of metabolic diseases, providing insights into their application in translational medicine.

Keywords: bile acids; metabolic diseases; obesity; takeda G protein-coupled receptor 5; type 2 diabetes mellitus; metabolic dysfunction-associated steatotic liver disease

Introduction

Metabolic diseases, such as type 2 diabetes mellitus (T2DM), obesity, dyslipidemias, and metabolic dysfunction-associated steatotic liver disease (MASLD, previously known as non-alcoholic fatty liver disease [NAFLD]), can lead to a range of long-term health complications, including cardiovascular disease, renal diseases, hypertension, and cancers [1]. Individuals with metabolic diseases often have multiple comorbid conditions, which significantly reduce the quality of life and complicate clinical care. Management of metabolic diseases usually requires lifelong treatment and lifestyle modifications, which not only pose significant challenges for patients to adhere to but also place a substantial financial burden on healthcare systems [2]. The prevalence and impact of metabolic diseases are disproportionately higher in specific populations, including racial and ethnic minorities, low-income groups, and those with limited access to healthcare [3]. Despite considerable progress in elucidating the underlying mechanism of metabolic disease in recent decades, the development of effective treatments remains challenging. This is mainly attributable to genetic, environmental, and lifestyle factors contributing to disease progression [4].

Metabolic diseases are characterized by metabolic dysfunction, including dysregulation of lipid, glucose, and energy metabolism. Beyond their classical role in facilitating dietary lipid digestion and absorption, bile acids, a class of cholesterol derivatives, emerge as critical players in maintaining metabolic homeostasis through intricate networks involving nuclear receptors and membrane-bound G protein-coupled receptors (GPCRs) [5, 6]. A growing body of evidence links the dysregulation of bile acid homeostasis to the pathogenesis of several metabolic diseases,

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underscoring its pivotal role in these diseases [7]. This review aimed to dissect the mechanisms by which alterations in bile acid homeostasis contribute to the development and progression of metabolic diseases, including obesity, T2DM, and MASLD. We will explore the regulatory pathways governing bile acid synthesis and enterohepatic circulation and the impact of dysregulation of bile acid homeostasis on systemic metabolism. A comprehensive understanding of bile acid-mediated regulation of metabolic processes will provide critical information for directing future research and developing effective therapeutics.

Bile acid homeostasis

Bile acid biosynthesis

Bile acid is synthesized from cholesterol in the hepatocytes *via* two major pathways: the classical pathway and the alternative pathway. As shown in Figure 1, the rate-limiting step in the classical pathway is catalyzed by cholesterol 7α-hydroxylase (CYP7A1), which converts cholesterol to 7α-cholesterol, which is converted to 7α-hydroxy-4-cholesten-3-one (C4) by 3β-hydroxy-Δ5-C27-steroid oxidoreductase (HSD3B7). C4 further undergoes hydroxylation by oxidation and shortening of the carbon side chain, leading to the formation of primary bile acids: cholic acid (CA) and chenodeoxycholic acid (CDCA). Both 12α-hydroxylase (CYP8B1) and sterol 27-hydroxylase (CYP27A1) are involved in this process. For the alternative pathway, the rate-limiting step is the hydroxylation of cholesterol at the C-27 position by CYP27A1, producing 27-hydroxycholesterol, followed by hydroxylating at the 7-alpha position to form 3β-hydroxy-5-cholestenoic acid by CYP7B1. After additional side-chain oxidation and cleavage, the primary bile acid formed in this pathway is CDCA. Although the alternative pathway only contributes to a smaller fraction (about 10 %) of total bile acid synthesis compared to the classical pathway, it plays an essential role in maintaining bile acid homeostasis under certain conditions [8, 9]. In rodents, CDCA and ursodeoxycholic acid (UDCA) are further converted into α-muricholic acid (MCA) and β-MCA by rodent-specific enzyme Cyp2c70, respectively [10]. After synthesis, bile acids are conjugated with amino acids, which increases their solubility. Glycine and taurine are the most common amino acids involved in bile acid conjugation. The conjugation process is facilitated by two enzymes: bile acid-CoA synthetase (also called Solute Carrier Family 27 Member 5, SLC27A5) and bile acid-CoA: amino acid N-acyltransferase (BAAT) [11]. Although BAAT performs an acyl-conjugation of the bile acids with either taurine or glycine, recent studies showed that BAAT mainly generates taurine-conjugated bile acids. Deletion of BAAT in mice significantly reduced taurine-conjugated bile acids but had no impact on glycine-conjugated bile acids, suggesting other enzymes may be involved in conjugating bile acid with glycine [12]. Glycine conjugation is an important phase II reaction and a central detoxification pathway. The key enzyme involved in this process is glycine N-acyltransferase (GLYAT), which is mainly expressed in the liver and kidney and conjugates glycine to xenobiotic and endogenous carboxylic acids. Although there is no direct evidence indicating that GLYAT mediates the conjugation of glycine to bile acids, our recent studies showed that downregulation of GLYAT is associated with the decrease of glycine-conjugated bile acids in cholangiocarcinoma patients (unpublished). A recent study also reported that bile acid can be conjugated with amino acids by bile salt hydrolase (BSH) acyltransferase in the gut microbiome, which significantly expanded the bile acid diversity [13].

Enterohepatic bile acid circulation

The enterohepatic circulation is an essential physiological process involving the recycling of bile acids between the liver and the small intestine, which play a crucial role in the digestion and absorption of dietary lipids. As shown in Figure 2, after synthesis, bile acids are secreted into the bile ducts and stored in the gallbladder. Bile will flow from the gallbladder through the common bile duct into the small intestine, where bile acids perform their critical role in emulsifying fats, breaking them down into smaller droplets, and enhancing the efficiency of fat digestion by pancreatic lipases [7, 14]. This emulsification process is vital for absorbing dietary fats and lipid-soluble vitamins across the intestinal epithelial. After completion of lipid digestion and absorption, the majority of bile acids (95 %) are reabsorbed by enterocytes in the terminal ileum *via* specific transporters and returned to the liver *via* the portal vein. Once returned to the liver, bile acids will be taken up by hepatocytes and reconjugated with glycine or taurine.

Primary bile acids secreted into the intestine will be deconjugated and dehydroxylated by intestinal bacteria, forming secondary bile acids, deoxycholic acid (DCA) from CA and lithocholic acid (LCA) from CDCA. In mice, Tα-MCA and Tβ-MCA are converted into murideoxycholic acid (MDCA) and hyodeoxycholic acid (HDCA), and ω-MCA [10]. About 5 % of bile acids lost in the fecal samples will be replaced by *de novo* synthesis from cholesterol in hepatocytes. The recycled and newly synthesized bile acids will be secreted back into the bile and stored in the gallbladder until the next meal to initiate another cycle of enterohepatic

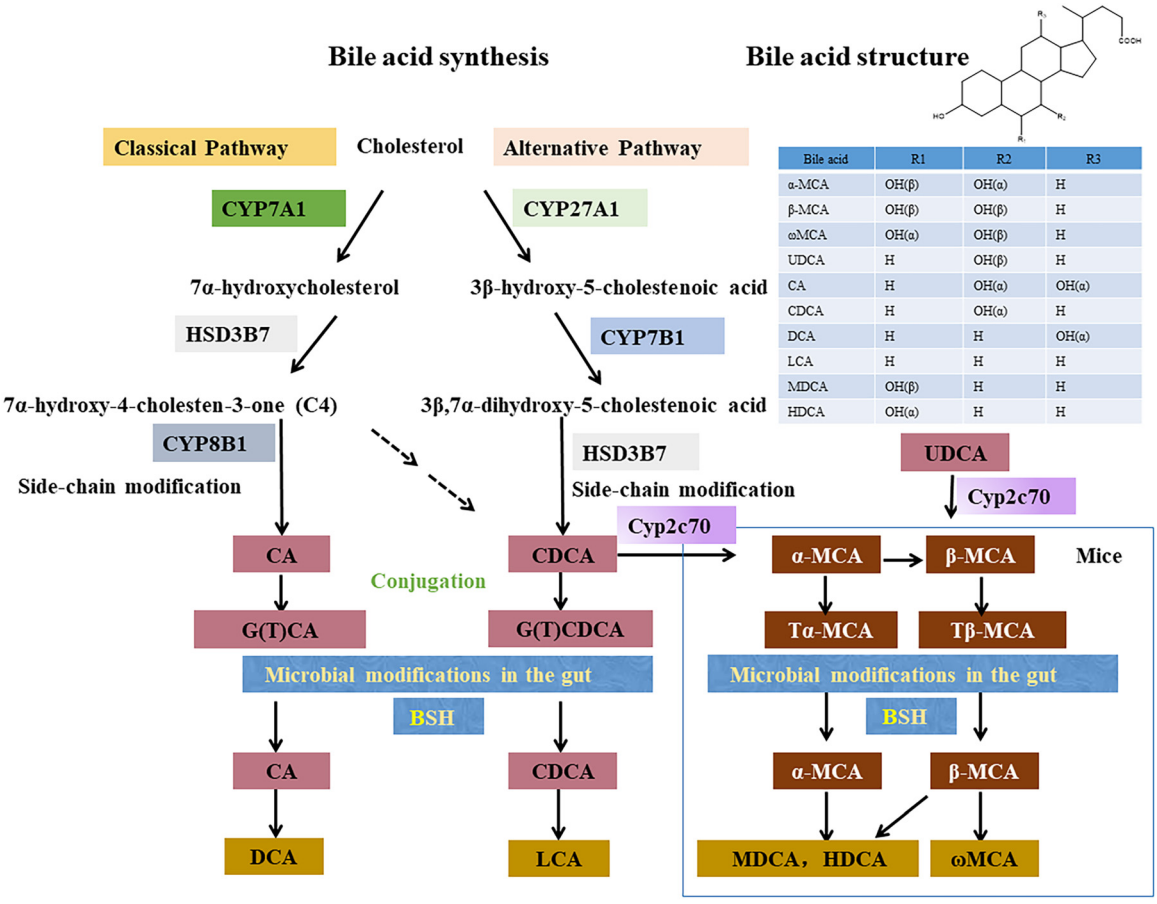

Figure 1: Synthetic pathways of bile acids. Two bile acid synthetic pathways have been identified. The classical pathway begins with converting cholesterol to 7α-hydroxycholesterol by CYP7A1, the rate-limiting step in this pathway. 7α-hydroxycholesterol is further converted into C4 by HSD3B7, followed by hydroxylation by CYP8B1 and side-chain modification to CA or CDCA. The alternative or acidic pathway starts with converting cholesterol to 27-hydroxycholesterol by the enzyme CYP27A1. This pathway leads to the production of CDCA through several steps involving different enzymes, including CYP7B1 and HSD3B7. CA and CDCA are two primary BAs found in humans, which are conjugated with glycine or taurine. In mice, CDCA and UDCA are further converted into α-MCA and β-MCA by Cyp2c70, respectively. α-MCA also can be converted into β-MCA. Both α-MCA and β-MCA are conjugated with taurine. The conjugated primary BAs are deconjugated and transformed into secondary BAs by gut microbiota. In humans, CA is converted into DCA and CDCA is converted into LCA. In mice, α-MCA and β-MCA are converted into MDCA, HDCA, and ω-MCA. LCA also can be converted into MDCA and HDCA. CYP7A1, cholesterol 7α-hydroxylase; C4, 7α-hydroxy-4-cholesterol-3-one; HSD3B7, 3β-hydroxy-Δ5-C27-steroid oxidoreductase; CYP8B1, 12α-hydroxylase (also called sterol 12α-hydroxylase); CA, cholic acid; CDCA, chenodeoxycholic acid; CYP27A1, sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; MDCA, murideoxycholic acid; HDCA, hyodeoxycholic acid.

circulation. This process is necessary not only to maintain a steady bile acid pool but also to play a crucial role in regulating cholesterol levels and lipid absorption and maintaining overall lipid homeostasis [7].

Mechanisms regulating bile acid homeostasis

Feedback inhibition of bile acid synthesis

Bile acid homeostasis is tightly regulated by a finely tuned regulatory system that involves the synthesis, secretion, absorption, and recycling of bile acids. Maintenance of bile acid homeostasis is crucial for nutrition absorption and lipid, glucose, and energy metabolism. High levels of bile acids in the liver inhibit their synthesis through negative feedback mechanisms. This is primarily mediated by the farnesoid X receptor (FXR), the first identified nuclear receptor activated by bile acids [15–17]. Activation of FXR induces the expression of the small heterodimer partner (SHP). SHP is an atypical nuclear receptor lacking a DNA binding domain. SHP does not directly bind to the CYP7A1 promoter. Instead, SHP represses CYP7A1 transcription indirectly by inhibiting the activities of positive transcription factors, such as liver receptor homolog-1 (LRH-1), and hepatocyte nuclear factor 4 alpha (HNF4α) [18].

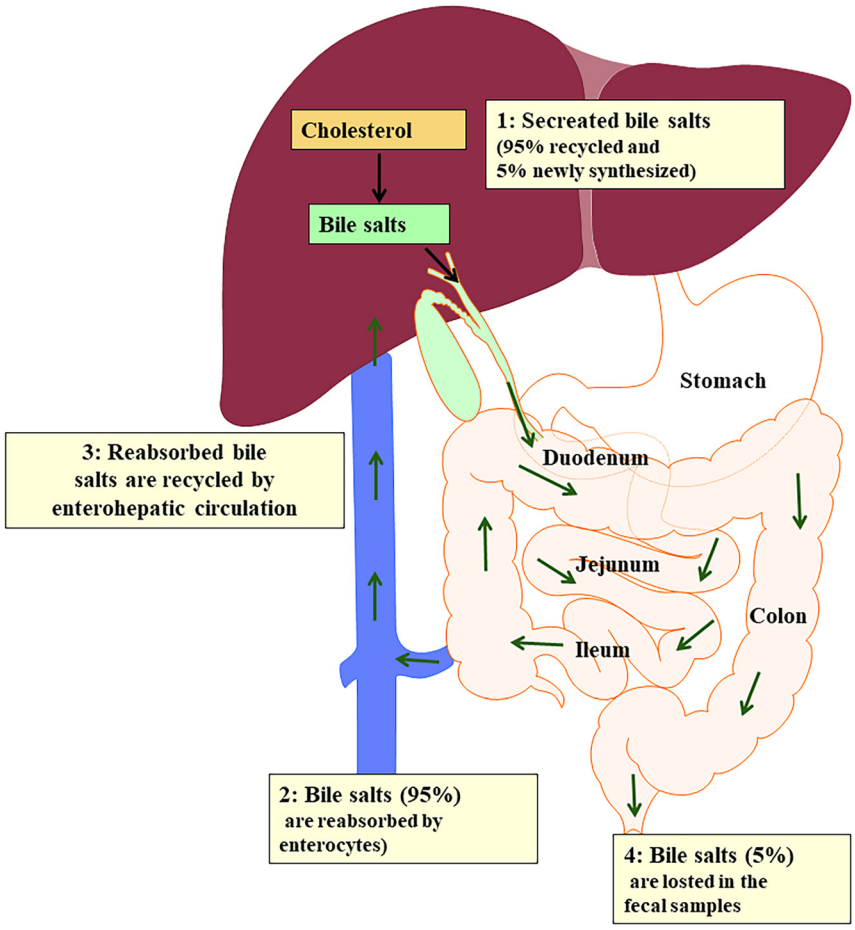

Figure 2: Enterohepatic bile acid circulation. Bile acids are synthesized from cholesterol in hepatocytes and stored in the gallbladder. Upon ingestion of food, bile acids are secreted into the duodenum. Bile acids emulsify lipids in the small intestine, facilitating the formation of micelles, which are necessary for the absorption of lipids and lipid-soluble vitamins. The majority (95 %) of bile acids are reabsorbed in the ileum and returned to the liver via portal vein. A small amount of bile acids (5 %) is lost in feces, which is replaced by *de novo* synthesis in the hepatocytes.

Hormonal control of bile acid synthesis

Bile acids released into the small intestine during digestion stimulate the secretion of fibroblast growth factor 19 (FGF19) in humans (or FGF15 in rodents). FGF19 travels *via* the portal blood to the liver, where it binds to the fibroblast growth factor receptor 4 (FGFR4) in hepatocytes, resulting in the inhibition of CYP7A1 expression. This endocrine feedback loop between the liver and the intestine is crucial in regulating systemic bile acid levels. In addition to FGF19/15, cholecystokinin, glucagon-like peptide-1 (GLP-1), and insulin are also involved in regulating bile acid synthesis, secretion, and recycling to maintain metabolic homeostasis [19]. However, FGF19/15 and the FXR pathway are the most directly involved in bile acid homeostasis.

Interaction with gut microbiota

The gut microbiota is integral to maintaining bile acid homeostasis, impacting the composition and function of bile acids. This relationship is characterized by a bidirectional interaction: bile acids modulate the gut microbiota, while the microbiota, in turn, influences bile acid metabolism [20]. In the distal part of the small intestine and the colon, the gut microbiota can deconjugate and modify primary bile acids through processes like dehydroxylation, deconjugation, and epimerization to form the secondary bile acids (e.g., DCA, LCA) by microbial action [21, 22]. Microbial enzymes, notably BSH, play a key role in breaking down bile salts into smaller, more easily absorbed molecules. This enzyme is produced by various BSH-positive gut bacteria, including species of *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, *Clostridium*, *Bacteroides*, and *Parabacteroides*. Moreover, bile acids possess antimicrobial properties that help shape the gut microbial landscape, selectively inhibiting the growth of certain bacterial species and thus influencing the overall composition of the microbial community [23]. Bile acids can activate receptors such as FXR and takeda G protein-coupled receptor 5 (TGR5) in the host cells. FXR, when activated by bile acids, can regulate the expression of antimicrobial peptides, further modulating the gut microbiota [24]. TGR5 activation, on the other hand, is implicated in regulating energy expenditure and intestinal motility. Gut microbes contribute to the deconjugation and alteration of bile acids,

which affects their reabsorption and recycling within the enterohepatic circulation, highlighting the crucial role of microbial metabolism in bile acid dynamics. Furthermore, the gut microbiome also contributes to bile acid conjugation, producing diverse bile acid species of microbially conjugated bile acids (MCBAs) by bile salt hydrolase/transferase (BSH/T), which has acyltransferase activity. This enzymatic function is not exclusive to one bacterial species but is found across various types, each producing a unique MCBA profile influenced by the specific amino acid sequence of their BSH/T. The first identified BSH/T with hydrolytic enzyme activity was in *Clostridium perfringens*. The BSH/T from *C. perfringens* demonstrated the ability to transfer acyl groups upon interacting with different amino acids and bile salts, significantly diversifying the range of bile acid derivatives and enriching their chemical diversity [13]. Changes in bile acid composition due to microbial activity can have a significant impact on gut permeability [25]. The intricate interplay between bile acids and the gut microbiota underscores its importance in maintaining metabolic homeostasis (Figure 3).

Bile acid transporters

Bile acid transporters play a crucial role in maintaining bile acid homeostasis by mediating the enterohepatic circulation of bile acids. These transporters are located in the liver, intestine, and kidney and are responsible for bile acid uptake, secretion, and reabsorption (Figure 4). In the liver, Na^+/taurocholate cotransporting polypeptide

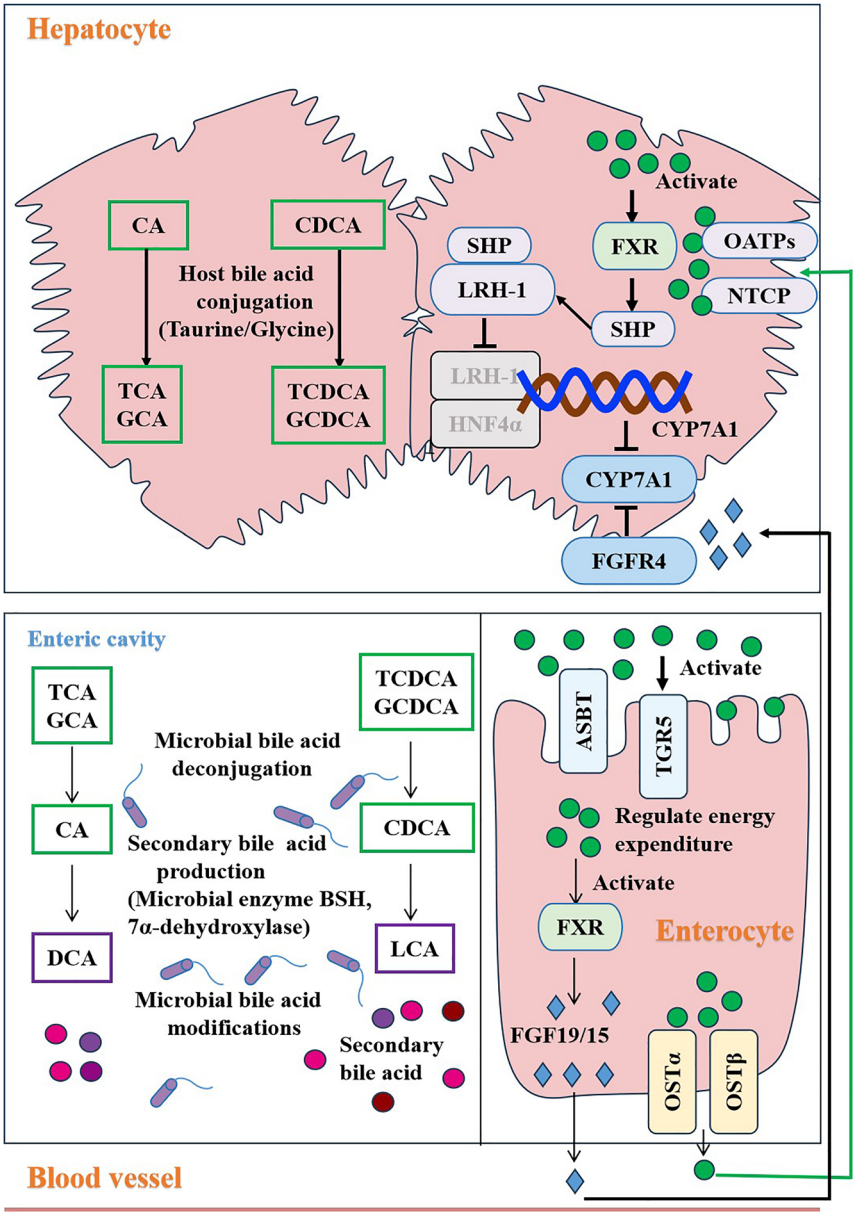

Figure 3: Mechanisms regulating bile acid homeostasis. Bile acid homeostasis is regulated by multiple mechanisms. The FXR-mediated feedback mechanism tightly regulates the synthesis of bile acids. Bile acid-mediated activation of FXR will upregulate the expression of SHP, inhibiting LRH-1 and HNF4α-mediated activation of CYP7A1 promoter activity. In human, gut microbiota further metabolizes primary bile acids to form secondary bile acids *via* dehydroxylation, deconjugation, and epimerization. The secondary bile acids can activate TGR5 and FXR in the intestinal enterocytes. Bile acid-mediated activation of FXR will release FGF19 or 15 into the portal vein and activate FGFR4 in hepatocytes, resulting in the inhibition of CYP7A1. Bile acid-mediated activation of TGR5 can regulate glucose and energy metabolism as well as intestinal barrier function. CA, cholic acid; CDCA, chenodeoxycholic acid; FXR, farnesoid X receptor; SHP, small heterodimer partner; LRH-1, liver receptor homolog-1; HNF4α, hepatocyte nuclear factor 4 alpha; CYP7A1, cholesterol 7α-hydroxylase; FGFR4, fibroblast growth factor receptor 4; OATPs, organic anion-transporting polypeptides; NTCP, sodium taurocholate co-transporting polypeptide; TGR5 (GPBAR1), takeda G protein-coupled receptor 5 (G protein-coupled bile acid receptor 1); FGF19/15, fibroblast growth factor 19/15; OSTα/OSTβ, organic solute transporter alpha and beta.

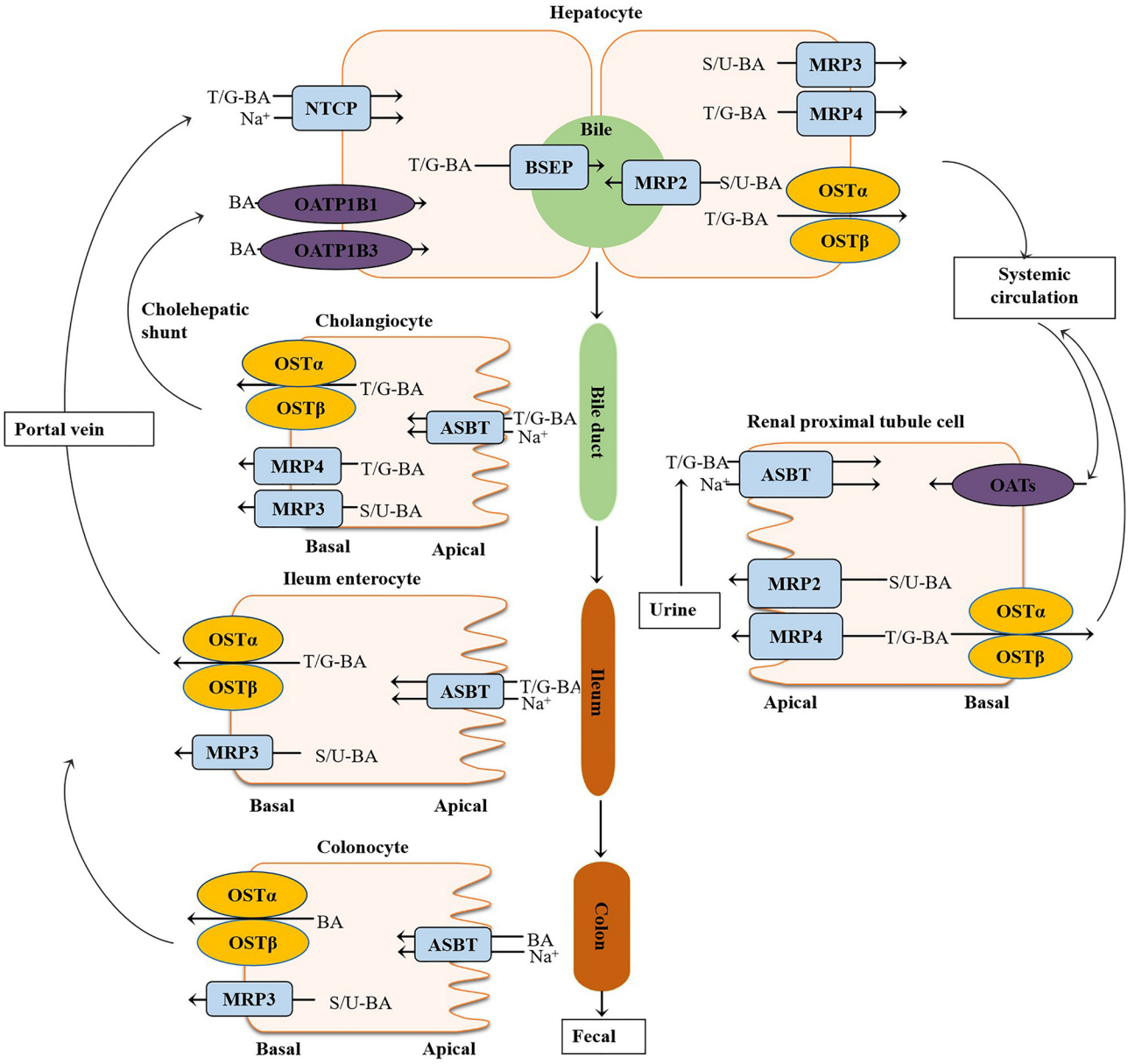

Figure 4: Bile acids transporters. This figure illustrates the role of bile acid transporters in hepatocytes, cholangiocytes, enterocytes in the ileum and colon, and cells in the renal proximal tubule. In hepatocytes, NTCP is key for bile acid uptake from portal blood, while OATP family members also contribute to this process. BSEP plays a crucial role in secreting conjugated bile acids into bile canaliculi, and MRP2 contributes to the efflux of sulfated or glucuronidated bile acids into bile. OSTα/OSTβ, MRP3, and MRP4 facilitate the efflux of bile acids and other organic anions from hepatocytes back into the systemic circulation. In the intestine, ASBT (IBAT) is responsible for the sodium-dependent reabsorption of primary bile acids from the lumen into the enterocytes. MRP3 and OSTα/OSTβ then transport bile acids from enterocytes into the portal vein. Similarly, these transporters are also involved in the bile acid uptake and efflux in colonocytes. In the kidney, OATs facilitate the uptake of bile acids from the systemic circulation into the cells. MRP2 and MRP4 are involved in the secretion of conjugated bile acids and other organic anions into the urine. ASBT contributes to the reabsorption of bile acids from the urine back into the renal tubule cell. OSTα/OSTβ are involved in the efflux of bile acid into the systemic circulation. Cholehepatic shunting is an alternative pathway for bile acids and other components of bile to return to the hepatocytes under pathological conditions. In the cholangiocytes, ASBT mediates the uptake of bile acids in the bile. Transporters, such as MRP3, MRP4, and OSTα/OSTβ, facilitate the efflux of bile acids from the cholangiocytes to the liver sinusoids, where bile acids can be taken up by hepatocytes and secreted back into bile. NTCP, sodium taurocholate co-transporting polypeptide; OATPs, organic anion transporting polypeptides; BSEP (ABCB11), bile salt export pump; MRP2 (ABCC2), multidrug resistance protein 2; OSTα/OSTβ, organic solute transporter alpha and beta; MRP3 (ABCC3), multidrug resistance protein 3; MRP4 (ABCC4), multidrug resistance protein 4; ASBT (IBAT, SLC10A2), apical sodium-dependent bile acid transporter; T/G-BA, taurine and glycine conjugated bile acid; S/U-BA, sulfated or glucuronidated bile acid.

(NTCP, SLC10A1) located on the basolateral (sinusoidal) membrane of hepatocytes is responsible for the uptake of bile acids from the portal blood into hepatocytes. Organic anion-transporting polypeptides (OATPs, e.g., OATP1B1/SLCO1B1 and OATP1B3/SLCO1B3) are located on the basolateral membrane of hepatocytes and participate in the hepatic uptake of bile acids, as well as other organic anions [26]. The bile salt export pump (BSEP, ABCB11) on the canalicular membrane of hepatocytes is involved in the secretion of bile acids into the bile canaliculi, a critical step in bile formation. Multidrug resistance protein 2 (MRP2/ABCC2) is another canalicular transporter that contributes to the efflux of sulfated or glucuronidated bile acids into the bile. It also plays a role in eliminating bilirubin and other organic anions. In addition, organic solute transporter alpha and beta (OSTα/OSTβ), MRP3 (ABCC3), and MRP4 (ABCC4) on the basolateral membrane of hepatocytes facilitate the efflux of bile acids and other organic anions from hepatocytes back into the bloodstream, especially under cholestatic conditions.

The bile acid transporters in the intestine also play a significant role in the enterohepatic circulation of bile acids. The apical sodium-dependent bile acid transporter (ASBT or IBAT, SLC10A2) is located on the apical membrane of enterocytes in the terminal ileum. ASBT/IBAT is responsible for reabsorbing primary bile acids from the intestinal lumen into the enterocytes. This process is sodium-dependent. After being absorbed by enterocytes, bile acids are transported across the basolateral membrane into the portal vein by MRP3 and Ostα/Ostβ [27]. Similarly, these transporters are also involved in the bile acid uptake and efflux in colonocytes.

Bile acid transporters in the kidney also play a critical role in the systemic circulation of bile acids, particularly when the enterohepatic circulation of bile acids is disrupted [28]. The kidney functions as an alternative pathway for bile acid excretion when the bile flow from the liver to the intestine is impaired. OATs, located on the basolateral membrane of renal tubular cells, facilitate the uptake of bile acids from the circulation into the cells. ASBT, MRP2, and MRP4 are expressed in the apical membrane of renal tubular cells. MRP2 and MRP4 are involved in the secretion of conjugated bile acids and other organic anions into the urine. ASBT contributes to the reabsorption of bile acids from the urine back into the renal tubule cell. OSTα/OSTβ in the basolateral membrane is involved in the efflux of bile acid into the systemic circulation.

Cholehepatic shunting is an alternative pathway for bile acids and other components of bile to return to the hepatocytes under pathological conditions, such as cholestasis [29]. In the cholangiocytes, ASBT in the apical membrane of cholangiocytes mediates the uptake of bile acids in the bile. The transporters expressed on the basolateral membranes of cholangiocytes, such as MRP3, MRP4, and OSTα/OSTβ, facilitate the efflux of bile acids from the cholangiocytes to the liver sinusoids, where bile acids can be taken up by hepatocytes and secreted back into bile.

Bile acid receptors

Bile acids, traditionally recognized as detergents for the digestion and absorption of dietary fats and lipid-soluble vitamins, have emerged as essential signaling molecules that regulate various metabolic pathways since the discovery of nuclear and membrane-bound receptors for bile acids. The discovery of bile acid receptors has revolutionized our understanding of bile acid physiology and the systemic effects of individual bile acid receptors on various metabolic processes. Dysregulation of bile acid receptor-mediated signaling pathways is closely related to metabolic disorders and various liver diseases. Exploring the complex molecular networks associated with different bile acid receptors offers the potential for novel therapeutic strategies for metabolic diseases [30–33].

FXR

The nuclear receptor FXR, initially identified in the mid-1990s *via* a search for orphan nuclear receptors, is a crucial regulator of bile acid metabolism. FXR is named for its ability to bind farnesol-derived sterols, but it was reported simultaneously by three research groups that its natural ligands were bile acids in 1999 [15–17]. This discovery of FXR as the first bile acid receptor markedly advanced the understanding of enterohepatic bile acid circulation, the feedback mechanisms that maintain bile acid homeostasis, and the impact of dysregulation of bile acid homeostasis on various metabolic processes. FXR is expressed predominantly in the liver and intestine, with lower levels in the kidney and adipose tissue, and activated by unconjugated bile acids [31, 34–37]. CDCA is recognized as one of the strongest natural agonists of FXR followed by DCA, LCA, CA and UDCA. Glycine-β-muricholic acid (Gly-MCA) has been identified as an intestine-specific FXR antagonist [38]. Furthermore, synthetic FXR agonists, including GW4064, WAY-362450, and GS9674, have been reported to offer therapeutic benefits by reducing liver inflammation and fibrosis, improving insulin resistance, and alleviating cholestatic liver injury [39–41].

FXR is a molecular sensor for bile acids and exerts a broad influence on metabolic processes. It plays an essential role in modulating bile acid synthesis, transport, and metabolism. Bile acid-mediated activation of FXR inhibits the expression of CYP7A1, the rate-limiting enzyme in the

classical pathway of bile acid synthesis. Activation of FXR induces the expression of SHP (also known as NR0B2), a nuclear receptor that lacks a DNA-binding domain and acts as a transcriptional repressor. SHP interacts with LRH-1 (also known as NR5A2) and inhibits its activity, preventing LRH-1 from binding to the CYP7A1 promoter region. The interaction between SHP and LRH-1 leads to the suppression of CYP7A1 transcription, resulting in decreased bile acid synthesis. This regulatory pathway exemplifies the negative feedback mechanism that controls bile acid synthesis and maintains bile acid homeostasis [42, 43].

Bile acid conjugation is a critical process of detoxification of bile acids, which is mediated by BAAT [44]. Activation of FXR leads to the upregulation of amino acid transporters that provide glycine or taurine for bile acid conjugation [45]. Conjugated bile acids are more water-soluble and less toxic than their unconjugated counterparts. Conjugation of bile acids enhances the secretion of bile acids into bile by BSEP. The expression of BSEP is also regulated by FXR [46, 47]. In the enterohepatic bile acid circulation, FXR also plays an essential role in regulating bile acid homeostasis by regulating the expression of FGF19/15 in the intestine. FGF19 is an important hormone that circulates *via* the portal vein back to the liver to suppress bile acid synthesis. FGF19 binds to Fibroblast Growth Factor Receptor 4 (FGFR4) through the assistance of the co-receptor β-Klotho, leading to the down-regulation of CYP7A1 expression. FXR also plays a multifaceted role in regulating lipid metabolism *via* regulating the expression of lipogenic genes and modulating very low-density lipoprotein (VLDL) secretion as well as phospholipid metabolism. In addition, bile acid-mediated activation of FXR has been linked to improved insulin sensitivity, energy expenditure, and anti-inflammatory effects (Figure 5) [48].

TGR5

TGR5 is the first identified GPCR activated by bile acid and is called G Protein-Coupled Bile Acid Receptor 1 (GPBAR1) [49, 50]. It was initially found in macrophages, but it plays diverse roles in regulating metabolism and immune response *via* its expression in various tissues and cells. However, it is not expressed in hepatocytes. Numerous studies have shown that TGR5 plays an important role in regulating energy homeostasis. Activation of TGR5 in enteroendocrine cells leads to the secretion of GLP-1, which

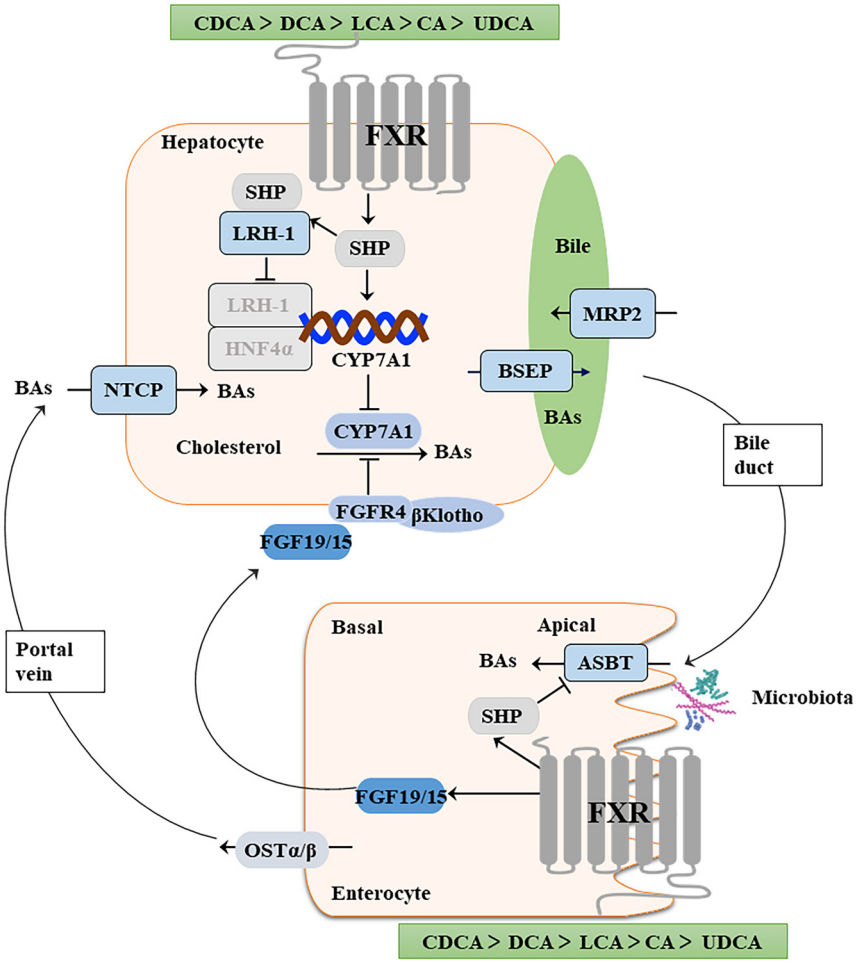

Figure 5: Bile acid-mediated activation of FXR. In the hepatocyte, bile acid-induced activation of FXR upregulates the expression of SHP, resulting in the inhibiting LRH-1 and HNF4α-mediated activation of CYP7A1 promoter activity. In the enterocyte, activation of FXR by bile acids results in the release FGF19/15 into the portal vein and activation of FGFR4 with the aid of its co-receptor β-Klotho in hepatocytes, which inhibit CYP7A1. BAs, bile acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; CA, cholic acid; UDCA, ursodeoxycholic acid; FXR, farnesoid X receptor; SHP, small heterodimer partner; LRH-1, liver receptor homolog-1; HNF4α, hepatocyte nuclear factor 4 alpha; CYP7A1, cholesterol 7α-hydroxylase; FGF19/15, fibroblast growth factor 19/15; FGFR4, fibroblast growth factor receptor 4; NTCP, sodium taurocholate co-transporting polypeptide; BSEP (ABCB11), bile salt export pump; MRP2(ABCC2), multidrug resistance protein 2; OSTα/OSTβ, organic solute transporter alpha and beta; ASBT (IBAT, SLC10A2), apical sodium-dependent bile acid transporter.

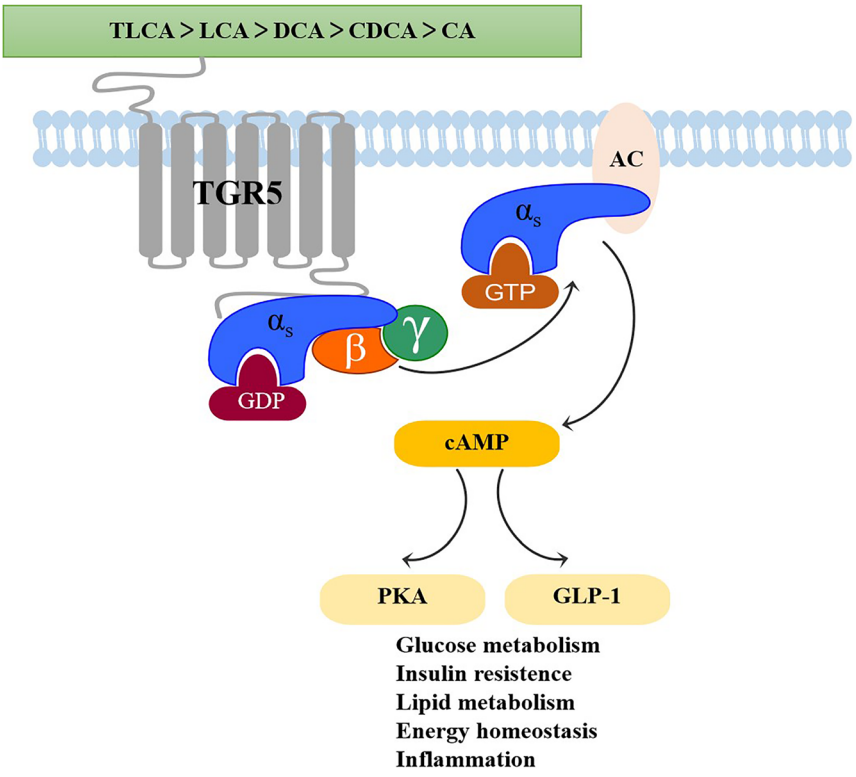

Figure 6: Bile acid-mediated activation of TGR5. The binding of bile acids (e.g., TLCA, LCA, DCA, CDCA, and CA) to TGR5 induces the activation of Gαs, which in turn stimulates adenylate cyclase (AC), leading to an increased in cyclic AMP levels. The increase in intracellular cAMP can activate PKA and stimulate the release of GLP-1, which are key players in various signaling pathways involved in regulation of glucose and lipid metabolism, insulin sensitivity, energy homeostasis and inflammation. TGR5 (GPBAR1), takeda G protein-coupled receptor 5 (G protein-coupled bile acid receptor 1); LCA, lithocholic acid; TLCA, taurolithocholic acid; GDP, guanine dinucleotide phosphate; GTP, guanine trinucleotide phosphate; AC, adenylate cyclase; PKA, protein kinase A; GLP-1, glucagon-like peptide-1; cAMP, cyclic adenosine phosphate.

contributes to increased insulin secretion and sensitivity and regulates glucose metabolism [51]. In addition, activation of TGR5 in brown adipose tissue and muscle enhances energy expenditure, which can contribute to preventing obesity and diabetes. Ligand specificity and affinity of TGR5 are different from FXR. LCA and its taurine-conjugated form (TLCA), are the most potent natural agonists and activate TGR5 at nanomolar concentrations. DCA, CDCA, and CA can activate TGR5 at micromolar concentrations [27]. Bile acid-mediated activation of TGR5 is mainly coupled with Gαs, which induces adenylate cyclase, increases cAMP level, and activates protein kinase A (PKA). PKA plays an important role in regulating glucose and lipid metabolism by phosphorylating various proteins involved in metabolic processes [52]. Activation of TGR5 in immune cells such as macrophages has been shown to inhibit the NF-κB pathway, a key regulator of inflammation (Figure 6) [53].

S1PR2

Sphingosine-1 phosphate receptor 2 (S1PR2), previously known as Endothelial Differentiation Gene-5 (EDG-5), was identified as a member of the S1PR family in the late 1990s [54]. The identification and characterization of S1PR2 and other S1P receptors were pivotal in understanding the complex signaling pathways mediated by sphingolipids, which are involved in regulating cell proliferation, survival, migration, and angiogenesis, as well as metabolism. The discovery of S1PR2 as a bile acid-activated GPCR contributed significantly to advancing the fields of sphingolipids and bile acids. It provided insights into the crosstalk between different signaling pathways in metabolic and inflammatory diseases. S1PR2 is widely expressed across various tissues in the body, indicating its involvement in numerous physiological and pathological processes. S1PR2 is highly expressed in the liver and gastrointestinal tract [55]. Unlike TGR5, S1PR2 is expressed in all hepatic cells and is the most abundant S1PR isoform in the hepatocytes. S1PR2 is activated by conjugated primary bile acids (TCA and GCA), but not by unconjugated bile acids and secondary bile acids [56]. Bile acid-mediated activation of S1PR2 in hepatocytes plays a critical role in regulating hepatic lipid metabolism *via* activating ERK and AKT pathways by coupling with Gαi [57]. In addition, conjugated bile acid-mediated activation of S1PR2 is a key regulator of sphingosine kinase 2 (SphK2) [58]. It also has been shown that activation of SphK2 plays a critical role in preventing hepatic steatosis under ER stress [59]. S1PR2 is also expressed in various immune cells, adipocytes, endothelial cells, smooth muscle cells, and epithelial cells. The diverse tissue distribution of S1PR2 underlines its multifaceted physiological and pathological roles (Figure 7) [60].

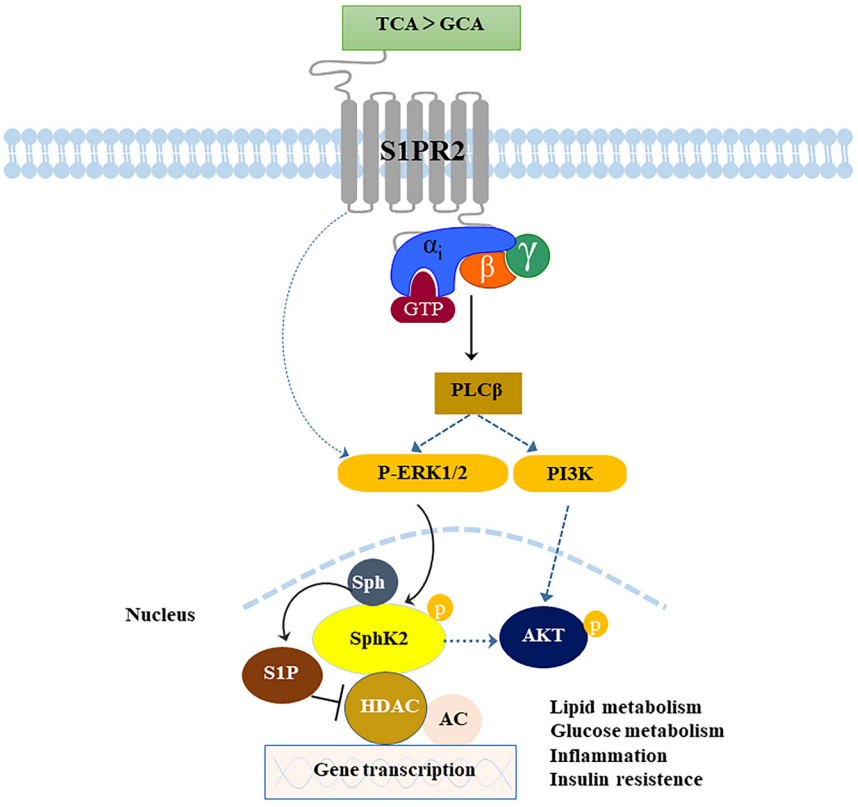

Figure 7: Bile acid-mediated activation of S1PR2. Bile acid-induced activation of S1PR2 in hepatocytes is coupled to the activation of the Gi protein, which in turn resulting in the activation of the ERK1/2 and PI3K-Akt pathways. The activated ERK1/2 pathway leads to activation of SphK2, resulting in the production of S1P within the nucleus. S1P acts as a potent inhibitor of histone deacetylase (HDAC), leading to increased histone acetylation. This epigenetic modification promotes the transcription of essential genes involved in lipid and glucose metabolism, inflammation, and insulin resistance. S1PR2, sphingosine-1-phosphate receptor 2; TCA, taurocholic acid; GCA, glycocholic acid; GDP, guanine dinucleotide phosphate; GTP, guanine trinucleotide phosphate; AC, adenylate cyclase; ERK, extracellular signal-regulated kinase; Sphk2, sphingosine kinase 2; HDAC, histone deacetylase.

Pathophysiology of dysregulated bile acid homeostasis

Mechanisms leading to dysregulation of bile acid homeostasis

Dysregulation of bile acid homeostasis arises from an intricate interplay of genetic predispositions, lifestyle factors, dietary patterns, and gut microbiome alternations [61]. Genetic variations, including mutations or polymorphisms in genes crucial for bile acid synthesis (such as CYP7A1), transport (like ASBT), and regulation (for instance, FXR), are pivotal in maintaining bile acid equilibrium [62–64]. These genetic factors can render individuals more prone to bile acid-related disorders. Lifestyle influences, including physical activity levels and stress, play critical roles in modulating bile acid metabolism. Regular physical activity has been demonstrated to modulate bile acid synthesis and circulation beneficially. In contrast, chronic stress may induce dysregulation through hormonal fluctuations that disrupt the homeostatic control of bile acid synthesis and enterohepatic circulation [65–67]. Dietary composition is a crucial determinant of bile acid metabolism. High-fat or Western diets have been shown to alter bile acid composition and levels, while dietary fiber intake can influence bile acid excretion and, subsequently, the gut microbiome, thereby affecting bile acid metabolism [68–70]. Excessive alcohol intake can also disrupt bile acid homeostasis by impairing bile acid synthesis, secretion, and metabolism [71, 72]. The gut microbiome is integral to bile acid metabolism. Dysbiosis, characterized by an imbalanced gut microbial community, can disrupt bile acid metabolism, affecting the regulatory feedback mechanisms of bile acid synthesis. Conditions affecting the gastrointestinal tract, like inflammatory bowel disease (IBD) or issues that compromise the integrity of the intestinal barrier, can negatively impact the absorption of bile acids [73]. Moreover, obesity and metabolic syndrome are linked with altered bile acid metabolism [74, 75].

Consequences of dysregulated bile acid homeostasis

Dysregulated bile acid homeostasis can significantly impact gastrointestinal health, liver function, and overall metabolic balance [76]. The consequences of such dysregulation encompass a spectrum of diseases, underscoring the essential role of bile acids in maintaining normal physiological functions. One of the primary consequences of disrupted bile acid homeostasis is liver disease. Bile acids are synthesized

in the liver, and the accumulation of bile acids due to impaired secretion and flow can lead to hepatotoxicity. Accumulation of hepatic bile acids can lead to liver inflammation, fibrosis, and, in severe cases, cirrhosis. Furthermore, bile acid-induced hepatocyte apoptosis can induce liver injury and dysfunction [77].

Bile acids are crucial for lipid digestion and absorption in the intestine. Dysregulated bile acid homeostasis can lead to malabsorption syndromes and nutritional deficiencies [78]. In addition, an imbalance in bile acid regulation can contribute to the development of IBD, such as Crohn's disease and ulcerative colitis, where altered bile acid composition and levels, as well as signaling pathways, may exacerbate intestinal inflammation and barrier dysfunction [79]. Certain secondary bile acids have been implicated in promoting carcinogenesis and increased risk of colorectal cancer [80–83].

Bile acids are not only important for digestion but also act as signaling molecules that regulate metabolism. Changes in bile acid metabolism can influence the growth and function of specific microbial populations, leading to dysbiosis. The altered microbial balance can further impact bile acid homeostasis and intestinal barrier function, creating a feedback loop that exacerbates homeostatic disruption of bile acid metabolism, contributing to metabolic diseases, obesity, and T2DM [84–86].

Bile acids and metabolic diseases

Obesity: role of bile acids in fat metabolism and energy expenditure

Obesity, a global metabolic epidemic, is intricately linked with dysregulation of bile acid metabolism [74, 87, 88]. Bile acids play an essential role in regulating fat metabolism and energy expenditure. It has been reported that key genes in bile acid synthesis are significantly elevated, showing a shift towards a 12α-hydroxylated bile acid profile in obese individuals [89]. The postprandial rise in serum bile acids is notably blunted in obesity, suggesting altered bile acid signaling. The hepatic DCA to CA ratios tended to be positively correlated with the GLP-1 serum concentrations, inversely related to hepatic triglyceride levels [90]. This suggests that increased DCA may enhance GLP-1 levels, potentially reducing hepatic steatosis [91]. Interestingly, hyocholic acid (HCA), a predominant bile acid in pigs, which is known for its exceptional resistance to spontaneous development of diabetic phenotypes, has been observed to have lower serum concentrations in individuals with obesity and diabetes [92]. Bile acid binding resins (BARs) have shown promise in obesity management by binding intestinal bile acid, thus lowering serum cholesterol. In animal studies, BARs have been shown to prevent obesity and boost energy expenditure in HFD-induced obesity in mice, partially by modulating intestinal microbiota [93]. In humans, Roux-en-Y gastric bypass (RYGB) surgery and exogenous CDCA administration have been linked to enhanced GLP-1 and glucagon secretion but not insulin secretion. However, the BA-sequestrant colesevelam reduced CDCA-stimulated GLP-1 secretion[94]. The rising rates of pathological obesity are leading to a greater need for weight loss interventions, including sleeve gastrectomy (SG). Changes in total CA and CDCA following SG have been directly linked to improvements in glycemic control and insulin resistance, as measured by the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)[95]. Lee and colleagues discovered that the accumulation of bile acids in the liver triggers the liver-specific expression of Orosomucoid (also known as alpha-1-acid glycoprotein), an acute-phase protein produced primarily in the liver. Furthermore, bile acid-induced secretion of Orosomucoid inhibits the differentiation of adipocytes, suggesting a potential mechanism through which bile acids might exert anti-obesity effects [96]. Castellanos-Jankiewicz and their team showed that activating TGR5 signaling in the brain can mitigate obesity induced by high-fat diets, whereas inhibiting TGR5 expression in the hypothalamus could contribute to obesity [97]. This mechanism adjusts food consumption and energy expenditure *via* the sympathetic nervous system, unveiling a sophisticated process by which bile acids influence the hypothalamus to control body weight in situations of obesity.

FXR, pivotal in cholesterol, triglyceride, and bile acid metabolism, offers a novel therapeutic avenue for metabolic disorders. Different from systemic treatments, the release of bile acids during digestion specifically triggers FXR in the intestine. Research by Fang and colleagues revealed that fexaramine (Fex), an FXR agonist confined to the intestine, significantly increases the production of intestinal FGF15. This leads to alterations in bile acid composition without influencing the activity of FXR-responsive genes in the liver [98]. Fex was shown to migrate HFD-induced weight gain, systemic inflammation, and hepatic glucose production while enhancing heat production and browning in white adipose tissue (WAT). UDCA, reported as an FXR antagonist attenuated the high-fat diet induced obesity *via* enhancing levels of non-12-OH BAs [99]. Similarly, the TGR5 agonist INT-777 was found to stimulate mitochondrial biogenesis and prevent renal oxidative stress and lipid accumulation, highlighting TGR5's potential in preventing kidney-related complications associated with obesity and diabetes [100].

In obesity, increased absorption of dietary fat disrupts lipid balance, a process in which bile acids play a crucial role. Specifically, ASBT is responsible for bile acid reabsorption in the terminal ileum. Studies in rat and mouse obesity models and human obese patients have shown an upregulation of ASBT in the ileal villi. This increase is likely driven by elevated expression of FXR in intestine cells during obesity, which upregulates ASBT levels [101]. Furthermore, higher FXR activity also upregulates proteins, such as ileal bile acid binding protein (IBABP, also known as fatty acid binding protein 6, FABP6) and OSTα/OSTβ, essential for bile acid recycling from the intestine to the liver [102]. IBABP is primarily expressed in the ileum and binds to bile acids within the enterocytes, helping solubilize and transport hydrophobic molecules through the aqueous environment. The dyslipidemia, associated with obesity and its resultant complications, could be attributed to disruption of intestinal bile acid absorption, at least partly [101]. Li et al. found that the circulating bile acids were increased in HFD-induced obese mice, and this increase was related to the activation of ileal TGR5 and the promotion of ASBT translocation to the plasma membrane, thus modulating bile acid transport, promoting lipolysis and energy consumption [103]. Tropifexor (LJN452) is a novel non-bile acid FXR agonist currently being tested in clinical trials for various chronic liver diseases. Single and multiple doses of Tropifexor showed dose-dependent increases in FGF19 in healthy volunteers [104]. No changes in serum lipids were observed in tropifexor- vs. placebo-treated obese subjects. In obese individuals, the protein levels of NTCP and BSEP were found to inversely correlate with body mass index [104]. The study by Donkers and colleagues demonstrated that blocking the NTCP transporter with Myrcludex B in specific genetically modified obese mice led to reduced bile acid removal from the blood by the liver, increased secretion of the hormone GLP-1, decreased body weight, and reduced liver fat [105]. Furthermore, Myrcludex B treatment was found to enhance the energy excreted in feces, shedding light on the noted weight reduction without alternations in dietary intake or physical activity levels [105].

Münzker et al. demonstrated that the gut microbiome plays a critical role in weight loss and metabolic success of gastric bypass surgery using a rat model [106]. Obesity has been associated with changes in gut microbiota composition, particularly a notable decrease in *Bacteroidetes* and an increase in *Firmicutes* among obese individuals [107]. Many studies indicate that these gut bacteria can influence intestinal barrier function, modulate bile acid metabolism, regulate immune response by impacting the production of host antigens, and can even indirectly influence dietary choices [108, 109]. Furthermore, the gut microbiota's composition is essential for how nutrients are accessed and utilized by the human body, making it a key factor in the development and progression of obesity and related diseases. The impact of these changes is also evident in the alteration of metabolites produced by gut bacteria during obesity's onset. In a placebo-controlled double-blind FMT pilot study of 22 obese patients without T2DM, MASLD, or metabolic syndrome using FMT capsules derived from a single lean donor, patients who received FMT were well tolerated and had sustained changes in the intestinal microbiome and bile acid profiles were shifted toward to those of the lean donor [110]. Allegretti and colleagues discovered that FMT improved the metabolism of bile acids by gut bacteria and delayed the onset of glucose intolerance compared to a placebo group [111]. Several candidate bacteria that might play a role in the metabolic improvements with FMT were identified, but how gut bacteria metabolize bile acids needs further functional validation [112]. *Clostridium scindens* had a positive correlation with UDCA and LCA. Gavage of *C. scindens* in mice increased the levels of hepatic non12-OH bile acids, accompanied by elevated serum C4 levels [99]. Treatment with *Parabacteroides distasonis* significantly changed the bile acid composition, leading to higher levels of LCA and UDCA [113]. Administering a combination of LCA and UDCA through gavage was effective in reducing hyperlipidemia by activating the FXR pathway and improving gut barrier integrity [113]. Additionally, A combination of *Litesse® Ultra™ polydextrose* (LU) and *Bifidobacterium animalis subsp. lactis* 420™(B420) led to an increase in the populations of *Akkermansia, Christensenellaceae, and Methanobrevibacter* and a decrease of *Paraprevotella* in overweight adults [114]. Consumption of B420 alone or in combination with LU led to changes in gut microbiota and bile acid metabolism. This could enhance gut barrier function and positively affect markers related to obesity. Consuming a Western diet along with *Clostridium* spp. led to elevated levels of the secondary bile acid, DCA, in the ileum. This increase adversely affected the function of Paneth cells [115].

T2DM: the effect of bile acids on insulin sensitivity and glucose metabolism

T2DM is a metabolic disease characterized by hyperglycemia. Bile acids play a significant role in the regulation of insulin sensitivity and glucose metabolism, which are critical aspects in the management of T2DM [116, 117]. Studies have shown that individuals with T2DM often exhibit higher levels of fasting total bile acid than those non-diabetic control subjects [118]. The fasting levels of total serum bile acids

correlate with insulin sensitivity, the functionality of islet β cells, and glucagon responses during glucose challenges in patients with T2DM [118, 119]. Moreover, elevated total serum bile acid levels have been consistently linked to a higher risk of developing T2DM and insulin resistance, as measured by HOMA-IR [120]. In black South African women, early T2DM stages have shown changes in the bile acid pool, alongside other metabolic shifts, indicating that bile acid profiles could serve as early indicators for T2DM risk, potentially over a decade before disease onset in this under-researched group [121]. In a rat T2DM model, the ratio of serum 12α-OH bile acids to non-12α-OH bile acids was increased due to the upregulation of Cyp8b1 in the liver. In addition, the altered bile acids negatively impact glucose metabolism by inhibiting TGR5/FXR-signaling pathways [122]. A clinical study reported that middle-aged women with gestational diabetes mellitus (GDM) with HOMA-IR > 2.8 have altered conjugated and non-12α-OH fractions of bile acids. However, it is not clear if the altered bile acid metabolism is a cause or a consequence of GDM [123].

TUDCA is an endogenous bile acid found in the bile of some species of bears [124]. A recent study reported that TUDCA attenuated hyperinsulinemia and improved glucose homeostasis in aged mice by enhancing liver insulin-degrading enzyme expression and insulin clearance [125]. In a double-blind, single-dose randomized controlled clinical trial, oral administration of delayed-release conjugated bile acids (ileoconic) for 28 days in patients with obesity and T2DM exerted a small lowering of postprandial glucose concentrations, improved LDL cholesterol, and altered fecal bile acid levels, but did not correct the FGF19 deficiency [126]. Pigs are routinely raised on obesogenic diets yet are resistant to the development of T2DM. Zheng et al. report that a group of bile acids, HCA, and its derivatives, the primary bile acids in pigs, improved serum GLP-1 secretion and glucose homeostasis to a greater extent than TUDCA in T2DM mouse model [127]. In humans, HCA and its derivatives are also present in the blood and urine, although relatively lower than in pigs. A recent clinical study demonstrated that serum HCA species are strong predictors for obesity and T2DM and can be used to assess the potential risk of developing metabolic disorders [92].

Colesevelam, a non-absorbed bile acid sequestrant, inhibits bile acid reabsorption in the intestines and has been used to lower cholesterol levels and control glucose levels in T2DM patients. Beysen and their team showed that Colesevelam increased circulating incretins and improved tissue glucose metabolism in both the fasting and postprandial states [128]. Elobixibat, a selective IBAT inhibitor, was recently approved in Japan for the treatment of chronic constipation. Elobixibat was found to benefit patients with T2DM by improving glucose metabolism by increasing plasma GLP-1[129].

The development of T2DM is closely related to the disturbances in the gut microbiome [130]. Differences in gut microbiota composition have been observed in both preclinical animal models and patients with T2DM. Specifically, the genera of *Akkermansia*, *Bifidobacterium*, *Faecalibacterium*, *Bacteroides*, and *Roseburia* were negatively associated with T2DM, while the genera of *Fusobacterium*, *Ruminococcus*, and *Blautia* were positively associated with T2DM [130]. Furthermore, T2DM is associated with dysregulation of bile acid metabolism by gut microbiota [117]. Remodeling of the gut microbiome by antibiotic treatment can modify the bile acid profiles. Different antibiotics have distinct effects on the composition of the bile acid pool [131]. T2DM patients who received metformin showed a decrease in *Bacteroides fragilis* accompanied by an increase of glycoursodeoxycholic acid (GUDCA) in the intestinal tract. GUDCA was identified as an intestinal FXR antagonist. Inhibition of FXR activation in the gut has a beneficial effect on improving metabolic dysfunction, including hyperglycemia [132]. GUDCA could regulate bile acids level and alter gut microbiota and glycolipid metabolism to attenuate T2DM [133]. The administration of GUDCA in db/db mice led to increased TLCA levels and a greater abundance of *Bacteroides vulgatus* in the gut. This change was associated with the activation of the TGR5 and an increased expression of UCP-1, indicating potential anti-diabetic effects of GUDCA are linked with the regulation of the bile acid and gut microbiota composition [133]. The intestinal flora of Uygur T2DM patients differed from that of normal glucose-tolerant people. FMT using Uygur T2DM patient fecal samples disrupts blood glucose and bile acid homeostasis in mice [134]. The relationship between bile acids, insulin sensitivity, and glucose metabolism in T2DM underscores the complexity of the disease and highlights the potential for targeting bile acid pathways as a therapeutic strategy. Given the intricate interplay between bile acids, gut microbiota, and metabolic regulation, further research is needed to fully understand these mechanisms and their implications for T2DM management.

MASLD and bile acid dysregulation

MASLD stands for metabolic dysfunction-associated steatotic liver disease and is the new name for NAFLD. The non-alcoholic steatohepatitis, known as NASH, is now called metabolic dysfunction-associated steatohepatitis, or MASH. The change of NAFLD and NASH to MASLD and MASH

emphasizes the role of metabolic risk factors, including obesity, T2DM, insulin resistance, and dyslipidemia, in the development of fatty liver disease [135]. Bile acid dysregulation has been implicated in the pathophysiology of MASLD [136]. Alterations of bile acid composition and concentration contribute to disease progression and are linked to the severity of liver steatosis and inflammation. A recent meta-analysis of 19 studies showed that total bile acid levels in MASLD patients are higher than those in healthy controls. In addition, the bile acid composition in MASLD patients was also significantly different from that in healthy controls [137]. The serum levels of total bile acids and conjugated bile acids elevated gradually in early MAFLD patients. The changes in ratios of glycine-conjugated bile acids to taurine-conjugated bile acids are linked with the severity of MAFLD, highlighting their potential role in the pathogenesis and progression of MASLD [138–140].

Bile acid-mediated activation of FXR activation plays a protective role by regulating lipid and glucose metabolism and reducing inflammation in MASLD [141]. In $FXR^{-/-}$ mice, HFD aggravated liver injury with diseased BSEP expression and increased lipogenesis as well as accumulation of hepatic bile acids and fatty acids [142]. Patients with MASLD are often at increased risk of developing cardiovascular diseases. In addition to FXR, TGR5 also plays an important role in maintaining metabolic homeostasis. The double knockout mice of FXR and TGR5 display hypotension and inflammation, along with disruption of intestinal barrier integrity and dysregulated bile acid synthesis [143]. This study suggests that a dual GPBAR1 (TGR5)/FXR ligand and atorvastatin hold potential in the treatment of MASLD with cardiovascular complications [143]. In a Western Diet (WD)-induced mouse metabolic dysfunction-associated steatohepatitis (MASH) model, INT-767, an FXR-TGR5 dual agonist, prevented the progression of WD-induced hepatic steatosis, inflammation, and fibrosis by modulating bile acid composition in the liver and intestine, leading to decreases in the hydrophobicity index of bile acids. Interestingly, the effects of INT-767 in attenuating MASH were absent in Fxr-null mice but still present in TGR5 null mice, indicating an FXR-dependent and TGR-independent effect of INT-767 [144]. The intracellular distribution of gut scavenger receptor class B type 1 (SR-B1) was highly sensitive to insulin levels [145]. HFD feeding impaired lipid homeostasis, including the stimulation of SR-B1 expression and elevated chylomicron secretion. Resveratrol intervention was reported to attenuate chylomicron secretion *via* repressing intestinal FXR-induced expression of gut SR-B1 [146]. Interestingly, Gly-MCA, an intestine-specific FXR antagonist, had beneficial effects on MASH *via* inhibiting the intestinal FXR-ceramide axis [147]. In addition to FXR, FGFR4 is also a key regulator of hepatic bile acid synthesis. Liver-specific FGFR4 silencing increased bile acid production and lowered serum cholesterol. Additionally, FGFR4 knockdown improved HFD-induced liver steatosis, primarily through activating the FXR-FGF15 pathway in intestinal cells rather than in hepatocytes [148]. Mice lacking TGR5 experienced more severe liver damage, higher levels of inflammatory markers, and increased M1 macrophage activation due to NLRP3 inflammasome stimulation. This highlights TGR5's role in mitigating liver steatosis and inflammation by inhibiting NLRP3-driven M1 macrophage polarization in MASH [149].

Inhibition of IBAT by IBATi enhances bile acid flow to the colon, improving MASLD by ameliorating gut microbiota dysbiosis [150]. Elobixibat, an IBAT inhibitor, reduced the serum bile acids, increased the fecal bile acid concentration, and ameliorated liver inflammation and fibrosis in MCD-fed mice [151]. Rao and colleagues found that a non-absorbable ASBT inhibitor (ASBTi; SC-435) significantly mitigated MASLD progression in mice on a choline-deficient, L-amino acid-defined (CDAA) diet, evidenced by decreases in intestinal fat absorption, body weight gain and hepatic lipid accumulation [152]. Our previous studies showed that bile acid-mediated activation of S1PR2 plays a crucial role in hepatic lipid and glucose metabolism [56, 58, 153]. Mice lacking S1PR2 or SphK2 showed increased susceptibility to HFD-induced fatty liver. Furthermore, we identified that TCA-mediated activation of S1PR2 led to the activation of SphK2 and the production of nuclear S1P, which has been identified as a potent inhibitor of HDACs [58, 154].

Gut microbiota and bile acid metabolism play crucial roles in MASLD's pathogenesis [155]. Studies have found that patients with MASLD have different degrees of intestinal flora disorder, which is characterized by the reduction of *Bacteroidetes* and the increase of *Firmicutes* [156]. A decrease in α-diversity and changes in gut microbiota abundance, characterized by increased levels of *Escherichia* and *Prevotella*, and decreased levels of *Akkermansia muciniphila* and *Faecalibacterium* were also found in patients with MASLD. Administration of vancomycin, which targets Gram-positive organisms, exacerbated liver damage, steatohepatitis, and fibrosis, in a high-fat/cholesterol/cholate-based diet, but not with metronidazole, which targets anaerobic microorganisms in the same mouse mode [157]. A recent study reported that *Lactiplantibacillus plantarum NKK20 strain* (NKK20) alleviated HFD-induced MASLD in mouse models *via* regulating bile acid anabolism [22]. Excessive fructose intake is closely associated with MASLD. A recent study done by Zhang et al. showed that chronic consumption of fructose impaired bile acid circulation with significant changes in the *Clostridium species* and LCA/TLCA levels [158]. A previous study reported that the *Lactobacillus*

casei YRL577 with high BSH activity improved MASLD by activating the intestinal bile acid FXR-FGF15 pathway[159]. Smirnova and colleagues demonstrated that circulating levels of the secondary bile acid, DCA, are associated with MASLD disease progression [160]. HDCA targeted intestinal FXR and promoted beneficial gut microbiomes, such as *P. distasonis*, leading to upregulation of hepatic CYP7B1, PPARα, and FXR and improvement of MAFLD [161]. Obeticholic acid (OCA), a novel bile acid derivative, has been demonstrated to ameliorate MASLD-related manifestations, but the mechanisms are not fully identified. A recent study by Liu et al. reported that OCA intervention altered gut microbiota composition with specially enriched gut microbes modulating host bile acids, thus effectively alleviating MASLD in the mice [162]. In addition, TUDCA has been reported to attenuate the progression of HFD-induced MAFLD in mice by ameliorating gut inflammation, improving intestinal barrier function, decreasing intestinal fat transport, and modulating intestinal microbiota composition [163]. In addition to the gut microbiome and bile acids, the mitochondria toxic CYP27A1-derived cholesterol metabolites also play an important role in the disease progression of MASH [164].

MASLD involves a complex interaction between bile acids, gut microbiome, and different bile acid receptors. Dysregulated bile acid metabolism and signaling contribute to dysregulation of hepatic lipid metabolism and inflammation, underscoring the importance of targeting these pathways for effective MASLD management.

Diagnostic and therapeutic implications

The role of bile acids as biomarkers for metabolic diseases

Bile acids have emerged as potential biomarkers for various metabolic diseases due to their involvement in metabolic processes and their interactions with specific receptors and pathways. Monitoring bile acid profiles can provide insights into the status of liver function, lipid metabolism, and overall metabolic health. Recent epidemiological studies have reported that specific changes in circulating bile acid profiles are associated with various metabolic diseases, including T2DM and MASLD [80]. Serum HCA levels can be used to predict the development of metabolic disorders in 5 or 10 years [92]. It has been demonstrated that elevated serum total bile acid levels are linked to an increased risk of developing T2DM [120]. Dysregulated bile acid metabolism has been associated with hepatic steatosis, inflammation, and fibrosis in MASLD. A recent clinical study with biopsy-proven MASLD patients showed serum secondary bile acid levels were significantly increased in MASLD, especially in those patients with mild fibrosis [165]. A recent meta-analysis showed that circulating levels of TCA, TDCA, TLCA, and GLCA have the potential to differentiate MASH [137]. In addition to serum bile acids, fecal bile acid composition and levels as well as serum C4 concentration, can be used to predict the disease severity of MASLD [166].

Current therapeutic strategies targeting bile acid pathways

Bile acids are important signaling molecules and play essential roles in maintaining metabolic homeostasis. Dysregulation of bile acid metabolism and enterohepatic circulation is closely associated with various metabolic diseases, including T2DM and MASLD. In the last decade, extensive effort has been put into developing therapeutic strategies targeting bile acid pathways for metabolic diseases, focusing on modulating bile acid receptors, such as FXR and TGR5, and bile acid synthesis and transport [167]. OCA, the first-in-class steroidal FXR agonist, has shown promise in MASH clinical trials, particularly in managing lipid metabolism, inflammation, and fibrosis. Its role in MASH treatment highlights the potential of targeting bile acid pathways for metabolic disease management. In addition to OCA, several non-steroidal FXR ligands with even higher affinity for FXR have been developed [168–171]. Interestingly, some specific antagonists of intestinal FXR, such as Gly-MCA [38, 147, 172] and GUDCA [132, 133, 173], have been reported as potential therapeutic agents for metabolic diseases. In addition, TGR5 agonists have also shown beneficial effects on metabolic diseases. Activation of TGR5 enhances energy expenditure, promoting GLP-1 secretion, improving insulin sensitivity, and reducing inflammation [174–179]. In addition to modulating bile acid-mediated signaling pathways, bile acid sequestrants, such as cholestyramine and colesevelam, have been used for the management of hypercholesterolemia and also has been investigated for their potential in metabolic diseases, including T2DM. Modulating the gut microbiota can indirectly affect bile acid metabolism, as the microbiota is involved in bile acid transformation. Exploring probiotics, prebiotics, and FMT offers the potential for managing bile acid-related conditions. Additionally, bariatric surgery, like RYGB, and using IBAT inhibitors may alter bile acid circulation and metabolism, potentially enhancing metabolic health (Figure 8) [106, 150, 162, 180–184].

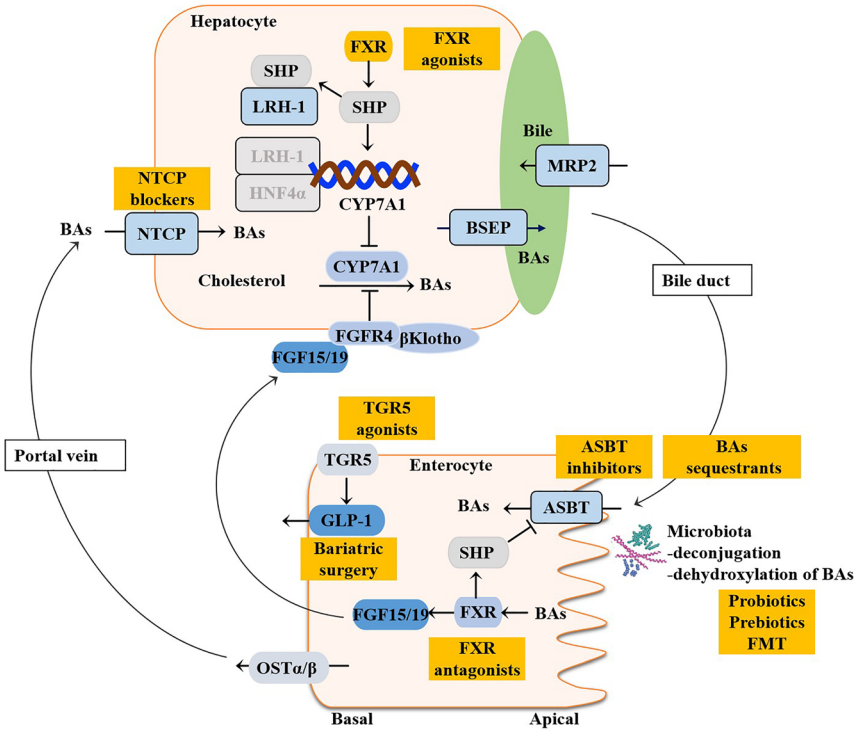

Figure 8: Therapeutic strategies targeting bile acid pathways for metabolic diseases. This figure outlines potential therapeutic interventions targeting bile acid receptors and transporters, modulating gut microbiota, utilizing bile acid sequestrants, and operating bariatric surgery. Modulation of bile acid metabolism and enterohepatic circulation offers therapeutic benefits for metabolic diseases, including obesity, T2DM and MASLD. FXR, farnesoid X receptor; TGR5 (GPBAR1), takeda G protein-coupled receptor 5 (G protein-coupled bile acid receptor 1); ASBT (IBAT, SLC10A2), apical sodium-dependent bile acid transporter; NTCP, sodium taurocholate cotransporting polypeptide; FMT, fecal microbiota transplantation.

Challenges and future directions

Our understanding of the role of bile acids in metabolic diseases has expanded significantly in the past decades. Recent studies highlight the multifaceted roles of bile acids in regulating lipid, glucose, and energy metabolism. Despite these advances, several challenges and problems persist in current bile acid research.

1) Complexity of bile acid-mediated signaling pathways. Bile acids can act as both agonists and antagonists for various receptors. The complexity of bile acid-mediated signaling pathways and their interconnections with other metabolic processes make it difficult to fully understand the precise mechanisms by which bile acids impact different metabolic diseases.
2) Individual variability in bile acid metabolism. The bile acid composition and signaling mechanisms vary significantly among individuals, influenced by genetics, gut microbiota, diet, and other environmental factors. This variability makes it difficult to interpret the research findings and the development of generalized therapeutic strategies.
3) The role of gut microbiota. Bile acid metabolism is intricately linked with gut microbiota. However, the complex interactions between bile acids, gut microbiota, and host metabolism is not fully understood. Disentangling these interactions is challenging but crucial for understanding the role of bile acids in metabolic diseases.
4) Limitation of animal models. Much of our current knowledge about bile acid metabolism and function comes from animal studies, particularly in rodents. However, significant differences in bile acid composition and metabolism between rodents and humans may limit the applicability of these findings to human diseases. The discovery of the rodent-specific enzyme Cyp2c70 and the development of mouse models with a humanized bile acid pool are steps toward bridging this gap.
5) Off-target and side effects of bile acid-targeted therapeutics. The development of therapies targeting bile acid metabolic and signaling pathways, such as FXR agonists, show promise for metabolic diseases. However, these agents often have off-target or adverse effects, which pose significant challenges in developing bile acid-based effective therapies for metabolic diseases.
6) Broad impact on metabolic pathways. Bile acids influence a broad range of metabolic processes, including lipid, glucose, and energy metabolism. In order to fully understand these interactions, more advanced and integrative approaches are needed. Addressing these challenges requires multidisciplinary approaches, including molecular biology, microbiology, genetics,

bioinformatics, and system biology. Recent advancements in these fields offer the potential to map interconnected metabolic networks and explore new therapeutic approaches by modulating bile acid metabolism. Moreover, the development of personalized medicine strategies that incorporate bile acid profiling has the potential to significantly improve treatment outcomes and provide valuable insights into preventive strategy. This underscores the importance of collaborative efforts across multiple disciplines.

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References

1. Kaya E, Yilmaz Y. Metabolic-associated fatty liver disease (MAFLD): a multi-systemic disease beyond the liver. J Clin Transl Hepatol 2022;10: 329–38.
2. McPhail S. Multimorbidity in chronic disease: impact on health care resources and costs. Risk Manag Healthc Pol 2016;9:143–56.
3. Washington TB, Johnson VR, Kendrick K, Ibrahim AA, Tu L, Sun K, et al. Disparities in access and quality of obesity care. Gastroenterol Clin N Am 2023;52:429–41.
4. Clemente-Suárez VJ, Martín-Rodríguez A, Redondo-Flórez L, López-Mora C, Yáñez-Sepúlveda R, Tornero-Aguilera JF. New insights and potential therapeutic interventions in metabolic diseases. Int J Mol Sci 2023;24:10672.
5. Chiang JYL, Ferrell JM. Bile acids as metabolic regulators and nutrient sensors. Annu Rev Nutr 2019;39:175–200.
6. Chiang JYL, Ferrell JM. Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. Am J Physiol Gastrointest Liver Physiol 2020;318:G554–73.
7. Li T, Chiang JYL. Bile acids as metabolic regulators: an update. Curr Opin Gastroenterol 2023;39:249–55.
8. Chiang JYL. Bile acid metabolism and signaling. Compr Physiol 2013;3: 1191–212.
9. Chiang JYL. Regulation of bile acid synthesis. Front Biosci 1998;3: d176–93.
10. Takahashi S, Fukami T, Masuo Y, Brocker CN, Xie C, Krausz KW, et al. Cyp2c70 is responsible for the species difference in bile acid metabolism between mice and humans. J Lipid Res 2016;57:2130–7.
11. Falany CN, Johnson MR, Barnes S, Diasio RB. Glycine and taurine conjugation of bile acids by a single enzyme. Molecular cloning and expression of human liver bile acid CoA: amino acid N-acyltransferase. J Biol Chem 1994;269:19375–9.
12. Alrehaili BD, Lee M, Takahashi S, Novak R, Rimal B, Boehme S, et al. Bile acid conjugation deficiency causes hypercholanemia, hyperphagia, islet dysfunction, and gut dysbiosis in mice. Hepatol Commun 2022;6:2765–80.
13. Guzior DV, Okros M, Shivel M, Armwald B, Bridges C, Fu Y, et al. Bile salt hydrolase acyltransferase activity expands bile acid diversity. Nature 2024;626:852–8.
14. Chiang JYL, Ferrell JM. Bile acid metabolism in liver pathobiology. Gene Expr 2018;18:71–87.
15. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science 1999;284: 1362–5.
16. Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: natural ligands for an orphan nuclear receptor. Science 1999;284:1365–8.
17. Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999;3: 543–53.
18. Kir S, Zhang Y, Gerard RD, Kliewer SA, Mangelsdorf DJ. Nuclear receptors HNF4α and LRH-1 cooperate in regulating Cyp7a1 *in vivo*. J Biol Chem 2012;287:41334–41.
19. Koop I, Schindler M, Bosshammer A, Scheibner J, Stange E, Koop H. Physiological control of cholecystokinin release and pancreatic enzyme secretion by intraduodenal bile acids. Gut 1996;39:661.
20. Hylemon PB, Su L, Zheng PC, Bajaj JS, Zhou H. Bile acids, gut microbiome and the road to fatty liver disease. Compr Physiol 2021;12: 2719–730.
21. Muthiah MD, Smirnova E, Puri P, Chalasani N, Shah VH, Kiani C, et al. Development of alcohol-associated hepatitis is associated with specific changes in gut-modified bile acids. Hepatol Commun 2022;6: 1073–89.
22. Sun C, Qiu C, Zhang Y, Yan M, Tan J, He J, et al. Lactiplantibacillus plantarum NKK20 alleviates high-fat-diet-induced nonalcoholic fatty liver disease in mice through regulating bile acid anabolism. Molecules 2023;28. https://doi.org/10.3390/molecules28104042.
23. Rimal B, Collins SL, Tanes CE, Rocha ER, Granda MA, Solanki S, et al. Bile salt hydrolase catalyses formation of amine-conjugated bile acids. Nature 2024;626:859–63.
24. Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall H-U, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metabol 2013;17:225–35.
25. Shi L, Jin L, Huang W. Bile acids, intestinal barrier dysfunction, and related diseases. Cells 2023;12:1888.
26. Dawson PA. Role of the intestinal bile acid transporters in bile acid and drug disposition. In: Handbook of experimental pharmacology. Berlin: Springer; 2011:169–203 pp.
27. Xue R, Su L, Lai S, Wang Y, Zhao D, Fan J, et al. Bile acid receptors and the gut-liver axis in nonalcoholic fatty liver disease. Cells 2021;10. https://doi.org/10.3390/cells10112806.
28. Ghallab A, Gonzalez D, Strangberg E, Hofmann U, Myllys M, Hassan R, et al. Inhibition of the renal apical sodium dependent bile acid transporter prevents cholemic nephropathy in mice with obstructive cholestasis. J Hepatol 2024;80:268–81.
29. Xia X, Francis H, Glaser S, Alpini G, LeSage G. Bile acid interactions with cholangiocytes. World J Gastroenterol 2006;12:3553–63.
30. Zeng J, Fan J, Zhou H. Bile acid-mediated signaling in cholestatic liver diseases. Cell Biosci 2023;13:77.
31. Yang J, de Vries HD, Mayeuf-Louchart A, Stroeve JH, Bloks VW, Koehorst M, et al. Role of bile acid receptor FXR in development and

function of brown adipose tissue. Biochim Biophys Acta Mol Cell Biol Lipids 2023;1868:159257.
32. Lin S, Wang S, Wang P, Tang C, Wang Z, Chen L, et al. Bile acids and their receptors in regulation of gut health and diseases. Prog Lipid Res 2023;89:101210.
33. Aseem SO, Hylemon PB, Zhou H. Bile acids and biliary fibrosis. Cells 2023;12:792.
34. Yang G, Jena PK, Hu Y, Sheng L, Chen SY, Slupsky CM, et al. The essential roles of FXR in diet and age influenced metabolic changes and liver disease development: a multi-omics study. Biomark Res 2023;11:20.
35. Li H, Zhu X, Xu J, Li L, Kan W, Bao H, et al. The FXR mediated anti-depression effect of CDCA underpinned its therapeutic potentiation for MDD. Int Immunopharm 2023;115:109626.
36. Guo Y, Xie G, Zhang X. Role of FXR in renal physiology and kidney diseases. Int J Mol Sci 2023;24:2408.
37. Chiang JYL, Ferrell JM. Discovery of farnesoid X receptor and its role in bile acid metabolism. Mol Cell Endocrinol 2022;548:111618.
38. Jiang J, Ma Y, Liu Y, Lu D, Gao X, Krausz KW, et al. Glycine-beta-muricholic acid antagonizes the intestinal farnesoid X receptor-ceramide axis and ameliorates NASH in mice. Hepatol Commun 2022; 6:3363–78.
39. Trauner M, Gulamhusein A, Hameed B, Caldwell S, Shiffman ML, Landis C, et al. The nonsteroidal farnesoid X receptor agonist cilofexor (GS-9674) improves markers of cholestasis and liver injury in patients with primary sclerosing cholangitis. Hepatology 2019;70: 788–801.
40. Ma Y, Huang Y, Yan L, Gao M, Liu D. Synthetic FXR agonist GW4064 prevents diet-induced hepatic steatosis and insulin resistance. Pharm Res 2013;30:1447–457.
41. Zhang S, Wang J, Liu Q, Harnish DC. Farnesoid X receptor agonist WAY-362450 attenuates liver inflammation and fibrosis in murine model of non-alcoholic steatohepatitis. J Hepatol 2009;51: 380–8.
42. Malerod L, Sporstol M, Juvet LK, Mousavi SA, Gjoen T, Berg T, et al. Bile acids reduce SR-BI expression in hepatocytes by a pathway involving FXR/RXR, SHP, and LRH-1. Biochem Biophys Res Commun 2005;336: 1096–105.
43. Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell 2000;6:517–26.
44. Shonsey EM, Sfakianos M, Johnson M, He D, Falany CN, Falany J, et al. Bile acid coenzyme A: amino acid N-acyltransferase in the amino acid conjugation of bile acids. Methods Enzymol 2005;400: 374–94.
45. Pircher PC, Kitto JL, Petrowski ML, Tangirala RK, Bischoff ED, Schulman IG, et al. Farnesoid X receptor regulates bile acid-amino acid conjugation. J Biol Chem 2003;278:27703–11.
46. Cui JY, Aleksunes LM, Tanaka Y, Fu ZD, Guo Y, Guo GL, et al. Bile acids via FXR initiate the expression of major transporters involved in the enterohepatic circulation of bile acids in newborn mice. Am J Physiol Gastrointest Liver Physiol 2012;302:G979–96.
47. Rizzo G, Renga B, Mencarelli A, Pellicciari R, Fiorucci S. Role of FXR in regulating bile acid homeostasis and relevance for human diseases. Curr Drug Targets Immune Endocr Metab Disord 2005;5: 289–303.
48. Xiang D, Yang J, Liu L, Yu H, Gong X, Liu D. The regulation of tissue-specific farnesoid X receptor on genes and diseases involved in bile acid homeostasis. Biomed Pharmacother 2023;168:115606.
49. Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). Biochem Biophys Res Commun 2002;298:714–9.
50. Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003; 278:9435–40.
51. Thomas C, Gioiello A, Noriega L, Strehle A, Oury J, Rizzo G, et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metabol 2009;10:167–77.
52. Pols TWH, Noriega LG, Nomura M, Auwerx J, Schoonjans K. The bile acid membrane receptor TGR5 as an emerging target in metabolism and inflammation. J Hepatol 2011;54:1263–72.
53. Ye D, He J, He X. The role of bile acid receptor TGR5 in regulating inflammatory signalling. Scand J Immunol 2024;99:e13361.
54. Ancellin N, Hla T. Differential pharmacological properties and signal transduction of the sphingosine 1-phosphate receptors EDG-1, EDG-3, and EDG-5. J Biol Chem 1999;274:18997–9002.
55. Nagahashi M, Yuza K, Hirose Y, Nakajima M, Ramanathan R, Hait NC, et al. The roles of bile acids and sphingosine-1-phosphate signaling in the hepatobiliary diseases. J Lipid Res 2016;57:1636–43.
56. Studer E, Zhou X, Zhao R, Wang Y, Takabe K, Nagahashi M, et al. Conjugated bile acids activate the sphingosine-1-phosphate receptor 2 in primary rodent hepatocytes. Hepatology 2012;55:267–76.
57. Kwong E, Li Y, Hylemon PB, Zhou H. Bile acids and sphingosine-1-phosphate receptor 2 in hepatic lipid metabolism. Acta Pharm Sin B 2015;5:151–7.
58. Nagahashi M, Takabe K, Liu R, Peng K, Wang X, Wang Y, et al. Conjugated bile acid-activated S1P receptor 2 is a key regulator of sphingosine kinase 2 and hepatic gene expression. Hepatology 2015;61:1216–26.
59. Lee SY, Hong IK, Kim BR, Shim SM, Sung Lee J, Lee HY, et al. Activation of sphingosine kinase 2 by endoplasmic reticulum stress ameliorates hepatic steatosis and insulin resistance in mice. Hepatology 2015;62: 135–46.
60. Adada M, Canals D, Hannun YA, Obeid LM. Sphingosine-1-phosphate receptor 2. FEBS J 2013;280:6354–66.
61. Hrncir T, Hrncirova L, Kverka M, Hromadka R, Machova V, Trckova E, et al. Gut microbiota and NAFLD: pathogenetic mechanisms, microbiota signatures, and therapeutic interventions. Microorganisms 2021;9:957.
62. Ho RH, Leake BF, Urquhart BL, Gregor JC, Dawson PA, Kim RB. Functional characterization of genetic variants in the apical sodium-dependent bile acid transporter (ASBT; SLC10A2). J Gastroenterol Hepatol 2011;26:1740–8.
63. Heni M, Wagner R, Ketterer C, Böhm A, Linder K, Machicao F, et al. Genetic variation in NR1H4 encoding the bile acid receptor FXR determines fasting glucose and free fatty acid levels in humans. J Clin Endocrinol Metab 2013;98:E1224–9.
64. Qayyum F, Lauridsen BK, Frikke-Schmidt R, Kofoed KF, Nordestgaard BG, Tybjærg-Hansen A. Genetic variants in CYP7A1 and risk of myocardial infarction and symptomatic gallstone disease. Eur Heart J 2018;39:2106–16.
65. Zhang M, Xiao B, Chen X, Ou B, Wang S. Physical exercise plays a role in rebalancing the bile acids of enterohepatic axis in non-alcoholic fatty liver disease. Acta Physiol 2024;240:e14065.
66. Mercer KE, Maurer A, Pack LM, Ono-Moore K, Spray BJ, Campbell C, et al. Exercise training and diet-induced weight loss increase markers of hepatic bile acid (BA) synthesis and reduce serum total BA concentrations in obese women. Am J Physiol Endocrinol Metab 2021; 320:E864–73.

67. Qu Y, Su C, Zhao Q, Shi A, Zhao F, Tang L, et al. Gut microbiota-mediated elevated production of secondary bile acids in chronic unpredictable mild stress. Front Pharmacol 2022;13:837543.
68. Wang Y, Tai Y-L, Zhao D, Zhang Y, Yan J, Kakiyama G, et al. Berberine prevents disease progression of nonalcoholic steatohepatitis through modulating multiple pathways. Cells 2021;10:210.
69. Lin H, An Y, Tang H, Wang Y. Alterations of bile acids and gut microbiota in obesity induced by high fat diet in rat model. J Agric Food Chem 2019;67:3624–32.
70. Yokota A, Fukiya S, Islam KS, Ooka T, Ogura Y, Hayashi T, et al. Is bile acid a determinant of the gut microbiota on a high-fat diet? Gut Microb 2012;3:455–9.
71. Kastl A, Zong W, Gershuni VM, Friedman ES, Tanes C, Boateng A, et al. Dietary fiber-based regulation of bile salt hydrolase activity in the gut microbiota and its relevance to human disease. Gut Microb 2022;14: 2083417.
72. He L, Vatsalya V, Ma X, Zhang J, Yin X, Kim S, et al. Metabolic profiling of bile acids in the urine of patients with alcohol-associated liver disease. Hepatol Commun 2021;5:798–811.
73. Duboc H, Rajca S, Rainteau D, Benarous D, Maubert MA, Quervain E, et al. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut 2013;62:531–9.
74. Osorio J. Obesity: gut-specific FXR agonism. Nat Rev Endocrinol 2015; 11:131.
75. Li T, Francl JM, Boehme S, Ochoa A, Zhang Y, Klaassen CD, et al. Glucose and insulin induction of bile acid synthesis: mechanisms and implication in diabetes and obesity. J Biol Chem 2012;287: 1861–73.
76. Xie C, Huang W, Young RL, Jones KL, Horowitz M, Rayner CK, et al. Role of bile acids in the regulation of food intake, and their dysregulation in metabolic disease. Nutrients 2021;13:1104.
77. Li Y, Lu LG. Therapeutic roles of bile acid signaling in chronic liver diseases. J Clin Transl Hepatol 2018;6:425–30.
78. Ticho AL, Malhotra P, Dudeja PK, Gill RK, Alrefai WA. Intestinal absorption of bile acids in health and disease. Compr Physiol 2019;10: 21–56.
79. Baars A, Oosting A, Knol J, Garssen J, van Bergenhenegouwen J. The gut microbiota as a therapeutic target in IBD and metabolic disease: a role for the bile acid receptors FXR and TGR5. Microorganisms 2015;3: 641–66.
80. Qi L, Chen Y. Circulating bile acids as biomarkers for disease diagnosis and prevention. J Clin Endocrinol Metab 2023;108:251–70.
81. Fu T, Coulter S, Yoshihara E, Oh TG, Fang S, Cayabyab F, et al. FXR regulates intestinal cancer stem cell proliferation. Cell 2019;176: 1098–12.e18.
82. Zimber A, Gespach C. Bile acids and derivatives, their nuclear receptors FXR, PXR and ligands: role in health and disease and their therapeutic potential. Anti Cancer Agents Med Chem 2008;8: 540–63.
83. De Gottardi A, Touri F, Maurer CA, Perez A, Maurhofer O, Ventre G, et al. The bile acid nuclear receptor FXR and the bile acid binding protein IBABP are differently expressed in colon cancer. Dig Dis Sci 2004;49:982–9.
84. Chiang JY, Ferrell JM, Wu Y, Boehme S. Bile acid and cholesterol metabolism in atherosclerotic cardiovascular disease and therapy. Cardiol 2020;5:159.
85. Chávez-Talavera O, Tailleux A, Lefebvre P, Staels B. Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. Gastroenterology 2017;152:1679–94.e3.
86. Zagoskin P, Erlykina E. Bile acids as a new type of steroid hormones regulating nonspecific energy expenditure of the body. Sovrem Tekhnol Med 2020;12:114–27.
87. Steenackers N, Eksteen G, Wauters L, Augustijns P, Van der Schueren B, Vanuytsel T, et al. Understanding the gastrointestinal tract in obesity: from gut motility patterns to enzyme secretion. Neuro Gastroenterol Motil 2024;36:e14758.
88. Lun W, Yan Q, Guo X, Zhou M, Bai Y, He J, et al. Mechanism of action of the bile acid receptor TGR5 in obesity. Acta Pharm Sin B 2024;14: 468–91.
89. Haeusler RA, Camastra S, Nannipieri M, Astiarraga B, Castro-Perez J, Xie D, et al. Increased bile acid synthesis and impaired bile acid transport in human obesity. J Clin Endocrinol Metab 2016;101:1935–44.
90. Ma H, Patti ME. Bile acids, obesity, and the metabolic syndrome. Best Pract Res Clin Gastroenterol 2014;28:573–83.
91. Nakade Y, Kitano R, Sakamoto K, Kimoto S, Yamauchi T, Inoue T, et al. Characteristics of bile acid composition in high fat diet-induced nonalcoholic fatty liver disease in obese diabetic rats. PLoS One 2021; 16:e0247303.
92. Zheng X, Chen T, Zhao A, Ning Z, Kuang J, Wang S, et al. Hyocholic acid species as novel biomarkers for metabolic disorders. Nat Commun 2021;12:1487.
93. Kusumoto Y, Irie J, Iwabu K, Tagawa H, Itoh A, Kato M, et al. Bile acid binding resin prevents fat accumulation through intestinal microbiota in high-fat diet-induced obesity in mice. Metabolism 2017;71:1–6.
94. Jonsson I, Bojsen-Moller KN, Kristiansen VB, Veedfald S, Wewer Albrechtsen NJ, Clausen TR, et al. Effects of manipulating circulating bile acid concentrations on postprandial GLP-1 secretion and glucose metabolism after roux-en-Y gastric bypass. Front Endocrinol 2021;12:681116.
95. De Vuono S, Ricci MA, Nulli Migliola E, Monti MC, Morretta E, Boni M, et al. Serum bile acid levels before and after sleeve gastrectomy and their correlation with obesity-related comorbidities. Obes Surg 2019; 29:2517–26.
96. Lee SH, Choi JM, Jung SY, Cox AR, Hartig SM, Moore DD, et al. The bile acid induced hepatokine orosomucoid suppresses adipocyte differentiation. Biochem Biophys Res Commun 2021;534:864–70.
97. Castellanos-Jankiewicz A, Guzman-Quevedo O, Fenelon VS, Zizzari P, Quarta C, Bellocchio L, et al. Hypothalamic bile acid-TGR5 signaling protects from obesity. Cell Metabol 2021;33:1483–92.e10.
98. Fang S, Suh JM, Reilly SM, Yu E, Osborn O, Lackey D, et al. Intestinal FXR agonism promotes adipose tissue browning and reduces obesity and insulin resistance. Nat Med 2015;21:159–65.
99. Wei M, Huang F, Zhao L, Zhang Y, Yang W, Wang S, et al. A dysregulated bile acid-gut microbiota axis contributes to obesity susceptibility. EBioMedicine 2020;55:102766.
100. Wang XX, Edelstein MH, Gafter U, Qiu L, Luo Y, Dobrinskikh E, et al. G protein-coupled bile acid receptor TGR5 activation inhibits kidney disease in obesity and diabetes. J Am Soc Nephrol 2016;27: 1362–78.
101. Sundaram S, Palaniappan B, Nepal N, Chaffins S, Sundaram U, Arthur S. Mechanism of dyslipidemia in obesity-unique regulation of ileal villus cell brush border membrane sodium-bile acid cotransport. Cells 2019;8:1197.
102. Stofan M, Guo GL. Bile acids and FXR: novel targets for liver diseases. Front Med 2020;7:544.
103. Li W, Zhuang T, Wang Z, Wang X, Liu L, Luo Y, et al. Red ginseng extracts ameliorate high-fat diet-induced obesity and insulin resistance by activating the intestinal TGR5-mediated bile acids signaling pathway. Phytomedicine 2023;119:154982.

104. Badman MK, Chen J, Desai S, Vaidya S, Neelakantham S, Zhang J, et al. Safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel non–bile acid FXR agonist tropifexor (LJN452) in healthy volunteers. Clin Pharmacol Drug Dev 2019;9:395–410.
105. Donkers JM, Roscam Abbing RLP, van Weeghel M, Levels JHM, Boelen A, Schinkel AH, et al. Inhibition of hepatic bile acid uptake by Myrcludex B promotes glucagon-like peptide-1 release and reduces obesity. Cell Mol Gastroenterol Hepatol 2020;10:451–66.
106. Munzker J, Haase N, Till A, Sucher R, Haange SB, Nemetschke L, et al. Functional changes of the gastric bypass microbiota reactivate thermogenic adipose tissue and systemic glucose control via intestinal FXR-TGR5 crosstalk in diet-induced obesity. Microbiome 2022;10:96.
107. Gomes AC, Hoffmann C, Mota JF. The human gut microbiota: metabolism and perspective in obesity. Gut Microb 2018;9:308–25.
108. Cai J, Sun L, Gonzalez FJ. Gut microbiota-derived bile acids in intestinal immunity, inflammation, and tumorigenesis. Cell Host Microbe 2022; 30:289–300.
109. Ghosh S, Whitley CS, Haribabu B, Jala VR. Regulation of intestinal barrier function by microbial metabolites. Cell Mol Gastroenterol Hepatol 2021;11:1463–82.
110. Allegretti JR, Kassam Z, Mullish BH, Chiang A, Carrellas M, Hurtado J, et al. Effects of fecal microbiota transplantation with oral capsules in obese patients. Clin Gastroenterol Hepatol 2020;18:855–63 e2.
111. Allegretti JR, Kassam Z, Hurtado J, Marchesi JR, Mullish BH, Chiang A, et al. Impact of fecal microbiota transplantation with capsules on the prevention of metabolic syndrome among patients with obesity. Hormones 2021;20:209–11.
112. Bustamante JM, Dawson T, Loeffler C, Marfori Z, Marchesi JR, Mullish BH, et al. Impact of fecal microbiota transplantation on gut bacterial bile acid metabolism in humans. Nutrients 2022;14:5200.
113. Wang K, Liao M, Zhou N, Bao L, Ma K, Zheng Z, et al. Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. Cell Rep 2019;26:222–35.e5.
114. Hibberd AA, Yde CC, Ziegler ML, Honore AH, Saarinen MT, Lahtinen S, et al. Probiotic or synbiotic alters the gut microbiota and metabolism in a randomised controlled trial of weight management in overweight adults. Benef Microbes 2019;10:121–35.
115. Liu TC, Kern JT, Jain U, Sonnek NM, Xiong S, Simpson KF, et al. Western diet induces Paneth cell defects through microbiome alterations and farnesoid X receptor and type I interferon activation. Cell Host Microbe 2021;29:988–1001.e6.
116. Ferrell JM, Chiang JYL. Understanding bile acid signaling in diabetes: from pathophysiology to therapeutic targets. Diabetes Metab J 2019; 43:257–72.
117. Gao R, Meng X, Xue Y, Mao M, Liu Y, Tian X, et al. Bile acids-gut microbiota crosstalk contributes to the improvement of type 2 diabetes mellitus. Front Pharmacol 2022;13:1027212.
118. Wang X, Chen C, Xie C, Huang W, Young RL, Jones KL, et al. Serum bile acid response to oral glucose is attenuated in patients with early type 2 diabetes and correlates with 2-hour plasma glucose in individuals without diabetes. Diabetes Obes Metabol 2022;24:1132–42.
119. Wang XH, Xu F, Cheng M, Wang X, Zhang DM, Zhao LH, et al. Fasting serum total bile acid levels are associated with insulin sensitivity, islet beta-cell function and glucagon levels in response to glucose challenge in patients with type 2 diabetes. Endocr J 2020;67:1107–17.
120. Zhu W, Wang S, Dai H, Xuan L, Deng C, Wang T, et al. Serum total bile acids associate with risk of incident type 2 diabetes and longitudinal changes in glucose-related metabolic traits. J Diabetes 2020;12: 616–25.
121. Zeng Y, Mtintsilana A, Goedecke JH, Micklesfield LK, Olsson T, Chorell E. Alterations in the metabolism of phospholipids, bile acids and branched-chain amino acids predicts development of type 2 diabetes in black South African women: a prospective cohort study. Metabolism 2019;95:57–64.
122. Zhang F, Yuan W, Wei Y, Zhang D, Duan Y, Li B, et al. The alterations of bile acids in rats with high-fat diet/streptozotocin-induced type 2 diabetes and their negative effects on glucose metabolism. Life Sci 2019;229:80–92.
123. Maghsoodi N, Shaw N, Cross GF, Alaghband-Zadeh J, Wierzbicki AS, Pinkney J, et al. Bile acid metabolism is altered in those with insulin resistance after gestational diabetes mellitus. Clin Biochem 2019;64: 12–7.
124. Zangerolamo L, Vettorazzi JF, Rosa LR, Carneiro EM, Barbosa HC. The bile acid TUDCA and neurodegenerative disorders: an overview. Life Sci 2021;272:119252.
125. Zangerolamo L, Carvalho M, Barssotti L, Soares GM, Marmentini C, Boschero AC, et al. The bile acid TUDCA reduces age-related hyperinsulinemia in mice. Sci Rep 2022;12:22273.
126. Calderon G, McRae A, Rievaj J, Davis J, Zandvakili I, Linker-Nord S, et al. Ileo-colonic delivery of conjugated bile acids improves glucose homeostasis via colonic GLP-1-producing enteroendocrine cells in human obesity and diabetes. EBioMedicine 2020;55:102759.
127. Zheng X, Chen T, Jiang R, Zhao A, Wu Q, Kuang J, et al. Hyocholic acid species improve glucose homeostasis through a distinct TGR5 and FXR signaling mechanism. Cell Metabol 2021;33:791–803 e7.
128. Beysen C, Murphy EJ, Deines K, Chan M, Tsang E, Glass A, et al. Effect of bile acid sequestrants on glucose metabolism, hepatic de novo lipogenesis, and cholesterol and bile acid kinetics in type 2 diabetes: a randomised controlled study. Diabetologia 2012;55: 432–42.
129. Yoshinobu S, Hasuzawa N, Nagayama A, Iwata S, Yasuda J, Tokubuchi R, et al. Effects of elobixibat, an inhibitor of ileal bile acid transporter, on glucose and lipid metabolism: a single-arm pilot study in patients with T2DM. Clin Therapeut 2022; 44:1418–26.
130. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine 2020;51:102590.
131. Ahmad TR, Haeusler RA. Bile acids in glucose metabolism and insulin signalling – mechanisms and research needs. Nat Rev Endocrinol 2019;15:701–12.
132. Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. Nat Med 2018;24:1919–29.
133. Chen B, Bai Y, Tong F, Yan J, Zhang R, Zhong Y, et al. Glycoursodeoxycholic acid regulates bile acids level and alters gut microbiota and glycolipid metabolism to attenuate diabetes. Gut Microb 2023;15:2192155.
134. Wang C, Wang Y, Yang H, Tian Z, Zhu M, Sha X, et al. Uygur type 2 diabetes patient fecal microbiota transplantation disrupts blood glucose and bile acid levels by changing the ability of the intestinal flora to metabolize bile acids in C57BL/6 mice. BMC Endocr Disord 2022;22:236.
135. Cusi K, Younossi Z, Roden M. From NAFLD to MASLD: promise and pitfalls of a new definition. Hepatology 2024;79:E13–15.
136. Chan WK, Chuah KH, Rajaram RB, Lim LL, Ratnasingam J, Vethakkan SR. Metabolic dysfunction-associated steatotic liver disease (MASLD): a state-of-the-art review. J Obes Metab Syndr 2023; 32:197–213.

137. Lai J, Luo L, Zhou T, Feng X, Ye J, Zhong B. Alterations in circulating bile acids in metabolic dysfunction-associated steatotic liver disease: a systematic review and meta-analysis. Biomolecules 2023;13. https://doi.org/10.3390/biom13091356.
138. Gottlieb A, Canbay A. Why bile acids are so important in non-alcoholic fatty liver disease (NAFLD) progression. Cells 2019;8:1358.
139. Puri P, Daita K, Joyce A, Mirshahi F, Santhekadur PK, Cazanave S, et al. The presence and severity of nonalcoholic steatohepatitis is associated with specific changes in circulating bile acids. Hepatology 2018;67:534–48.
140. Nimer N, Choucair I, Wang Z, Nemet I, Li L, Gukasyan J, et al. Bile acids profile, histopathological indices and genetic variants for non-alcoholic fatty liver disease progression. Metabolism 2021;116:154457.
141. Clifford BL, Sedgeman LR, Williams KJ, Morand P, Cheng A, Jarrett KE, et al. FXR activation protects against NAFLD via bile-acid-dependent reductions in lipid absorption. Cell Metabol 2021;33:1671–84.e4.
142. Liu X, Wang J, Li M, Qiu J, Li X, Qi L, et al. Farnesoid X receptor is an important target for the treatment of disorders of bile acid and fatty acid metabolism in mice with nonalcoholic fatty liver disease combined with cholestasis. J Gastroenterol Hepatol 2023;38:1438–46.
143. Marchiano S, Biagioli M, Bordoni M, Morretta E, Di Giorgio C, Vellecco V, et al. Defective bile acid signaling promotes vascular dysfunction, supporting a role for G-protein bile acid receptor 1/farnesoid X receptor agonism and statins in the treatment of nonalcoholic fatty liver disease. J Am Heart Assoc 2023;12:e031241.
144. Wang XX, Xie C, Libby AE, Ranjit S, Levi J, Myakala K, et al. The role of FXR and TGR5 in reversing and preventing progression of Western diet-induced hepatic steatosis, inflammation, and fibrosis in mice. J Biol Chem 2022;298:102530.
145. Hayashi AA, Webb J, Choi J, Baker C, Lino M, Trigatti B, et al. Intestinal SR-BI is upregulated in insulin-resistant states and is associated with overproduction of intestinal apoB48-containing lipoproteins. Am J Physiol Gastrointest Liver Physiol 2011;301:G326–37.
146. Pang J, Raka F, Heirali AA, Shao W, Liu D, Gu J, et al. Resveratrol intervention attenuates chylomicron secretion via repressing intestinal FXR-induced expression of scavenger receptor SR-B1. Nat Commun 2023;14:2656.
147. Gonzalez FJ, Jiang C, Patterson AD. An intestinal microbiota–farnesoid X receptor axis modulates metabolic disease. Gastroenterology 2016;151:845–59.
148. Moreau F, Brunao BB, Liu XY, Tremblay F, Fitzgerald K, Avila-Pacheco J, et al. Liver-specific FGFR4 knockdown in mice on an HFD increases bile acid synthesis and improves hepatic steatosis. J Lipid Res 2023;64:100324.
149. Shi Y, Su W, Zhang L, Shi C, Zhou J, Wang P, et al. TGR5 regulates macrophage inflammation in nonalcoholic steatohepatitis by modulating NLRP3 inflammasome activation. Front Immunol 2020;11:609060.
150. Matsui M, Fukunishi S, Nakano T, Ueno T, Higuchi K, Asai A. Ileal bile acid transporter inhibitor improves hepatic steatosis by ameliorating gut microbiota dysbiosis in NAFLD model mice. mBio 2021;12:e0115521.
151. Yamauchi R, Takedatsu H, Yokoyama K, Yamauchi E, Kawashima M, Nagata T, et al. Elobixibat, an ileal bile acid transporter inhibitor, ameliorates non-alcoholic steatohepatitis in mice. Hepatol Int 2021;15:392–404.
152. Rao A, van de Peppel IP, Gumber S, Karpen SJ, Dawson PA. Attenuation of the hepatoprotective effects of ileal apical sodium dependent bile acid transporter (ASBT) inhibition in choline-deficient L-amino acid-defined (CDAA) diet-fed mice. Front Med 2020;7:60.
153. Cao R, Cronk ZX, Zha W, Sun L, Wang X, Fang Y, et al. Bile acids regulate hepatic gluconeogenic genes and farnesoid X receptor via G(alpha)i-protein-coupled receptors and the AKT pathway. J Lipid Res 2010;51:2234–44.
154. Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science 2009;325:1254–7.
155. Zhao Q, Wu J, Ding Y, Pang Y, Jiang C. Gut microbiota, immunity, and bile acid metabolism: decoding metabolic disease interactions. Life Metabol 2023;2:load032.
156. Campo L, Eiseler S, Apfel T, Pyrsopoulos N. Fatty liver disease and gut microbiota: a comprehensive update. J Clin Transl Hepatol 2019;7:56.
157. Kasai K, Igarashi N, Tada Y, Kani K, Takano S, Yanagibashi T, et al. Impact of vancomycin treatment and gut microbiota on bile acid metabolism and the development of non-alcoholic steatohepatitis in mice. Int J Mol Sci 2023;24:4050.
158. Zhang D, Wang H, Liu A, Wang S, Xu C, Lan K, et al. The chronic consumption of dietary fructose promotes the gut Clostridium species imbalance and bile acid alterations in developing nonalcoholic fatty liver disease. J Nutr Biochem 2023;121:109434.
159. Zhang Z, Zhou H, Zhou X, Sun J, Liang X, Lv Y, et al. Lactobacillus casei YRL577 ameliorates markers of non-alcoholic fatty liver and alters expression of genes within the intestinal bile acid pathway. Br J Nutr 2021;125:521–9.
160. Smirnova E, Muthiah MD, Narayan N, Siddiqui MS, Puri P, Luketic VA, et al. Metabolic reprogramming of the intestinal microbiome with functional bile acid changes underlie the development of NAFLD. Hepatology 2022;76:1811–24.
161. Kuang J, Wang J, Li Y, Li M, Zhao M, Ge K, et al. Hyodeoxycholic acid alleviates non-alcoholic fatty liver disease through modulating the gut-liver axis. Cell Metabol 2023;35:1752–66.e8.
162. Liu J, Sun J, Yu J, Chen H, Zhang D, Zhang T, et al. Gut microbiome determines therapeutic effects of OCA on NAFLD by modulating bile acid metabolism. npj Biofilms Microbiomes 2023;9:29.
163. Wang W, Zhao J, Gui W, Sun D, Dai H, Xiao L, et al. Tauroursodeoxycholic acid inhibits intestinal inflammation and barrier disruption in mice with non-alcoholic fatty liver disease. Br J Pharmacol 2018;175:469–84.
164. Kakiyama G, Rodriguez-Agudo D, Pandak WM. Mitochondrial cholesterol metabolites in a bile acid synthetic pathway drive nonalcoholic fatty liver disease: a revised “two-hit” hypothesis. Cells 2023;12:1434.
165. Liu AN, Xu CF, Liu YR, Sun DQ, Jiang L, Tang LJ, et al. Secondary bile acids improve risk prediction for non-invasive identification of mild liver fibrosis in nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2023;57:872–85.
166. Kasai Y, Kessoku T, Tanaka K, Yamamoto A, Takahashi K, Kobayashi T, et al. Association of serum and fecal bile acid patterns with liver fibrosis in biopsy-proven nonalcoholic fatty liver disease: an observational study. Clin Transl Gastroenterol 2022;13:e00503.
167. Trauner M, Fuchs CD. Novel therapeutic targets for cholestatic and fatty liver disease. Gut 2022;71:194–209.
168. Yao Z, Chen L, Hu M, Meng F, Chen M, Wang G. The discovery of a new potent FXR agonist based on natural product screening. Bioorg Chem 2024;143:106979.
169. Wang K, Zhang Y, Wang G, Hao H, Wang H. FXR agonists for MASH therapy: lessons and perspectives from obeticholic acid. Med Res Rev 2024;44:568–86.

170. Huang W, Cao Z, Wang W, Yang Z, Jiao S, Chen Y, et al. Discovery of LH10, a novel fexaramine-based FXR agonist for the treatment of liver disease. Bioorg Chem 2024;143:107071.
171. Di Pasqua LG, Cagna M, Palladini G, Croce AC, Cadamuro M, Fabris L, et al. FXR agonists INT-787 and OCA increase RECK and inhibit liver steatosis and inflammation in diet-induced ob/ob mouse model of NASH. Liver Int 2024;44:214–27.
172. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. Nat Commun 2015;6:10166.
173. Cheng L, Chen T, Guo M, Liu P, Qiao X, Wei Y, et al. Glycoursodeoxycholic acid ameliorates diet-induced metabolic disorders with inhibiting endoplasmic reticulum stress. Clin Sci 2021; 135:1689–706.
174. Ding L, Yang Q, Zhang E, Wang Y, Sun S, Yang Y, et al. Notoginsenoside Ft1 acts as a TGR5 agonist but FXR antagonist to alleviate high fat diet-induced obesity and insulin resistance in mice. Acta Pharm Sin B 2021; 11:1541–54.
175. Iracheta-Vellve A, Calenda CD, Petrasek J, Ambade A, Kodys K, Adorini L, et al. FXR and TGR5 agonists ameliorate liver injury, steatosis, and inflammation after binge or prolonged alcohol feeding in mice. Hepatol Commun 2018;2:1379–91.
176. Lamers C, Merk D, Gabler M, Flesch D, Kaiser A, Schubert-Zsilavecz M. SAR studies on FXR modulators led to the discovery of the first combined FXR antagonistic/TGR5 agonistic compound. Future Med Chem 2016;8:133–48.
177. Gertzen CG, Spomer L, Smits SH, Haussinger D, Keitel V, Gohlke H. Mutational mapping of the transmembrane binding site of the G-protein coupled receptor TGR5 and binding mode prediction of TGR5 agonists. Eur J Med Chem 2015;104:57–72.
178. Roda A, Pellicciari R, Gioiello A, Neri F, Camborata C, Passeri D, et al. Semisynthetic bile acid FXR and TGR5 agonists: physicochemical properties, pharmacokinetics, and metabolism in the rat. J Pharmacol Exp Therapeut 2014;350:56–68.
179. Sato H, Macchiarulo A, Thomas C, Gioiello A, Une M, Hofmann AF, et al. Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure-activity relationships, and molecular modeling studies. J Med Chem 2008;51:1831–41.
180. Zhou X, Zhang X, Yu J. Gut mycobiome in metabolic diseases: mechanisms and clinical implication. Biomed J 2023:100625. https://doi.org/10.1016/j.bj.2023.100625.
181. Xu H, Fang F, Wu K, Song J, Li Y, Lu X, et al. Gut microbiota-bile acid crosstalk regulates murine lipid metabolism via the intestinal FXR-FGF19 axis in diet-induced humanized dyslipidemia. Microbiome 2023;11:262.
182. Yang JY, Liu MJ, Lv L, Guo JR, He KY, Zhang H, et al. Metformin alleviates irradiation-induced intestinal injury by activation of FXR in intestinal epithelia. Front Microbiol 2022;13:932294.
183. Jian YP, Yang G, Zhang LH, Liang JY, Zhou HL, Wang YS, et al. Lactobacillus plantarum alleviates irradiation-induced intestinal injury by activation of FXR-FGF15 signaling in intestinal epithelia. J Cell Physiol 2022;237:1845–56.
184. Monaghan T, Mullish BH, Patterson J, Wong GK, Marchesi JR, Xu H, et al. Effective fecal microbiota transplantation for recurrent Clostridioides difficile infection in humans is associated with increased signalling in the bile acid-farnesoid X receptor-fibroblast growth factor pathway. Gut Microb 2019;10:142–8.