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SECONDARY BILE ACIDS AND HOST METABOLISM: CROSSTALK, SIGNALING PATHWAYS AND THERAPEUTIC FRONTIERS

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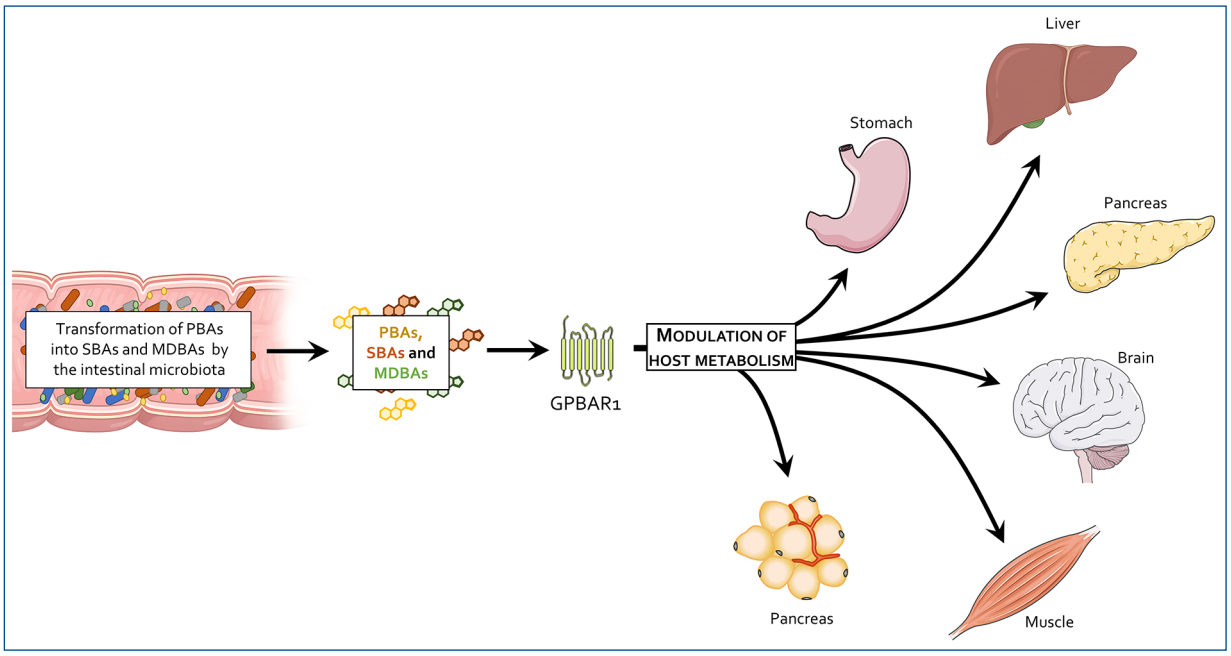
ABSTRACT – Bile acids, traditionally regarded as detergents essential for lipid solubilization and absorption, are now recognized as potent endocrine and immunomodulatory molecules that integrate metabolic, microbial, and inflammatory pathways. Primary bile acids synthesized from cholesterol in the liver are transformed by gut microbiota into an extensive repertoire of secondary and microbially derived bile acids (MDBAs) that act as signaling mediators across multiple organs. These metabolites regulate host metabolism and immune homeostasis through activation of nuclear and membrane receptors, including FXR, GPBAR1, VDR, CAR, and RORγt. FXR–FGF19 and GPBAR1–GLP-1 pathways mediate enterohepatic and entero-pancreatic communication, modulating glucose and lipid metabolism, while GPBAR1 activation in thermogenic tissues promotes thyroid hormone conversion and energy expenditure. Moreover, lithocholic acid and related secondary bile acids engage AMPK–sirtuin signaling, mimicking the systemic benefits of caloric restriction and contributing to longevity. By shaping the gut microbial ecosystem and influencing host physiology, bile acids constitute a molecular bridge between the microbiota and systemic metabolism. In this context, understanding the signaling landscape of secondary bile acids provides crucial insights into host–microbiota communication and unveils innovative therapeutic perspectives for metabolic, immune, and age-related disorders.

KEYWORDS: Secondary bile acids, Gut microbiota, FXR, GPBAR1, Host metabolism.

INTRODUCTION

Bile acids are atypical steroids generated in the mammalian liver from cholesterol exerting a non-dispensable role in lipid absorption. Bile acid represents the driving force for bile formation by hepatocytes and because their amphipathic structure is essential for assembly of micelles, supramolecular structures contributed by phospholipids and the breakdown products of fat digestion (fatty acids and monoglycerides) in the small intestine. This process is essential for lipid absorption, since the micelle's hydrophilic outer layer allows the water-insoluble lipids to be transported through the watery milieu of small intestine to the surface of the enterocytes for fatty acid and monoglyceride absorption[1].

Chemically, bile acids represent a widely differentiated family of steroids. In relation to the main site of their biotransformation, these steroids are classified as primary and secondary bile acids. Primary bile acids, that are formed in the liver, from cholesterol while secondary bile acids are generated in the intestine by the activity of the intestinal microbiota[2]. While only two main primary bile acids have been identified in mammalians, cholic



Graphical Abstract. Primary bile acids (PBAs) are transformed by the intestinal microbiota into secondary bile acids (SBAs) and microbiota-derived bile acids (MDBAs). These metabolites act on GPBAR1 to regulate key metabolic pathways in several organs including liver, stomach, pancreas, brain, adipose tissue, and muscle.

acid (CA) and chenodeoxycholic acids (CDCA), the extensive utilization of mass spectrometry technologies combined with informatic approaches has led to the identification of a large number (over 200) secondary bile acids and microbially derived bile acids (MDBA)[2]. Collectively, these molecules represent an essential component of the chemical network that mediates mutual interactions between the intestinal microbiota and the host immune system[2], contributing to the regulation of host immune functions and metabolism in non-entero-hepatic tissues[3-5].

These regulatory effects are mediated by activation of an heterogenous group of cell membrane and nuclear receptors, collectively known as bile acids regulated receptors (BARR)[6]. BARR are ubiquitously expressed receptors, although their higher expression occurs in the liver and gastrointestinal tract[7]. The two best receptors characterized of BARR are the Farnesoid X receptor (FXR)[8] and G protein coupled bile acid receptor (GPBAR)-1[9]. Both receptors are expressed in metabolically competent tissues, including adipose tissues, muscles, pancreatic β-cells and L cells, and intestinal I endocrine cells contributing to the regulation of intestinal-liver and intestinal-pancreas axes essential for glucose and lipid metabolism[10,11]. BARRs are also expressed in various immune cells, and it is now well established that engagement of FXR and GPBAR1 by their ligands activates a counter-regulatory program that help the intestinal/liver immune system to maintain a tolerogenic phenotype toward intestinal bacteria[12].

In addition to FXR and GPBAR1, secondary bile acids regulate several other receptors, such as the Vitamin D Receptor (VDR), the Constitutive Androstane Receptor (CAR), the Retinoic Acid Receptor–related Orphan Receptor γt (RORγt), a nuclear transcription factor expressed by Th17 cells and type 3 innate lymphoid cells (ILC3)[3,13-17], and the Leukemia Inhibitory Factor (LIF) Receptor[18,19], thereby mediating some of the biological functions of the intestinal microbiota[6,13,20,21]. Secondary bile acids and MDBA could be considered under several aspects as postbiotics and dissecting their regulatory effects might open the path to the discovery of novel therapeutic approaches to immune and metabolic disorders[22].

BILE ACID SYNTHESIS AND ENTEROHEPATIC CIRCULATION

Bile acids are amphipathic steroid molecules derived from cholesterol that combine the capacity to solubilize dietary lipids with the ability to act as endocrine signaling molecules. In hepatocytes, bile acid synthesis begins when cholesterol is converted into primary bile acids through two interconnected metabolic routes: the classical (or neutral) pathway and the alternative (or acidic) pathway (Figure 1)[23,24]. The classical pathway accounts for roughly 90% of bile acid production under physiological conditions and is initiated by the microsomal cytochrome P450 enzyme cholesterol-7α-hydroxylase (CYP7A1) (Figure 1). CYP7A1 introduces a hydroxyl group at carbon 7, forming 7α-hydroxycholesterol, which is further oxidized

and isomerized by 3β-hydroxysteroid dehydrogenase and Δ^4-isomerase[24]. Subsequent side-chain oxidation by CYP27A1 and 12α-hydroxylation by sterol 12α-hydroxylase (CYP8B1) yields cholic acid (CA). In the absence of CYP8B1 activity, the pathway produces chenodeoxycholic acid (CDCA); thus, CYP8B1 activity determines the CA/CDCA ratio in the bile acid pool[25]. The alternative pathway begins with mitochondrial sterol 27-hydroxylase (CYP27A1), which oxidizes cholesterol at the side chain to form 27-hydroxycholesterol; this metabolite is hydroxylated at the 7α-position by oxysterol 7α-hydroxylase (CYP7B1), eventually giving rise predominantly to CDCA (Figure 1)[23,24,26].

Newly synthesized bile acids possess a hydrophobic steroid nucleus and a hydrophilic side chain terminating in a carboxyl group. To increase water solubility and prevent passive reuptake across membranes, hepatocytes conjugate bile acids with amino acids[27]. This conjugation involves two enzymes: bile acid–CoA synthetase (BACS), which forms bile acid–CoA thioesters, and bile acid–CoA:amino-acid N-acyltransferase (BAAT), which transfers the CoA-activated bile acid to glycine or taurine[24]. In humans, approximately 75% of bile acids are amidated with glycine and 25% with taurine, whereas in rodents, taurine conjugates predominate[28]. Recent metabolomic studies

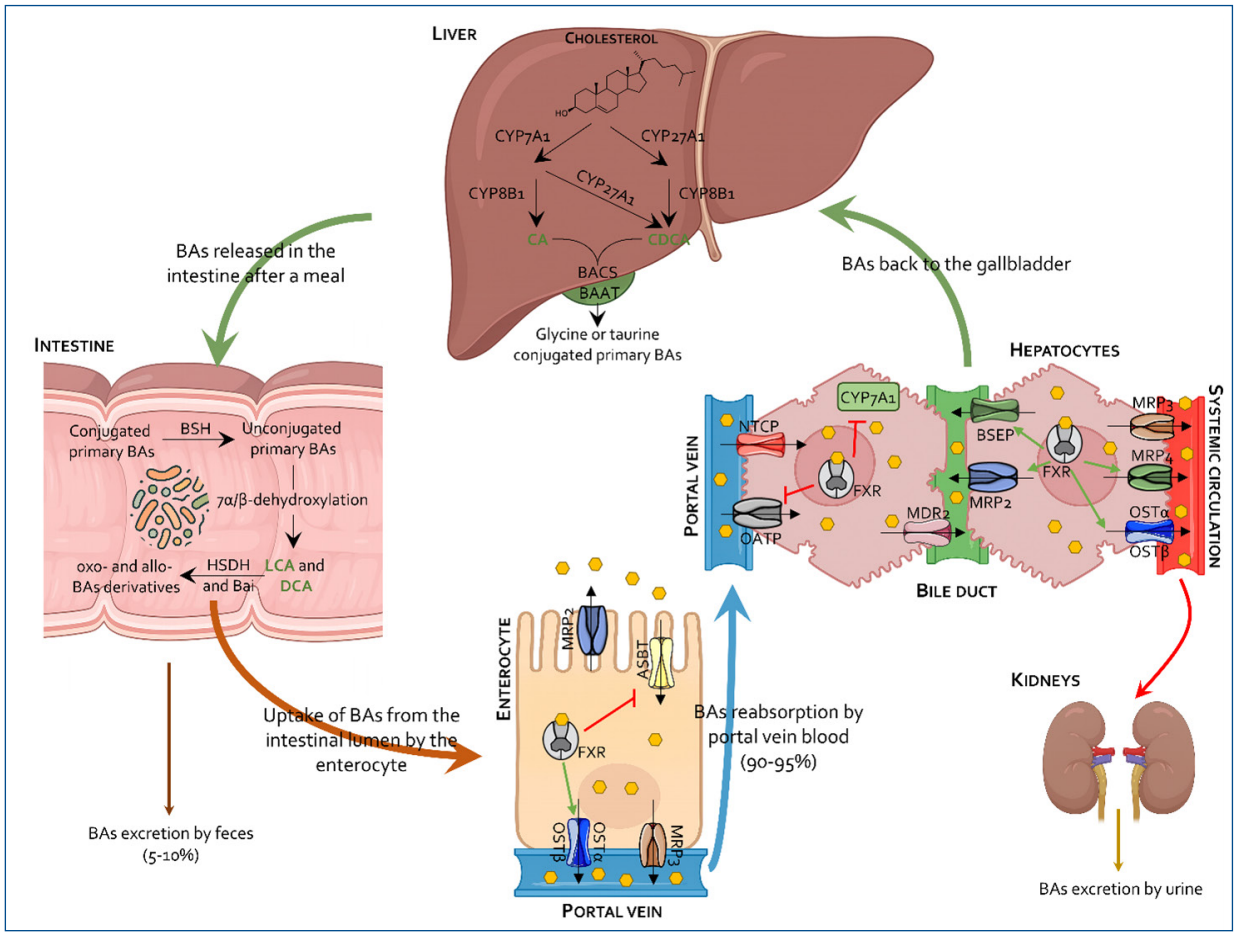


Figure 1. Bile acid synthesis and enterohepatic circulation. Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), together with their taurine- and glycine-conjugated forms, are synthesized in hepatocytes from cholesterol through two main biosynthetic routes: the classical (neutral) and the alternative (acidic) pathways. Once formed, these bile acids are secreted into the biliary canaliculi and stored in gallbladder. Following food intake, bile acids are discharged into the small intestine via the common bile duct, where they play a crucial role in lipid emulsification and absorption. Within the intestinal lumen, bile acids undergo extensive biotransformation by the gut microbiota, which enzymatically convert them into secondary bile acids, deoxycholic acid (DCA) and lithocholic acid (LCA), as well as several related derivatives. In the ileum, bile acids are actively reabsorbed by enterocytes through the apical sodium-dependent bile acid transporter (ASBT). Their uptake triggers farnesoid X receptor (FXR) activation, which, in turn, downregulates ASBT expression to limit further absorption. Only 5-10% of bile acids are not reabsorbed and excreted in the feces. Bile acids exit enterocytes through MRP3 and OSTα/β transporters, entering the portal vein and returning to the liver, where they are taken up again by hepatocytes via NTCP and OATP transporters. Elevated intrahepatic bile acid concentrations repress their own synthesis through activation of the FXR/SHP regulatory cascade and reduce the uptake. In the liver, additional transporters are localized to distinct membrane domains: MRP2, BSEP, and MDR2 are situated on the canalicular membrane, whereas MRP3, MRP4, and OSTα/β are expressed on the basolateral membrane. Finally, bile acids undergo glomerular filtration in the kidneys and are subsequently reabsorbed in the renal tubules, thereby minimizing their urinary excretion and conserving the bile acid pool.

have identified atypical bile acid conjugates with phenylalanine, leucine and tyrosine, but their physiological significance is not fully understood. After conjugation, bile acids are actively secreted across the canalicular membrane by the bile salt export pump (BSEP/ABCB11) and the phospholipid flippase MDR3 (ABCB4) (Figure 1)[6,25]. Bile acids drive bile flow and extraction of cholesterol and phosphatidylcholine into bile, forming mixed micelles that protect the biliary epithelium from detergent damage. Bile flows through the hepatic bile ducts into the gallbladder, where it is concentrated five- to ten-fold by active transport of water and electrolytes. During fasting, the gallbladder stores bile acids; upon ingestion of a meal rich in fat or protein, cholecystokinin is released, causing gallbladder contraction and relaxation of the sphincter of Oddi, discharging concentrated bile into the duodenum[29].

As conjugated bile acids pass through the distal small intestine, they become substrates for an array of microbial enzymes (Figure 1). The initial step in this microbial conversion is the hydrolysis of the amide bond linking glycine or taurine to the sterol backbone by bile-salt hydrolases (BSHs)[30-33]. The free bile acids liberated by BSHs are then transformed into secondary bile acids through 7α/β-dehydroxylation catalyzed by proteins encoded by the *bai* operon and by hydroxysteroid dehydrogenases (HSDHs), which oxidize and epimerize hydroxyl groups on the steroid nucleus. In humans these reactions yield deoxycholic acid (DCA), lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) as the principal secondary bile acids, whereas hyodeoxycholic acid (HDCA) is present in smaller amounts. Some microbes also re-amidate unconjugated bile acids with non-canonical amino acids, creating microbially conjugated bile acids (MCBAs), which include derivatives of DCA, LCA, and UDCA. Such re-amidation and further ring or side-chain modifications greatly expand bile acid diversity; metabolomic have documented more than twenty distinct ring/core modifications and over a hundred carboxyl-tail variants, yielding a repertoire of roughly seven hundred bile acid molecules[2,34]. While many MCBAs are present only in trace amounts and likely exert local effects within the intestinal lumen, others contribute meaningfully to the circulating bile acid pool and are thought to influence the composition of the gut microbiota and the balance of mucosal and systemic immunity[14].

Within the intestinal lumen, bile acids act as emulsifiers, forming mixed micelles with dietary triacylglycerols, cholesterol, phospholipids and fat-soluble vitamins. Bile acids also promote absorption of fat-soluble vitamins A, D, E and K. More than 95% of the bile acid load entering the ileum is reabsorbed. This reabsorption is mediated by the apical sodium-dependent bile acid transporter (ASBT/SLC10A2) located on the brush border membrane of ileal enterocytes. ASBT couples bile acid uptake to the inward sodium gradient maintained by the Na^+/K^+-ATPase. Inside enterocytes, bile acids bind the ileal bile acid-binding protein (IBABP/FABP6), a cytosolic carrier that transports them to the basolateral membrane. Efflux into the portal blood is mediated by the heterodimeric organic solute transporter (OSTα/β) and multidrug resistance-associated protein 3 (MRP3/ABCC3)[35]. Portal venous blood carries reabsorbed bile acids back to the liver, where they are extracted by hepatocytes *via* the sodium taurocholate cotransporting polypeptide (NTCP/SLC10A1) and organic anion-transporting polypeptides (OATPs) (Figure 1). This sinusoidal uptake completes the enterohepatic cycle. The liver thus sees a continuous flux of bile acids; hepatocytes re-conjugate and secrete them, while only a small fraction of non-reabsorbed bile acids enters the colon or is excreted in urine after renal filtration and tubular reabsorption. Because the daily hepatic secretion of bile acids (12-18 grams) far exceeds the pool size, the efficiency of reabsorption is critical to avoid depleting cholesterol stores and maintain lipid absorption.

The synthesis and enterohepatic circulation of bile acids is finely controlled. Farnesoid X receptor (FXR) is a ligand-activated nuclear receptor expressed in hepatocytes and enterocytes that senses intracellular bile acid levels (Figure 1). Activation of FXR in hepatocytes induces the atypical nuclear receptor small heterodimer partner (SHP) which suppresses the transcription of CYP7A1 and CYP8B1, limiting bile acid synthesis. FXR also up-regulates BSEP, OSTα/β and MRP2/3, enhancing bile acid export from hepatocytes and enterocytes[36]. In the ileum, FXR activation suppresses ASBT inhibiting intestinal reabsorption of bile acid. Furthermore, the activation of FXR at intestinal level induces the release of fibroblast growth factor 19 (FGF19). FGF19 *via* the portal vein reaches the liver, binds the FGFR4/β-Klotho receptor complex on hepatocytes and activates signaling pathways that repress CYP7A1, an essential enzyme in the synthesis of bile acids. This FXR-FGF19 axis constitutes a negative feedback loop that couples intestinal bile acid flux to hepatic synthesis[37]. In mice, the orthologous hormone FGF15 performs a similar function. Disruption of this pathway, whether by genetic mutations, cholestatic diseases or pharmacologic inhibition, leads to dysregulated bile acid synthesis and can contribute to diarrhea or hepatic injury. Other nuclear receptors, including pregnane X receptor (PXR), constitutive androstane receptor (CAR), and liver X receptor (LXR) respond to bile acid overload by inducing detoxification enzymes, phase II conju-

gating enzymes and alternative transporters. Their cooperation with FXR ensures that hepatocytes can adapt to fluctuations in bile acid levels and protect against toxicity.

In humans, the primary bile acids CA and CDCA dominate the pool; CDCA is the most potent endogenous FXR agonist, whereas CA has lower affinity. Murine species, however, convert CDCA into α- and β-muricholic acids (MCA) *via* CYP2C70-mediated 6β-hydroxylation and 6β-epimerization. These muricholic acids are more hydrophilic and less cytotoxic than CDCA and act as partial FXR antagonists. As a result, the major FXR ligand in mice is taurocholic acid rather than CDCA. The murine bile acid pool has a lower detergent capacity and a different receptor activation profile, leading to species-specific differences in lipid absorption and metabolic regulation.

Beyond digestion, bile acids serve as endocrine mediators that coordinate metabolism across organs. Activation of intestinal and hepatic FXR induces FGF19 release, which travels to the liver and suppresses bile acid synthesis and gluconeogenesis. Enteroendocrine L-cells secrete glucagon-like peptide 1 (GLP-1) in response to nutrients and bile acids. Activation of the G-protein-coupled receptor (GPBAR1) by secondary bile acids and their derivatives enhances GLP-1 release, linking bile acid flux to insulin secretion, appetite control and glucose homeostasis. GPBAR1 signaling in brown adipose tissue also increases energy expenditure by promoting thyroid hormone activation and thermogenesis. These endocrine loops illustrate how bile acids integrate digestion with systemic metabolic regulation; perturbations of bile acid synthesis or enterohepatic circulation can therefore influence body weight and metabolic health.

In summary, bile acid synthesis and enterohepatic circulation constitute a dynamic axis that links cholesterol catabolism to nutrient absorption, endocrine signaling and systemic homeostasis.

ENZYMATIC TUNING OF BILE ACID DIVERSITY: HOW GUT MICROBES SHAPE AND ARE SHAPED BY THE BILE ACID POOL

Bile acids are unusual metabolites: synthesized from cholesterol in the liver, secreted into the intestine as glycine or taurine conjugated detergents, and then continuously recycled through the enterohepatic loop. Yet once they encounter the gut microbiota, they cease to be just host molecules. At the molecular level, the first and arguably rate-determining step in the microbial reshaping of bile chemistry is carried out by the bile salt hydrolases (BSHs). BSHs catalyze the hydrolytic cleavage of the C-24 N-acyl amide bond linking taurine or glycine to the steroid nucleus of primary bile acids. This apparently simple amide hydrolysis has disproportionately large consequences because it not only generates free (unconjugated) bile acids with altered solubility and detergent activity, but it also creates the chemical precondition for all downstream biotransformations[38]. Consequently, the set of bile acids that reach the intestine is inherently complex: primary bile acids (CA, CDCA, and species-specific analogues such as HCA, α/βMurocholic acids; and their taurine/glycine conjugates) are supplemented by a growing set of microbially generated derivatives, oxidized, dehydroxylated, allo-, iso- and Δ4/Δ4,6 modified forms, and by the recently described class of microbially-conjugated bile acids (MCBAs) formed *via* BSH-linked trans-amidation reactions. Therefore, BSHs are now recognized not only as hydrolases but also as aminotransferases that can replace taurine/glycine with other amino acids such as phenylalanine, leucine, tyrosine, tryptophan or threonine[39,40]. More than 2,456 BSH enzymes have already been annotated from human metagenomes[38], BSH-encoding taxa include Lactobacillaceae, *Clostridium, Bacteroides, Bifidobacterium, Enterococcus, Eubacterium, Listeria, Ruminococcus, Streptococcus, Roseburia, Parabacteroides, Butyricimonas, Barnesiella intestinihominis,* and *Anaerostipes hadrus*[33,41]. Once unconjugated, bile acids become competent substrates for 7α-dehydroxylation — the canonical pathway that generates the two predominant secondary bile acids in humans: DCA (from CA) and LCA (from CDCA or UDCA). This pathway is executed by a multi-enzyme, inducible operon known as the bai operon, which encodes at least seven enzymes required for the multi-step conversion of a 7α-hydroxy group into a "stripped" steroid lacking the 7-OH. 7α-dehydroxylation is phylogenetically narrow: only a few organisms in the gut community carry a complete bai gene cluster — including *Clostridium scindens*, *C. hylemonae*, *C. hiranonis* (reclassified as *Peptacetobacter hiranonis*), *C. leptum*, *Eggerthella lenta*, *Eggerthella spp*. and *Proteocatella sphenisci*. This extreme functional sparsity explains why adding only one bai-positive species to a synthetic community can dramatically shift the bile acid pool[42,43]. The third axis of diversification is carried out by hydroxysteroid dehydrogenases (HSDHs). HSDHs catalyze oxidation or reduction of hydroxyl groups at positions C3, C7, and C12, generating oxo-bile acid intermediates that accumulate to 20-30% of the luminal bile pool. These oxo species are not inert intermediates: several of them have been shown to bind the retinoid-related orphan receptor RORγt, making them direct immunomodulators[21,44]. HSDHs occur in multiple substrate/stereoselective flavours, 3α-HSDH, 3β-HSDH, 7α-HSDH, 7β-HSDH, 12α-HSDH, and each isoactivity is supported by different

Table I. Microbial bile acid transformation.

BSHs Deconjugation and reconjugation The BSHs remove the C-24 N-acyl bond that binds CDCA and CA to glycine and taurine. The microbial BSHs also possess aminotransferase activities promoting the generating of several non-glycine or taurine CA and CDCA amidated derivatives.	- CDCA and CA - Microbially conjugated bile acids: Glu-CA, Ile-CA, Leu-CA, Phe-CA, Thr-CA, Trp-CA, Tyr-CA, Gly-CA, Tau-CA, Glu-CDCA, Met-CDCA, Phe-CDCA, Trp-CDCA, Tyr-CDCA, Gly-CDCA, Tau-CDCA Glu-DCA, Met-DCA, Phe-DCA, Gly-DCA, Tau-DCA
BA-inducible operon 7α-dehydroxylation The dehydroxylation at C-7 is carried out by seven different enzymes, encoded by the 9 genes of *bai* operon.	DCA, LCA
HSDHs Oxidation/reduction The oxidation/reduction of hydroxy groups at the 3-, 7-, and 12-carbons is carried out by hydroxysteroid dehydrogenases (HSDHs) giving rise to stable oxo-bile acids intermediates	3 oxo DCA 7 oxo DCA 12 oxo DCA 3 oxo LCA 7 oxo LCA 12 oxo LCA
HSDHs Epimerization. HSDHs carry out various epimerization reactions including the C_7, C_3 and C_{12} epimerization of CA; the C_7, C_3 epimerization of CDCA and C_3 epimerization of LCA	UCA, isoCA, ECA UDCA, isoCDCA isoLCA

microbial species such as *Eggerthella lenta, Ruminococcus gnavus,* and *Clostridium scindens*. The cumulative effect of HSDH diversification is that the microbial community can selectively enrich signaling-active steroids with different receptor preferences[43].

Finally, HSDHs also mediate epimerization: C7 epimerization of CA generates UCA; C3 epimerization generates isoCA; C12 epimerization generates epicholic acid (ECA). CDCA can become UDCA (C7 epimerization) or isoCDCA (C3 epimerization), and LCA can become isoLCA. These epimers have distinct physicochemical properties, and in several cases altered receptor preferences[43]. Together, unconjugation, dehydroxylation, oxidation/reduction and epimerization constitute an intertwined metabolic network, through which microbiota expands a relatively small set of liver-derived precursors into a chemically rich signaling language that feeds back on host metabolism and immunity (Table I).

The relationship between bile acids and microbes is decidedly bidirectional: just as microbes transform bile acids, bile acids shape which microbes thrive. At high enough concentrations, bile acids behave like detergents, disrupting bacterial membranes, collapsing proton motive force, damaging DNA, and denaturing proteins. Sensitivity varies by species as well as by bile acid conjugation state and hydrophobicity. *In vitro*, unconjugated and secondary bile acids are often more inhibitory than conjugated primary species. As a result, changes in the concentration and composition of luminal bile acids select for bile-tolerant taxa and suppress others, reconfiguring the ecosystem[45,46].

Pathophysiology provides natural experiments, if bile flow into the intestine is interrupted, such as after bile duct ligation, small-intestinal bacterial overgrowth (SIBO) becomes common, highlighting the antimicrobial role of bile in the proximal gut[47,48]. In chronic liver disease, altered bile acid secretion and microbial dysbiosis are tightly linked: a clinical study in cirrhosis correlated fecal bile acid concentrations with microbiome composition, observing decreases in Gram-positive taxa with 7α-dehydroxylating capacity (Ruminococcaceae, Blautia/Clostridia) and expansions of Enterobacteriaceae as fecal bile acids fell[49,50]. These observations reinforce the concept that diminished delivery of bile acids to the lumen favors Gram-negative outgrowth and loss of secondary-BA producers.

Interventional studies strengthen the causal picture. In rats, oral cholic acid simplifies the microbiota and enriches Firmicutes, especially Clostridia and Erysipelotrichi, while reducing Bacteroidetes and Actinobacteria; these shifts accompany increased conversion of CA to DCA[7]. More broadly, feeding bile acids or increasing endogenous BA flux has repeatedly been shown to remodel the gut

community at the phylum, class, and genus levels, with Blautia and related 7α-dehydroxylators often expanding under bile-rich conditions[51]. FXR signaling adds an indirect, host-mediated layer. Beyond their direct antimicrobial actions, bile acids activate the farnesoid X receptor (FXR), which regulates epithelial defenses, barrier integrity, antimicrobial peptides, and bile acid transport[48,52]. Activating FXR protects against intestinal inflammation and modulates community structure; conversely, targeted manipulations of intestinal FXR can reshape microbiota composition and metabolic signaling in mice[52]. Thus, bile acids influence microbes not only as detergents but also *via* host transcriptional programs that adjust the intestinal habitat.

SECONDARY BILE ACIDS AND METABOLISM REGULATION

Secondary bile acids, formed by microbial modification of primary bile acids, have emerged as key modulators of host metabolism. Beyond their classical roles in digestion, these molecules engage nuclear and membrane receptors to influence glucose homeostasis, stimulate hormonal and neuronal circuits that regulate energy expenditure, and activate stress-responsive pathways that mimic the benefits of caloric restriction.

Secondary bile acids and glucose metabolism

The large abundance of molecularly distinct microbiota-derived bile acids highlighted in the

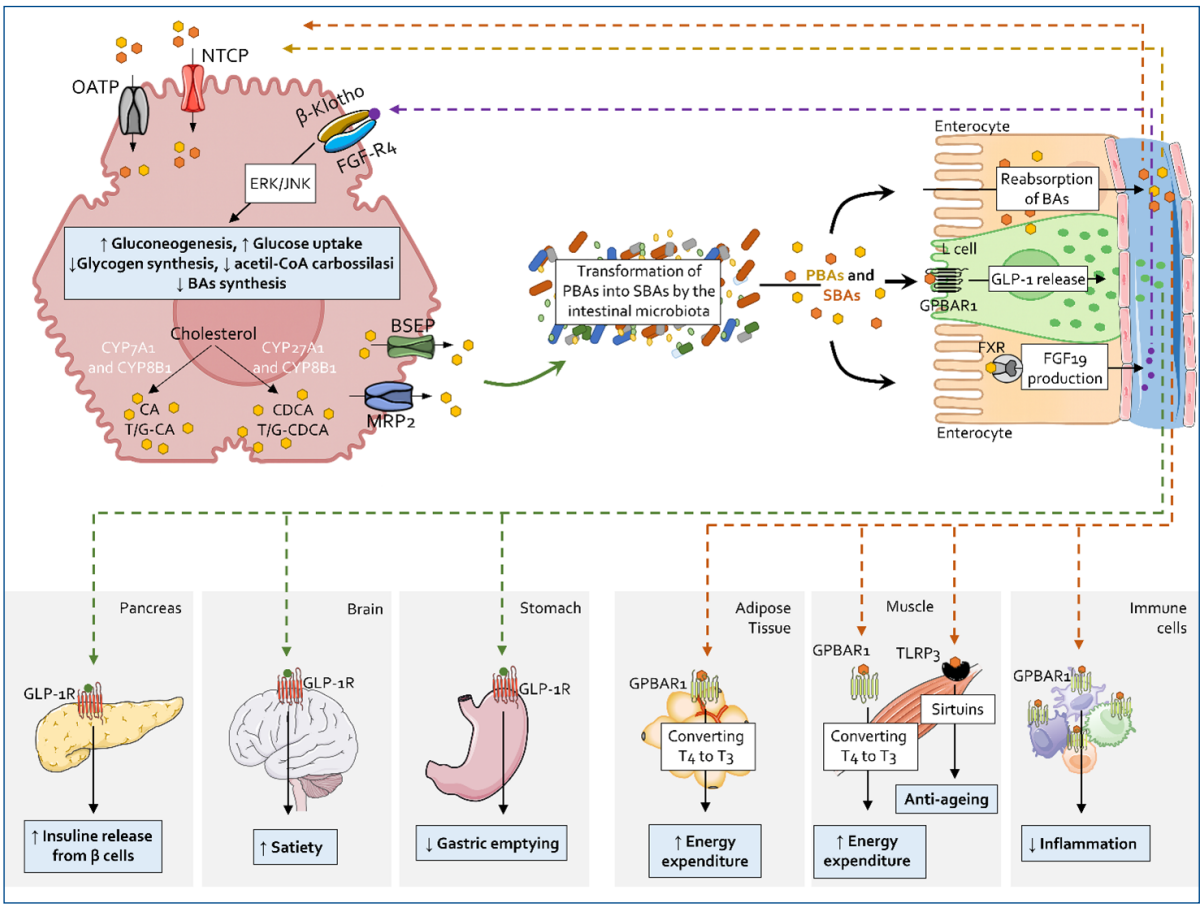


Figure 2. Metabolic effects of secondary bile acids. Primary bile acids, such as cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized in the liver and conjugated with glycine or taurine. These conjugates are secreted into bile ducts *via* transporters (e.g., BSEP, MRP2) and released into the small intestine after food intake, where they aid in lipid digestion and absorption. In the intestine, gut microbiota converts primary bile acids into secondary bile acids. Approximately 90% of bile acids are reabsorbed in the ileum and transported back to the liver *via* the portal vein, mediated by OATP and NTCP transporters. In enterocytes, bile acids activate FXR, inducing FGF19 secretion, which suppresses hepatic bile acid synthesis and regulates glucose metabolism by increasing GLUT4 expression while inhibiting glycogen synthesis and acetyl-CoA carBOXylase activity. In the colon, secondary bile acids activate GPBAR1 on L-cells, promoting GLP-1 release. GLP-1 enhances insulin secretion, satiety, and delays gastric emptying *via* interactions with pancreatic beta cells, neurons, and the stomach. Systemically, secondary bile acids bind GPBAR1 in adipose and muscle tissue, stimulating energy expenditure through T4-to-T3 conversion and exerting anti-inflammatory effects. Recent findings highlight lithocholic acid (LCA) as a modulator of TLRP3 in muscle, promoting anti-aging effects *via* sirtuin activation.

previous sections strongly advocates a function for these steroids as signaling molecules regulating gut microbiota communications with liver, adipose tissues, pancreas, muscles, brain, and cardiovascular system in normal and pathologic states[5]. Activation of intestinal FXR and GPBAR1, induces the release of two potent enterokines: the Fibroblast Growth Factor (FGF)19[53] and the Glucagon Like Peptide (GLP)-1[54], that mediates entero-hepatic and entero-pancreatic axes. In the feed state, primary bile acids activate intestinal FXR promoting the release of FGF19 from ileal enterocytes[55], while activation of GPBAR1 by secondary bile acids stimulates the release of GLP-1 from the intestinal L cells in the ileum and colon[56,57] (Figure 2). In the liver, FGF19 binds to the FGF receptor (FGFR)/Beta klotho complex located to the cell membrane of hepatocytes, repressing the activity of CYP7A1 (Figure 2). Since FGF19 release takes place in the late postprandial states, this pathway contributes to feed-back inhibition of bile acids synthesis[58] and negative regulation of liver autophagy in response to feeding[59,60].

FGFs constitute a family of 22 human polypeptides that play roles in a wide range of biological processes, including embryonic development, tissue repair, and, notably, metabolic regulation. The FGF19 class that includes FGF21 and FGF23 act as classical endocrine mediators and bind and activate the transmembrane proteins α-Klotho or β-Klotho as specific co-receptors to bind FGFRs and initiate signaling. The endocrine FGF subgroup plays a major role in metabolic regulation, controlling the homeostasis of glucose, lipids, bile acids, and phosphate.

In addition to bile acid synthesis regulation, FGF19 (and its murine homolog FGF15), that is released from the terminal ileum in the late post-prandial phase, contributes to glucose and lipid metabolism and promotes weight loss. As such, FGF19 have been developed for treating MASH.

FGF21 that is primarily produced in the liver during fasting (or under metabolic stress), is a key regulator of fasting adaptation. FGF21 enhances insulin sensitivity, fatty acid oxidation, ketogenesis, and energy expenditure, thereby promoting weight loss and improving the overall metabolic profile—mainly by acting on adipose tissue and the central nervous system. FGF21 analogs, pegozafermin and efruxifermin, are currently trialed for the treatment of MASH[61-63]. FGF23, which is produced mainly by osteocytes, regulates phosphate and active vitamin D homeostasis by inhibiting renal phosphate reabsorption and the production of calcitriol (active vitamin D). Elevated FGF23 levels are associated with cardiovascular disease and impaired glucose homeostasis[64].

GLP-1 is an incretin that promotes insulin secretion from pancreatic β-cells along with delayed gastric emptying and appetite suppression. Since GLP-1 analogues or GLP-1 receptor agonists represent one of the main treatment options for obesity and diabetes, the regulatory effects exerted by secondary bile acids on this incretin have attracted a robust interest[6], but the translational relevance of this finding remains poorly demonstrated.

In addition to the indirect, GLP-1–mediated effects, bile acids act on pancreatic β-cells to directly stimulate insulin secretion. Various studies have shown that both GPBAR1 and FXR are expressed by human pancreatic β-cells, and that their activation promotes insulin transcription and secretion[65-67]. However, it is worth noting that, while animal studies have consistently shown a beneficial effect of FXR and GPBAR1 agonism in rodent models of insulin resistance and metabolic associated liver diseases[6], it is still controversial whether these preclinical findings translate into clinically relevant effects[68].

Secondary bile acids, thyroid hormones and energy metabolism

There is a consensus that GPBAR1 agonism increases energy expenditure by promoting intracellular thyroid hormone conversion in thermogenically competent tissues. GPBAR1 is abundantly expressed in adipose tissue and muscle, and its agonism induces a cAMP-dependent increase in the expression and activity of the thyroid hormone–activating enzyme type 2 iodothyronine deiodinase (DIO2), which converts tetraiodothyronine (T4) into the active tri-iodothyronine (T3)[69]. Intracellular accumulation of T3 in muscle cells and adipocytes promotes a thyroid hormone receptor (THR) dependent increase in energy expenditure (Figure 2). In the white (WAT) brown (BAT) adipocytes and skeletal muscles, where GPBAR1 and DiO2 co-localize, bile acids promote a T3-dependent activation of THR that in, in its turn, acts as a transcription factor for genes with Thyroid Response Elements (TREs). In the WAT and BAT, GPBAR1 activation by DCA induces the expression of Uncoupling Protein 1 (UCP1), which by establishing a futile cycle of mitochondrial proton shuttling, increases energy expenditure *via* thermogenesis[70,71] and increases basal metabolic rate.

Secondary bile acids as calorie restriction mimetic and anti-aging agents

Caloric restriction is a dietary intervention that promotes overall health and can extend lifespan in diverse species including primates. Caloric restriction induces a range of molecular, cellular and physiological changes to maintain mitochon-

drial quality and quantity, reduce oxidative damage and suppress inflammation, all of which are commonly dysregulated in age-associated metabolic disorders including type 2 diabetes, obesity, immune mediated diseases and cancers[72]. The AMP-activated protein kinase (AMPK) has emerged as a critical mediator of the beneficial effects of caloric restriction[73]. AMPK regulates a large number of signaling pathways that impede ageing, such as target of rapamycin complex 1 (TORC1) and forkhead BOX O (FOXO) proteins to mimic the reduction in insulin–IGF-1 signaling[74]. A major advance in decoding signals promoted by calorie restriction has been the discovery that LCA is one of the metabolites that alone can recapitulate the beneficial effects of caloric restriction in animal models[75]. Thus, not only LCA accumulates in tissues in response to calorie restriction, but feeding mice with LCA activates AMPK in muscle cells, enhances muscle regeneration and grip strength and running capacity in old mice[75]. LCA also activates AMPK and induces life-extending and health-extending effects in *Caenorhabditis elegans* and *Drosophila melanogaster*[75]. Further supporting a mechanistic role for the LCA-AMPK pathway in mediating systemic beneficial effects of calorie restriction, knocking down AMPK abrogates LCA-induced phenotypes in the three animal models[75].

It has been proposed that LCA might function as an indirect modulator of AMPK. Indeed, exposure of muscle cells to LCA enhances the activity of sirtuin 1 to deacetylate and subsequently inhibits vacuolar H^+-ATPase (v-ATPase), which leads to AMPK activation through the lysosomal glucose-sensing pathway[76]. Proteomics analyses of proteins that co-immunoprecipitated with sirtuin 1 identified TUB-like protein 3 (TULP3), a sirtuin-interacting protein[76], as a LCA receptor. In detail, LCA-bound TULP3 allosterically activates sirtuins, which then deacetylate the V1E1 subunit of v-ATPase. Muscle-specific expression of a V1E1 mutant which mimics the deacetylated state, strongly activates AMPK in aged mice. Taken together these findings suggest that administration of LCA, a microbial derived secondary bile acid recapitulate the beneficial effects of calorie restriction.

Partially consistent with these findings are results obtained from analysis of bile acids composition in centenarians[43]. A study carried out in Japanese centenarians found that, in comparison with other age cohorts, feces of centenarians are enriched in secondary bile acids including iso-LCA, 3-oxo-LCA, allo-LCA, 3-oxoallo-LCA and isoallo-LCA. It has been shown that bacteria of the *Odoribacteraceae* family can carry out the modification that produces isoallo-LCA in this population. Since isoallo-LCA exerts potent antimicrobial effects against Gram-positive (but not Gram-negative) multidrug-resistant pathogens, including *Clostridioides difficile* and *Enterococcus faecium*, it has been suggested that isoallo-LCA-producing bacteria might help to promote or maintain intestinal homeostasis and that this mechanism could contribute to the healthy ageing of centenarians[43]. Despite these data are of interest, it should be noted that isoallo-LCA does not reproduce the effects of LCA as caloric restriction mimetic[75], and therefore the two mechanisms seem to be independent. Further on, these findings should be taken cautiously, since isoallo-LCA is extremely hydrophobic and others have shown that accumulation microbial-derived metabolites (secondary bile acids, trimethylamine-N-oxide (TMAO), tryptophan derivatives and others), generated by dysbiosis microbiota might represent a risk factor for age-related cognitive decline[77].

Secondary bile acids and cardiovascular system

Secondary bile acids have several functions that may be relevant for their pharmacological effects in MASH. One of these functions is their ability to modulate the production of gaseous mediators in the cardiovascular (CV) system, mainly through receptor-mediated pathways, such as GPBAR1 and FXR, and by directly interacting with ion channels. In particular secondary bile acids can influence the synthesis and availability of gaseous mediators such as nitric oxide (NO) and hydrogen sulfide (H_2S)[78-82]. Secondary bile acids also regulate levels of NO, an essential vasodilator that controls vascular tone and endothelial function. They promote NO production by enhancing the phosphorylation and activation of endothelial nitric oxide synthase (eNOS), the enzyme responsible for NO generation in endothelial cells[83]. This results in increased NO production and subsequent vasodilation[84].

In cholestatic disorders, bile acids are thought to contribute to systemic vasodilation. This effect is partly attributed to an increased NO production by endothelial cells, which promotes the relaxation of vascular smooth muscle cells. The same mechanism is considered relevant in reversing liver microvascular vasoconstriction seen in portal hypertension. In this context, both FXR and GPBAR1 agonists have been shown to improve hepatic blood flow[84,85]. Secondary bile acids modulate the expression/function of cystathionine-gamma-lyase (CSE), an enzyme involved H2S generation, in aGPBAR1-dependent manner[79]. Interestingly, CSE gene ablation attenuates the vasodilatory effects of DCA and LCA, suggesting that

the transsulfuration pathway contributes to their vascular actions[86].

Furthermore, bile acids can activate the S1P receptor 2 (S1PR2)[87] in vascular smooth muscle cells. This activation inhibits inducible NO synthase (iNOS) and reduces NO levels, particularly under conditions of vascular injury or inflammation. Together these effects might have relevance for treating metabolic conditions associated with insulin resistance and hypertension.

CONCLUSIONS

Here we have reviewed how secondary bile acids act as metabolic, vascular and immunoregulatory agents. Secondary bile acids are emerging as the largest family of chemical mediators that modulates interaction of the intestinal microbiota with the host. The extensive use of mass spectrometry methodology and advanced bioinformatic approaches has revealed that the microbiota generates remarkably diverse array of these steroidal molecules. While the concentrations of some of these steroids in the gastrointestinal tract and systemic circulation might be too low to exert measurable functional effects, others are found in sufficient quantities to bind and activate target receptors, thereby influencing host physiology.

This intricate network is only partially characterized, and extensive investigations are ongoing. The discovery that secondary bile acids might mediate some of the beneficial effects of caloric restriction in animal models adds an additional layer of interest, though confirmation in humans is still required. Nonetheless, this possibility opens an exciting arena for drug discovery, including strategies aimed at selectively modifying bile acid composition, engineering microbiota-derived enzymatic pathways, or targeting receptor-specific signaling axes. As our understanding expands, secondary bile acids may increasingly be recognized not only as metabolic indicators but also as actionable therapeutic targets in major human diseases.

Conflict of interest

The authors declare they have no conflict of interest.

Data Availability

Not applicable.

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