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Gut microbiome and bile acids in obesity-related diseases

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Dysbiosis has been implemented in the etiologies of obesity-related chronic diseases such as type 2 diabetes, NAFLD and cardiovascular diseases. Bile acids, a class of amphipathic steroids produced in the liver and extensively modified by the microbiome, are increasingly recognized as actors in onset and progression of these diseases. Indeed, human obesity is associated with altered bile acid metabolism. Bile acids facilitate intestinal fat absorption but also exert hormone-like functions through activation of nuclear and membrane-bound receptors and thereby modulate glucose, lipid and energy metabolism, intestinal integrity and immunity. Bile acid-signaling pathways have thus been identified as potential pharmacological targets for obesity-related diseases. Interfering with microbiome composition may also be considered, as liver- and microbiome-derived bile acid species have different signaling functions. This review summarizes recent developments in this rapidly expanding field of research and addresses potential clinical prospects of interference with bile acid signaling pathways in human diseases.

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Introduction

The World Health Organization (WHO) reported in 2016 that more than 1.9 billion adults were overweight, of which over 650 million were obese, accounting for 39% and 13% of adult population, respectively [1]. Obesity increases the risk of metabolic disorders, such as type 2 diabetes (T2D), non-alcoholic fatty liver disease (NAFLD) and cardiovascular diseases (CVD). These obesity-related diseases carry a huge economic burden and strongly reduce quality of life. Active research on the treatment of obesity, T2D, NAFLD and CVD has recently highlighted the importance of the human gut microbiome and of bile acids (BAs), which appear to play a role in the onset and progression of these diseases.

Imbalance of the gut microbiome, generally referred to dysbiosis, has repeatedly been reported in patients with obesity-related diseases [2]. Impact of microbiome on disease etiology is thought to occur primarily via actions of microbiome-derived metabolites, such as short chain fatty acids (SCFAs), ethanol, trimethylamine (TMA), lipopolysaccharide (LPS) and also of BAs, which are the focus of this review. BAs are synthesized in the liver and efficiently maintained within the enterohepatic circulation. During their intestinal passage, gut bacteria can metabolize liver-derived primary BAs into so-called secondary BAs, which constitute an important but highly variable fraction of the human BA pool [3].

Aside from aiding dietary fat absorption by acting as "intestinal soaps", BAs exert endocrine actions within and outside of the enterohepatic system via multiple nuclear receptors (FXR, farnesoid X receptor; VDR, vitamin D receptor; PXR, pregnane X receptor; CAR, constitutive androstane receptor) and membrane-bound receptors (TGR5, Takeda G protein-coupled receptor 5; S1PR2, sphingosine-1-phosphate receptor 2; M3R, muscarinic acetylcholine receptor M3), leading to a broad spectrum of physiological effects [4]. Of note, BA concentrations show large differences in various compartments of the body, with millimolar concentrations in bile and intestine and micromolar concentrations in blood and lymph [5] (Fig. 1). Moreover, different BA species have divergent affinities for the receptors mentioned. Interestingly, obesity is associated with altered BA metabolism and a wealth of pre-clinical studies indicate important roles of BAs, the gut microbiome and their crosstalk in disease etiology.

In this review, we aim to provide a brief overview of BA metabolism in the human body and of recent findings on BA-metabolizing bacteria and the enzymes involved. We will highlight the microbiome signature and BA profile in patients with obesity-related diseases, including insulin resistance, T2D and NAFLD, and describe how BAs might contribute to these obesity-related diseases. Finally, we will discuss possibilities to manipulate this system to fight obesity-associated diseases. Clinical studies describing effects of novel pharmacological agents that target BA signaling pathways will not be addressed since this has been subject of several recent reviews e.g., [5,6].

Hepatic bile acid synthesis and microbial modulation

BAs are cholesterol metabolites that are exclusively produced in the liver by a complex, multiple-step process, involving cytosolic, mitochondrial and peroxisomal enzymes [7]. In short, primary BAs cholic acid (CA) and chenodeoxycholic acid (CDCA) can be synthesized via two well—known pathways, i.e., the "classical" and "alternative" pathways, respectively (Fig. 1). In the first, BA synthesis is initiated by the rate-controlling enzyme cytochrome P450 cholesterol 7α-hydroxylase (*CYP7A1*), while sterol 12α-hydroxylase (*CYP8B1*) is required to produce CA. The alternative pathway only produces CDCA via sterol 27α-hydroxylase (*CYP27A1*) and 25-hydroxycholesterol 7α-hydroxylase (*CYP7B1*). CA and CDCA are conjugated with either glycine or taurine prior to their secretion into bile by the bile salt-export pump (*BSEP* or *ABCB11*) and stored in the gallbladder. Upon ingestion of a meal, conjugated CA and CDCA are discharged into the intestinal lumen to aid fat absorption in the small intestinal tract. Most BAs are efficiently reabsorbed in the ileum by the apical sodium-dependent BA transporter (*ASBT*), while a small amount enters the colon where gut microbiota can convert primary BAs into more hydrophobic secondary BAs deoxycholic acid (DCA) and lithocholic acid (LCA). These secondary BAs can passively be reabsorbed from the colon. In general, about 95% of BAs is reabsorbed in the ileum or colon, returning to the liver through the portal vein for re-secretion into bile, which maintains the BA enterohepatic circulation. The ~5% fecal BA loss is balanced by *de novo* synthesis of BAs in the liver to maintain BA pool size (Fig. 1). Hepatocytic reuptake of BAs from portal blood occurs mainly through the basolateral transporter Na^+-taurocholic acid co-transporting polypeptide (*NTCP*). Meanwhile,

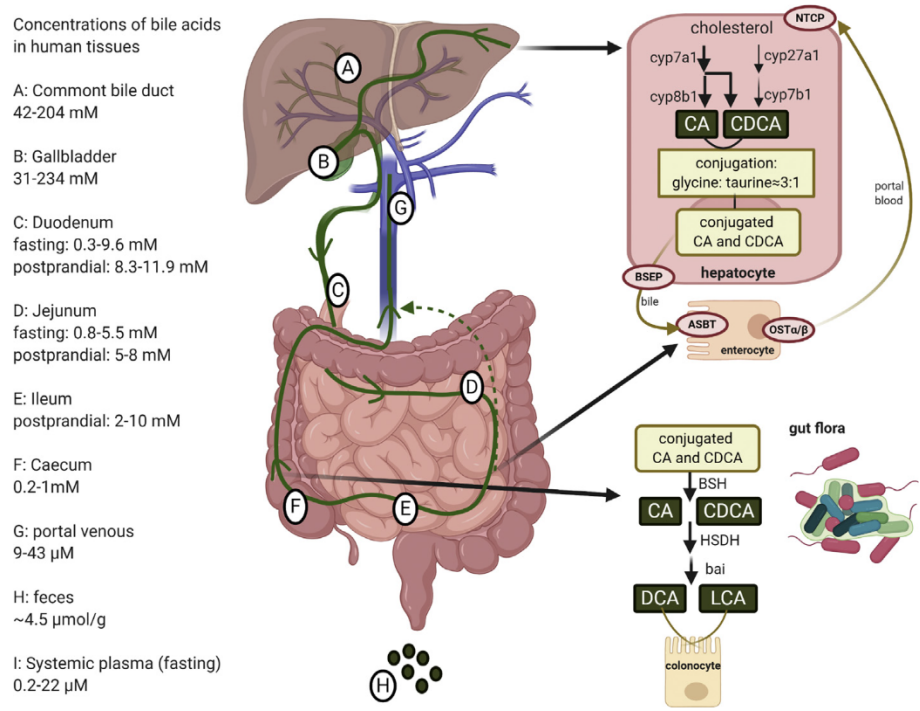


Fig. 1. Hepatic BA synthesis, microbial modulation and concentration of BAs in the human body [5]. CA, Cholic acid; CDCA, Chenodeoxycholic acid; DCA, Deoxycholic acid; LCA, Lithocholic acid; NTCP, Na^+-taurocholic acid co-transporting polypeptide; BSEP: bile salt-export pump; ASBT, apical sodium-dependent BA transporter; OSTα/β: Organic solute transporter α/β; BSH, bile salt hydrolases; HSDH, hydroxysteroid dehydrogenases; bai, BA inducible.

relatively small amounts of BAs escape reuptake by the liver and, particularly during postprandial periods, spill over to the systemic circulation to reach the peripheral tissues and organs such as adipose tissue, muscle and pancreas. Therefore, BAs are predominantly present in bile ducts, gallbladder and intestine, but also in the systemic blood compartment and in peripheral organs in relatively low concentrations [5,8].

Conversion of primary BAs into secondary BAs by gut microbiota involves three major groups of bacterial enzymes (Fig. 1). The bile salt hydrolases (*BSHs*) represent a widespread gene family within the gut microbiome and are commonly found in two taxonomic domains, archaea and bacteria [9]. In the latter, *BSHs* have been identified in over 100 different genera [10]. *BSHs* catalyze the deconjugation of primary BAs by hydrolysis of the glycine or taurine moieties from the C_{24} position at the side chain of the BA molecules [11]. Recent studies classify this gene family into 7 [12] or 8 [10] sub-groups. These sub-groups seem to vary in substrate-specificity [10] and are differentially associated with various diseases [12]. Bacteria may also oxidize and epimerize BAs by means of hydroxysteroid dehydrogenases (*HSDHs*). *HSDHs* act on the C_3-, C_7- or C_{12}-hydroxy groups at the steroid nucleus of the BA molecules. These reactions require two distinct *HSDHs*, which can be expressed either by different or by the same bacteria. The oxidation step of the reaction is dependent on the prevailing redox potential in the environment [11]. For instance, oxidation of LCA at C_3 may produce oxo-LCA and other intermediates that act as strong agonists of intestinal VDR [13]. The third major reaction is dehydroxylation of unconjugated BAs in the colon. Many bacterial species carry out different dehydroxylation reactions, however, only 7-α/β dehydroxylation results in the formation of the major secondary BAs, i.e., DCA and LCA. The enzymes that are responsible for this process are located in an operon known as BA inducible (*bai*), an evolutionary conserved operon composed of 8 genes, from which 6 are required to produce

secondary BAs [14]. In addition to the abovementioned major reactions, bacteria also carry out some other reactions on the colonic BA content. For example, sulfated BAs need to be de-sulfated before further bacterial transformations can take place [15,16]. This reaction is known to occur in the large bowel [16]. The species involved are not well-known in humans, while species from the genus *Clostridium* are shown to express this activity in rats [17]. Glucuronidated BAs can also be metabolized, after which the glucose moieties can be used as an additional energy source by the respective bacterial taxa [18]. BA metabolizing genes have been identified in several gut bacterial taxa (Fig. 2). Moreover, an *in-silico* assessment has shown that 37% of 693 analyzed microbial genomes have the potential to deconjugate or biotransform BAs, including 29% of the genomes with BSH activity and 7% of the genomes with HSDH activity, mainly 7α-HSDH, and three taxa with the bai operon [19].

Microbiome signatures in obesity-related diseases

Gut microbiome dysbiosis appears to be present in obese subjects with and without co-morbidities. For instance, over 40 observational studies showed some consistent microbial associations with T2D [21]. Moreover, 14 microbial features were found to predict T2D risk [22]. Interestingly, many of these T2D-associated bacteria show BA metabolizing capacities. At the genus level, *Blautia, Fusobacterium* and *Ruminococcus* are positively associated with T2D. Both *Blautia* and *Ruminococcus* comprise species harboring 3α-HSDH activity. Indeed, *R. gnavus* is an important producer of iso-DCA [23]. Moreover, *Akkermansia, Bacteroides, Bifidobacterium, Faecalibacterium* and *Roseburia* are decreased in their abundance in T2D patients. *Bifidobacterium* is a genus known to carry several *BSH* genes and in the genus *Bacteroides* the species *B. fragilis* can have 7α-HDSH activity. It has also been reported that *Roseburia hominis* and *Roseburia inulinivorans* are differently associated with fecal BA content, the former with higher amounts of conjugated DCA and deconjugated CA, and the latter with less conjugated CA and DCA [3]. This suggests that different *Roseburia* species have differential roles in BA deconjugation.

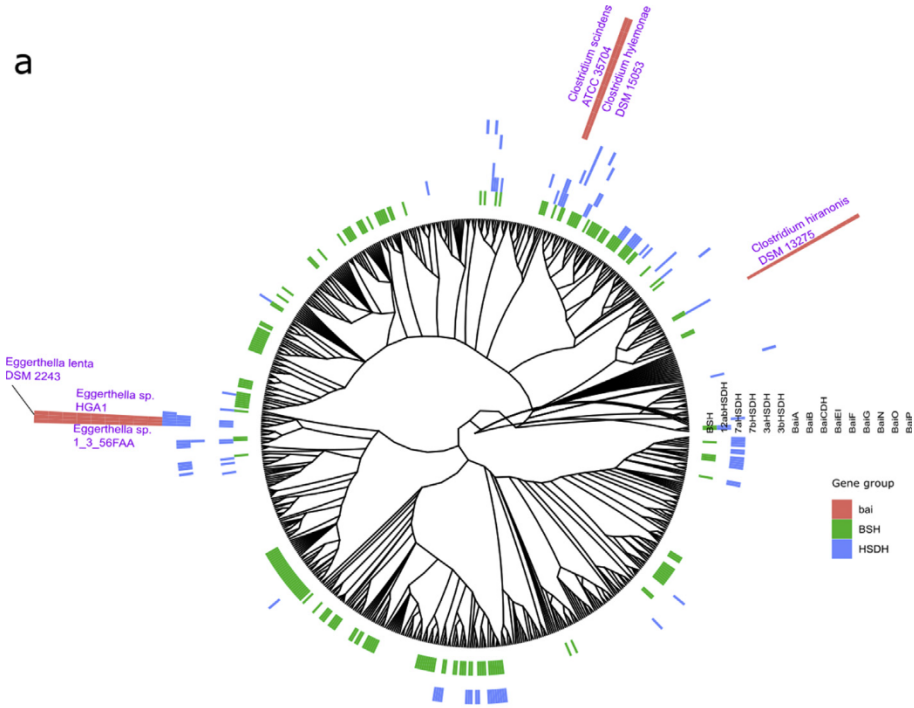
Fewer studies have linked NAFLD and its more severe state non-alcoholic steatohepatitis (NASH) to microbiome. Bacterial changes associated with NAFLD/NASH have been previously reviewed in [2]. Common NAFLD and NASH microbial signatures include increased abundance of the phylum *Proteobacteria* and its family *Enterobacteriaceae* and decreased abundance of *Rikenellaceae* and *Ruminococcaceae* families. Genera changes in NAFLD include increased *Escherichia*, *Dorea*, *Peptoniphilus* and decreased *Anaerosporobacter*, *Coprococcus*, *Eubacterium*, *Faecalibacterium* and *Prevotella*. Similarly, *Dorea* appears to be increased in NASH while *Faecalibacterium*, *Coprococcus* and *Anaerosporobacter* are decreased. From the NASH-associated genera, only *Eubacterium* is known to contain 3α-HSDH activity.

Given the existence of gut dysbiosis in obesity, T2D, fatty liver disease and their comorbidities, disease-specific microbial signatures will be hard to disentangle. NAFLD and T2D appear to present a common decrease of the genus *Lactobacillus*, while the genus *Roseburia* is seen to decrease in T2D but to increase in NAFLD. At the species level, *E. coli* is consistently reported to be increased in both conditions [2].

Bile acid changes in obesity-related diseases

Altered plasma BA levels have been reported in obesity and obesity-related diseases [6,24–26]. However, these observations were not conclusive due to relatively small sample sizes of the studies. In line with this, we have recently reported an unexpectedly large inter-individual variability in fasting plasma BA profiles in obese individuals [3]. In general, total plasma BA concentrations appear to be positively correlated with obesity, T2D and NAFLD as evidenced by higher fasting or postprandial plasma BAs levels [6]. Yet, it is difficult to establish an independent association between BA alterations and individual diseases. For example, Legry et al. [27] recently reported that BA alterations in obese subjects with advanced NAFLD, i.e., NASH, were related to the prevailing insulin resistance rather than to the features of liver disease. The relevance of insulin resistance in BA metabolism has been shown in multiple studies. For instance, the ratio of 12α-hydroxylated to non-12α-hydroxylated BAs has universally been reported to be elevated in T2D patients compared to non-T2D patients [28,29]. Preclinical studies have demonstrated a role of insulin in control of *Cyp8b1* expression [30], which is required for

a



b

Gene	Phylum	Genera	Species
BSH	Actinobacteria	Bifidobacterium	
	Firmicutes	Clostridium	
	Firmicutes	Enterococcus	
	Firmicutes	Listeria	
	Firmicutes	Lactobacillus	
3-α HSDH	Firmicutes	Blautia	Blautia produta
	Firmicutes	Clostridium	Clostridium sp.
	Actinobacteria	Eggerthella	Eggerthella lenta
	Firmicutes	unclassified Lachnospira	Lachnospiraceae sp
	Firmicutes	Mediterraneibacter	Ruminococcus gnavus
7-α HSDH	Bacteroidetes	Bacteroides	Bacteroides fragilis
	Firmicutes	Clostridium	Clostridium sordelli
	Actinobacteria	Collinsella	Collinsella aerofaciens
	Firmicutes	Eubacterium	Eubacterium sp.
12-α HSDH	Firmicutes	Clostridium	Clostridium sp
	Actinobacteria	Eggerthella	Eggerthella lenta
7-α dehydroxylation	Firmicutes	Clostridium	Clostridium scindents
	Firmicutes	Clostridium	Clostridium hiranonis

Fig. 2. Bile-acid metabolizing prokarya. (**a**) Phylogenetic tree presents the *in-silico* prediction of BA metabolizing genes in 668 genomes from [19]. Phylogenetic tree was extracted from the Open Tree of life [20] in matching taxa names, which resulted in 668/693 taxa. Heatmap surrounding the tree indicates presence or absence of BA metabolizing genes. Color indicates the family of reactions the gene belongs to. Taxa containing *bai* genes are labelled. (**b**) Table of most important bacterial taxa contributing to each BA-metabolizing reaction. Given the widespread nature of *BSH* genes, species-level contribution in this reaction is not indicated. Color represents bacterial phyla.

the production of 12α-hydroxylated BAs (CA and DCA). More recently, glucose signaling via *Chrebp* has also been implicated in regulation of *Cyp8b1* [31]. Furthermore, low-abundant hyocholic acid (HCA), which was reported to stimulate glucagon-like peptide-1 (GLP-1) secretion and to maintain glucose homeostasis *in vivo*, was shown to be decreased in plasma of diabetic patients [32,33].

Besides case–control based association analyses, the potential impact of the BAs in these diseases has also been evaluated in several human intervention studies. For instance, bariatric surgery for morbid obesity ameliorates glucose and lipid metabolism in obese patients and, as reviewed in [34], is accompanied by elevated plasma BA levels and, hence, potentially enhanced peripheral FXR and TGR5

signaling. In addition, the BA-binding resin colesevelam, which prevents intestinal BA reabsorption and thereby induces hepatic BA synthesis and lowers plasma BA levels, reduces Hemoglobin A1C (HbA1c) and fasting plasma glucose in T2D patients [29,35]. Importantly, Brufau et al. [29] were able to demonstrate that colesevelam, surprisingly, did not alter the size of the circulating BA pool but did change its composition towards more CA and less CDCA and DCA, i.e., a pool with less FXR activating potency. Therefore, changes in human BA physiology and, consequently, receptor activities likely contribute to metabolic improvements in obesity-related diseases.

Potential roles of bile acids contributing to improving obesity-related diseases

Despite widely observed associations between BAs and obesity-related diseases, it remains to be determined unequivocally whether altered BA metabolism is a result of disease development in humans or has a causal role in pathophysiology. Several potential underlying mechanisms of BAs and their signaling pathways involved in the improvement of obesity-related diseases have been proposed. Herein, we summarize the potential roles of BAs on energy metabolism, gut peptides and insulin secretion as well as on intestinal integrity and immune system (Fig. 3).

Regulation of energy metabolism

It is now well-recognized that, within their enterohepatic circulation, BAs function as "metabolic integrators" of lipid and glucose metabolism and of energy expenditure [36–38]. BAs modulate triglyceride production by both inhibiting hepatic fatty acid and triglyceride biosynthesis via a BAs-FXR-SHP-Srebp1c (sterol-regulatory-element-binding protein 1c) pathway and by stimulating fatty acid oxidation via a BAs-FXR-PPARα pathway [39]. In addition, BAs promote plasma triglyceride clearance through increasing lipoprotein lipase (LPL) activity [40]. At the same time, BAs may decrease hepatic gluconeogenesis as evidenced by down-regulation of PEPCK and G6pase in the liver [41]. Other proposed modes of action include decreasing hepatic glycolytic gene expression and increasing hepatic glycogen synthesis via a FGF15/19-dependent pathway [42]. More recently, conjugated BAs have been suggested to regulate genes encoding enzymes involved in hepatic lipid and glucose metabolism via a S1PR2-SphK2 dependent pathway [43]. BAs have also been reported to increase oxygen consumption and energy expenditure in mice via a BAs-TGR5-Dio2 (type 2 iodothyronine deiodinase)-UCP1 pathway in rodent brown adipose tissue and in human skeletal myocytes [44]. Recently, CDCA was reported to increase human brown adipose tissue activity under thermoneutral conditions: upon 24 h intervention with CDCA in healthy female subjects, basal metabolic rate was significantly increased by ~5%–6%, but 24 h energy expenditure remained unaltered [45].

Regulation of gut peptides and insulin secretion

BAs can both directly and indirectly stimulate gut peptide secretion, including GLP-1 and PYY, and subsequently reduce food intake, delay gastric motility, and increase insulin secretion. Enteroendocrine L-cells are specialized cells that produce gastrointestinal hormones and peptides, which can sense the presence of BA via TGR5 [8] or fatty acids and monoacylglycerols that are via GPR119 [46]. Both BAs -TGR5 and fatty acid-GPR119 pathways increase cyclic AMP and activate PKA in L-cells to induce GLP-1 and PYY release [34,47]. Higuchi et al. [46] showed that a lower level of 12α-hydroxylated BAs in *Cyp8b1*$^{-/-}$ mice impaired triglyceride hydrolysis and absorption and allowed access of monoacylglycerol and free fatty acids to the distal intestine. Subsequently, the fat receptor GPR119 mediated GLP-1 and PYY secretion, leading to decreased gastric emptying and reduced body weight development of high fat diet-fed *Cyp8b1*$^{-/-}$ mice. Next to gut peptide release, BAs also directly act on pancreatic β-cells to stimulate glucose-induced insulin secretion in an FXR-dependent manner. Activation of FXR stimulated AKT phosphorylation to increase translocation of glucose transporter 2 (GLUT2) to the plasma membrane to increase insulin secretion in a pancreatic β-cell line and human islets [48]. Furthermore, TCDCA was reported to depolarize membrane potential to enhance cytosolic Ca^{2+} concentration and to induce insulin secretion, thereby modulating glucose and lipid metabolism [49]. In line with this, studies in subjects undergoing bariatric surgery confirmed the role of BAs in

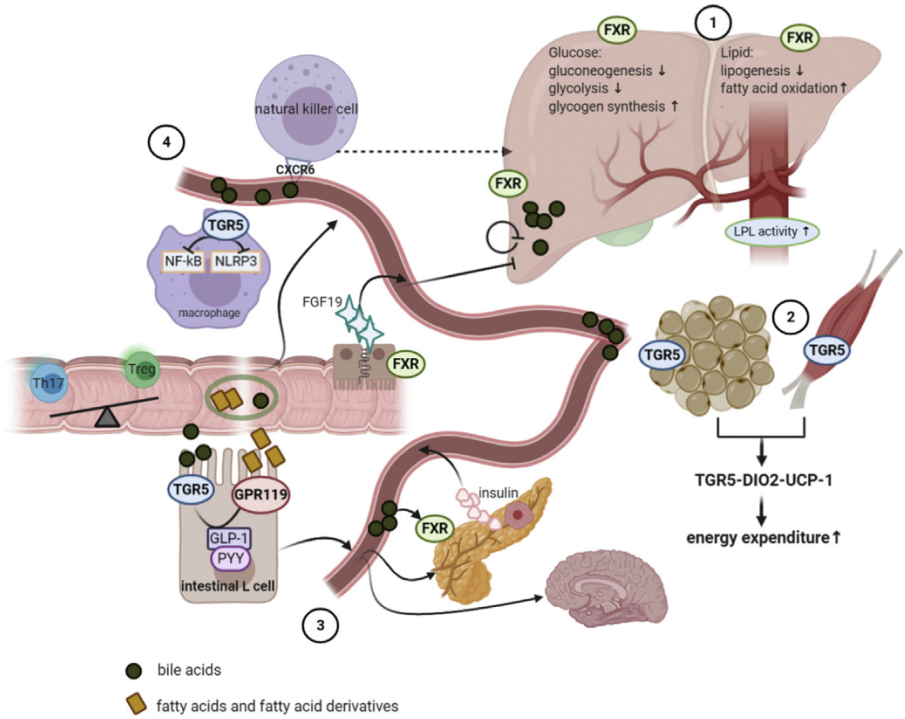


Fig. 3. Potential roles of BAs on energy metabolism (1–2), gut peptides and insulin secretion (3) as well as intestinal integrity and immune system (4). BAs are mainly present in the liver and intestine, affecting lipid and glucose metabolism. Together with BA stimulated hormone secretion such as GLP-1 and FGF19, low levels of BAs in systemic plasma also exert metabolic effects on brown adipose, muscle, pancreas, brain and immune system, especially the intestinal immune responses. See text for details. FXR, farnesoid X receptor; TGR5, Takeda G protein-coupled receptor 5; GPR119, G protein-coupled receptor 119; LPL, lipoprotein lipase; GLP-1, Glucagon-like peptide-1; PYY, Peptide YY; FGF19, Fibroblast Growth Factor 19; CXCR6, C-X-C Motif Chemokine Receptor 6.

release of gut peptides and insulin recreation in humans [34]. Elevated plasma BAs as well as increased intestinal BA signaling to promote GLP-1 release have been implicated in the beneficial health effects of bariatric surgery [34]. On the other hand, post-bariatric hypoglycaemia in subjects who experienced successful weight loss upon Roux-en-Y gastric bypass surgery (RYGB) may also be attributed to the enhanced release of BAs, gut peptide and insulin secretion, indicating elevated BAs might contribute to both beneficial effects and unwanted side effects of bariatric surgery on glucose regulation [50].

Regulation of intestinal integrity and immune system

Chronic low-grade inflammation is a crucial component in obesity-related diseases [51]. This phenomenon is associated with interruption of intestinal barrier function and involves both innate and adaptive immune cells [52,53]. Therefore, targeting intestinal permeability and inflammatory responses might represent an important step in design of novel therapies for obesity-related diseases.

FXR, that is expressed along the entire length of the gastrointestinal tract, was reported to protect the distal small intestine from bacterial overgrowth and translocation across the mucosal barrier in bile duct-ligated (BDL) mice. FXR-deficient mice showed a leaky gut as evidenced by impaired epithelial barrier in ileum, bacterial translocation to lymphatic vessels, as well as strong neutrophil infiltration [54]. Recently, the protective effect of FXR in intestinal integrity was demonstrated to prevent NAFLD development in preclinical studies. In both high fat diet-induced or methionine/choline-deficient diet

induced NAFLD models, 1–2 weeks of administration of the FXR agonist Obeticholic acid protected against gut barrier disruption and therefore reduced bacterial translocation and inhibited NASH development [55,56]. Similarly, in a recent study from our laboratory, the hydrophilic, FXR-antagonistic BA UDCA was found to revert increased intestinal permeability in a humanized mouse model (*Cyp2c70*-deficient mice) with a hydrophobic BA pool. Improved intestinal function coincided with normalization of plasma AST and ALT levels, indicative for improved liver function [57]. Clearly, the role of FXR in control of intestinal permeability needs further investigation. Importantly, both *in vivo* and *in vitro* studies suggest that BAs can exert anti-inflammatory responses via TGR5 activation in macrophages, suppressing both NF-kB signaling pathways [58] and NLRP3-dependent inflammasome activities [59]. Interestingly, CDCA increased NKT cell recruitment in the liver via induction of CXCL16, while its metabolite GLCA showed opposite effects [60]. However, this study was performed in multiple mouse models with liver tumors. Whether NKT cells contribute to metabolic inflammation and whether this phenomenon also occurs in humans remains to be seen.

Except for the innate immune system, BAs were also reported to control host adaptive immune responses in mice by directly modulating the balance of Th17 and T_{reg} cells in the intestinal lamina propria. Both primary and secondary BA supplementation increased T_{reg} cell counts in the colon in a VDR-dependent manner [61]. Even more interestingly, iso-allo-LCA was identified to increase the differentiation of T_{reg} cells, possibly through the production of mito-ROS, hence protecting mice from developing colitis [62]. In apparent contrast, germ-free mice colonized with feces from coronary artery disease (CAD) patients showed increased ratio of Th17/T_{reg}, accompanied with elevated secondary BA levels in plasma compared to the control group, leading to worsened gut barrier permeability and vascular dysfunction [63]. Furthermore, host scaled immune responses to reduced food intake during infection in an FXR-dependent manner. Deletion of FXR in T cells prevented starvation-induced loss of effector T cells *in vivo* [64]. The signaling functions of BAs and their intermediates formed by bacterial metabolism in control of the immune system are beginning to be unraveled. Thus, their role on metabolic inflammation is pending to be elucidated.

Of note, most mechanisms described so far have been revealed in studies applying rodent models. However, rodent-specific muricholic acids (MCAs) account for 35% of the total BA pool in mice, making the murine pool more hydrophilic than the human BA pool [65]. Given that BA species have dissimilar affinities for the activation of BA receptors (FXR: CDCA>LCA=DCA>CA; TGR5: LCA>DCA>CDCA>CA) while MCAs act as FXR and TGR5 antagonists [66], differences in BA composition between mice and humans likely differentially affect metabolism of nutrients, hormone secretion and immune functions. Therefore, translation of results from rodent studies to the human situation is challenging. Recently, a novel mouse model with human-like BA pool has been developed by depleting the MCA-generating enzyme *Cyp2c70* [67]. *Cyp2c70*-deficient mice presumably serve as a better model in research on the role of BAs in metabolic diseases [57,68,69].

A micoribome-to-bile acid link in disease and treatment

As summarized above, BAs act as important hormonal signals that modulate metabolism and immunity. As the microbiome is an important player in BA metabolism and both microbiome and BA composition have been implemented in obesity and its associated diseases, it is sensible to consider the potential links between these entities. It appears that only a few human studies have addressed concomitant changes in BA parameters and microbiome in a systematic manner (Table 1).

Fecal microbiota transplantation (FMT) is an intervention in which feces from a healthy donor, in this case a lean subject, are transferred to the gut of an obese recipient. A recent clinical trial showed bacterial engraftment of over 20 different bacterial strains, making the recipient's microbial community resemble that of the donor [70]. A particular observation was that several strains of the butyrate-producing genus *Faecalibacterium* were engrafted and increased their abundance. This genus is known to harbor BSH activity, which might explain the observed decrease of primary BAs, particularly of TCA [70]. Despite the transition towards the donor's microbial composition and BA profile, there were no changes in plasma concentrations of GLP-1 or BMI. Nevertheless, in other studies, transfer of intestinal microbiota from lean donors increased insulin sensitivity in individuals with metabolic syndrome [71] and improved hepatic inflammation in NASH patients [72]. In those studies, however, plasma or fecal

Table 1
Summary of therapeutic studies showing effects on both microbiome and bile acids in obesity-related diseases.

Author	Study	Obesity related disease	Intervention	Bile acid effect	Bacterial effect	Physiological effect
Allegretti et al., 2020	Effects of Fecal Microbiota Transplantation With Oral Capsules in Obese Patients [70]	Obesity	FMT from lean donor	Decrease: TCA (fecal) Increase: -	Decrease: - Increase: 200 OTUs. Faecalibacterium	No effect on GLP-1 or body mass index.
Gu et al., 2017	Analyses of Gut Microbiota and Plasma Bile Acids Enable Stratification of Patients for Antidiabetic Treatment [76]	Type 2 diabetes	Acarbose	Decrease: secondary/ primary; Conjugated DCA; Taurine conjugates; 12α-OH/non 12α-OH. Increased: CA, CDCA. Unconjugated primary BAs. Unconjugated/conjugated	Decrease: Bacteroides (*Bacteroides plebeius*, *Bacteroides dorei*/vulgatus -bai), Alistipes and Clostridium (*Clostridium bolteae*- bai), Increase: Lactobacillus (*Lactobacillus gasseri*), bifidobacterium (bifidobacterium longum).	Reduced glucose
Hibberd et al., 2019	Probiotic or Synbiotic Alters the Gut Microbiota and Metabolism in a Randomised Controlled Trial of Weight Management in Overweight Adults [74]	Overweight and obesity	B420 (probiotic) + LU (prebiotic)	Decrease: GCA, GUDCA, THDCA and TUDCA. Increase: LCA, HDCA	Decrease:Turicibacter, Lachnospira, (Ruminococcus)spp., unclassified Clostridiales. Increase: *Christensenellaceae* spp, Bifidobacterium (OTU), S24-7, (*Barnesiellaceae*) spp., Parabacteroides, *Rickenellaceae* spp., Akkermansia, Streptococcus, Lactobacillus	Unchanged body weight; *Christensenellaceae* correlated negatively to waist-hip ratio and energy intake at baseline, and waist-area body fat mass after 6 months treatment with LU + B420
Kim et al., 2018	Ursodeoxycholic Acid Improves Liver Function via Phenylalanine/ tyrosine Pathway and Microbiome Remodelling in Patients with Liver Dysfunction [80]	Obesity + Liver dysfunction	UDCA	Decrease: hydrophobic BA, DCA (plasma) Increase: -	Decrease: Bifidobactrium, Lactobacillus and Lactobacillaceae (Urine) Increase: -	UDCA improved liver function
Mayengbam et al., 2019	Impact of Dietary Fiber Supplementation on Modulating Microbiota–Host–Metabolic Axes in Obesity [73]	Obesity	Fiber supplementation	Decrease: fecal BA concentration, CA, DCA Increase:-	Decrease: Actinomycetace. Actinomyces, Holdermania, Oscillospira. Increase: *Barnesiellaceae*. Lachnospira, lachnospira	Body weight changes
Sun et al., 2018	Gut Microbiota and Intestinal FXR Mediate the Clinical Benefits of Metformin [77]	Type 2 diabetes	Metformin	Decrease: - Increase: GUDCA and TUDCA; conjugated/ unconjugated (fecal and plasma)	Decrease: Bacteroides, Bacteroides fragili. Increase: -	Decreased fasting glucose and HOMA-IR, increased energy expenditure in mice; not changed in humans after 3 days' treatment

BAs were not measured, which made it unclear whether improvements in insulin sensitivity and hepatic inflammation upon FMT were related to BA changes.

Diet intervention is considered to provide an efficient means to regulate microbiome and to prevent obesity-related diseases. A recent trial used pea fiber, which has been shown to reduce body weight and increase glucose tolerance, as prebiotic dietary supplement. It has been hypothesized that the effect of pea fiber might be mediated by microbial changes [73]. Longitudinal follow-up of overweight participants with pea fiber supplementation showed an increased abundance of *Lachnospira, Barnesiellaceae* and *Oscillospira* genera. *Lachnospira* is a SCFA producer, which was found to be positively associated with fecal acetate levels. On the other hand, the increase in *Oscillospira* was seen to correlate with decreased relative abundances of fecal DCA and iso-LCA. An overall decrease in BA concentration in feces was also observed, particularly for CA, CDCA and DCA. This study indicated a potential role of microbiota in modulating both SCFA and BA metabolism contributing to metabolic benefits of fiber supplementation.

Probiotics for treatment of metabolic diseases have also gained broad interest. The probiotic ***Bifidobacterium animalis*** **subsp. Lactis 420**™(B420) was administered with and without a prebiotic consisting of fiber [74]. The results showed a synergistic effect on both microbial and BA changes. The treatment increased *Christensenellaceae* spp., the introduced *Bifidobacterium strain S24-7, Barnesiellaceae* spp., *Parabacteroides, Rickenellaceae* spp., *Akkermansia, Streptococcus* and *Lactobacillus*. *Bifidobacterium* and *Lactobacillus* are genera with a high abundance of BSH genes, which may thus enable a higher BA deconjugation rate. This might explain the observed decrease of conjugated BAs, including GCA, GUDCA, THDCA, and TUDCA in plasma. Although deconjugation is the first step to produce secondary BAs, DCA and LCA were not affected, possibly due to a decrease of *Clostridiales*, which contains *Clostridium*, a genus in which dehydroxylation-responsible genes (*bai*) are present. In addition, increased abundance of *Christensenellaceae* was correlated negatively to waist-area body fat mass after 6 months of treatment with the probiotics/prebiotics, indicating beneficial effects on weight management, in line with previous observations [75].

It is well known that the microbiome can contribute to therapeutic effects of **medication**. For instance, conventional T2D drugs may partially act via the microbiome-BA axis. **Acarbose** was shown to induce microbial alterations while reducing blood fasting glucose levels and body weights in T2D patients [76]. Acarbose decreased the bai-containing species *Bacteroides plebeius* and *Bacteroides dorei/vulgatus* and the BSH-containing *Clostridium bolteae*. At the same time, it increased the BSH-containing species *Lactobacillus gasseri* and *Bifidobacterium longum*. These changes were accompanied by increases in the primary to secondary BA ratio, which was negatively related to plasma cholesterol and triglyceride levels as well as to C-peptide and insulin levels. In this study, it was demonstrated that bai-harboring species decreased in their abundance, particularly taxa containing baiE and baiI. Simultaneously, abundances of BSH and 7β-HSD expressing taxa were increased. **Metformin** has also been shown to induce bacterial and BA changes [77]. In a short-term experiment (3 days), T2D patients taking metformin showed a reduction of both *Bacteroides fragili* and overall *BSH* gene content. This was linked with an increase of GUDCA and inhibition of intestinal FXR signaling, confirming GUDCA as an FXR antagonist. Metformin was found to inhibit the growth of *B. fragilis* through modification of folate and methionine metabolism, which are both essential for bacterial growth and survival. *In vitro*, it was demonstrated that *B. fragili*'s BSH activity could deconjugate GUDCA and abrogate the improvement of glucose tolerance by metformin. Therefore, metformin was proposed to act in part through a gut microbiota–GUDCA–intestinal FXR axis to improve hyperglycemia, possibly via elevated GLP-1 production, independent of AMPK signaling. However, the long-term effect of metformin treatment on the microbiota-BA axis in T2D patients needs further investigation.

Moreover, BAs themselves, e.g., UDCA, which is effective in treating NAFLD patients [78,79], may modulate microbiome composition. In patients suffering from obesity and liver disease [80], UDCA not only improved liver function but also affected BA and microbial abundances. After UDCA treatment for 4 and 8 weeks, plasma levels of UDCA and its conjugates were increased, while hydrophobic BAs, such as DCA, were reduced. UDCA treatment significantly reduced the BSH-containing genus *Bifidobacterium* and *Lactobacillus*. DCA is known to be associated with NAFLD [81], thus a decrease in DCA concentration might be considered an indication of a healthier BA profile.

Summary

BAs are synthesized in the liver and modulated by gut bacteria in the intestine. BAs are absorbed efficiently and circulate within the enterohepatic system, while only small amounts spill over to the systemic circulation to reach peripheral organs and tissues, such as adipose tissue, muscle and pancreas. Beyond fat absorption, BAs exert hormone-like functions mainly through FXR, TGR5 and VDR, regulating energy metabolism, gut peptides and insulin secretion, intestinal integrity and immune response. In recent years, the gut microbiome and BA signaling pathways have emerged as attractive therapeutic targets for prevention and treatment of obesity-related morbidities. Indeed, the current treatments for T2D, including acarbose, metformin and fiber supplementation, are reported to modulate the microbiome-BA axis, thereby contributing to their beneficial effects. However, insight in the direct interaction between microbiome and BAs in disease development or during treatments is still lacking. The safety and efficacy of fecal microbiota transplantation or probiotics/synbiotics in patients thus remain to be further explored. Clinical trials of microbiome-based treatments (such as fecal microbiota transplantation and probiotics/synbiotics) and BA-based treatments (such as FXR agonist, TGR5 agonist) are currently ongoing as promising therapies for obesity-related diseases. The generation of the *Cyp2c70*-deficient mice with a human-like BA pool might be helpful to identify underlying mechanisms and to accelerate translation.

Practice points

- Both dysbiosis and changes in BA profile are repeatedly reported in patients with obesity, T2D and/or NAFLD.
- BAs facilitate intestinal fat absorption but also exert hormone-like functions through activation of nuclear and membrane-bound receptors and thereby modulate glucose, lipid and energy metabolism, intestinal integrity and immunity.
- Gut microbiome contributes to regulation of BA pool composition in humans: this regulation is disturbed in obesity.
- Conventional T2D treatment including acarbose, metformin and fiber supplementation modulates the microbiome-BA-metabolic axis.
- Microbiome-based and BAs-based therapies are being tested for the management of obesity-related diseases.

Research agenda

- Accurately define reciprocal interactions between gut bacteria and BAs in human obesity-related diseases.
- Define mode of action and impact of microbiome-BAs-immune response in human obesity-related diseases.
- Further identify of (patho)physiological roles of individual (rare) BA species and BA-metabolizing bacteria in obesity-related diseases.
- Determine safety and efficacy of fecal microbiota transplantation and probiotics/synbiotics in patients with obesity-related diseases.
- Definition of exact modes of action and safety/efficacy of pharmacological modulators of BA signaling pathways in subjects suffering from different obesity-related diseases.

Declaration of competing interest

No conflicts declared.

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