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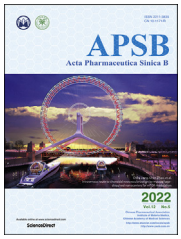

REVIEW

Bile acid coordinates microbiota homeostasis and systemic immunometabolism in cardiometabolic diseases

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Abstract Cardiometabolic disease (CMD), characterized with metabolic disorder triggered cardiovascular events, is a leading cause of death and disability. Metabolic disorders trigger chronic low-grade inflammation, and actually, a new concept of metaflammation has been proposed to define the state of metabolism connected with immunological adaptations. Amongst the continuously increased list of systemic metabolites in regulation of immune system, bile acids (BAs) represent a distinct class of metabolites implicated in the whole process of CMD development because of its multifaceted roles in shaping systemic immunometabolism. BAs can directly modulate the immune system by either boosting or inhibiting inflammatory responses *via* diverse mechanisms. Moreover, BAs are key determinants in

Abbreviations: AS, atherosclerosis; ASBT, apical sodium-dependent bile salt transporter; BAs, bile acids; BSEP, bile salt export pump; BSH, bile salt hydrolases; CA, cholic acid; cAMP, cyclic adenosine monophosphate; CAR, constitutive androstane receptor; CDCA, chenodeoxycholic acid; CCs, cholesterol crystals; CMD, cardiometabolic disease; CVDs, cardiovascular diseases; CYP7A1, cholesterol 7 alpha-hydroxylase; CYP8B1, sterol 12α-hydroxylase; DAMPs, danger-associated molecular patterns; DCA, deoxycholic acid; DCs, dendritic cells; ERK, extracellular signal-regulated kinase; FA, fatty acids; FGF, fibroblast growth factor; FFAs, free fatty acids; FMO3, flavin-containing monooxygenase 3; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide 1; HCA, hyocholic acid; HDL, high-density lipoprotein; HFD, high fat diet; HNF, hepatocyte nuclear receptor; IL, interleukin; IR, insulin resistance; JNK, c-Jun N-terminal protein kinase; LCA, lithocholic acid; LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; LPS, lipopolysaccharide; mTOR, mammalian target of rapamycin; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NF-κB, nuclear factor-κB; NLRP3, NLR family pyrin domain containing 3; OCA, obeticholic acid; ox-LDL, oxidated low-density lipoprotein; PKA, protein kinase A; PPARα, peroxisome proliferator-activated receptor alpha; PXR, pregnane X receptor; RCT, reverses cholesterol transportation; ROR, retinoid-related orphan receptor; SCFAs, short-chain fatty acids; SHP, small heterodimer partner; S1PR2, sphingosine-1-phosphate receptor 2; TGR5, takeda G-protein receptor 5; TLR, toll-like receptor; TMAO, trimethylamine *N*-oxide; TG, triglyceride; UDCA, ursodeoxycholic acid; VDR, vitamin D receptor.

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maintaining the dynamic communication between the host and microbiota. Importantly, BAs *via* targeting Farnesoid X receptor (FXR) and diverse other nuclear receptors play key roles in regulating metabolic homeostasis of lipids, glucose, and amino acids. Moreover, BAs axis *per se* is susceptible to inflammatory and metabolic intervention, and thereby BAs axis may constitute a reciprocal regulatory loop in metaflammation. We thus propose that BAs axis represents a core coordinator in integrating systemic immunometabolism implicated in the process of CMD. We provide an updated summary and an intensive discussion about how BAs shape both the innate and adaptive immune system, and how BAs axis function as a core coordinator in integrating metabolic disorder to chronic inflammation in conditions of CMD.

1. Introduction

Cardiometabolic diseases (CMD), a group of common conditions including heart attack, stroke, diabetes, insulin resistance and non-alcoholic fatty liver disease (NAFLD), are a leading contributor to death and disability in the world[1]. The term of CMD is to reinforce the pathological significances of metabolic disorders, including but not limited to obesity, hyperlipidemia, insulin resistance, diabetes, and NAFLD, which may ultimately lead to cardiovascular events. Metabolic disorders trigger chronic low-grade inflammation, and actually, a new concept of metaflammation has been proposed to define the state of metabolism connected with immunological adaptations[2]. It has been now widely acknowledged that metaflammation represents the most predominant pathological feature in the whole process of CMD development due to a disarrangement of immune signaling[3]. Hence, it is of paramount importance to better understand how metabolic dysregulation chronically triggers aberrant immunological responses leading to inflammatory injury of cardiovascular systems, from which appropriate therapeutic strategies can be exploited.

The mechanisms of metabolites in regulation of immune system, albeit very complex, can be classified into two types. One is that metabolites serve as nutrients, energy resources, and building blocks which are necessary for the development, differentiation, proliferation, and functional activities of immune cells. The other is that metabolites function as signaling molecules that can be directly detected by immune cells *via* receptors expressed in diverse subcellular organelles including but not limited to the membrane, cytoplasm, mitochondrion, and nucleus. *Via* these two types of mechanisms, it has been now well accepted that cell-specific and systemic metabolism are highly integrated with immunological responses, and the appropriate function of each is dependent on the other. Therefore, the immunometabolism interface, which may be classified into local metabolism of immune cells and systemic metabolism in regulation of immune system, has now been exploited as potential therapeutic avenues for diverse chronic diseases including CMD. This review intends to focus in discussing how systemic immunometabolism[4], defined as the communication circuits that underlie the reciprocal regulatory loop between metabolism and inflammatory responses across organs, is implicated and coordinated in the whole process of CMD for exploiting better therapeutic strategies and innovative drug discovery.

Both host and microbiota derived metabolites, and also those from nutrients, have been explored for their activities in regulation of immune system and have been validated for their involvement in the pathological development of CMD. For example, oxidated low-density lipoprotein (ox-LDL) uptake *via* scavenger receptors, including low-density lipoprotein receptor (LDLR), CD-36, class-A scavenger receptors, and lectin-like oxidized low-density lipoprotein receptor-1, can directly activate macrophages. Cholesterol crystals (CCs) have been widely validated to be a type of danger-associated molecular patterns (DAMPs) that can activate NLR family pyrin domain containing 3 (NLRP3) inflammasome for triggering chronic inflammation[5]. Saturated fatty acids elicit direct cytotoxic effects *via* diverse mechanisms and can also regulate toll-like receptor (TLR) 4 signals in triggering inflammatory responses[6]. More recently, comprehensive researches in bridging gut microbiota to the cardiovascular system and also the systemic immunometabolism have expanded our understanding in this exciting area. For example, trimethylamine *N*-oxide (TMAO) derived from dietary L-carnitine and phosphatidylcholine *via* microbial fermentation and hepatic oxidation has been proven to be an atherosclerosis (AS) and thrombosis-promoting metabolic product[7]. Metabolic homeostasis involves the coordination of multiple levels of crosstalk and communication at the organ, tissue, and cell levels[8]. Systemic immunometabolism, uncovering how systemic metabolites regulate multiple facets of immune system as well as interorgan communications, is thus pivotal in understanding the pathological processes of CMD. In physiological condition, systemic metabolites serve as both nutrients and signaling molecules to coordinate the organismal homeostasis. However, this homeostasis is disrupted in conditions of CMD due to dysregulated metabolism across different organs including the liver, adipose tissue, gastrointestinal tract, and pancreatic islet, and thereby the aberrant systemic immunometabolism (Fig. 1).

Amongst the continuously increased list of systemic metabolites in regulation of immune system, bile acids (BAs) represent a distinct class of metabolites implicated in the whole process of CMD development because of its multifaceted roles in shaping systemic immunometabolism. BAs can directly modulate the immune system by either boosting or inhibiting inflammatory responses *via* diverse mechanisms. Importantly, in addition to their direct effects in immune system, BAs are key species in controlling diverse metabolic homeostasis of lipids, glucose, and even amino acids across multiple metabolic associated organs, and thus may represent a driving factor in coordinating systemic immunometabolism. Moreover, BAs are key determinants in maintaining the dynamic communication between the host and microbiota. Of interest, BAs homeostasis, which should be tightly

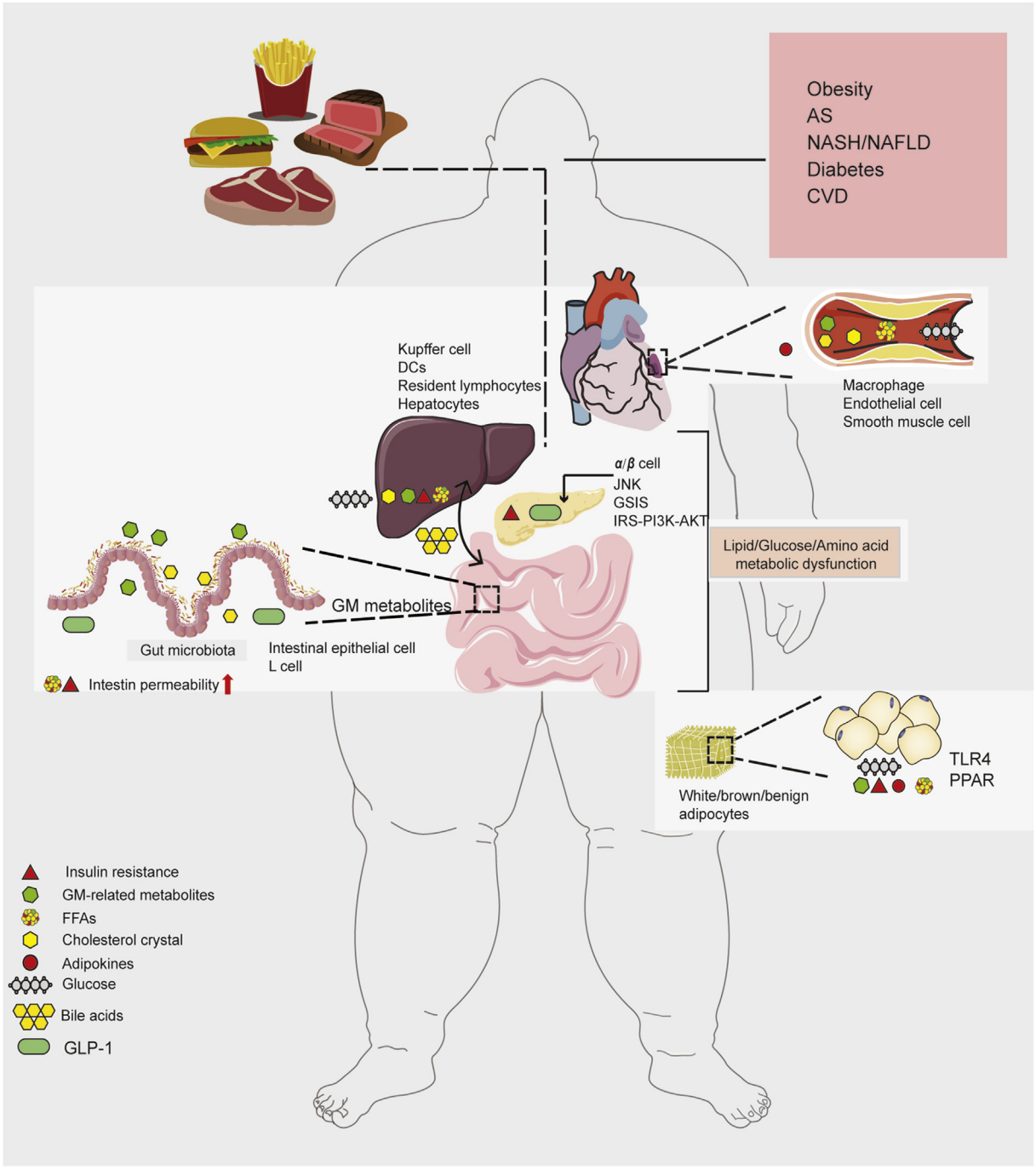

Figure 1 Dysregulated metabolic adaptation and the aberrant systemic immunometabolism in CMD. Metabolic homeostasis involves the coordination of multiple levels of crosstalk and communication at the organ system, tissue, and cell levels. Endogenous metabolites serve as both nutrients and signal molecules to coordinate the organismal homeostasis. The target organs of CMD include but not limit to the liver, gut, pancreatic islet, adipose tissue, heart, and blood vessels. However, this homeostasis is disrupted in conditions of CMD due to dysregulated metabolic adaptation and thereby the aberrant systemic immunometabolism.

controlled, is susceptible to be disrupted in various conditions of CMD. Several previous reviews had already provided a comprehensive summary of how BAs axis is implicated in CMD[9–12]; however, it remains unclear about how BAs axis might be involved in bridging the complex systemic immunometabolism system in CMD. On the basis of our recent research findings and the fast-expanding field of systemic immunometabolism, we propose in this review that BAs axis may represent a core coordinator of systemic immunometabolism in conditions of CMD. We provide an updated summary and an intensive discussion

about how BAs shape immune system, and how BA axis function as a nexus in integrating metabolic disorder with chronic inflammation in conditions of CMD.

2. BAs axis as a nexus in orchestrating systemic metabolism

The metabolic network is very complex involving diverse species of lipids, amino acids, and glucose, and multiple organs and tissues are intricately connected with the disruption of metabolic homeostasis in CMD. An intriguing question is that whether there is a coordinating factor that can integrate and/or bridge such a complex metabolic disorder and thereby the aberrant systemic immunometabolism in CMD.

BAs, functioning as metabolic regulators by engaging diverse BA receptors, playing fundamental roles in coordinating diverse metabolic pathways of lipids, glucose, amino acids, and also in maintaining homeostasis of gut microbiota metabolism. It has now been widely accepted that BAs, in addition to its solubilizing effects, are signaling molecules impacting both metabolic and immune system through several membrane and nuclear receptors such as G protein-coupled bile acid receptor 1 (aka Takeda G-protein receptor 5, TGR5), vitamin D receptor (VDR), pregnane X receptor (PXR), constitutive androstane receptor (CAR) and the farnesoid X receptor (FXR). Meanwhile, novel physiological functions of basal PXR and CAR on the gut microbiome were revealed[13]. These receptors are ubiquitously expressed across the whole body and are particular high in gastrointestinal tract and the liver. Importantly, receptors of BAs including TGR5 and FXR are also expressed in diverse kinds of immune cells, such as macrophages, dendritic cells (DCs) and natural killer T cells. BAs are circulating metabolites that can reach most, if not all, of the organs, tissues and cells where their receptors are expressed. Thus, it is not a surprise to find that BAs play important roles in regulating systemic metabolism, immune system, neurological system, and can also determine cell fates. Moreover, BAs dysregulation has been connected with an increasing list of pathological conditions, including but not limited to metabolic syndrome, cancer, cardiovascular diseases (CVDs), and colitis, all of which are characterized with chronic inflammation. Conversely, BAs axis is also vulnerable to be dysregulated by inflammatory challenge. Therefore, BAs axis may represent a coordinator in bridging metabolic disruption to aberrant immunological responses in CMD.

2.1. *Homeostatic regulation of BAs axis*

BAs are *de novo* synthesized in the liver and are regulated by a very precising and comprehensive feedback system to maintain homeostasis. The main two BA synthesis enzymes are cholesterol 7 alpha-hydroxylase (*CYP7A1*) and sterol 27-hydroxylase (*CYP27A1*), deficient of which disturb BA homeostasis[14]. FXR, the first receptor identified for BAs, has been extensively studied for its role in metabolic regulation of lipids, glucose, amino acids, and also the feedback regulation of BAs *per se*. Hepatic FXR *via* its effect on transcription of small heterodimer partner (SHP) suppresses the expression of sterol 12α-hydroxylase (*CYP8B1*) and to a lesser extent, *CYP7A1*, while intestinal FXR activation results in the production of fibroblast growth factor (FGF) 19 (15 in rodents) that upon binding to hepatic FGFR4/β-Klotho activates c-Jun N-terminal protein kinase/extracellular signal-regulated kinase (JNK/ERK) signaling and suppresses both hepatic *CYP7A1* and *CYP8B1* expression[15]. FXR promotes BAs secretion by upregulating bile salt export pump (BSEP, the principal BA efflux transporter in the liver) and multidrug related protein 2. BAs are reabsorbed from the gut back to the liver *via* the enterohepatic circulation by several active transporters on ileal enterocytes [apical sodium-dependent bile salt transporter (ASBT) and organic solute transporter alpha and beta (OSTα/β)] and hepatocytes [sodium taurocholate cotransporting polypeptide (NTCP) and organic anion transporting polypeptide (OATP)]. FXR activation inhibit BAs reabsorption by downregulating ASBT and NTCP. FXR can be activated by specific BA metabolites, including chenodeoxycholic acid (CDCA), cholic acid (CA), deoxycholic acid (DCA) and lithocholic acid (LCA), and their glycine and taurine-conjugated metabolites. The potency of various BA species to activate FXR is CDCA $>$ DCA $=$ LCA $>$ CA[16]. DCA, LCA, and their derivatives are major components of the recirculating BAs pool[17]. Excessive accumulation of BAs in both the liver and the intestine can activate FXR in a feedback mode to repress *de novo* hepatic synthesis of BAs. Of interest, FXR can also be antagonized by several BA species such as T-βMCA, Gly-MCA and ursodeoxycholic acid (UDCA). Therefore, the co-existence of agonists and antagonists of FXR in both the liver and intestine may constitute a precising regulatory machinery for tightly controlling the homeostasis of BAs.

2.2. *Role of BAs axis in orchestrating lipid and glucose metabolism*

BAs, as a class of cholesterol metabolites *per se*, play fundamental roles in regulation of lipid homeostasis, and also more recently are connected with metabolic regulation of glucose and amino acids. BAs synthesis and excretion are major pathways of cholesterol catabolism. As polar derivatives of cholesterol, BAs are classically considered to be detergents in the small intestine, directly incorporating dietary lipids, cholesterol and fat-soluble vitamins into mixed micellar solutions, which was also called lipid emulsification[18]. Thus, this amphipathic nature of BAs is pivotal to lipid absorption and systemic energy balance.

The metabolic regulatory effects of BAs in lipids and glucose metabolism and insulin sensitivity have been supposed to be dependent of its receptor FXR and TGR5 in the liver, intestine, adipose tissue and pancreas[19,20]. Mechanistically, FXR is essential for the cholesterol catabolism, transport, lipogenesis, and triglyceride (TG) metabolism. Firstly, FXR both takes part in cholesterol transportation and reverses cholesterol transportation (RCT) *via* acting on high-density lipoprotein (HDL) metabolism[21]. FXR agonist obeticholic acid (OCA) increased fecal cholesterol excretion and macrophage RCT, and targeting hepatic FXR and/or BAs may be useful for boosting RCT and preventing the development of AS[22]. FXR−SHP pathway plays a key role in lipogenesis and *de novo* cholesterol synthesis *via* the interference of sterol regulatory element-binding protein-1c expression[23]. FXR takes part in fatty acids (FA) oxidation though peroxisome proliferator-activated receptor alpha (PPARα) and its target genes[24]. FXR activation by both natural agonist CDCA or taurocholic acid suppresses hepatic Apo CIII expression[25,26]. Thus, FXR activity is effective against FAs induced TG accumulation and the prevention of the FA-induced lipotoxicity[27,28]. However, another panel of researchers found FXR overexpression in adipose tissue impaired lipid storage capacity, leading to elevated plasma free fatty acids (FFAs) and ectopic fat deposition in the liver and

muscles as well as the whole-body insulin resistance (IR)[29]. Meanwhile, FXR signalling in ileum biopsies of humans positively correlated with body mass index, and high-affinity FXR antagonists can prevent, or reverse, high fat diet (HFD)-induced and genetic obesity, IR and hepatic steatosis in mice[30,31]. Furthermore, mouse models of metabolic diseases demonstrated that the inhibition of intestinal FXR signalling by TβMCA reduces obesity, IR and NAFLD by modulation of hepatic and gut bacteria-mediated BA metabolism, and intestinal ceramide synthesis[32]. The discrepancy of FXR modulation in mediating lipid metabolism and related pathophysiological processes could be due to its different effects in different tissues, and other confounding factors including dietary patterns and therapeutic conditions. In addition to the typical BAs receptor FXR, TGR5, another important BAs receptor, has been also found playing key roles in metabolic regulation of lipids. LCA was identified as the strongest BA species in activating TGR5, followed by DCA, CDCA and CA. Activated TGR5 promotes mitochondrial fission and beige remodeling of white adipose tissue as well as provokes FFAs release through lipolysis, hence inducing β-oxidation and thermogenic activity in adipocytes[33]. In rodents, TGR5-cyclic adenosine monophosphate (cAMP)-enzyme type 2 iodothyronine deiodinase (D2) signaling pathway in brown adipose tissues is activated by BAs, which prevents obesity and resistance to insulin[34]. In addition, TGR5 activation contributed to skeletal muscle function as well as skeletal muscle activity in mice[35]. Similarly, in humans, administration of CDCA promotes expansion of brown adipocytes and increases energy expenditure *via* a TGR5-D2 dependent mechanism[36] (Fig. 2).

BAs activated FXR and TGR5 signaling is also important for glucose metabolism. The downstream target of BA-activated FXR, FGF15/19, is pivotal for glucose/insulin homeostasis. Activated FXR in a SHP dependent manner also enhanced the expression of gluconeogenic genes[37,38]. Meanwhile, intestine-restricted activation of FXR induces TGR5 to stimulate glucagon-like peptide 1 (GLP-1) secretion to improve insulin sensitivity and hepatic metabolism[39]. In diabetes, binding and activating FXR in the liver and intestine as well as TGR5, in enteroendocrine cells and pancreatic β-cells were proven to be useful in establishing glycemic control. Agonism of intestinal FXR activates TGR5/GLP-1 signaling to improve hepatic glucose and insulin sensitivity and increase adipose tissue browning[39]. FXR positively regulates hepatic glucose production, which integrates the glucagon/cAMP signal and the forkhead box protein O1/forkhead box A2 signal, *via* post-translational modification and engagement of protein−protein interactions, respectively[38]. In the skeletal muscle, activated TGR5 induces muscle hypertrophy and further increases glucose utilization by activating glycolytic flux, contributing to ameliorated glucose metabolism dysfunction due to diet-induced obesity and aging[40]. Alternative mechanism by which TGR5 activation may influence glucose metabolism involves protein kinase A (PKA)-dependent stimulation of insulin secretion in both alpha and beta cells[41,42]. FXR expressed in beta cell may stimulate insulin secretion *via* K_{ATP} channel inhibition and activation of TGR5 modulates pancreatic islet alpha-cells to promote glucose homeostasis[41].

2.3. *Mutual regulation between BAs and gut microbiota*

The gut is lined by a large barrier surface, which is a major mediator of crosstalk between microbes in the lumen and host cells, including immune cells in the lamina propria. Gut microbiota influences the immune homeostasis by regulating through its composition and its metabolites and are concomitantly shaped by the immune system. Indeed, the gut and its inhabited microbiota have now been widely acknowledged as a central hub in dictating the systemic immunometabolism, *via* the host-microbiota co-metabolites which can transport and distribute across the whole body.

The bidirectional crosstalk between the host gut system and microbiota has now been believed is mostly mediated by the co-metabolites. BAs, because of their distinctive features, represent a class of important mediator in microbiota−host crosstalk. Gut microbiota play important roles in expanding the chemical diversity, composition, and maintaining the intrinsic homeostasis of BAs. The microbial enzyme bile salt hydrolases (BSH) play a key role in deconjugation of BAs, acting as an essential upstream step of BA metabolism[43]. BSH activity has already been identified in several microbial genera, presenting in all major bacterial divisions and archaeal species in the human gut including members of *Lactobacilli*, *Bifidobacteria*, *Clostridium* and *Bacteroides*. Recently, the analysis of BSH distribution patterns also provides a novel insight of a reasonable relationship between BA metabolism and CMD[44]. Apart from deconjugation, BAs undergo epimerization and oxidation of specific hydroxyl groups (C3, C7, C12) by hydroxysteroid dehydrogenases, another microbial catalytic enzyme, leading to the formation of secondary BAs (*e.g.*, DCA and LCA)[45]. More recently, a metabolic pathway for BAs dihydroxylation by the gut microbiota has been identified, and established a set of six enzymes for the conversion of CA to DCA[46]. Gut microbiota perturbations not only influence the metabolic composition of BAs, but may also induce impairment in the ileal absorption *via* regulating the apical-sodium BA transporter, resulting in decreased expression of FXR and FGF19 and an imbalance of BAs, notably characterized by an increase in colonic primary conjugated BAs[47]. Thus, the levels and composition of BA metabolites are markedly influenced by gut bacteria, which produce enzymes that carry out dehydroxylation and deconjugation of BAs produced in the liver[48].

Conversely, BAs can also modulate gut microbial communities and functions directly or indirectly. Studies showed that when blocking the bile flow into the gut, microbial overgrew and translocated into the circulating system, which can be reversed with administration of conjugated BAs[49]. BAs and particularly the unconjugated BAs such as DCA and CDCA are direct antibacterial agents, changing or destroying the bacterial cell membrane[50]. The bactericidal activities of BAs are likely to be linked to the hydrophobic properties, which increased the adhesion of BA molecules to the phospholipid of membrane inducing cellular damage[51]. Interestingly, variations in BA compositions may have the potential to protect against pathogens such as *Clostridium difficile*[52]. Moreover, BAs may regulate gut microbiota *via* the activation of FXR, which protect against bacterial proliferation through triggering expression of antimicrobial agents, such as inducible nitric oxide synthase and interleukin (IL)-18.

The mutual regulation of BAs and gut microbiota plays a critical role in the establishment of immunological memory. For instance, previous infection promoted the generation of taurine derived from BAs, leading to long-term functional remodeling of host microbiota, which, in turn, stimulate subsequent antimicrobial defenses[53]. Acting as active signaling molecules for multiple types of immune cells contained in intestine mucosa, BAs are also involved in mucosal immune regulation[54]. Dietary lard and primary BA-fed mice were characterized by shifts in dominant gut bacterial

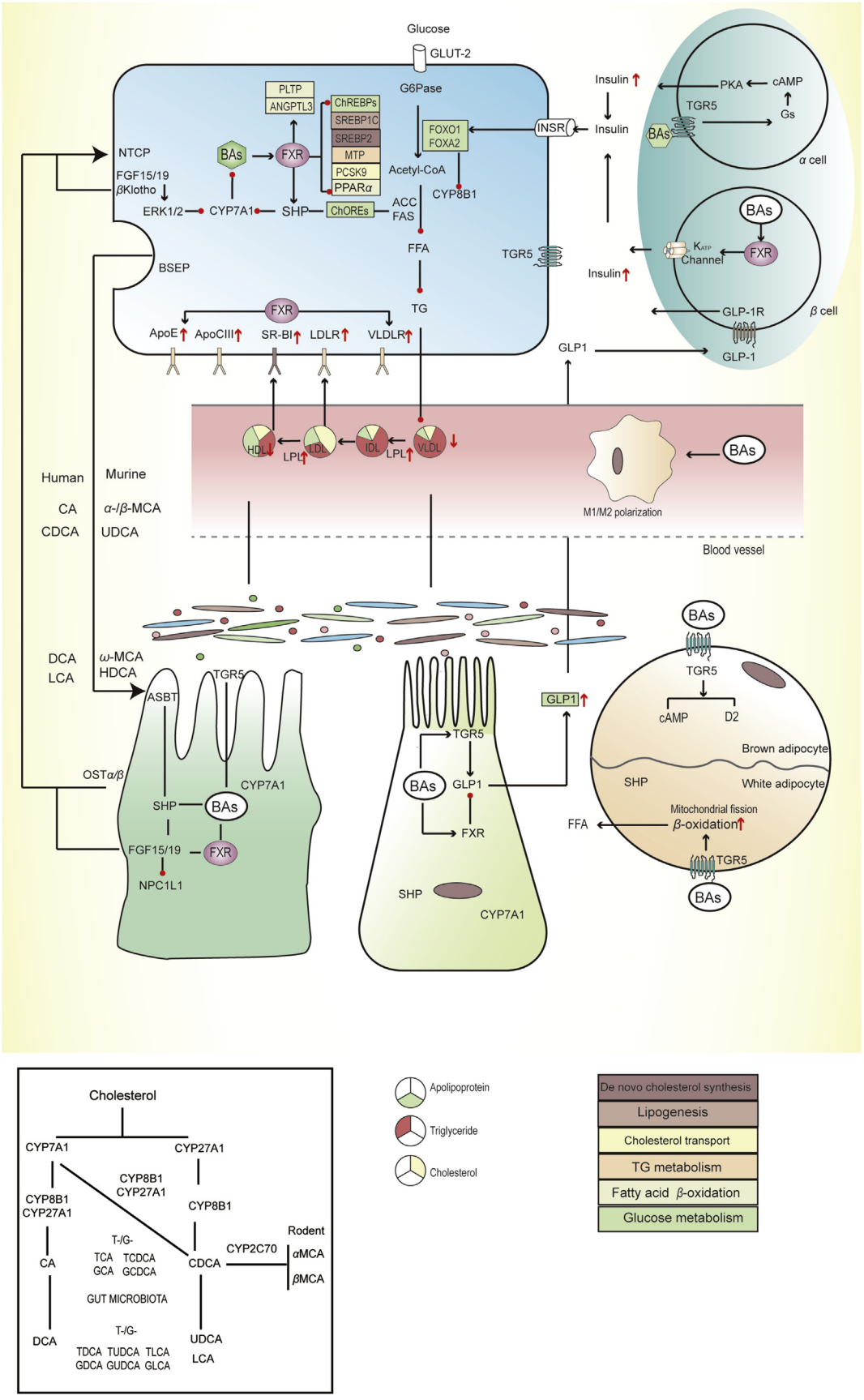

Figure 2 BAs play central roles in orchestrating lipid and glucose metabolism. BAs are important metabolic regulators of lipids and glucose *via* targeting FXR and other nuclear receptors. BAs activate hepatic FXR−SHP pathway, preventing hepatic triglyceride (TG) accumulation *via*

communities, including decreased relative abundances of Lachnospiraceae and increased occurrence of Desulfovibrionaceae and the species *Clostridium lactatifermentans* and *Flintibacter butyricus*[55]. Importantly, BAs also regulate other gut microbiota-derived metabolites such as short-chain fatty acids (SCFAs) and TMAO, some of which have key roles in immunological efficacy. FXR signaling is associated with TMAO generation; it has been shown that flavin-containing monooxygenase 3 (FMO3) generating proatherogenic TMAO was under the regulation of FXR[56,57]. Treated with natural (CA) or synthetic (GSK2324 or GW4064) FXR ligands exhibited induction of FMO3 expression and increased TMAO levels[58] in wild-type mice but not FXR-deficient mice[58]. Furthermore, they showed that hepatic FMO3 expression is regulated by hepatic FXR[56,58]. Of interest, TMAO may also regulate BAs signals *via* differential ways. Inhibition of gut microbial choline trimethylamine lyase was found to be of benefit to the homeostasis of host cholesterol and BA metabolism[59]. TMAO aggravated liver steatosis by increasing BA synthesis and shifting hepatic BA toward FXR-antagonistic activity in NAFLD[60,61]. A diet supplemented with carnitine or TMAO-induced AS was associated with BA dysmetabolism, characterized with the prominent increase of tauromuricholic acid, DCA and CA[62]. SCFAs (mainly butyrate, propionate, and acetate) are end products of bacterial fermentation of dietary fiber and present in high concentrations (20−140 mmol/L per day depending on the diet) in the human cecum and the ascending and transverse colon. Increasing evidence indicate that the homeostasis between SCFAs and BAs is reciprocally connected in the interface of gut microbiota and the host intestinal environment. The butyrate-producing bacteria *Clostridium butyricum* decreased BA-biotransforming bacteria[63], and butyrate supplementation reversed dysregulated BA synthesis including hepatic β-muricholic acid and bacteria-generated DCA, and its associated hepatitis in the $FXR^{-/-}$ mice[64]. Prebiotic fiber, the source of SCFAs, modulated gut microbiota, such as *Bifidobacterium* and various phyla of *Lactobacillus*, as well as the intestinal pH, which led to increased BA degradation[65,66]. Reciprocally, FXR activation by BAs and its synthetic agonists decreased L-cell GLP-1 secretion in response to inulin-derived SCFA by reducing FFAR2 expression and signaling[67]. In view that BAs possess strong activities in regulation of gut microbiota and relevant host-microbiota co-metabolism as those for the illustrated cases of TMAO and SCFAs, it is reasonable to predict that BAs may play key roles in shaping the metabolic system of gut microbiota, which warrants future research to explore how BAs is connected with other host and microbiota co-metabolites.

3. BAs axis coordinates systemic immunometabolism in CMD

BAs axis represent a nexus on the crossroad of metabolic homeostasis, because of its distinctive feature in engaging various receptors involved in metabolism regulation and also its bidirectional interactions with microbiota. CMD is a group of conditions characterized with metabolic disturbance of BAs, glucose, lipids, and insulin resistance, and the accompanying chronic metabolic inflammation. The disruption of systemic immunometabolism is likely to be a major cause underlying the pathological development of CMD. We propose here that BAs axis represent a core coordinator in integrating the signals of systemic immunometabolism in the context of CMD.

3.1. *Perturbed BAs axis in CMD*

BAs axis, as a master regulator in metabolism homeostasis and immunological responses, should be tightly controlled. It is thus not a surprise to observe that BAs axis is largely disrupted in conditions of CMD, including obesity, NAFLD, nonalcoholic steatohepatitis (NASH), diabetes, and CVD[47,68−71]. BA pool and the total concentrations of circulating BAs were also found increased in obese patients, and were positively correlated with the body mass index. In comparison with non-diabetic controls, the total concentrations of BAs were found increased in most of the previous studies. The levels of CDCA and CA, and to a lesser extent DCA were positively associated to IR in obese and T2D patients. Novel evidence about the perturbed hyocholic acid (HCA) profile was associated with diabetes, and assessed as the future risk of developing metabolic abnormalities[72].

The *de novo* synthesis, catabolism, transport, and reabsorption of BAs involve a very complex system that is constituted by various nuclear receptors, enzymes, transporters, and also the gut microbiota. Therefore, the disrupted homeostasis of BA axis observed in conditions of CMD should be expected to be mechanistically related to this complex system. In NAFLD, the hepatic gene expression pattern and the gut microbiome composition consistently support a pattern of elevated BA production, characterized with increased mRNA expression of *CYP7A1* but not SHP and BSEP, and reduced level of serum FGF19 hinting to a repressed negative feedback inhibition of BAs synthesis[73]. Consistently, the BA synthesizing enzyme *CYP7A1* was found significantly up-regulated in obese patients[74]. Notably, the transporters of BAs, such as BSEP and ASBT, were found decreased in

inhibiting hepatic lipogenesis by interfering with the promoters (carbohydrate response elements, ChOREs) of glucose-regulated genes and SREBP-1C. SREBP-1C induces acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl CoA desaturase (SCD). In addition, FXR-SHP pathway acts on *de novo* cholesterol synthesis by inhibiting SREBP-2. FXR activation regulates cholesterol uptake by inhibiting PCSK9; alleviates the very low-density lipoprotein (VLDL) and TG secretion by repressing the expression of microsomal TG transfer protein (MTP); induces phospholipid transfer protein (PLTP) and angiopoietin-like protein 3 (ANGPTL3); promotes FA β-oxidation though engaging PPARα. Intestinal FXR activation leads to FGF15/19 secretion and thereby inhibiting expression of NPC1-like intracellular cholesterol transporter 1 (NPC1L1) in intestine and cholesterol absorption. In addition, FXR induces ApoE but suppresses hepatic ApoC-III expression and thus inhibiting lipoprotein lipase (LPL). For glucose metabolism, BA−FXR signaling inhibits gluconeogenesis and promotes glycogen synthesis by negative regulation of G6Pase and carbohydrate responsive element-binding protein (ChREBP). In intestinal L cells, BA−TGR5 signaling leads to GLP-1 expression and secretion, whereas BA−FXR signaling inhibits GLP-1 production. Activation of TGR5 in brown adipocytes positively regulates cAMP−D2 signaling pathway, promoting mitochondrial fission and beige remodeling of white adipose tissue as well as provoking FFAs release. FXR in β cell stimulates insulin secretion *via* K_{ATP} channel inhibition. TGR5 activation augmented a hyperglycemia-induced switch from glucagon to GLP-1 synthesis in islet α cells by GS/cAMP/PKA/cAMP-response element-binding protein-dependent activation of polycystin-1 (PC1) to promote glucose homeostasis.

NAFLD and NASH, hinting to decreased efflux of BAs and thereby the increased retention of BAs in the liver and circulating system[75]. As a further support, OSTα/β, which facilitates the recirculation of BAs from the gut to the liver, was found upregulated in the patients with NASH, obesity and diabetes, leading to the increased accumulation of circulating BAs.

Notably, conditions of CMD are characterized with chronic inflammation and nuclear receptors including FXR, PPARs, PXR, and VDR were found vulnerable to be disrupted by inflammation. Nuclear factor-κB (NF-κB), that is activated in inflammation, binds directly to the promoter of FXR and inhibit its transcription, promoting the loss of BA transporters, increased biosynthesis of BAs, and ultimately accumulation of BAs in the liver. The downstream signal of NF-κB, myeloid differentiation primary-response gene 88 in the hepatocytes also affect BAs profile and FXR expression[76]. PPARs and mammalian target of rapamycin (mTOR), important modulators in metaflammation involved in conditions of CMD, can also perturbed BAs homeostasis. PPARα modulated numerous genes associated with lipid homeostasis and BA biosynthesis *via* inhibiting *CYP7A1* transcription. Inflammatory stress disrupted PPAR−LXR−*CYP7A1*/ATP-binding cassette transporter A1-mediated BA synthesis and cholesterol efflux. During the acute phase response, cytokines tumor necrosis factor and IL-1 decreased PPARα/β in liver cells, and thereby disrupting BA homeostasis. In CMD conditions, cholesterol-induced lysosomal stress feed-forward may activate transcriptional factor EB-FGF15/19 regulatory loop, acting *via* mTOR/ERK signaling and transcriptional factor EB phosphorylation, and thereby controlling hepatic cholesterol and BA homeostasis[77]. Hepatocyte nuclear receptor (HNF)-1α, usually disrupted in CMD condition, is also an essential regulator of BA and plasma cholesterol[78]. Transforming growth factor-beta released by hepatic stellate cells during chronic liver injury plays a critical role in liver inflammation, fibrogenesis, and BAs homeostasis. Prostaglandin E2, an important lipid mediator of inflammation, promotes hepatic BA synthesis by an E prostanoid receptor 3-mediated HNF-4α/*CYP7A1* pathway in mice[79]. Hepatic E prostanoid receptor 3 deficiency decreased *CYP7A1* expression by elevating PKA-dependent Ser143 phosphorylation of HNF4α. IL-1β inhibits *CYP8B1* gene transcription *via* a mitogen-activated protein kinase/JNK pathway that inhibited *HNF4α* gene expression and its DNA-binding ability. Glucagon and cAMP inhibit *CYP7A1* gene expression in human hepatocytes *via* PKA phosphorylation of HNF4α, unveiled a discordant regulation of BA synthesis and gluconeogenesis by glucagon in human livers during fasting[80]. FXR transcriptional activity is regulated by the glucagon/PKA and the forkhead box A2 signaling pathways, which act on FXR through phosphorylation and protein−protein interactions, respectively, to increase hepatic glucose synthesis[38]. Because BA homeostasis involves a very complex regulatory system, previous reports about the dysregulation BA axis in conditions of CMD were sometimes controversial. It is important to analyze and understand BA axis in a context dependent manner, and more intensive studies to delineate the compositions, rather than just the levels, of BAs, together with the multiple signals involved, are warranted for deeper understanding of BA axis in conditions of CMD.

3.2. *BAs and relevant metabolites in remodeling immune system in CMD*

CMD is characterized with the disruption of metabolic homeostasis and the resultant dysregulated systemic immunometabolism, which may represent the most dominant causes for triggering pathological development of ultimate cardiovascular events. Panels of signaling metabolites, such as BAs, SCFAs, and various lipid species, derived from the host and the microbiota system have been validated for their effects in modulating the immune system. The homeostasis of such metabolites was found largely disturbed in conditions of CMD resulting in chronic metaflammation. Therefore, it is important to delineate how the disturbed metabolites remodel the immune system for exploiting better targeting strategy to the immunometabolism system.

Accumulating evidence support that BAs have very comprehensive effects in both innate and adaptive immune system, and different species may exhibit differential and even converse effects. B and T cells are most common members of adaptive immune system. Various efforts have been made to identify immune-modulating therapies targeting either T or B cells with a potential anti-atherosclerotic impact[81−83]. 24-nor-UDCA affected the surface expression level of class II major histocompatibility complex of macrophages and DCs as well as the activation/proliferation of T-lymphocyte[84]. Meanwhile, CDCA, as interferon, activated protein kinase C and PKA, resulting in the induction of class one major histocompatibility complex expression[85]. More recently, it has been found that BAs pool may modulate gut retinoid-related orphan receptor (ROR) γ^{+} regulatory Treg cell homeostasis *via* the BA−VDR axis[86]. 3-oxoLCA inhibited differentiation of Th17 cells by directly binding to the key transcription factor ROR-γt (RORγt), while isoalloLCA increased the differentiation of Treg cells which was enhanced by forkhead box protein 3 and mitoROS[87]. Similarly, 3β-hydroxydeoxycholic acid (isoDCA) increase forkhead box protein 3 induction by acting on DCs to diminish their immunostimulatory properties to potentiate peripheral Treg cell differentiation[88]. Notably, Tregs in the heart acted as a critical factor to protect the heart against myocardial infarction[89]. It is thus of interest to determine whether and how the BAs shaped Tregs are involved in the pathological development of CMD.

In addition to adaptive immunity, innate immune system which initiates sterile inflammation *via* sensing metabolic disorder and DAMPs play essential roles in the pathological development of CMD. DCA may serve as an initiator to activate macrophages, dose-dependently promoting M1 macrophage polarization and pro-inflammatory cytokines production, at least partially through TLR2 transactivated by M2 muscarinic acetylcholine receptor (M2-mAchR)/Src pathway[90]. Moreover, NF-κB/ERK/JNK signaling downstream of TLR2 was involved in the DCA-induced macrophage polarization[90], while UDCA alleviated the macrophages-induced inflammation in obese mice[91]. Taurochenodeoxycholic acid induced monocyte differentiation toward IL-12 hypo-producing DCs *via* a TGR5-dependent pathway[92].

As an integral part of innate immunity, the inflammasome recognize a wide range of DAMPs and then trigger further metabolic disorders indirectly through the secretion of active cytokines[93]. By far, accumulating evidence has highlighted that NLRP3 inflammasome activated by BAs is a cardinal feature of systemic chronic low-grade inflammation. Lipopolysaccharide (LPS)-induced down-regulation of hepatic FXR and the resultant reverse transport of BAs, leading to increased levels of BAs, such as DCA and CDCA, in circulating system for the activation of NLRP3 inflammasome in the macrophages *via* activating both signal 1 and signal 2[94]. Accompanying with the increase of accumulating levels, DCA and CDCA may further elicit mitochondrial permeability transition and thereby activating the non-

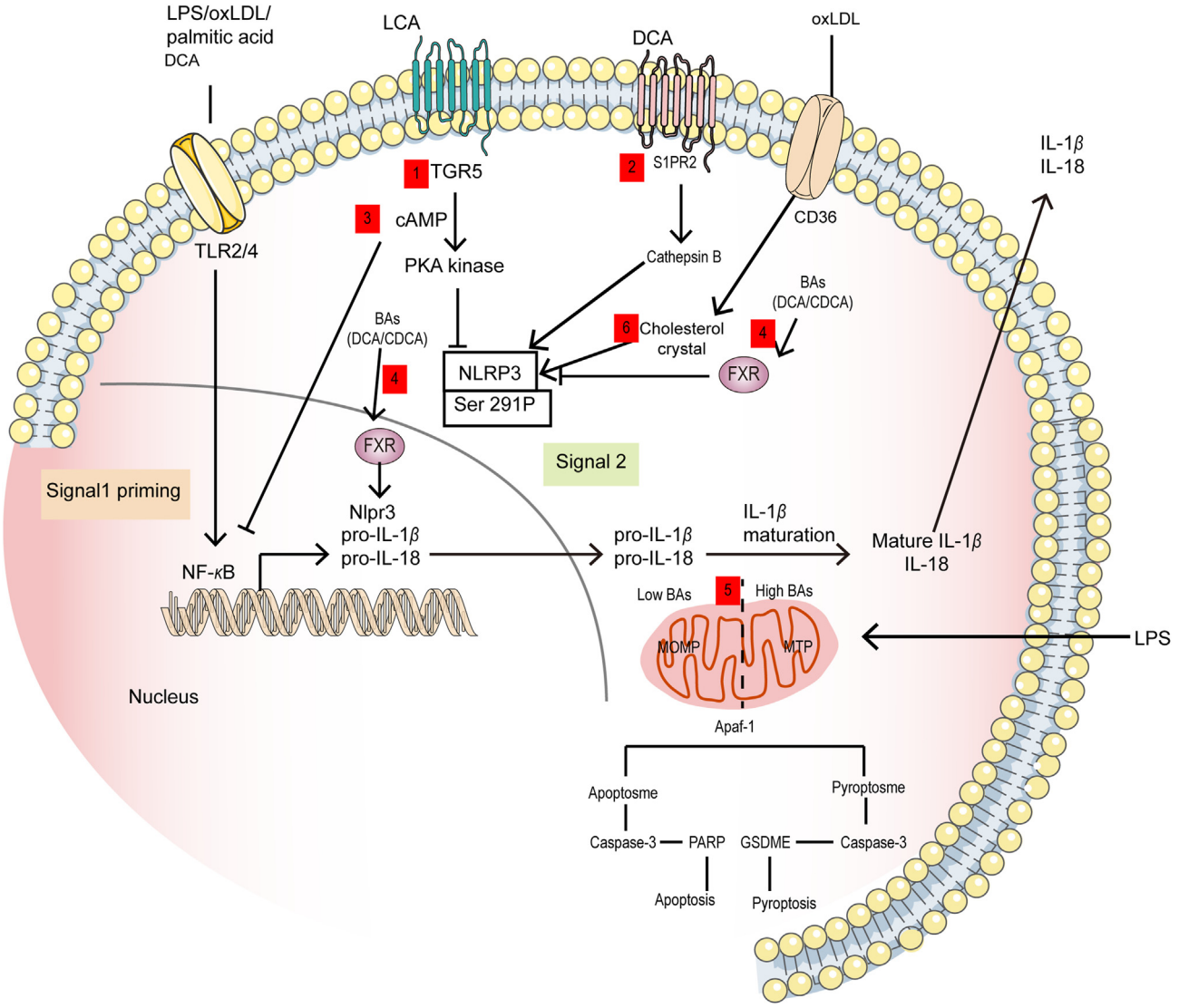

Figure 3 BAs and other relevant lipids serve as DAMPs and direct inflammatory triggers. BAs (such as DCA and CDCA) are metabolic DAMPs that can activate both signal 1 and 2 of the NLRP3 inflammasome and thereby promoting the secretion of IL-1β, and at high levels, BAs can further open the mitochondrial permeability transition pore and facilitates a very fast pryoprototic death of immune cells. In contrast, some BA species like LCA can also repress NLRP3 inflammasome *via* activation of TGR5 signals through the cAMP−NF-κB signaling pathway, and can decrease oxidized LDL uptake in macrophages and thereby protecting against on atherosclerotic plaque. Cholesterol crystal (CC) and oxLDL, both of which are regulated by BA signals, are also important DAMPs in activating NLRP3 inflammasome. CC causes lysosomal damage, and thereby activating NLRP3 inflammasome; CC also activate the complement system, which promotes macrophage priming (signal 1) as well as CC phagocytosis and hence, NLRP3 activation. Besides, BAs associated metabolites such as ox-LDL, palmitic acid and saturated fatty acid (SFA) contribute to the pro-inflammation of macrophages through TLR2/4 mediated activation of NLRP3 inflammasome.

canonical inflammasome caspase-4 *via* promoting the assembly of a protein complex apoptotic protease activating factor-1 pyroptosome for the execution of facilitated pyroptosis[95]. Meanwhile, BAs-related DAMPs synergize with other DAMPs, such as adenosine triphosphate, participate in the process of NLRP3 inflammasome activation. DCA may trigger NLRP3 inflammasome *via* the sphingosine-1-phosphate receptor 2 (S1PR2)−cathepsin B pathway, leading to subsequent IL-1β maturation and release[96,97]. Controversially, BAs also exhibit anti-inflammatory actions in part by repressing NLRP3 inflammasome. As endogenous regulators, LCA inhibits NLRP3 inflammasome activation through the TGR5 signaling in bone marrow-derived macrophage[98]. To be specific, LCA exert their inhibitory effects by inducing NLRP3 ubiquitination and degradation, which is associated with the Ser291 phosphorylation driven by PKA.

It seems that different BA species might have differential modulating effects in NLRP3 inflammasome. One possible explanation of the discrepancy is that BAs may have dual regulatory effect on inflammasome activation by different mechanisms, which may depend on BA concentration, specific BA species, receptors involved, and the presence or absence of other DAMPs. The BAs and their receptor TGR5 activation blocked NLRP3 inflammasome-dependent inflammasome, inducing LPS-induced systemic inflammation, alum-induced peritoneal

inflammation and type2 diabetes related inflammasome through a TGR5−cAMP−PKA axis[98]. In NASH, TGR5 expression was decreased in liver tissues, of which deficiency facilitated M1 macrophage polarization by promoting NLRP3 inflammasome activation and caspase-1 cleavage[99]. In the development of AS, activated TGR5 may inhibit cytokine production through the cAMP−NF-κB signaling pathway and decreases ox-LDL uptake in macrophages and thereby protecting against on atherosclerotic plaque[100]. The net effects of BAs in regulation of NLRP3 inflammasome in conditions of CMD remains unclear. However, in view that in conditions of AS and CMD, the levels of BA species such as CDCA and DCA and their taurine conjugates, which are potential DAMPs, are increased in the circulating system and thus may promote activation, rather than repression, of NLRP3 inflammasome and thereby contributing to chronic low-grade inflammation. Future research is warranted to explore whether targeting BAs metabolism and their signals, *via* modulating inflammasome activation, would be of an alternative approach to the prevention of therapy for CMD (Fig. 3).

As discussed earlier in this review, BAs axis functions as a nexus in integrating the metabolic homeostasis of various metabolites including lipids, amino acids, and glucose *via* engaging diverse receptors and the bidirectional interactions with the microbiota. Thus, the disruption of BAs axis in conditions of CMD is likely to initiate consequential metabolic change of other signaling metabolites which are also key players in remodeling the immune system. Systemic immunometabolism initiated by these metabolites have been comprehensively reviewer elsewhere, we thus make only an insightful summary of how BAs axis altered and its relevant metabolites are implicated in immunological regulation in CMD. BAs can determine the homeostasis of FFA[101−103], ceramides[104], and TG, all of which have been validated to play key roles in driving pathogenesis of AS, the major causes of cardiovascular events, *via* triggering chronic and low-grade inflammation. In AS, systemic signal molecules produced with the help of BAs signaling such as cholesterol-containing lipoproteins accumulated in arterial wall and recruited several types of adaptive immune cells. For example, contained in very low-density lipoprotein and low-density lipoprotein (LDL), apo B-100 contribute to vascular inflammation by triggering the response of T and B cells locally, which may influence the vascular inflammation plaques stability[105]. CCs are key component in atherosclerotic lesions which is eliminated by macrophages. As well known, CCs play a key role in AS, *via* serving as DAMPs in activation of NLRP3 inflammasome[106,107]. Additionally, ox-LDL and palmitic acid also contribute to the pro-inflammation of macrophages through TLR2/4 mediated NLRP3 inflammasome activation[108,109]. It is importantly to note that BAs are *de novo* synthesized from cholesterol, and *via* targeting FXR and other nuclear receptors, regulate the homeostasis of lipids. Thus, in conditions of AS and CMD, it is reasonable to expect that BAs axis play detrimental roles in lipids triggered chronic inflammation *via* regulating the disposition of various lipid species like cholesterol, ox-LDL, and FFAs, all of which may represent DAMPs in triggering chronic inflammation in CMD.

3.3. *Signaling pathways of BAs axis in shaping immune responses in CMD*

The different profile and species of BAs shape the immune system in a systemic and/or an organ-associated manner by changing the metabolic microenvironment and/or *via* their receptors, which is expressed in a wide range of immune cells. However, the molecular mechanisms and targets underlying BAs-remodeling immune system are largely unclear. Some effects were found dependent of their typical receptors like FXR and TGR5 but some others not. Moreover, FXR and TGR5 may also possess BAs independent effects in shaping the immune system.

During adipose tissue inflammation, activation of the TGR5−AKT−mTOR complex 1 in macrophage was shown to reduce chemokine expression, hence ameliorating obesity-induced IR and macrophage migration[110]. In hepatic inflammation status, TGR5 activation suppresses the phosphorylation of NF-κB inhibitor IκBα, the translocation of p65, NF-κB DNA-binding activity, and its transcription activity[111]. In support of this TGR5-dependent role in diminishing inflammatory liver injury, researchers found that macrophage migration, infiltration and pro-inflammatory M1 activation were attenuated and macrophage M2 polarization was facilitated *via* a TGR5−Cat E-dependent pathway[112]. TGR5 signaling involves cAMP−PKA-mediated inhibition of NF-κB and directly regulate IL-10 expression by a cAMP/PKA/phosphorylated cAMP response element-binding protein pathway[113]. Of interest, HCA was found to promote GLP-1 production and secretion in enteroendocrine cells *via* simultaneously activating TGR5 while inhibiting FXR[114] to improve glucose homeostasis in diabetes. Increasing evidence support that TGR5 may serve as a potential target in developing therapeutics against metaflammation.

The immunological modulating effects of FXR have been extensively studied. However, most of the studies observed from the use of FXR agonists were hard to discriminate the direct immunological effects and the indirect effects from the regulation of metabolic homeostasis. In one hand, upon agonists ligation, FXR may directly repress the transcription of proinflammatory cytokines and thereby the inhibition of inflammatory responses. In the other hand, FXR activation *in vivo* may restore the metabolic homeostasis of BAs, lipids, and glucose, and also insulin resistance, thereby may constitute a feedforward loop to repress metaflammaiton. In the context of obesity, elevated acetylation of FXR appears to play a causative role in promoting hepatic inflammation and abnormal metabolism, while agonist-activated FXR is SUMOylated and exerts anti-inflammatory response by trans-repressing NF-κB target inflammatory genes[115], indicating that the dynamic post-translational modification might represent an important mechanism in determining the differential effects of FXR in different context. Notably, ligand independent non-canonical effects of FXR have been reported to show that FXR is a negative regulator of NLRP3 and NF-κB *via* a mechanism of protein−protein interaction. Furthermore, because the protein of FXR is liable to be degraded by inflammatory stimulation, the decreased FXR protein levels seen in NASH and other CMD conditions may compromise both the ligands dependent and independent effects of FXR in combating CMD.

VDR, which can be also engaged by BAs, has been reported to have a role of anti-inflammation *via* regulation of macrophages[116], DCs[117] or T cells[118]. A recent study showed that LCA impairs Th1 activation at physiological relevant concentrations *via* VDR signaling[119]. Another study indicates that LCA has a significant protective effect on TNF-α-induced injury of intestinal barrier function through the VDR suppressing NF-κB signaling and activating the silent information regulator 1/nuclear factor erythroid2-related factor 2 pathway might be one of the mechanisms underlying the protective effect of LCA[120]. However, there is still a lack of research on the direct effect of LCA−VDR signal

Table 1 Therapeutic opportunities to targeting BAs/lipids immunometabolism for CMD.

Treatment	Clinical development phase	Biological effect	Therapeutic effect	Ref.
FXR/TGR5 agonist				
Natural				
1 CDCA	/	Potential DAMPs, increased in the circulating system and thus may promote activation, rather than repression, of NLRP3 inflammasome and thereby contributing to chronic low-grade inflammation. Increase brown adipocytes and energy expenditure *via* a TGR5-D2 dependent mechanism; activate protein kinase C and A (PKC and PKA), resulting in the induction of major histocompatibility complex class I (MHC-I) expression; in circulating system for the activation of NLRP3 inflammasome in the macrophages *via* both signal 1 and signal 2; Activated PKC and PKA, resulting in the induction of class one major histocompatibility complex (MHC) expression; further elicit mitochondrial permeability transition (MPT) and thereby activating the non-canonical inflammasome caspase-4 *via* promoting the assembly of a protein complex APAF-1 pyroptosome for the execution of facilitated pyroptosis	Anti-obesity, anti-inflammation	36, 94, 95
2 DCA	/	Serve as an initiator to activate macrophages, dose-dependently promoted M1 macrophage polarization and pro-inflammatory cytokines production at least partially through TLR2 transactivated by M2 muscarinic acetylcholine receptor (M2-mAchR)/Src pathway, NF-κB/ERK/JNK signaling downstream of TLR2 are involved in the DCA-induced macrophage polarization; trigger NLRP3 inflammasome *via* the sphingosine-1-phosphate receptor 2 (S1PR2) −cathepsin B pathway, leading to subsequent IL-1β maturation and release, in circulating system for the activation of NLRP3 inflammasome in the macrophages *via* both signal 1 and signal 2, activates TGR5 signaling pathway, contributing to decreasing inflammation and ameliorating heart function post-infarction	Anti-obesity, anti-systemic inflammation	90, 94, 96, 97,136
3 LCA	/	Inhibit NLRP3 inflammasome activation through the TGR5 signaling in bone marrow-derived macrophage, exert their inhibitory effects by inducing NLRP3 ubiquitination, which is associated with the Ser291 phosphorylation driven by PKA. Impairs Th1 activation at physiological relevant concentrations *via* VDR signaling since VDR RNA silencing abrogated these effects, controls adaptive immunity *via* inhibition of Th1 activation,has a significant protective effect on TNF-α-induced injury of intestinal barrier function through the VDR and suggests that suppressing NF-κB signaling and activating the silent information regulator 1/nuclear factor erythroid 2-related factor 2 pathway might be one of the mechanisms underlying the protective effect of LCA. Resulting in increased secretion of GLP-1 that improves insulin sensitivity and decreases obesity through white adipose browning	Anti-obesity, anti-systemic inflammation	98, 119, 120

(*continued on next page*)

Table 1 (*continued*)

Treatment	Clinical development phase	Biological effect	Therapeutic effect	Ref.
4 CA	/	Exhibited induction of FMO3 expression and increased TMAO level		58
Synthetic				
Steroidal				
1 Fexaramine	/	Resulted in decreased HFD- induced metabolic phenotypes in mice. Increased FGF15 synthesis, which is delivered to the liver where it decreases expression of the hepatic BA synthesis enzyme CYP7A1; adipose browning was also elevated, alters the gut microbiota composition is by increasing *Bacteroides* and *Acetatifactor*. Accompanied by activation of both intestinal FXR signalling and TGR5 signalling resulting from elevated LCA	Intestine-FXR restricted agonist, anti-obesity and metabolic syndrome	39, 154
2 INT-747 (OCA)	PHASE 4	Improved fibrosis and key components of NASH disease activity among patients with NASH, which showed clinically significant histological improvement with predictable clinical benefit. Weight loss in up to 44% of patients with NASH, and OCA therapy and weight loss had additive benefits on serum aminotransferases and histology. Decreased high-sensitivity C-reactive protein level in primary biliary cholangitis patients. Decreased HDL cholesterol and increased LDL cholesterol. Decreased cholesterol solubility in bile by increasing human gallbladder cholesterol saturation and BA hydrophobicity *via* increasing hepatobiliary levels of FGF19	Anti-AS, treatment of NASH/NAFLD, diabetes, and primary biliary cholangitis	155−165
3 EDP-305	PHASE 2	Activate FXR has demonstrated by increased levels of FGF19 and decreases BA synthesis, drug for NASH	Potential therapeutic effects of NASH	167
Non-steroidal				
1 Tropifexor	PHASE 2	Reducing the liver fat content with NASH and fibrosis, associated with a mild increase in LDL and a decrease in HDL and pruritus was reported	Potential therapeutic of NASH and primary biliary cholangitis	168−170
2 GS-9674/cilofexor	PHASE 3	Well-tolerated and provided significant reductions in hepatic steatosis, liver biochemistry, and serum BAs in patients with NASH	Therapeutic effects of NASH	171, 172
3 MET409	PHASE 2	Lowered liver fat content (LFC) in patients with NASH and delivered a differentiated pruritus and LDL-C profile	Therapeutic effects of NASH	173
4 PX-102/Phenex	PHASE 2A	Improve NAFLD in animal models and to prevent atherosclerosis in $LDLR^{-/-}$/cholesteryl ester transfer protein-transgenic mice	Potential therapeutic effects of NAFLD	174, 175
5 Notoginsenoside Ft1	/	Alleviate high fat diet-induced obesity and insulin resistance in mice	TGR5 agonist but FXR antagonist. Anti-obesity, and anti-insulin resistance	176
FXR antagonists				
Natural				
1 UDCA	PHASE 4	Suppress inflammation by inhibiting the function of DCs through the FXR, alleviate the macrophages-induced inflammation in obese mice; promote the M1 to M2 polarization of macrophages in patients post liver IR injury, involved in the crosstalk of inflammatory and metabolic pathways *via* the blockade of the NOTCH1 inflammatory response and improvement of macrophage infiltration, exhibits anti-atherogenic activity *via* inhibiting the disturbed flow- or cholesterol crystals-induced inflammatory responses, alters BA and fatty acid profile in a mouse model of diet induced obesity	Anti-cholestatic properties, anti-AS, potential treatment of diabetes, and NAFLD/NASH	91, 112, 121−124, 132−135

Table 1 (*continued*)

Treatment	Clinical development phase	Biological effect	Therapeutic effect	Ref.
2 Gly-MCA	/	Cause the biosynthesis reduction of intestinal-derived ceramides	Candidate for the treatment of metabolic disorder	30
3 TβMCA	/	Selectively suppresses intestinal FXR signaling, thus regulated hepatic gluconeogenesis; decrease obesity and IR	Anti-obesity and diabetes	138, 139
TGR5 agonists				
Synthetic				
1 INT-777	/	Protected against high glucose-induced cardiomyocyte inflammation by suppressing the expression of proinflammatory cytokines and NF-κB, shown by reduction of reactive oxygen species production and inhibition of NLRP3 inflammasome activation	Anti-inflammation and AS	144, 145
2 BAR501 AND 502	/	Attenuates liver fat deposition and fibrosis in mice fed a high-fat die, reverse liver and vascular damage, also regulate the M1/M2 phenotype of intestinal macrophage from murine colitis	Potential treatment of NASH	113, 149−151
3 RDX8940	/	Improves liver steatosis and insulin sensitivity in a mouse model of NAFLD and does not inhibit gallbladder emptying in mice	Potential treatment of NAFLD	152
Dual FXR and TGR5 agonists				
1 INT-767	/	Directly down-regulates the expression of Ly6C on bone marrow-derived monocytes and decreases production of proinflammatory cytokines by macrophages, attenuates the pro-inflammatory responses by suppression of NF-κB signaling pathway	Anti-inflammation, protective effects on NASH and AS	146−148
BA sequestrants	/	Have beneficial effects on lipid metabolism, glycemic control and insulin sensitivity. Cholestyramine is well tolerated and to exert cholesterol-lowering effects as well as to reduce CVD mortality. Improve glycemic control	Potential treatment of AS, T2DM, CVD in men with phenotypic familial hypercholesterolaemia	125−131

/, Not applicable.

on immunometabolism in CMD. Thus, whether physiological or pathological levels of LCA and its derivatives modulate immunometabolism *via* VDR signal *in vivo* remains to be determined.

A panel of other receptors CAR, LXRs, PXR, RORγT, and S1PR2 has been shown to be involved in BAs signaling, albeit to a lesser extent of affinity in comparison with their specific ligands. Moreover, it seems that there are still unknown targets of BAs, particularly considering the chemical diversity of BA species, which might be involved in regulation of immunometabolism involved in CMD. It warrants future research to explore the causal link of signaling targets of BAs to the immunometabolic homeostasis in conditions of CMD.

4. Therapeutic opportunities to targeting BAs signals for CMD

Interventions that target BAs or BA signaling pathways are currently in use or being investigated for immune and metabolic indications. BA replacement therapy, such as UDCA, has been used to alter inner BAs and microbiota composition, resulting in the reduction of toxic BA metabolites concentrations that accumulate in diabetes, adiposity, NASH and AS[121−124]. Chemical synthesized compounds targeting FXR and TGR5 have been developed in preclinical and clinical studies. Phytochemicals, especially polyphenolic compounds, have been described to markedly alter microbiota compositions linked to BA metabolic and immune functions. In view from the increasing evidence supporting the fundamental roles of endogenous BAs signaling in CMD, it is expected that targeting BAs signaling can be exploited as promising targets and/or strategies for developing therapeutics against CMD (Table 1).

4.1. *BA sequestrants and BA derivates*

BA sequestrants, including colesevelam, colestimide, cholestyramine, and colestipol, have beneficial effects on lipid metabolism, glycemic control and insulin sensitivity in AS and diabetic rodents[125,126]. Cholestyramine is well tolerated and to exert cholesterol-lowering effects as well as to reduce CVD mortality[127,128]. A randomized clinical trial demonstrated that together with a fat-modified diet, cholestyramine could improve the course of CVD in men with phenotypic familial hypercholesterolaemia[129]. Recently, an ongoing randomized clinical trial protocol designed to investigate the combined efficacy and safety of Elobixibat and cholestyramine for NAFLD[130], and a recent meta-analysis of randomized controlled trials unequivocally demonstrated that BA sequestrants also improve glycemic control, all of which are important risk factors for CMD[131].

Indeed, numerous evidences demonstrated that specific BAs replacement is helpful for CMD treatment.

UDCA, a critical endogenous agonist for TGR5, is clinically used for treating cholestatic liver disease. Clinical studies indicated that UDCA may promote the M1 to M2 polarization of macrophages in patients post liver IR injury[112]. In a rodent study of obesity, UDCA is directly involved in the crosstalk of inflammatory and metabolic pathways *via* the blockade of the NOTCH1 inflammatory response and improvement of macrophage infiltration[91]. Other evidence indicates that UDCA exhibits anti-atherogenic activity *via* inhibiting the disturbed flow- or cholesterol crystals-induced inflammatory responses[132,133]. However, a small study that included patients with chronic heart failure failed to find any anti-inflammatory effects of UDCA[134]. By exerting FXR-antagonistic effects, UDCA treatment in NAFLD patients strongly impacts on cholesterol and BA synthesis and induces neutral lipid accumulation in both liver and visceral white adipose tissue[135]. Thus, it remains uncertain about whether UDCA is of benefit to the therapy of CMD and more evidence is warranted to support possible larger clinical trials to validate the potential benefit of UDCA in CMD.

DCA administration activates TGR5 signaling pathway, contributing to decreasing inflammation and ameliorating heart post-infarction[136]. HCA species improve glucose homeostasis through a distinct TGR5 and FXR signaling mechanism[114]. Hyodeoxycholic acid, a natural secondary BA, improved HDL function and inhibited AS formation by increasing the expression of genes involved in cholesterol efflux, such as ATP-binding cassette transporter A1, ABCG1, and apolipoprotein E[137]. Gly-MCA is a selective high-affinity FXR antagonist and thereby reducing the biosynthesis of intestinal-derived ceramides, representing a candidate for the treatment of metabolic disorder[30]. Notably, TβMCA in mice and glycoursodeoxycholic acid in humans are primary BAs produced in the liver as FXR antagonists that can be deconjugated by BSH expressed by certain species of gut bacteria[138,139]. Supplementation of glycoursodeoxycholic acid could inhibit endoplasmic reticulum stress to treat the diet-induced metabolic disorders, including insulin resistance and hepatic steatosis[140]. In wild-type mice, shifted microbial community composition with lowered BSH activity increased intestinal taurine-conjugated BA levels, which resulted in markedly elevated intestinal TβMCA levels and inhibition of FXR signaling for combating obesity and IR[139]. A novel BA analog, A17, ameliorated NASH in high-fat diet-fed hamsters[141].

4.2. *FXR/TGR5 synthetic agonists*

For BAs receptors, FXR and TGR5 are the most two extensively studied receptors and have been exploited as potential targets for developing therapeutics against CMD. The activation of FXR expressed in cardiomyocytes, endothelial cells and vascular smooth muscle cells may elicit beneficial effects against pathogenesis of CMD *via* repressing endothelin 1 transcription, NF-κB activation, and angiotensin II-induced ERK activation and growth proliferation. Conversely, FXR deficiency might attenuate AS development, most likely resulting from reduced oxLDL-cholesterol uptake by macrophages[142]. As a support, a more recent finding indicated that suppression of intestinal FXR leads to improved AS in ApoE$^{-/-}$ mice fed a high-cholesterol diet[143]. The TGR5-selective agonist INT-777, a widely used as an experimental drug, protected against high glucose-induced cardiomyocyte inflammation by suppressing the expression of proinflammatory cytokines and NF-κB[144]. INT-777 may have anti-inflammatory effects in pancreatic acinar cells, shown by reduction of reactive oxygen species production and inhibition of NLRP3 inflammasome activation[145]. INT-767, a dual FXR/TGR5 agonist, directly down-regulates the expression of Ly6C on bone marrow-derived monocytes and decreases production of proinflammatory cytokines by macrophages[146]. INT-767 also attenuates the pro-inflammatory responses by suppression of NF-κB signaling pathway, hence exerting protective effects against NASH and AS[147,148]. A study showed FXR and TGR5 dual deficiency exacerbated the development of AS and the anti-atherogenic effect of INT-767 requires the anti-inflammatory effect but not the lipid-lowering effect through the simultaneous activation of FXR and TGR5[148]. TGR5 agonist BAR501 (a selective TGR5 ligand) and BAR502 (a dual FXR and TGR5 ligand) both from BAR Pharmaceuticals attenuated liver fat deposition and fibrosis in mice fed a high-fat diet[149–151]. A study showed BAR501 reversed liver and vascular damage, highlighting a potential role for TGR5 agonism in treating the liver and vascular component of NASH[149]. In addition, BAR501 also regulated the M1/M2 phenotype of intestinal macrophage from murine colitis[113]. RDX8940, a potent, selective, minimally systemic oral TGR5 agonist, improves liver steatosis and insulin sensitivity in a mouse model of NAFLD and does not inhibit gallbladder emptying in mice[152]. Klemens and colleges[153] identified isonicotinamides exemplified by compound **3** as nonsteroidal TGR5 agonists, reduced the production of proinflammatory cytokines and stabilized the alterative macrophage phenotype.

FXR has been extensively validated to be a possible target to developing therapeutics against NASH, for which CVD represents the most dominant cause for the death of patients. Therefore, it is important to test whether FXR agonists, currently developed as possible therapeutics against NASH, can also be evaluated for their potential benefit to CMD. Fexaramine, an intestine-FXR restricted agonist, resulted in decreased HFD-induced metabolic phenotypes in mice[39,154]. Mechanistically, fexaramine increased FGF15 synthesis, which is delivered to the liver where it decreases expression of the hepatic BA synthesis enzyme *CYP7A1* and increases adipose browning. Fexaramine alters the gut microbiota composition by increasing *Bacteroides* and *Acetatifactor*, which are the predominant bacteria that convert CDCA and UDCA to LCA, a secondary BA identified as a TGR5 agonist, resulting in increased secretion of GLP-1 that improves insulin sensitivity and decreases obesity through white adipose browning. Hence, the beneficial metabolic effects of fexaramine are accompanied by activation of both intestinal FXR signaling and TGR5 signaling. These pronounced metabolic improvements suggest tissue-restricted FXR activation as a new approach in the treatment of obesity and metabolic syndrome in animal models[154].

Orally OCA activated FXR and protected mice from LPS-induced inflammatory infiltration[155] and has been shown to have clinical benefits of NASH[156–160], primary biliary cholangitis[161], NAFLD and diabetes[162]. In 2015 and 2019, two multicenter randomized clinical trial reported that OCA 25 mg significantly improved fibrosis and key components of NASH disease activity among patients with NASH, which showed clinically significant histological improvement with predictable clinical benefit[156,157]. Another randomized clinical trial found OCA led to weight loss in up to 44% of patients with NASH, and OCA therapy and weight loss had additive benefits on serum aminotransferases and histology[159]. Meanwhile, a double-blind, placebo-controlled, phase 3 trial of OCA reported markedly decreased high-sensitivity CRP

level in primary biliary cholangitis patients treated with OCA for 12 months[161]. In addition, there are also several ongoing trials measured the role of OCA in the patients of NAFLD with raised alanine transaminase (NCT03836937), lipodystrophy (phase 2, NCT02430077), morbidly obesity (phase 1, NCT02532335), NASH with fibrosis (NCT02548351), and compensated cirrhosis cue to NASH (NCT03439254). However, in healthy volunteers and NASH patients, OCA dose-independently decreased HDL-cholesterol and increased LDL-cholesterol[163,164]. This LDL-cholesterol increasing phenotype also existed in patients with NASH, which was proven to be safely mitigated with atorvastatin by a randomized phase 2 study[158]. And OCA decreased cholesterol solubility in bile by increasing human gallbladder cholesterol saturation and BA hydrophobicity *via* increasing hepatobiliary levels of FGF19[165]. Moreover, pruritus represents a key side effect of OCA, which may explain why FDA had not approved OCA as a NASH therapeutics, in spite of that OCA had reached the clinical endpoint in the phase 3 clinical trial. In addition to OCA, there have been many other FXR agonists with different chemical structures undergoing different stage of clinical trials for the therapy of NASH. Interestingly, *in vivo* study showed that precise delivery of OCA *via* nanoapproach triggered natural killer T cells population and thus eliciting anti-tumor effects[166]. EDP-305 is a steroidal, non-BA, FXR agonist. In a phase 1 multiple ascending dose study, EDP-305 was shown to activate FXR as demonstrated by increased levels of FGF19 and decreases BA synthesis[167]. An ongoing clinical trial has been designed to measure the clinical benefit of EDP-305 in NASH (NCT04378010). Tropifexor (NOVARTIIS), previously known as LJN452, is currently investigated in combination with cenicriviroc in patients with NASH and fibrosis (the TANDEM study)[168]. Tropifexor has advanced into phase 2 human clinical trials in patients with NASH and primary biliary cholangitis[169]. Results from a stand-alone therapy in patients with NASH, the FLIGHT-FXR Phase 2 study (NCT02855164), has shown efficacy in reducing the liver fat content by 5.4% and 10.7% at 60 and 90 μg doses, respectively[170]. Tropifexor administration was associated with a mild increase in LDL and a decrease in HDL and pruritus was reported in 14% and 8% of subjects at the 60-μg and 90-μg doses, respectively, compared to 7% in the placebo group[170]. Cilofexor, previously known as GS-9674, is a nonsteroidal FXR agonist, which showed well-tolerated and provided significant reductions in hepatic steatosis, liver biochemistry, and serum BAs in patients with NASH[171,172]. In a 12-week randomized clinical trial, MET409, a non-BA FXR agonist with a unique chemical scaffold, lowered liver fat content in patients with NASH and delivered a differentiated pruritus and LDL-C profile at 50 mg, providing the first clinical evidence that the risk−benefit profile of FXR agonists can be enhanced through structural optimization[173]. Px-102 from Phenex, synthetic FXR agonists, induces HDL-mediated transhepatic cholesterol efflux in mice and monkeys and prevent AS in cholesteryl ester transfer protein transgenic $LDLR^{-/-}$ mice[174], has been advanced to a phase 2a in NAFLD patients from phase 1[175]. Notoginsenoside Ft1 acts as a TGR5 agonist but FXR antagonist to alleviate HFD-induced obesity and insulin resistance in mice[176]. Accumulating evidence support that FXR is a promising target for developing novel therapeutics against CMD. However, because of the complexity of FXR signaling, future studies are warranted to explore the differential roles of FXR expressed in different organs/tissues, the dynamic features in protein−protein interactions and posttranslational modifications, and the canonical and noncanonical efficacies, to better exploit FXR as a potential target for developing therapeutics against CMD.

4.3. Targeting other signals involved in BAs axis

Besides the extensively exploited receptors FXR and TGR5, BAs have been increasingly found to be engaged with diverse other receptors and signals, which could be also expected and exploited to the development of CMD therapeutics. PPARs are closely associated with BAs axis. PPAR ligands have been extensively validated both preclinically and clinically for the prevention of CVD[177], diabetes and NAFLD[178]. Recent researches found the combination of PPAR agonist bezafibrate and UDCA exhibited specific anticholestatic properties[179]. Transgenic expression of *CYP7A1* in the liver prevented HFD-induced obesity and IR in mice[180]. Metformin, which is also relevant to BAs axis regulation, and ASBT inhibitor IMB-1715[181], have been tested for their effects in modulating systemic immunometabolism in conditions of CMD[182]. The pleiotropic effects of FGF-19, including inhibition of BA synthesis from cholesterol *via CYP7A1*, and inhibition of insulin-induced hepatic lipogenesis, making FGF-19 agonism an attractive potential therapeutic mechanism[183]. Other receptors including VDR, CAR, LXRs, PXR, RORγT, and S1PR2 can be also engaged by BAs, and their effects in modulating systemic immunometabolism in the process of CMD conditions have been reported, and thus, it is reasonable to expect these BAs engaged targets can be exploited for their potentials in developing therapeutics against CMD.

5. Concluding remarks and future perspectives

We propose BAs as a core coordinator in integrating and orchestrating the comprehensive systemic immunometabolism implicated in conditions of CMD. CMD is a typical complicate and chronic disease that is closely connected with metabolic disorder initiated inflammatory damage. In this review, we propose that BAs relevant systemic immunometabolism represent an integrated signal in the whole pathological process of CMD, building a bridge in linking metabolic disorder to the immunological dysregulation, and shedding lights in better understanding the pathological mechanisms of CMD and thereby exploiting BAs axis and the signals involved as therapeutic opportunities.

Much work remains to be done to unravel the complex and dynamical BAs related metabolic homeostasis. The chemical diversity of BAs needs to be further delineated in view that more and more new BA species have been recently identified. Numerous receptors have been identified to be engaged in BAs signaling and this list is still increasing. The exact working mechanism of such a precising BAs-receptors interacting network, and particularly how different BA species engaged with differential receptors for culminating specific immunological effects, remains largely unexplored. Moreover, BAs axis is closely bridged with other systemic metabolites initiated immunological adaptations. For instance, during abnormal glucose and lipid metabolism and other environmental factors (hypoxia, cytokines), it is clear that immune cells, including but not limiting to macrophage, T cells, and DCs, exhibit metabolic rewiring in response to microbial BA metabolites, and these changes will, in turn, impact the functions of tissues or cells. Further studies are warranted to analyze how immune cells prioritize the different BAs profile and BA signals that they are exposed to and adopt metabolic signals that meet the needs of host immune response at specific conditions and contexts. In summary, a better and more intensive understanding of the immunomodulatory function of BAs axis will be of fundamental importance in the establishment of novel therapeutic approaches to CMD.

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Author contributions

Anlu Wang, Haiping Hao, Keji Chen and Hao Xu were responsible for the conception and design of the review. Baoyi Guan and Jinlin Tong analyzed the literatures, summarized the results and drafted the manuscript. Anlu Wang, Haiping Hao, Keji Chen and Hao Xu revised the manuscript. All authors have read and approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Sattar N, Gill JMR, Alazawi W. Improving prevention strategies for cardiometabolic disease. *Nat Med* 2020;**26**:320−5.
2. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;**444**:860−7.
3. Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. *Nature* 2017;**542**:177−85.
4. Lercher A, Baazim H, Bergthaler A. Systemic immunometabolism: challenges and opportunities. *Immunity* 2020;**53**:496−509.
5. Mridha AR, Wree A, Robertson AAB, Yeh MM, Johnson CD, Van Rooyen DM, et al. NLRP3 inflammasome blockade reduces liver inflammation and fibrosis in experimental NASH in mice. *J Hepatol* 2017;**66**:1037−46.
6. Lancaster GI, Langley KG, Berglund NA, Kammoun HL, Reibe S, Estevez E, et al. Evidence that TLR4 is not a receptor for saturated fatty acids but mediates lipid-induced inflammation by reprogramming macrophage metabolism. *Cell Metabol* 2018;**27**:1096−1110.e5.
7. Heianza Y, Ma W, DiDonato JA, Sun Q, Rimm EB, Hu FB, et al. Long-term changes in gut microbial metabolite trimethylamine *N*-oxide and coronary heart disease risk. *J Am Coll Cardiol* 2020;**75**:763−72.
8. Lee YS, Wollam J, Olefsky JM. An integrated view of immunometabolism. *Cell* 2018;**172**:22−40.
9. Vítek L. Bile acids in the treatment of cardiometabolic diseases. *Ann Hepatol* 2017;**16**:s43−52.
10. Ryan PM, Stanton C, Caplice NM. Bile acids at the cross-roads of gut microbiome-host cardiometabolic interactions. *Diabetol Metab Syndrome* 2017;**9**:102.
11. Đanić M, Stanimirov B, Pavlović N, Goločorbin-Kon S, Al-Salami H, Stankov K, et al. Pharmacological applications of bile acids and their derivatives in the treatment of metabolic syndrome. *Front Pharmacol* 2018;**9**:1382.
12. Chiang JYL, Ferrell JM, Wu Y, Boehme S. Bile acid and cholesterol metabolism in atherosclerotic cardiovascular disease and therapy. *Cardiol* 2020;**5**:159−70.
13. Little M, Dutta M, Li H, Matson A, Shi X, Mascarinas G, et al. Understanding the physiological functions of the host xenobiotic-sensing nuclear receptors PXR and CAR on the gut microbiome using genetically modified mice. *Acta Pharm Sin B* 2022;**12**:801−20.
14. Rizzolo D, Kong B, Taylor RE, Brinker A, Goedken M, Buckley B, et al. Bile acid homeostasis in female mice deficient in *Cyp7a1* and *Cyp27a1*. *Acta Pharm Sin B* 2021;**11**:3847−56.
15. Kim I, Ahn SH, Inagaki T, Choi M, Ito S, Guo GL, et al. Differential regulation of bile acid homeostasis by the farnesoid X receptor in liver and intestine. *J Lipid Res* 2007;**48**:2664−72.
16. 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.
17. Arab JP, Karpen SJ, Dawson PA, Arrese M, Trauner M. Bile acids and nonalcoholic fatty liver disease: molecular insights and therapeutic perspectives. *Hepatology* 2017;**65**:350−62.
18. de Aguiar Vallim TQ, Tarling EJ, Edwards PA. Pleiotropic roles of bile acids in metabolism. *Cell Metabol* 2013;**17**:657−69.
19. Rajani C, Jia W. Bile acids and their effects on diabetes. *Front Med* 2018;**12**:608−23.
20. Zhang Y, Lee FY, Barrera G, Lee H, Vales C, Gonzalez FJ, et al. Activation of the nuclear receptor FXR improves hyperglycemia and hyperlipidemia in diabetic mice. *Proc Natl Acad Sci U S A* 2006;**103**:1006−11.
21. de Boer JF, Schonewille M, Boesjes M, Wolters H, Bloks VW, Bos T, et al. Intestinal farnesoid X receptor controls transintestinal cholesterol excretion in mice. *Gastroenterology* 2017;**152**:1126−1138.e6.
22. Xu Y, Li F, Zalzala M, Xu J, Gonzalez FJ, Adorini L, et al. Farnesoid X receptor activation increases reverse cholesterol transport by modulating bile acid composition and cholesterol absorption in mice. *Hepatology* 2016;**64**:1072−85.
23. Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, et al. Bile acids lower triglyceride levels *via* a pathway involving FXR, SHP, and SREBP-1c. *J Clin Invest* 2004;**113**:1408−18.
24. Pineda Torra I, Claudel T, Duval C, Kosykh V, Fruchart JC, Staels B. Bile acids induce the expression of the human peroxisome proliferator-activated receptor alpha gene *via* activation of the farnesoid X receptor. *Mol Endocrinol* 2003;**17**:259−72.
25. Ghosh Laskar M, Eriksson M, Rudling M, Angelin B. Treatment with the natural FXR agonist chenodeoxycholic acid reduces clearance of plasma LDL whilst decreasing circulating PCSK9, lipoprotein(a) and apolipoprotein C-III. *J Intern Med* 2017;**281**:575−85.
26. Claudel T, Inoue Y, Barbier O, Duran-Sandoval D, Kosykh V, Fruchart J, et al. Farnesoid X receptor agonists suppress hepatic apolipoprotein CIII expression. *Gastroenterology* 2003;**125**:544−55.
27. Wu K, Zhao T, Hogstrand C, Xu YC, Ling SC, Chen GH, et al. FXR-mediated inhibition of autophagy contributes to FA-induced TG accumulation and accordingly reduces FA-induced lipotoxicity. *Cell Commun Signal* 2020;**18**:47.
28. Kim YC, Byun S, Seok S, Guo G, Xu HE, Kemper B, et al. Small heterodimer partner and fibroblast growth factor 19 inhibit expression of NPC1L1 in mouse intestine and cholesterol absorption. *Gastroenterology* 2019;**156**:1052−65.
29. van Zutphen T, Stroeve JHM, Yang J, Bloks VW, Jurdzinski A, Roelofsen H, et al. FXR overexpression alters adipose tissue architecture in mice and limits its storage capacity leading to metabolic derangements. *J Lipid Res* 2019;**60**:1547−61.
30. 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.
31. Parséus A, Sommer N, Sommer F, Caesar R, Molinaro A, Ståhlman M, et al. Microbiota-induced obesity requires farnesoid X receptor. *Gut* 2017;**66**:429−37.
32. Jiang C, Xie C, Li F, Zhang L, Nichols RG, Krausz KW, et al. Intestinal farnesoid X receptor signaling promotes nonalcoholic fatty liver disease. *J Clin Invest* 2015;**125**:386−402.
33. Velazquez-Villegas LA, Perino A, Lemos V, Zietak M, Nomura M, Pols TWH, et al. TGR5 signalling promotes mitochondrial fission and beige remodelling of white adipose tissue. *Nat Commun* 2018;**9**:245.
34. Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 2006;**439**:484−9.

35. Sasaki T, Kuboyama A, Mita M, Murata S, Shimizu M, Inoue J, et al. The exercise-inducible bile acid receptor Tgr5 improves skeletal muscle function in mice. *J Biol Chem* 2018;**293**:10322−32.
36. Broeders EP, Nascimento EB, Havekes B, Brans B, Roumans KH, Tailleux A, et al. The bile acid chenodeoxycholic acid increases human brown adipose tissue activity. *Cell Metabol* 2015;**22**:418−26.
37. Seok S, Sun H, Kim YC, Kemper B, Kemper JK. Defective FXR−SHP regulation in obesity aberrantly increases miR-802 expression, promoting insulin resistance and fatty liver. *Diabetes* 2021;**70**:733−44.
38. Ploton M, Mazuy C, Gheeraert C, Dubois V, Berthier A, Dubois-Chevalier J, et al. The nuclear bile acid receptor FXR is a PKA- and FOXA2-sensitive activator of fasting hepatic gluconeogenesis. *J Hepatol* 2018;**69**:1099−109.
39. Pathak P, Xie C, Nichols RG, Ferrell JM, Boehme S, Krausz KW, et al. Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. *Hepatology* 2018;**68**:1574−88.
40. Sasaki T, Watanabe Y, Kuboyama A, Oikawa A, Shimizu M, Yamauchi Y, et al. Muscle-specific TGR5 overexpression improves glucose clearance in glucose-intolerant mice. *J Biol Chem* 2020;**296**:100131.
41. Maczewsky J, Kaiser J, Gresch A, Gerst F, Düfer M, Krippeit-Drews P, et al. TGR5 activation promotes stimulus-secretion coupling of pancreatic β-cells *via* a PKA-dependent pathway. *Diabetes* 2019;**68**:324−36.
42. Kumar DP, Asgharpour A, Mirshahi F, Park SH, Liu S, Imai Y, et al. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet α cells to promote glucose homeostasis. *J Biol Chem* 2016;**291**:6626−40.
43. Foley MH, O'Flaherty S, Allen G, Rivera AJ, Stewart AK, Barrangou R, et al. Lactobacillus bile salt hydrolase substrate specificity governs bacterial fitness and host colonization. *Proc Natl Acad Sci U S A* 2021:118.
44. Song Z, Cai Y, Lao X, Wang X, Lin X, Cui Y, et al. Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. *Microbiome* 2019;**7**:9.
45. Kriaa A, Bourgin M, Potiron A, Mkaouar H, Jablaoui A, Gérard P, et al. Microbial impact on cholesterol and bile acid metabolism: current status and future prospects. *J Lipid Res* 2019;**60**:323−32.
46. Funabashi M, Grove TL, Wang M, Varma Y, McFadden ME, Brown LC, et al. A metabolic pathway for bile acid dehydroxylation by the gut microbiome. *Nature* 2020;**582**:566−70.
47. Molinaro A, Wahlström A, Marschall HU. Role of bile acids in metabolic control. *Trends Endocrinol Metabol* 2018;**29**:31−41.
48. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metabol* 2016;**24**:41−50.
49. Lorenzo-Zúñiga V, Bartolı x, Ramón Planas R, Hofmann AF, et al. Oral bile acids reduce bacterial overgrowth, bacterial translocation, and endotoxemia in cirrhotic rats. *Hepatology* 2003;**37**:551−7.
50. Tian Y, Gui W, Koo I, Smith PB, Allman EL, Nichols RG, et al. The microbiome modulating activity of bile acids. *Gut Microb* 2020;**11**:979−96.
51. Watanabe M, Fukiya S, Yokota A. Comprehensive evaluation of the bactericidal activities of free bile acids in the large intestine of humans and rodents. *J Lipid Res* 2017;**58**:1143−52.
52. Jukes CA, Ijaz UZ, Buckley A, Spencer J, Irvine J, Candlish D, et al. Bile salt metabolism is not the only factor contributing to *Clostridioides (Clostridium) difficile* disease severity in the murine model of disease. *Gut Microb* 2020;**11**:481−96.
53. Stacy A, Andrade-Oliveira V, McCulloch JA, Hild B, Oh JH, Perez-Chaparro PJ, et al. Infection trains the host for microbiota-enhanced resistance to pathogens. *Cell* 2021;**184**:615−627.e17.
54. Chen ML, Takeda K, Sundrud MS. Emerging roles of bile acids in mucosal immunity and inflammation. *Mucosal Immunol* 2019;**12**:851−61.
55. Just S, Mondot S, Ecker J, Wegner K, Rath E, Gau L, et al. The gut microbiota drives the impact of bile acids and fat source in diet on mouse metabolism. *Microbiome* 2018;**6**:134.
56. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;**19**:576−85.
57. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;**472**:57−63.
58. Bennett Brian J, Vallim Thomas Qde A, Wang Z, Shih Diana M, Meng Y, Gregory J, et al. Trimethylamine-*N*-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. *Cell Metabol* 2013;**17**:49−60.
59. Pathak P, Helsley RN, Brown AL, Buffa JA, Choucair I, Nemet I, et al. Small molecule inhibition of gut microbial choline trimethylamine lyase activity alters host cholesterol and bile acid metabolism. *Am J Physiol Heart Circ Physiol* 2020;**318**:H1474−86.
60. Tan X, Liu Y, Long J, Chen S, Liao G, Wu S, et al. Trimethylamine *N*-oxide aggravates liver steatosis through modulation of bile acid metabolism and inhibition of farnesoid X receptor signaling in nonalcoholic fatty liver disease. *Mol Nutr Food Res* 2019;**63**:e1900257.
61. León-Mimila P, Villamil-Ramírez H, Li XS, Shih DM, Hui ST, Ocampo-Medina E, et al. Trimethylamine *N*-oxide levels are associated with NASH in obese subjects with type 2 diabetes. *Diabetes Metabol* 2021;**47**:101183.
62. Ding L, Chang M, Guo Y, Zhang L, Xue C, Yanagita T, et al. Trimethylamine-*N*-oxide (TMAO)-induced atherosclerosis is associated with bile acid metabolism. *Lipids Health Dis* 2018;**17**:286.
63. Chen D, Jin D, Huang S, Wu J, Xu M, Liu T, et al. *Clostridium butyricum*, a butyrate-producing probiotic, inhibits intestinal tumor development through modulating Wnt signaling and gut microbiota. *Cancer Lett* 2020;**469**:456−67.
64. Sheng L, Jena PK, Hu Y, Liu HX, Nagar N, Kalanetra KM, et al. Hepatic inflammation caused by dysregulated bile acid synthesis is reversible by butyrate supplementation. *J Pathol* 2017;**243**:431−41.
65. Meslier V, Laiola M, Roager HM, De Filippis F, Roume H, Quinquis B, et al. Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake. *Gut* 2020;**69**:1258−68.
66. Green M, Arora K, Prakash S. Microbial medicine: prebiotic and probiotic functional foods to target obesity and metabolic syndrome. *Int J Mol Sci* 2020:21.
67. Ducastel S, Touche V, Trabelsi MS, Boulinguiez A, Butruille L, Nawrot M, et al. The nuclear receptor FXR inhibits glucagon-like peptide-1 secretion in response to microbiota-derived short-chain fatty acids. *Sci Rep* 2020;**10**:174.
68. 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**:1679−1694.e3.
69. Sun L, Cai J, Gonzalez FJ. The role of farnesoid X receptor in metabolic diseases, and gastrointestinal and liver cancer. *Nat Rev Gastroenterol Hepatol* 2021;**18**:335−47.
70. Fiorucci S, Distrutti E. Bile acid-activated receptors, intestinal microbiota, and the treatment of metabolic disorders. *Trends Mol Med* 2015;**21**:702−14.
71. Qi L, Tian Y, Chen Y. Circulating bile acid profiles: a need for further examination. *J Clin Endocrinol Metabol* 2021;**106**:3093−112.
72. 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.
73. Jiao N, Baker SS, Chapa-Rodriguez A, Liu W, Nugent CA, Tsompana M, et al. Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut* 2018;**67**:1881−91.
74. Bechmann LP, Kocabayoglu P, Sowa JP, Sydor S, Best J, Schlattjan M, et al. Free fatty acids repress small heterodimer partner

(SHP) activation and adiponectin counteracts bile acid-induced liver injury in superobese patients with nonalcoholic steatohepatitis. *Hepatology* 2013;**57**:1394−406.
75. Okushin K, Tsutsumi T, Enooku K, Fujinaga H, Kado A, Shibahara J, et al. The intrahepatic expression levels of bile acid transporters are inversely correlated with the histological progression of nonalcoholic fatty liver disease. *J Gastroenterol* 2016;**51**:808−18.
76. Duparc T, Plovier H, Marrachelli VG, Van Hul M, Essaghir A, Ståhlman M, et al. Hepatocyte MyD88 affects bile acids, gut microbiota and metabolome contributing to regulate glucose and lipid metabolism. *Gut* 2017;**66**:620−32.
77. Wang Y, Gunewardena S, Li F, Matye DJ, Chen C, Chao X, et al. An FGF15/19-TFEB regulatory loop controls hepatic cholesterol and bile acid homeostasis. *Nat Commun* 2020;**11**:3612.
78. Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, et al. Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat Genet* 2001;**27**:375−82.
79. Yan S, Tang J, Zhang Y, Wang Y, Zuo S, Shen Y, et al. Prostaglandin E_2 promotes hepatic bile acid synthesis by an E prostanoid receptor 3-mediated hepatocyte nuclear receptor 4α/cholesterol 7α-hydroxylase pathway in mice. *Hepatology* 2017;**65**:999−1014.
80. Song KH, Chiang JY. Glucagon and cAMP inhibit cholesterol 7alpha-hydroxylase (*CYP7A1*) gene expression in human hepatocytes: discordant regulation of bile acid synthesis and gluconeogenesis. *Hepatology* 2006;**43**:117−25.
81. Douna H, Amersfoort J, Schaftenaar FH, Kröner MJ, Kiss MG, Slütter B, et al. B- and T-lymphocyte attenuator stimulation protects against atherosclerosis by regulating follicular B cells. *Cardiovasc Res* 2020;**116**:295−305.
82. Lü SL, Dang GH, Deng JC, Liu HY, Liu B, Yang J, et al. Shikonin attenuates hyperhomocysteinemia-induced $CD4^+$ T cell inflammatory activation and atherosclerosis in $ApoE^{-/-}$ mice by metabolic suppression. *Acta Pharmacol Sin* 2020;**41**:47−55.
83. Tabas I, Lichtman AH. Monocyte-macrophages and T cells in atherosclerosis. *Immunity* 2017;**47**:621−34.
84. Sombetzki M, Fuchs CD, Fickert P, Österreicher CH, Mueller M, Claudel T, et al. 24-Nor-ursodeoxycholic acid ameliorates inflammatory response and liver fibrosis in a murine model of hepatic schistosomiasis. *J Hepatol* 2015;**62**:871−8.
85. Hillaire S, Boucher E, Calmus Y, Gane P, Ballet F, Franco D, et al. Effects of bile acids and cholestasis on major histocompatibility complex class I in human and rat hepatocytes. *Gastroenterology* 1994;**107**:781−8.
86. Song X, Sun X, Oh SF, Wu M, Zhang Y, Zheng W, et al. Microbial bile acid metabolites modulate gut $ROR\gamma^+$ regulatory T cell homeostasis. *Nature* 2020;**577**:410−5.
87. Hang S, Paik D, Yao L, Kim E, Trinath J, Lu J, et al. Bile acid metabolites control T_H17 and T_{reg} cell differentiation. *Nature* 2019;**576**:143−8.
88. Campbell C, McKenney PT, Konstantinovsky D, Isaeva OI, Schizas M, Verter J, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature* 2020;**581**:475−9.
89. Xia N, Lu Y, Gu M, Li N, Liu M, Jiao J, et al. A unique population of regulatory T cells in heart potentiates cardiac protection from myocardial infarction. *Circulation* 2020;**142**:1956−73.
90. Wang L, Gong Z, Zhang X, Zhu F, Liu Y, Jin C, et al. Gut microbial bile acid metabolite skews macrophage polarization and contributes to high-fat diet-induced colonic inflammation. *Gut Microb* 2020;**12**:1−20.
91. Chen YS, Liu HM, Lee TY. Ursodeoxycholic acid regulates hepatic energy homeostasis and white adipose tissue macrophages polarization in leptin-deficiency obese mice. *Cells* 2019;**8**.
92. Ichikawa R, Takayama T, Yoneno K, Kamada N, Kitazume MT, Higuchi H, et al. Bile acids induce monocyte differentiation toward interleukin-12 hypo-producing dendritic cells *via* a TGR5-dependent pathway. *Immunology* 2012;**136**:153−62.
93. Stienstra R, Joosten LA, Koenen T, van Tits B, van Diepen JA, van den Berg SA, et al. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. *Cell Metabol* 2010;**12**:593−605.
94. Hao H, Cao L, Jiang C, Che Y, Zhang S, Takahashi S, et al. Farnesoid X receptor regulation of the NLRP3 inflammasome underlies cholestasis-associated sepsis. *Cell Metabol* 2017;**25**:856−867.e5.
95. Xu W, Che Y, Zhang Q, Huang H, Ding C, Wang Y, et al. Apaf-1 pyroptosome senses mitochondrial permeability transition. *Cell Metabol* 2021;**33**:424−436.e10.
96. Zhao S, Gong Z, Zhou J, Tian C, Gao Y, Xu C, et al. Deoxycholic acid triggers NLRP3 inflammasome activation and aggravates DSS-induced colitis in mice. *Front Immunol* 2016;**7**:536.
97. Zhao S, Gong Z, Du X, Tian C, Wang L, Zhou J, et al. Deoxycholic acid-mediated sphingosine-1-phosphate receptor 2 signaling exacerbates DSS-induced colitis through promoting cathepsin B release. *J Immunol Res* 2018;**2018**:2481418.
98. Guo C, Xie S, Chi Z, Zhang J, Liu Y, Zhang L, et al. Bile acids control inflammation and metabolic disorder through inhibition of NLRP3 inflammasome. *Immunity* 2016;**45**:802−16.
99. 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.
100. Pols TW, Nomura M, Harach T, Lo Sasso G, Oosterveer MH, Thomas C, et al. TGR5 activation inhibits atherosclerosis by reducing macrophage inflammation and lipid loading. *Cell Metabol* 2011;**14**:747−57.
101. Mao Y, Luo W, Zhang L, Wu W, Yuan L, Xu H, et al. STING−IRF3 triggers endothelial inflammation in response to free fatty acid-induced mitochondrial damage in diet-induced obesity. *Arterioscler Thromb Vasc Biol* 2017;**37**:920−9.
102. Meng H, Matthan NR, Wu D, Li L, Rodríguez-Morató J, Cohen R, et al. Comparison of diets enriched in stearic, oleic, and palmitic acids on inflammation, immune response, cardiometabolic risk factors, and fecal bile acid concentrations in mildly hypercholesterolemic postmenopausal women-randomized crossover trial. *Am J Clin Nutr* 2019;**110**:305−15.
103. Wang Y, Qian Y, Fang Q, Zhong P, Li W, Wang L, et al. Saturated palmitic acid induces myocardial inflammatory injuries through direct binding to TLR4 accessory protein MD2. *Nat Commun* 2017;**8**:13997.
104. Choi RH, Tatum SM, Symons JD, Summers SA, Holland WL. Ceramides and other sphingolipids as drivers of cardiovascular disease. *Nat Rev Cardiol* 2021;**18**:701−11.
105. Ketelhuth DF, Hansson GK. Adaptive response of T and B cells in atherosclerosis. *Circ Res* 2016;**118**:668−78.
106. Duewell P, Kono H, Rayner KJ, Sirois CM, Vladimer G, Bauernfeind FG, et al. NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature* 2010;**464**:1357−61.
107. Janoudi A, Shamoun FE, Kalavakunta JK, Abela GS. Cholesterol crystal induced arterial inflammation and destabilization of atherosclerotic plaque. *Eur Heart J* 2016;**37**:1959−67.
108. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, et al. Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell* 2018;**172**:162−175.e14.
109. Miura K, Yang L, van Rooijen N, Brenner DA, Ohnishi H, Seki E. Toll-like receptor 2 and palmitic acid cooperatively contribute to the development of nonalcoholic steatohepatitis through inflammasome activation in mice. *Hepatology* 2013;**57**:577−89.
110. Perino A, Pols TW, Nomura M, Stein S, Pellicciari R, Schoonjans K. TGR5 reduces macrophage migration through mTOR-induced C/EBPβ differential translation. *J Clin Invest* 2014;**124**:5424−36.
111. Wang YD, Chen WD, Yu D, Forman BM, Huang W. The G-protein-coupled bile acid receptor, Gpbar1 (TGR5), negatively regulates hepatic inflammatory response through antagonizing nuclear factor κ light-chain enhancer of activated B cells (NF-κB) in mice. *Hepatology* 2011;**54**:1421−32.
112. Zhou H, Zhou S, Shi Y, Wang Q, Wei S, Wang P, et al. TGR5/cathepsin E signaling regulates macrophage innate immune activation

in liver ischemia and reperfusion injury. *Am J Transplant* 2020;**21**:1453−64.
113. Biagioli M, Carino A, Cipriani S, Francisci D, Marchianò S, Scarpelli P, et al. The bile acid receptor GPBAR1 regulates the M1/M2 phenotype of intestinal macrophages and activation of GPBAR1 rescues mice from murine colitis. *J Immunol* 2017;**199**:718−33.
114. 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.
115. Kim DH, Xiao Z, Kwon S, Sun X, Ryerson D, Tkac D, et al. A dysregulated acetyl/SUMO switch of FXR promotes hepatic inflammation in obesity. *EMBO J* 2015;**34**:184−99.
116. Dong B, Zhou Y, Wang W, Scott J, Kim K, Sun Z, et al. Vitamin D receptor activation in liver macrophages ameliorates hepatic inflammation, steatosis, and insulin resistance in mice. *Hepatology* 2020;**71**:1559−74.
117. Karthaus N, van Spriel AB, Looman MWG, Chen S, Spilgies LM, Lieben L, et al. Vitamin D controls murine and human plasmacytoid dendritic cell function. *J Invest Dermatol* 2014;**134**:1255−64.
118. Fernandez Lahore G, Raposo B, Lagerquist M, Ohlsson C, Sabatier P, Xu B, et al. Vitamin D3 receptor polymorphisms regulate T cells and T cell-dependent inflammatory diseases. *Proc Natl Acad Sci U S A* 2020;**117**:24986−97.
119. Pols TWH, Puchner T, Korkmaz HI, Vos M, Soeters MR, de Vries CJM. Lithocholic acid controls adaptive immune responses by inhibition of Th1 activation through the vitamin D receptor. *PLoS One* 2017;**12**:e0176715.
120. Yao B, He J, Yin X, Shi Y, Wan J, Tian Z. The protective effect of lithocholic acid on the intestinal epithelial barrier is mediated by the vitamin D receptor *via* a SIRT1/Nrf2 and NF-κB dependent mechanism in Caco-2 cells. *Toxicol Lett* 2019;**316**:109−18.
121. Sun L, Pang Y, Wang X, Wu Q, Liu H, Liu B, et al. Ablation of gut microbiota alleviates obesity-induced hepatic steatosis and glucose intolerance by modulating bile acid metabolism in hamsters. *Acta Pharm Sin B* 2019;**9**:702−10.
122. Chung J, An SH, Kang SW, Kwon K. Ursodeoxycholic acid (UDCA) exerts anti-atherogenic effects by inhibiting RAGE signaling in diabetic atherosclerosis. *PLoS One* 2016;**11**:e0147839.
123. Mooranian A, Raj Wagle S, Kovacevic B, Takechi R, Mamo J, Lam V, et al. Bile acid bio-nanoencapsulation improved drug targeted-delivery and pharmacological effects *via* cellular flux: 6-months diabetes preclinical study. *Sci Rep* 2020;**10**:106.
124. Li M, Wang Q, Li Y, Cao S, Zhang Y, Wang Z, et al. Apical sodium-dependent bile acid transporter, drug target for bile acid related diseases and delivery target for prodrugs: current and future challenges. *Pharmacol Ther* 2020;**212**:107539.
125. Kuipers F, Bloks VW, Groen AK. Beyond intestinal soap—bile acids in metabolic control. *Nat Rev Endocrinol* 2014;**10**:488−98.
126. Zhou E, Hoeke G, Li Z, Eibergen AC, Schonk AW, Koehorst M, et al. Colesevelam enhances the beneficial effects of brown fat activation on hyperlipidaemia and atherosclerosis development. *Cardiovasc Res* 2020;**116**:1710−20.
127. The lipid research clinics coronary primary prevention trial results. I. Reduction in incidence of coronary heart disease. *JAMA* 1984;**251**:351−64.
128. Watts GF, Lewis B, Brunt JN, Lewis ES, Coltart DJ, Smith LD, et al. Effects on coronary artery disease of lipid-lowering diet, or diet plus cholestyramine, in the St Thomas' Atherosclerosis Regression Study (STARS). *Lancet* 1992;**339**:563−9.
129. Watts GF, Pang J, Chan DC, Brunt JNH, Lewis B. Angiographic progression of coronary atherosclerosis in patients with familial hypercholesterolaemia treated with non-statin therapy: impact of a fat-modified diet and a resin. *Atherosclerosis* 2016;**252**:82−7.
130. Kessoku T, Kobayashi T, Ozaki A, Iwaki M, Honda Y, Ogawa Y, et al. Rationale and design of a randomised, double-blind, placebo-controlled, parallel-group, investigator-initiated phase 2a study to investigate the efficacy and safety of elobixibat in combination with cholestyramine for non-alcoholic fatty liver disease. *BMJ Open* 2020;**10**:e037961.
131. Hansen M, Sonne DP, Mikkelsen KH, Gluud LL, Vilsbøll T, Knop FK. Bile acid sequestrants for glycemic control in patients with type 2 diabetes: a systematic review with meta-analysis of randomized controlled trials. *J Diabet Complicat* 2017;**31**:918−27.
132. Chung J, Kim KH, Lee SC, An SH, Kwon K. Ursodeoxycholic acid (UDCA) exerts anti-atherogenic effects by inhibiting endoplasmic reticulum (ER) stress induced by disturbed flow. *Mol Cell* 2015;**38**:851−8.
133. Bode N, Grebe A, Kerksiek A, Lütjohann D, Werner N, Nickenig G, et al. Ursodeoxycholic acid impairs atherogenesis and promotes plaque regression by cholesterol crystal dissolution in mice. *Biochem Biophys Res Commun* 2016;**478**:356−62.
134. von Haehling S, Schefold JC, Jankowska EA, Springer J, Vazir A, Kalra PR, et al. Ursodeoxycholic acid in patients with chronic heart failure: a double-blind, randomized, placebo-controlled, crossover trial. *J Am Coll Cardiol* 2012;**59**:585−92.
135. Mueller M, Thorell A, Claudel T, Jha P, Koefeler H, Lackner C, et al. Ursodeoxycholic acid exerts farnesoid X receptor-antagonistic effects on bile acid and lipid metabolism in morbid obesity. *J Hepatol* 2015;**62**:1398−404.
136. Wang J, Zhang J, Lin X, Wang Y, Wu X, Yang F, et al. DCA−TGR5 signaling activation alleviates inflammatory response and improves cardiac function in myocardial infarction. *J Mol Cell Cardiol* 2021;**151**:3−14.
137. Shih DM, Shaposhnik Z, Meng Y, Rosales M, Wang X, Wu J, et al. Hyodeoxycholic acid improves HDL function and inhibits atherosclerotic lesion formation in LDLR-knockout mice. *FASEB J* 2013;**27**:3805−17.
138. Sayin SI, Wahlström A, Felin J, Jäntti S, Marschall HU, 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.
139. 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.
140. 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 (Lond)* 2021;**135**:1689−706.
141. Wang Y, Zhu Y, Niu J, Deng Q, Guo S, Jiang H, et al. A novel bile acid analog, A17, ameliorated non-alcoholic steatohepatitis in high-fat diet-fed hamsters. *Toxicol Appl Pharmacol* 2020;**404**:115169.
142. Guo GL, Santamarina-Fojo S, Akiyama TE, Amar MJA, Paigen BJ, Brewer B, et al. Effects of FXR in foam-cell formation and atherosclerosis development. *Biochim Biophys Acta* 2006;**1761**:1401−9.
143. Wu Q, Sun L, Hu X, Wang X, Xu F, Chen B, et al. Suppressing the intestinal farnesoid X receptor/sphingomyelin phosphodiesterase 3 axis decreases atherosclerosis. *J Clin Invest* 2021;**131**:e142865.
144. Deng L, Chen X, Zhong Y, Wen X, Cai Y, Li J, et al. Activation of TGR5 partially alleviates high glucose-induced cardiomyocyte injury by inhibition of inflammatory responses and oxidative stress. *Oxid Med Cell Longev* 2019;**2019**:6372786.
145. Li B, Yang N, Li C, Li C, Gao K, Xie X, et al. INT-777, a bile acid receptor agonist, extenuates pancreatic acinar cells necrosis in a mouse model of acute pancreatitis. *Biochem Biophys Res Commun* 2018;**503**:38−44.
146. McMahan RH, Wang XX, Cheng LL, Krisko T, Smith M, El Kasmi K, et al. Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease. *J Biol Chem* 2013;**288**:11761−70.

147. Hu YB, Liu XY, Zhan W. Farnesoid X receptor agonist INT-767 attenuates liver steatosis and inflammation in rat model of nonalcoholic steatohepatitis. *Drug Des Dev Ther* 2018;**12**:2213−21.
148. Miyazaki-Anzai S, Masuda M, Kohno S, Levi M, Shiozaki Y, Keenan AL, et al. Simultaneous inhibition of FXR and TGR5 exacerbates atherosclerotic formation. *J Lipid Res* 2018;**59**:1709−13.
149. Carino A, Marchianò S, Biagioli M, Bucci M, Vellecco V, Brancaleone V, et al. Agonism for the bile acid receptor GPBAR1 reverses liver and vascular damage in a mouse model of steatohepatitis. *FASEB J* 2019;**33**:2809−22.
150. Carino A, Cipriani S, Marchianò S, Biagioli M, Scarpelli P, Zampella A, et al. Gpbar1 agonism promotes a Pgc-1α-dependent browning of white adipose tissue and energy expenditure and reverses diet-induced steatohepatitis in mice. *Sci Rep* 2017;**7**:13689.
151. Carino A, Marchianò S, Biagioli M, Fiorucci C, Zampella A, Monti MC, et al. Transcriptome analysis of dual FXR and GPBAR1 agonism in aodent model of NASH reveals modulation of lipid droplets formation. *Nutrients* 2019;**11**:1132.
152. Finn PD, Rodriguez D, Kohler J, Jiang Z, Wan S, Blanco E, et al. Intestinal TGR5 agonism improves hepatic steatosis and insulin sensitivity in Western diet-fed mice. *Am J Physiol Gastrointest Liver Physiol* 2019;**316**:G412−24.
153. Högenauer K, Arista L, Schmiedeberg N, Werner G, Jaksche H, Bouhelal R, et al. G-protein-coupled bile acid receptor 1 (GPBAR1, TGR5) agonists reduce the production of proinflammatory cytokines and stabilize the alternative macrophage phenotype. *J Med Chem* 2014;**57**:10343−54.
154. 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.
155. Xiong X, Ren Y, Cui Y, Li R, Wang C, Zhang Y. Obeticholic acid protects mice against lipopolysaccharide-induced liver injury and inflammation. *Biomed Pharmacother* 2017;**96**:1292−8.
156. Younossi ZM, Ratziu V, Loomba R, Rinella M, Anstee QM, Goodman Z, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet* 2019;**394**:2184−96.
157. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *Lancet* 2015;**385**:956−65.
158. Pockros PJ, Fuchs M, Freilich B, Schiff E, Kohli A, Lawitz EJ, et al. CONTROL: a randomized phase 2 study of obeticholic acid and atorvastatin on lipoproteins in nonalcoholic steatohepatitis patients. *Liver Int* 2019;**39**:2082−93.
159. Hameed B, Terrault NA, Gill RM, Loomba R, Chalasani N, Hoofnagle JH, et al. Clinical and metabolic effects associated with weight changes and obeticholic acid in non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2018;**47**:645−56.
160. Loomba R, Neuschwander-Tetri BA, Sanyal A, Chalasani N, Diehl AM, Terrault N, et al. Multicenter validation of association between decline in MRI-PDFF and histologic response in NASH. *Hepatology* 2020;**72**:1219−29.
161. Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, et al. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. *N Engl J Med* 2016;**375**:631−43.
162. Mudaliar S, Henry RR, Sanyal AJ, Morrow L, Marschall HU, Kipnes M, et al. Efficacy and safety of the farnesoid X receptor agonist obeticholic acid in patients with type 2 diabetes and nonalcoholic fatty liver disease. *Gastroenterology* 2013;**145**:574−582.e1.
163. Pencek R, Marmon T, Roth JD, Liberman A, Hooshmand-Rad R, Young MA. Effects of obeticholic acid on lipoprotein metabolism in healthy volunteers. *Diabetes Obes Metabol* 2016;**18**:936−40.
164. Siddiqui MS, Van Natta ML, Connelly MA, Vuppalanchi R, Neuschwander-Tetri BA, Tonascia J, et al. Impact of obeticholic acid on the lipoprotein profile in patients with non-alcoholic steatohepatitis. *J Hepatol* 2020;**72**:25−33.
165. Al-Dury S, Wahlström A, Panzitt K, Thorell A, Ståhlman M, Trauner M, et al. Obeticholic acid may increase the risk of gallstone formation in susceptible patients. *J Hepatol* 2019;**71**:986−91.
166. Ji G, Ma L, Yao H, Ma S, Si X, Wang Y, et al. Precise delivery of obeticholic acid *via* nanoapproach for triggering natural killer T cell-mediated liver cancer immunotherapy. *Acta Pharm Sin B* 2020;**10**:2171−82.
167. Ahmad A, Sanderson K, Dickerson D, Adda N. Pharmacokinetics, pharmacodynamics, and safety of EDP-305, in healthy and presumptive NAFLD subjects. *J Hepatol* 2018;**68**:S584.
168. Pedrosa M, Seyedkazemi S, Francque S, Sanyal A, Rinella M, Charlton M, et al. A randomized, double-blind, multicenter, phase 2b study to evaluate the safety and efficacy of a combination of tropifexor and cenicriviroc in patients with nonalcoholic steatohepatitis and liver fibrosis: study design of the TANDEM trial. *Contemp Clin Trials* 2020;**88**:105889.
169. Tully DC, Rucker PV, Chianelli D, Williams J, Vidal A, Alper PB, et al. Discovery of tropifexor (LJN452), a highly potent non-bile acid FXR agonist for the treatment of cholestatic liver diseases and nonalcoholic steatohepatitis (NASH). *J Med Chem* 2017;**60**:9960−73.
170. Fiorucci S, Biagioli M, Sepe V, Zampella A, Distrutti E. Bile acid modulators for the treatment of nonalcoholic steatohepatitis (NASH). *Expet Opin Invest Drugs* 2020;**29**:623−32.
171. Patel K, Harrison SA, Elkhashab M, Trotter JF, Herring R, Rojter SE, et al. Cilofexor, a nonsteroidal FXR agonist, in patients with noncirrhotic NASH: a phase 2 randomized controlled trial. *Hepatology* 2020;**72**:58−71.
172. Loomba R, Noureddin M, Kowdley KV, Kohli A, Sheikh A, Neff G, et al. Combination therapies including cilofexor and firsocostat for bridging fibrosis and cirrhosis attributable to NASH. *Hepatology* 2021;**73**:625−43.
173. Harrison SA, Bashir MR, Lee K-J, Shim-Lopez J, Lee J, Wagner B, et al. A structurally optimized FXR agonist, MET409, reduced liver fat content over 12 weeks in patients with non-alcoholic steatohepatitis. *J Hepatol* 2021;**75**:25−33.
174. Hambruch E, Miyazaki-Anzai S, Hahn U, Matysik S, Boettcher A, Perović-Ottstadt S, et al. Synthetic farnesoid X receptor agonists induce high-density lipoprotein-mediated transhepatic cholesterol efflux in mice and monkeys and prevent atherosclerosis in cholesteryl ester transfer protein transgenic low-density lipoprotein receptor (−/−) mice. *J Pharmacol Exp Therapeut* 2012;**343**:556−67.
175. Al-Khaifi A, Rudling M, Angelin B. An FXR agonist reduces bile acid synthesis independently of increases in FGF19 in healthy volunteers. *Gastroenterology* 2018;**155**:1012−6.
176. 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.
177. Vosper H, Khoudoli GA, Graham TL, Palmer CN. Peroxisome proliferator-activated receptor agonists, hyperlipidaemia, and atherosclerosis. *Pharmacol Ther* 2002;**95**:47−62.
178. Kaul U, Parmar D, Manjunath K, Shah M, Parmar K, Patil KP, et al. New dual peroxisome proliferator activated receptor agonist-Saroglitazar in diabetic dyslipidemia and non-alcoholic fatty liver disease: integrated analysis of the real world evidence. *Cardiovasc Diabetol* 2019;**18**:80.
179. Mazzetti M, Marconi G, Mancinelli M, Benedetti A, Marzioni M, Maroni L. The management of cholestatic liver diseases: current

therapies and emerging new possibilities. *J Clin Med* 2021;**10**: 1763.

180. Li T, Owsley E, Matozel M, Hsu P, Novak CM, Chiang JY. Transgenic expression of cholesterol 7alpha-hydroxylase in the liver prevents high-fat diet-induced obesity and insulin resistance in mice. *Hepatology* 2010;**52**:678−90.
181. Ge MX, Niu WX, Ren JF, Cai SY, Yu DK, Liu HT, et al. A novel ASBT inhibitor, IMB17-15, repressed nonalcoholic fatty liver disease development in high-fat diet-fed Syrian golden hamsters. *Acta Pharmacol Sin* 2019;**40**:895−907.
182. Kårhus ML, Brønden A, Sonne DP, Vilsbøll T, Knop FK. Evidence connecting old, new and neglected glucose-lowering drugs to bile acid-induced GLP-1 secretion: a review. *Diabetes Obes Metabol* 2017;**19**:1214−22.
183. Charlton M. FGF-19 agonism for NASH: a short study of a long disease. *Lancet* 2018;**391**:1124−6.