

## Diversification of host bile acids by members of the gut microbiota

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### ABSTRACT

Bile acid biotransformation is a collaborative effort by the host and the gut microbiome. Host hepatocytes synthesize primary bile acids from cholesterol. Once these host-derived primary bile acids enter the gastrointestinal tract, the gut microbiota chemically modify them into secondary bile acids. Interest into the gut-bile acid-host axis is expanding in diverse fields including gastroenterology, endocrinology, oncology, and infectious disease. This review aims to 1) describe the physiologic aspects of collaborative bile acid metabolism by the host and gut microbiota; 2) to evaluate how gut microbes influence bile acid pools, and in turn how bile acid pools modulate the gut microbial community structure; 3) to compare species differences in bile acid pools; and lastly, 4) discuss the effects of ursodeoxycholic acid (UDCA) administration, a common therapeutic bile acid, on the gut microbiota-bile acid-host axis.

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*C. difficile*; bile acids; gut microbiota; ursodeoxycholic acid (UDCA); FXR

### Introduction

Roughly a liter of bile is produced by human hepatocytes daily.<sup>1</sup> Bile acids constitute about 50% of the organic component of bile.<sup>1</sup> The primary bile acids produced in humans are cholic acid (CA) and chenodeoxycholic acid (CDCA). Once primary bile acids enter the gastrointestinal tract, over 50 chemically distinct secondary bile acids are produced.<sup>2</sup> The chemical diversification of bile acids is a collaborative effort by the host (production of primary bile acids) and the gut microbiota (production of secondary bile acids). Some postulate that the gut microbiota act as an “endocrine organ” by altering host physiology via the production of metabolites, such as microbially derived secondary bile acids.<sup>3</sup> Recently, interest in the gut microbiota-bile acid-host axis is expanding in diverse fields including gastroenterology, endocrinology, oncology, and infectious disease.<sup>1,3–12</sup>

### Host bile acid metabolism

Bile acids are water-soluble, cholesterol derived amphipathic molecules of saturated hydroxylated C-24 sterols that are synthesized by hepatocytes.<sup>13</sup> The liver is the only organ that contains all 14 enzymes that are required for *de novo* synthesis of

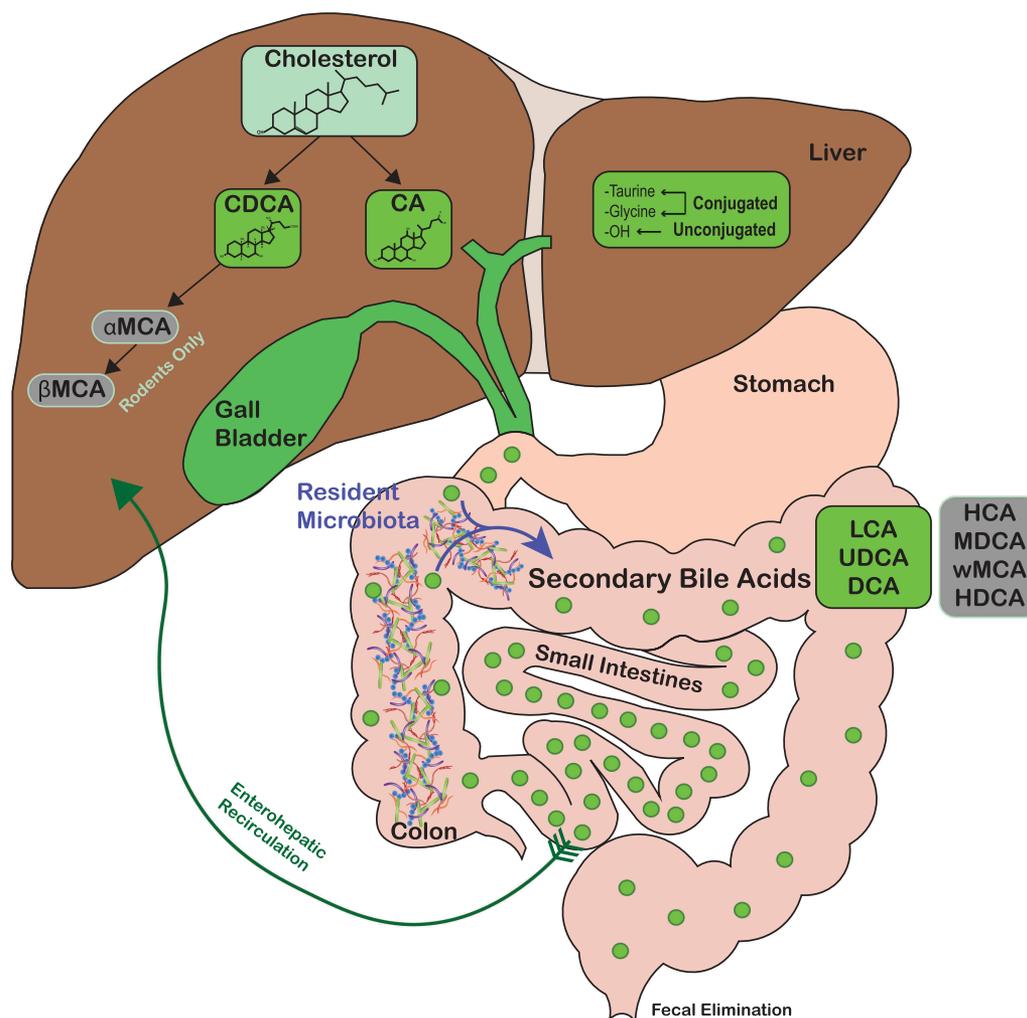
bile acids.<sup>14,15</sup> Cholic acid and CDCA are the main primary bile acids synthesized in humans and rodents (Figure 1).<sup>13,16,17</sup> In rodents, a significant quantity of CDCA is converted by 6 $\beta$ -hydroxylation to muricholic acids, which are not detected in humans (Figure 1).<sup>3,18</sup> These primary bile acids are further metabolized via *N-acyl* amidation to glycine or taurine producing conjugated bile acids, such as taurocholic acid (TCA).<sup>13</sup> Conjugation is important for solubility of bile acids; at physiologic pH, unconjugated bile acids are only sparingly soluble.<sup>1,19</sup> The ratio of glycine to taurine conjugated bile acids varies across mammals.<sup>16,20,21</sup> Conjugated bile acids are actively secreted across the canalicular membranes into the bile ducts, which converge and empty into the gallbladder (except in species that lack a gallbladder such as horses, rats, and rabbits). Bile acids are just one component in bile, which also includes phospholipids, biliverdin/bilirubin, immunoglobulin A, mucus, various endogenous products (such as lipovitamins, corticosteroids, progesterone, testosterone), trace metals, and xenobiotics.<sup>1</sup> Within the gallbladder, bile is concentrated 5–10 times, via removal of water and electrolytes, and acidified via Na<sup>+</sup>/H<sup>+</sup> exchangers.<sup>1,22</sup> Only about 50% of bile

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**Figure 1.** Overview of bile acid metabolism and enterohepatic recirculation.

Host-derived primary bile acids are synthesized by hepatocytes (CA and CDCA in humans, and CA, CDCA, αMCA, and βMCA in rodents) and conjugated with either taurine or glycine. Primary bile acids are then secreted into the bile and stored in the gallbladder until secreted in the duodenum. Resident gut microbiota biotransform primary bile acids into secondary bile acids such as LCA, UDCA, and DCA (green shaded circles) and HCA, MDCA, ωMCA, and HDCA in rodents (gray-shaded circles). Abbreviations: CA, cholate; CDCA, chenodeoxycholate; DCA, deoxycholate; HCA, hyocholate; HDCA, hyodeoxycholate; LCA, lithocholate; MDCA, murideoxycholate; UDCA, ursodeoxycholate; αMCA, α-muricholate; βMCA, β-muricholate; ωMCA, ω-muricholate.

enters into the gallbladder, the remaining enters directly into the gastrointestinal tract.<sup>1</sup> During a meal, cholecystokinin from the duodenum causes contraction of the gallbladder and concentrated bile is released into the proximal gastrointestinal tract.

High concentrations of conjugated primary bile acids are noted within the duodenum, jejunum, and proximal ileum.<sup>13</sup> The primary role of bile acids in the small intestine is to aid in fat emulsification and absorption. Bile acids undergo enterohepatic recirculation, a process which involves: (1). Passive absorption of conjugated and unconjugated bile acids in the

small intestine and colon; (2) High-affinity active transport in the distal ileum.<sup>1,17,23</sup> Absorbed bile acids enter into the portal bloodstream and are rapidly taken up by hepatocytes and resecreted into bile (Figure 1). A small fraction of bile acids escape enterohepatic recirculation and spill into systemic circulation, which allows bile signaling to occur in other organs and tissues.<sup>24,25</sup> Enterohepatic recirculation is extremely efficient, with 95% of bile acids reabsorbed and only 5% lost into the feces.<sup>1</sup> Hepatocytes maintain the bile acid pool by synthesizing bile acids to make up for fecal loss. In healthy humans, the total bile acid

pool cycles about 10 times each day, which requires enterocytes and hepatocytes to transport about 20 g of bile acids every hour.<sup>5,26</sup>

Bile acids regulate their own synthesis and transport via the nuclear farnesoid X receptor (FXR; NR1H4), thus acting as hormones.<sup>27–30</sup> Binding of bile acids to ileal FXR induces expression of fibroblast growth factor (FGF15/19).<sup>31</sup> FGF15/19 travels via the portal bloodstream and binds to cell surface receptors on hepatocytes to repress bile acid synthesis (feedback inhibition) by inhibiting the rate-limiting enzyme cholesterol 7 $\alpha$ -hydroxylase (CYP7A1) which allows the host to synthesize primary bile acids from cholesterol.<sup>28</sup> Different bile acids have varying affinities to FXR.<sup>3,28,32</sup> The bile acid-FXR pathway is also important for glucose homeostasis, lipid metabolism, protein synthesis, inflammation, and liver regeneration; however, this is beyond the scope of this review.<sup>6,27,28,33</sup> Bile acids also interact with pregnane X receptors (PXR; NR1I2) and vitamin D receptors (VDR; NR1I1), which are reviewed elsewhere.<sup>4,28,34</sup>

Bile acids are biological detergents and can act as a host physicochemical defense system within the gut directly against both commensal resident microbes and enteric pathogens.<sup>1</sup> Bile acids, via stimulation of FXR, can also induce expression of antimicrobial peptides.<sup>31</sup> Therefore, bile acids are a major survival and colonization challenge to gut microbes. Regardless, gut commensals and some pathogens can elude the detrimental effects of bile acids and in some cases secure a fitness advantage.<sup>1</sup>

### Diversification of host bile acids by gut microbes

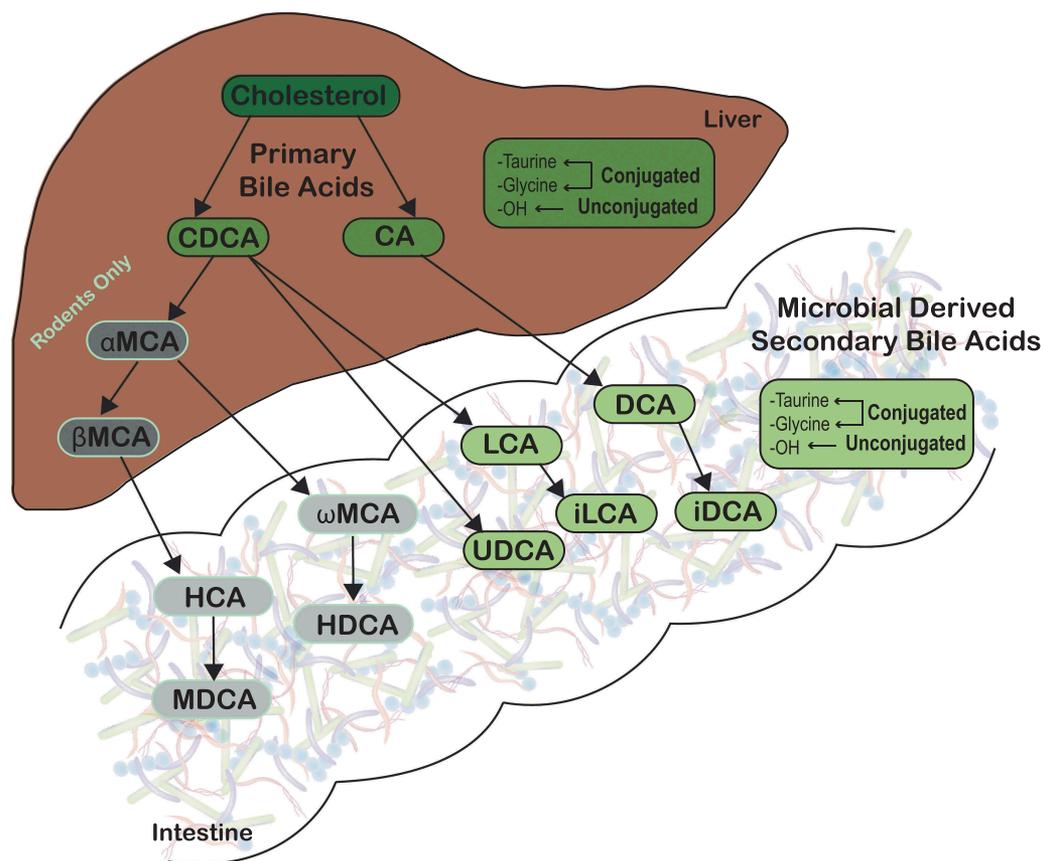
The gastrointestinal microbiome is the most densely populated natural ecosystem and is comprised of over 10<sup>14</sup> bacterial cells.<sup>14,35</sup> Originally it was thought that microbial cells outnumbered human cells in the body by a ratio of 10:1;<sup>14,35</sup> however, recent revised estimates suggest closer to 1.3–2.3:1.<sup>36</sup> There are thousands of different bacterial species in the human gut.<sup>37</sup> In healthy humans and animals, over 90–99% of the microbial community are dominated by two phyla, Firmicutes and Bacteroidetes, with fewer members in Proteobacteria, Actinobacteria, Verrucomicrobia, and Cyanobacteria.<sup>38,39</sup> Diversity within this community is mainly at the genus, species, and strain level.<sup>14</sup> It is estimated that 99% of

functional genes in the human body are of microbial origin.<sup>40</sup> By acting in an endocrine fashion, the gut microbiome can alter host physiology by producing metabolites, such as secondary bile acids.<sup>3,41</sup>

Secondary bile acids are produced by gut microbes via biotransformation of host-derived primary bile acids (Figure 2). When the small fraction (approximately 5%) of unabsorbed bile acids enters the distal ileum, cecum, and colon they undergo chemical diversification via three main microbial pathways: deconjugation, dehydrogenation, and dehydroxylation reactions.<sup>13</sup>

Deconjugation of host-derived primary bile acids occurs rapidly and via bile salt hydrolases (BSH), which are widespread in members of the gut microbiota.<sup>1,13,42</sup> Based on metagenomic screening, three major phyla have BSHs: Firmicutes (30%), Bacteroidetes (14.4%), and Actinobacteria (8.9%).<sup>42</sup> Within these phyla, the following genera are heavily studied: *Clostridium*, *Bacteroides*, *Lactobacillus*, *Bifidobacterium*, and *Enterococcus* (reviewed in Begley et al., 2005).<sup>1</sup> The physiologic function of BSH is debated and the current three hypotheses are: (1) BSHs provide a nutritional advantage by liberating amino acids that can be used for carbon/nitrogen sources and energy generation via taurine as a terminal electron acceptor; (2) BSHs aid in incorporation of cholesterol and bile components into bacterial membranes; (3) BSHs provide a detoxification mechanism to diminish the inherent detergent properties of bile acids.<sup>1,43</sup> Bile salt hydrolases appear to enhance bacterial colonization within the lower gastrointestinal tract, but appears to be strain specific.<sup>13</sup> Some probiotic *Lactobacillus* strains have several BSH genes, thus highlighting the importance of deconjugation of bile acids for gut microbes.<sup>44–46</sup> For the host, deconjugation of bile acids by gut microbes has several consequences. Unconjugated bile acids result in less effective emulsification of fat, less efficient enterohepatic recirculation of bile acids due to reduced distal ileal transporter affinity, and lowering of serum cholesterol levels.<sup>1,47,48</sup> Despite the host impacts of microbial BSH activity, the bacterial physiologic advantage of BSHs remains elusive.<sup>13</sup>

Three distinct microbial hydroxysteroid dehydrogenases (HSDH), 3 $\alpha$ -, 7 $\alpha$ -, and 12 $\alpha$ -, which result in oxidization and epimerization of specific



**Figure 2.** Microbial metabolism of host-derived primary bile acids.

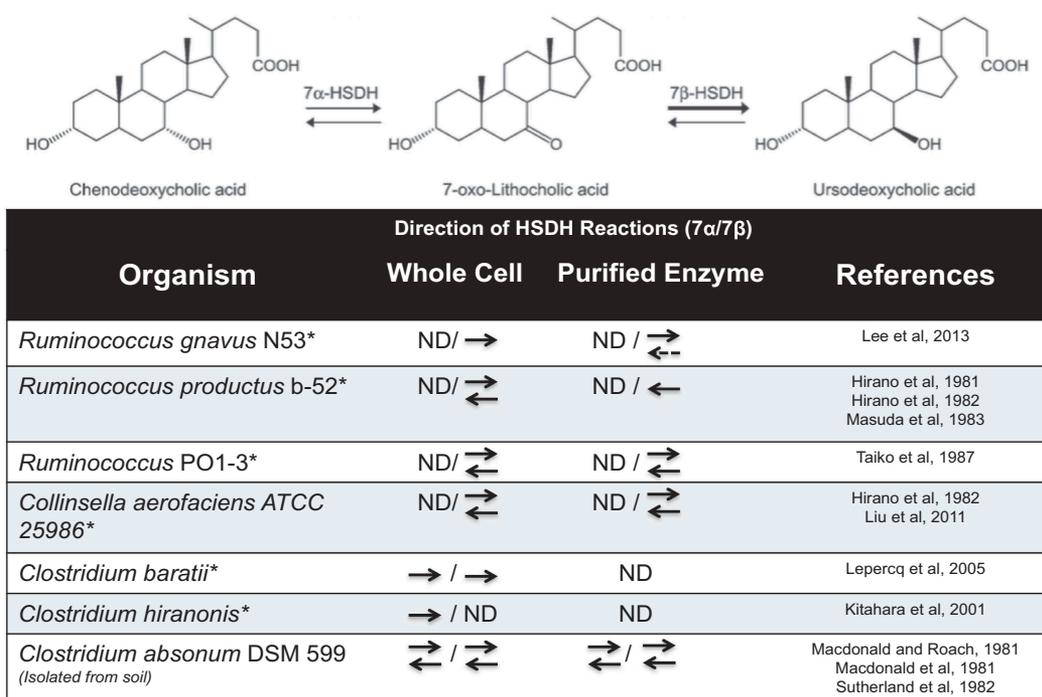
Primary bile acids are synthesized from cholesterol by hepatocytes. Primary bile acids are then biotransformed by resident bacteria in the gastrointestinal tract to secondary bile acids, such as LCA and DCA, which predominate in humans. Abbreviations: CA, cholate; CDCA, chenodeoxycholate; DCA, deoxycholate; HCA, hyocholate; HDCA, hyodeoxycholate; LCA, lithocholate; MDCA, murideoxycholate; UDCA, ursodeoxycholate;  $\alpha$ MCA,  $\alpha$ -muricholate;  $\beta$ MCA,  $\beta$ -muricholate;  $\omega$ MCA,  $\omega$ -muricholate.

hydroxyl groups on bile acids are present in gut microbes.<sup>13</sup> These HSDHs can lead to the formation of over 20 diverse metabolites from the host-derived primary bile acids alone.<sup>13,14</sup> Ridlon et al. speculated that these bile acid metabolites evolved as signaling molecules for microbes to communicate with other gut microbes (via microbe-microbe interactions) and/or alter host physiology (via microbe-host interactions).<sup>3</sup>

In the colon, nearly 100% of bile acids are microbially derived, and unconjugated bile acids undergo dehydrogenation carried out by a broad spectrum of bacteria.<sup>13,49</sup> However, 7 $\alpha$ -dehydroxylation is performed by only a few anaerobic species representing less than 0.025% of total gut microbiome and 0.0001% of total colonic microbiota.<sup>3,13,50</sup> These are largely *Clostridium* spp. (*C. hiranonis*, *C. hylemonae*, *C. sordelli*, and *C. scindens*), which are members of the Firmicutes phyla.<sup>13,14,51–57</sup> Removal of the 7 $\alpha$ -

hydroxyl group from host-derived primary bile acids requires multiple intracellular enzyme steps, which are encoded on the *bai* (bile acid inducible) operon.<sup>4,13,14,56,58,59</sup> These reactions ultimately lead to formation of the secondary bile acids, deoxycholate (DCA) from CA and lithocholate (LCA) from CDCA (Figure 2).<sup>5,49,60</sup> Additionally, DCA and LCA can be modified into other secondary bile acids by microbes, such as isoDCA (iDCA) and isoLCA (iLCA),<sup>49</sup> however it is only partially understood which microbes conduct this conversion (Figures 2 and 3).<sup>5,60,62</sup> Secondary bile acids can also undergo enterohepatic recirculation by passive colonic absorption and thus can be found in the liver and bile.<sup>3,23</sup> In human feces, although secondary bile acids DCA and LCA predominate, there are over 50 different microbially derived secondary bile acids present.<sup>2</sup>

The microbial physiologic role of secondary bile acids remains elusive. It is suggested that microbially



**Figure 3.** Directions of 7α/7β-HSDH reactions form UDCA-producing bacteria.

Epimerization reaction from CDCA to UDCA, which is catalyzed by the 7α-HSDH and 7β-HSDH enzymes. ND: Not determined; \*: Found in the gastrointestinal tract. Dashed line denotes weak reaction. Figure modified from Lee et al. 2013.<sup>61</sup>

derived secondary bile acids are used in energy production as terminal electron acceptors, aid in formation of less membrane damaging bile acid pools, and alter enteric pathogen virulence, such as germination and outgrowth of *Clostridioides difficile*.<sup>1,9,13,63–65</sup> In the host, secondary bile acids such as DCA and LCA can be cytotoxic molecules leading to oxidative stress, membrane damage, and colonic carcinogenesis (reviewed in Barrasa et al.).<sup>66</sup> However, the secondary bile acid UDCA can protect colonic cells against apoptosis and oxidative damage.<sup>66</sup> Additionally, in a colitis model, UDCA and LCA exerted anti-inflammatory properties.<sup>67</sup> Thus, collectively highlighting the diverse and potentially divergent roles of microbially derived secondary bile acids. It is remarkable how little is known about secondary bile acids, including which gut microbes produce them, their microbial biologic function, and their impacts on host health and disease.

### Gut microbial influence on composition of the host bile acid pools

When comparing gnotobiotic and conventional mice, bile acid pool size, composition, and concentrations

are directly influenced by the gut microbiota and their metabolism of host-derived primary bile acids.<sup>68</sup> The bile acid pools of conventional mice are more chemically diverse than gnotobiotic mice.<sup>68</sup> The greatest diversification of bile acids was noted in biogeographic regions with dense and diverse microbial communities such as the cecum, colon, and feces.<sup>13,68</sup>

Disruptions in gut microbial communities result in derangements in bile acid metabolism.<sup>8,65,69</sup> A classic example occurs after administration of antibiotics, which alter the gut microbiota community structure and function.<sup>18,65,68,70</sup> Various antibiotics lead to rapid shifts in bile acid pools with host-derived primary bile acids predominating over microbially derived secondary bile acids in the large intestine and feces.<sup>18,65,70</sup> This is not surprising since antibiotic treatment leads to depletion of 7α-dehydroxylation activity by the fecal microbiota.<sup>71</sup> Subsequent bile acid dysmetabolism is also observed in liver cirrhosis and inflammatory bowel disease (IBD) patients with altered microbial ecosystems.<sup>8,72,73</sup> Gut microbes are not only involved in formation of secondary bile acids but are also important for regulation of bile acid synthesis in hepatocytes.<sup>18</sup> Therefore, it is

not surprising that diseases, which alter the gut microbial ecosystem will also result in bile acid dysmetabolism.

Bile acid pool composition is modulated by interactions of microbially derived secondary bile acids with FXR.<sup>68</sup> Recall, that bile acids differ in their activation of FXR (CDCA > DCA > LCA > CA), and thus, their ability to participate in feedback inhibition of host synthesis of primary bile acids.<sup>33</sup> When comparing wild type and FXR-deficient mice raised conventionally versus rederived as gnotobiotic, the presence of the gut microbiota resulted in bile acid pools which were more potent activators of FXR and thus harbored more potential to inhibit host primary bile acid production.<sup>68</sup> By altering host production of primary bile acids via FXR, the overall composition of the bile acid pool is modified.

The administration of probiotics also modifies the bile acid composition. Oral administration of *Lactobacillus reuteri*, a BSH-active microbe, resulted in increased intraluminal and circulating unconjugated bile acids, in addition to decreased inflammatory markers in hypercholesterolemic patients.<sup>74</sup> Also, when mice were given VSL#3, comprised of eight different probiotics strains (*Bifidobacterium breve*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, *Streptococcus thermophiles*), an increase in fecal bile acid deconjugation, fecal excretion of bile acids, and hepatic bile acid synthesis were noted.<sup>29</sup> Using FXR- and FGF15-deficient mice, it was demonstrated that these changes in bile acid metabolism were dependent on the presence of the FXR-FGF system.<sup>29</sup>

These examples provide evidence that bile acid composition is influenced and dependent on the presence of the gut microbiota. Researchers propose that development of targeted microbial therapies could be used to manipulate host bile acid pools.<sup>75</sup> For example, by coupling the bile acid metabolism of bacteria with the *bai* operon (found in *C. scindens* and *C. hiranonis*) to biotransform CA into DCA, one could then use other bacteria (such as *Eggerthella lenta* and *Ruminococcus gnavus*) to biotransform DCA into iso-DCA, which is considered to be less toxic to the host.<sup>49</sup> Additional studies to orchestrate collaborative bile acid metabolism could provide novel therapeutic strategies in diseases associated with

bile acid dysmetabolism, such as metabolic disease, obesity, IBD, and infectious enteric diseases such as *C. difficile* infection.<sup>3,4,7,8,50,65,73,76</sup>

### Impact of bile acids on the gut microbiota community structure

As demonstrated, bile acid pools are a function of collaborative metabolism of the host and the gut microbiota. Resultant chemically diverse bile acids may function to directly shape the gut microbial community structure. Due to their lipophilic nature, bile acids display antimicrobial activity with bacterial membranes being their main targets.<sup>1</sup> In addition to membrane damage, bile acids impose a fitness challenge to gut microbes through disruption of macromolecule stability by interfering with RNA secondary structures, causing DNA damage and promoting protein misfolding (reviewed in Begley et al. and more recently in Bustos et al.).<sup>1,43</sup>

Evidence of the direct antimicrobial effects of bile acids can be gleaned from murine models of biliary obstruction, which exhibit dramatic gut microbial community proliferation and increased bacterial translocation.<sup>31,77</sup> These effects can be ameliorated with administration of bile acids resulting in inhibition of bacterial overgrowth.<sup>31</sup> Bile acids also have indirect antimicrobial effects via FXR-induced antimicrobial peptide production and FXR-induced regulation of the host immune response.<sup>78,79</sup>

Significant changes in the microbiota community structure have been described in rats that were fed the primary bile acid, CA.<sup>80</sup> CA supplemented in the feed resulted in expansion of the Firmicutes from 54% relative abundance in controls to 93–98% in CA-fed rats. This mainly consisted of an increase in *Clostridium spp.* from 39% relative abundance in controls to 70% in CA-fed rats.<sup>80</sup> In general, a decrease in bile acid pools appears to favor Gram-negative outgrowth, which are capable of producing lipopolysaccharide (LPS) and some members harbor pathogenic potential.<sup>4,14,23,80</sup> In contrast, outgrowth of Gram-positive Firmicutes, including some with 7 $\alpha$ -dehydroxylation capabilities, is observed with increased bile acid pools and thus promotes secondary bile acid production.<sup>4,14,23,73,80</sup>

Another example of bile acids shaping the gut microbial ecosystem is in relation to colonization resistance against the enteric pathogen *C. difficile*. In humans with recurrent *C. difficile* infection (CDI), bile acid dysmetabolism, represented by an increase in primary bile acids and a decrease in secondary bile acids, is documented.<sup>81</sup> Fecal microbiota transplantation (FMT), which leads to restoration of bile acid pools, particularly the microbially derived secondary bile acids, results in a 95% cure rate in recurrent CDI patients.<sup>81,82</sup> Secondary bile acids directly inhibit spore germination and outgrowth of *C. difficile*.<sup>65,83,84</sup> Administration of *C. scindens*, a 7 $\alpha$ -dehydroxylating bacterium, partially restored colonization resistance against CDI in mice.<sup>56</sup> Furthermore, administration of UDCA (Ursodiol) to a human patient with recurrent *C. difficile* ileal pouchitis prevented recurrence of CDI.<sup>85</sup> Although the exact mechanisms of colonization resistance are unknown, it is apparent that secondary bile acids play an important role.<sup>7</sup>

It is evident that bile acids can alter the gut microbiota community structure through various mechanisms. In turn, the gut microbial communities can modulate bile acid pools, thus highlighting the interconnectedness of the gut microbiota-bile acid-host axis. Dysfunction in any of these components will have ramifications on the others. Numerous disease states are affected by the gut microbiota-bile acid-host axis and investigation into novel therapeutic strategies to modulate this axis are needed.<sup>1,3-12</sup>

### Differences in bile acids profile between species

A major limitation in bile acid research is differences in bile acid profiles and metabolism among animals, therefore making it challenging to compare across species.<sup>5,16,60</sup> A key example is the dramatic differences in UDCA in various species. This bile acid is considered to be the primary bile acid made by hepatocytes in bears, nutria, and beavers.<sup>16,60,86,87</sup> However, UDCA has long been considered a secondary bile acid that is microbially synthesized in the gut of humans and rodents.<sup>15,86,88</sup> Endogenous UDCA can be formed in two ways: (1) Epimerization of 7 $\alpha$ -hydroxyl by 7 $\alpha$ -hydroxysteroid dehydrogenase and 7 $\beta$ -hydroxysteroid dehydrogenase found in single species such as *Ruminococcus gnavus*, *Clostridium absonum* (only found in soil),

or *Clostridium baratii*; or (2) By two separate bacterial species expressing one or the other HSDH enzyme (Figure 3).<sup>27,52,55,61,88-98</sup> It remains unknown what additional gut microbes are also capable of converting CDCA into 7-oxo-LCA and then into UDCA.

Although the majority (>90%) of bile acid profiles in murine liver and bile are composed of muricholic acid (MCA) and CA, there is also evidence of UDCA and conjugated UDCA (with glycine and taurine).<sup>99</sup> Recently, several murine studies reported that after antibiotic treatment there is an increase in total UDCA (combination of UDCA + taurine conjugated, TUDCA) in the large intestine and liver.<sup>18,68</sup> Since antibiotic administration significantly reduces gut microbial populations, it was suggested that UDCA might be a primary bile acid synthesized by murine hepatocytes. This was further supported in gnotobiotic mice, which have TUDCA present in the absence of the microbiota.<sup>18,68</sup> However, studies in conventional and gnotobiotic rats revealed hepatic UDCA only in conventional rats with intact gut microbiota and no TUDCA (which would be host conjugated).<sup>100</sup> Unfortunately, this study did not evaluate bile acid profiles from intestinal luminal contents or feces.

Another key finding is that murine bile acid synthesis may be under potent positive feedback control via MCA and UDCA acting as antagonists of FXR/FGF15 system.<sup>101,102</sup> Therefore, MCA and UDCA may counter the negative feedback inhibition effects of CDCA and CA on FXR/FGF15 in mice.<sup>101</sup> It remains unclear if positive feedback on bile acid synthesis occurs in humans.

These studies highlight the inter-species differences in bile acid composition and abundance. Since there are differences in bile acid profiles and feedback responses between mice and humans, researchers have questioned if studies on the murine gut microbiota-bile acid-host axis can be utilized to gain insight into human diseases.<sup>6,103</sup> Currently, this is a limitation of bile acid metabolism research, and the physiologic significance of inter-species variations remains unclear.

### Ursodeoxycholic acid modulation of microbiota-bile acid-host axis

Bile from animals, including bear bile, have been extensively used therapeutically in China for over

2500 years.<sup>60</sup> Globally, close to 1000 metric tons of UDCA are produced annually for pharmaceutical and nutraceutical use.<sup>86</sup> Originally UDCA was obtained from bear bile, which is a primary bile acid in this species.<sup>60</sup> Today, the most common formulation of UDCA is Ursodiol, which is chemically synthesized from CA.<sup>104</sup> There are various other trade names globally for this product including Actigall, Deursil, and Urso. Currently, the Food and Drug Administration (FDA) approved formulation of UDCA, Ursodiol, is used to treat a variety of diseases including: cholesterol gallstones, primary biliary cirrhosis, primary sclerosing cholangitis, nonalcoholic fatty liver disease, chronic viral hepatitis C, recurrent colonic adenomas, cholestasis of pregnancy, and recurrent pancreatitis.<sup>3,102,105–112</sup> UDCA has vast beneficial effects (anticholestatic, antifibrotic, antiproliferative, and anti-inflammatory) but the major effect on bile acid physiology is an increase in the hydrophilic bile acid pool by diluting the concentration of hydrophobic toxic secondary bile acids, DCA and LCA.<sup>3,113</sup>

In healthy humans administered UDCA (15 mg/kg/day) for 3 weeks, biliary and duodenal bile acid concentrations of UDCA and its conjugates (GUDCA and TUDCA) increased by 40% compared to baseline.<sup>114</sup> A decrease in primary bile acids (CA and CDCA) and their conjugates as well as a decrease in the secondary bile acid DCA and its conjugates (GDCA and TDCA) was observed within biliary and duodenal bile.<sup>114</sup> An increase in conjugates of the secondary bile acid LCA (GLCA and TLCA) was observed after UDCA treatment within biliary and duodenal bile samples.<sup>114</sup> The gut microbiota was not evaluated in this study. As observed in the above study, LCA can increase during UDCA administration. This is thought to be from microbial conversion of UDCA to the hydrophobic toxic bile acid, LCA.<sup>88</sup> Not only does this limit the UDCA available to provide beneficial therapeutic effects, it could be potentially harmful to the host, not to mention the gut microbiota. It has recently been suggested that pharmacological inhibition of microbial *baiI* gene, a component of the *bai* operon, could limit microbial conversion of UDCA into LCA and thus increase availability of UDCA.<sup>3</sup> Impacts of higher concentrations of UDCA on the host and gut microbiota have not been investigated.

UDCA is incompletely absorbed in the small intestine, thus only a fraction reaches the large intestine where the greatest consortium of microbes are present.<sup>13,115,116</sup> Several formulations of UDCA have been developed to try to increase colonic delivery, including conjugation with glutamate, nanosuspensions/nanoparticles, and microbial derivation.<sup>116–118</sup> The UDCA-glutamate prevents absorption and bio-transformation in the small intestine but takes advantage of the peptide bond cleavage within the colonic brush boarder enzymes to remove the glutamate thus allowing for colonic delivery of UDCA.<sup>116</sup> UDCA nanosuspensions encapsulate this bile acid and allow for increased colonic transit time.<sup>117,118</sup> UDCA could also be microbially derived from administration of UDCA-producing bacteria, such as *Clostridium baratii* or *Ruminococcus gnavus* N53 (Figure 3).<sup>61,90</sup> Additional research is required to investigate the use of UDCA-producing bacteria *in vivo*. Lastly, 24-*nor*-UDCA is a shortened side chain of UDCA that undergoes chole-hepatic shunting, meaning that it passes through cholangiocytes into sinusoids via periductular capillary plexus.<sup>119,120</sup> This formulation of UDCA is thought to enhance ductal targeting of UDCA administration. Effects of 24-*nor*-UDCA on bile acid profiles and the gut microbiota need to be characterized.

It remains unclear if and how exogenously administered UDCA shapes the gut microbial community structure and/or intestinal and fecal bile acid metabolome. It is evident that UDCA can alter liver and biliary bile acid pools, but intestinal contents and feces have not been fully evaluated. The host-derived primary bile acids within the small intestine are substrates for the microbially derived secondary bile acids. Therefore, characterization of this bile acid pool is imperative to accurately reflect physiologic effects of administering bile acids, such as UDCA. Further studies employing multi-omics approaches are required to investigate the impact of UDCA administration on the gut microbial community structure, bile acid metabolome, and the host.

## Conclusions

In conclusion, there are two main sites of bile acid biosynthesis: hepatocytes within the host and microbes within the gastrointestinal tract. Bile acid pool diversity is attributed to a collaborative

metabolism of the host and gut microbiota. As demonstrated, microbially derived secondary bile acids can not only affect the composition and function of the gut microbiota but also can modulate host physiology. Thus, secondary bile acids are hypothesized to be inter-kingdom signaling molecules.<sup>3</sup> By understanding the dynamic intricacies in the gut microbiota-bile acid-host axis, insight into a variety of disease states (such as enteric pathogenesis, metabolic disease, obesity, IBD, and other chronic inflammatory conditions) may be elucidated. Expanding our knowledge may contribute to precision medicine through the development of novel targeted therapeutic strategies to manipulate the gut microbiota-bile acid-host axis during disease states with the ultimate goal of restoring health to the host and gastrointestinal ecosystem.

### Disclosure of potential conflicts of interest

CMT is a scientific advisor to Locus Biosciences, a company engaged in the development of antimicrobial technologies. CMT is a consultant for Vedanta Biosciences.

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