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Interaction of Gut Microbiota with Bile Acid Metabolism and its Influence on Disease States

Christopher Staley[1], **Alexa R. Weingarden**[1], **Alexander Khoruts**[#1,2], and **Michael J. Sadowsky**[#1,3,*]

[1]BioTechnology Institute, Center for Immunology University of Minnesota, Minneapolis, MN

[2]Division of Gastroenterology, Department of Medicine, Center for Immunology University of Minnesota, Minneapolis, MN

[3]Department of Soil, Water and Climate, University of Minnesota, St. Paul, MN

[#] These authors contributed equally to this work.

Abstract

Primary bile acids serve important roles in cholesterol metabolism, lipid digestion, host-microbe interactions, and regulatory pathways in the human host. While most bile acids are reabsorbed and recycled via enterohepatic cycling, ~5% serve as substrates for bacterial biotransformation in the colon. Enzymes involved in various transformations have been characterized from cultured gut bacteria and reveal taxa-specific distribution. More recently, bioinformatic approaches have revealed greater diversity in isoforms of these enzymes, and the microbial species in which they are found. Thus, the functional roles played by the bile acid-transforming gut microbiota and the distribution of resulting secondary bile acids, in the bile acid pool, may be profoundly affected by microbial community structure and function. Bile acids and the composition of the bile acid pool have historically been hypothesized to be associated with several disease states, including recurrent *Clostridium difficile* infection, inflammatory bowel diseases, metabolic syndrome, and several cancers. Recently, however, emphasis has been placed on how microbial communities in the dysbiotic gut may alter the bile acid pool to potentially cause or mitigate disease onset. This review highlights the current understanding of the interactions between the gut microbial community, bile acid biotransformation, and disease states, and addresses future directions to better understand these complex associations.

Keywords

Bile acids; microbial metabolism; host-interactions; dysbiosis; *C. difficile*

[*]Correspondence: Michael J. Sadowsky, 1479 Gortner Ave., 140 Gortner Labs, St. Paul, MN 5508; sadowskyumn.edu; Phone: 612-624-2706 .

Conflict of interest

The authors declare that they have no conflicts of interests.

Compliance with ethical standards

Ethical approval

Any studies with humans or animals performed by any of the authors were approved by the University of Minnesota Institutional Review Board (IRB) and Institutional Animal Care and Use Committee (IACUC). As is known by the authors, Referenced studies adhere to applicable international, national and/or institutional guidelines for the care and use of animals.

Introduction

The composition of the bile acid pool is mediated by bacterial metabolism in the intestinal tract and is intrinsically linked to host physiology. This occurs via a variety of regulatory processes, variation in toxicity among the bile acids, and the microbial ecology of the gut (Ridlon et al. 2014). As such, the function of bile acids as potential mechanisms and treatments for disease states is receiving increasing attention. In this review we focus on bile acid-mediated regulation associated with bacterial community structure, dysbiosis, and disease states. We provide an overview of the current understanding of the regulation and biotransformation of the bile acid pool by the gut microbial community and address how the complex interactions between bile acids and the gut microbiota influence the onset and treatment of disease states.

The bile acid pool size and composition are inherently linked with microbial community presence, and, presumably, composition. Therefore, it seems likely that specific assemblages of microorganisms may result in distinct variations in the bile acid pool. Thus, the current knowledge of the taxa-specific distributions of enzymes involved in bile acid transformation is addressed followed by a discussion of disease states associated with bile acids. To the extent available, information regarding relationships between the gut microbial community, bile acid transformation, and disease state will be highlighted, along with emerging technologies that allow for a better characterization of the complex interactions between the gut microbiota and the human host associated with bile acid production and metabolism.

Overview of bile acid metabolism

Bile acids are amphipathic biological detergents that primarily function in lipid metabolism, but also serve a wide range of regulatory functions throughout the body (Chiang 2009). The primary bile acids, chenodeoxycholic acid (CDCA) and cholic acid (CA), are synthesized from cholesterol in the liver, in a process involving at least 14 enzymes (Russell and Setchells 1992; Chiang 2009). The CDCA molecule has two α-hydroxy groups at the C-3 and C-7 positions, and CA has an additional α-hydroxy group at position C-12 (Hofmann 1999) (Figure 1). There are two primary pathways by which bile acids are synthesized which have been reviewed in detail (Chiang 2004; Chiang 2009) and are summarized in Figure 2. The classical bile acid synthesis pathway occurs only in hepatocytes and is controlled by the rate limiting step converting cholesterol to 7α-hydroxycholesterol (Chiang 2004). Cholic acid is formed through subsequent modification by the enzyme cascades shown in Figure 1, and CDCA is formed by a similar pathway excluding 12α-hydroxylation by CYP8B1 (Chiang 2004). Alternatively, in a variety of tissues, cholesterol may be oxidized to 27-hydroxycholesterol, which is further modified by the oxysterol hydroxylase CYP7B1 and further converted to primary bile acids (Figure 1) (Chiang 2004).

In the liver, primary bile acids are conjugated to either glycine (predominantly in humans) or taurine by the enzymes bile acid-CoA synthase (BACS) and bile acid-amino acid transferase (BAT). Conjugation increases solubility of these hydrophobic (non-polar) bile acids and concurrently decreases potential cell membrane disruption. Conjugated bile acids

subsequently accumulate in the gallbladder (Hofmann 1999; Chiang 2009). Following food intake, the gallbladder releases bile acids into the duodenum, where they contribute to the solubilization of ingested lipids as the bile acids proceed through the small intestine and colon (Hofmann 1999). Bile acids are reabsorbed in the distal ileum, primarily through active transport by the apical sodium-dependent bile salt transporter (ASBT) or the ileal bile acid transporter (IBAT). Typically this results in a ~50 to 90% recovery of conjugated bile acids (Hofmann 1999), via enterohepatic circulation, with and an overall bile acid recovery efficiency of ~95% (Ridlon et al. 2006; Chiang 2009). The cycle of enterohepatic circulation is shown in Figure 2.

Bile acids that are not reabsorbed can serve as substrates for microbial metabolism and undergo biotransformation to secondary bile acids (Figure 3) (Hofmann 1999). The predominant secondary bile acids, formed via 7α-dehydroxylation by gut bacteria, are deoxycholic acid (DCA), formed from CA, and lithocholic acid, formed from CDCA. Ursodeoxycholic acid (UDCA) has a structure similar to CDCA and this molecule is also present in small quantities in the total bile acid pool (Hofmann 1999). Once recirculated to the liver, DCA and LCA are both conjugated to glycine or taurine, similar to primary bile acids, with conjugated DCA contributing approximately 20% to the bile acid pool (Hofmann 1999). In contrast, LCA, a highly toxic bile acid, is subsequently sulfated at the C-3 position, after which it is poorly reabsorbed and excreted in the feces (Hofmann 1999).

Host regulatory pathways of bile acids

Bile acid synthesis is naturally regulated by feedback inhibition from bile acids being recirculated to the liver. What we present here is a brief overview of the regulation of this pathway, focusing on the aspects that are likely to be affected by microbial processes and gut microbial composition, discussed in the following section.

The farnesoid X receptor (FXRα) is the primary regulator of bile acid synthesis in the liver, which inhibits the enzymes that synthesize bile acids from cholesterol precursors (Figure 2) (Chiang et al. 2000; Yang et al. 2002; Chen and Chiang 2003). The FXRα is activated by hydrophobic (non-polar) primary and secondary bile acids (*i.e.* CDCA, CA, DCA, LCA) (Makishima et al. 1999; Hu et al. 2014; Ridlon et al. 2014) and binds to regulatory regions of DNA as a heterodimer with retinoid X receptors (Edwards et al. 2002). The mechanisms of inhibition vary depending on whether FXRα in the liver or intestine is activated (Kim et al. 2007; Chiang 2009), with FXRα working through a small heterodimer partner (SHP) in the liver or fibroblast growth factor 19 (FGF-19; FGF-15 is an ortholog in mice) (Holt et al. 2003; Inagaki et al. 2005; Kim et al. 2007; Chiang 2009). Recently bile acids have been postulated to regulate proliferation of intestinal cells via FXRα and epidermal growth factor receptor signaling pathways (Dossa et al. 2016). Repression of bile acid synthesis has also been suggested to be coordinately regulated by both hepatic and intestinal FXRα, where intestinal FXRα has a strong effect on CYP7A1, but not CYP8B1. In contrast hepatic FXRα also inhibits CYP8B1 (Kim et al. 2007).

Bile acid pool size has been recently demonstrated, in mice, to depend on the microbial community present in the intestines (Sayin et al. 2013). It should be noted, however, that

mice produce primarily hydrophilic (polar) muricholic acids. Consequently, conclusions generated from mouse models must be separately validated in humans, whose bile acid pool is more hydrophobic (Chiang 2009). When compared to germ-free mice, conventional mice have an overall bile acid pool that is significantly reduced (~71%). Furthermore, the presence of microbiota was found to have an influence on the site-specific bile acid concentrations in the gallbladder, small intestine, cecum, colon, and feces (Sayin et al. 2013). The authors hypothesized that differences in bile acid concentrations may be due to FXR-mediated reabsorption via the ASBT (Sinha et al. 2008; Sayin et al. 2013), and the disruption of the gut microbiota has been recently shown to increase efficiency of reabsorption (Hu et al. 2014). Similarly, the presence of microbiota resulted in suppression of CYP7A1 and BACS, but not CYP8B1 and BAT, and expression of FXRα, SHP, and FGF-15 were up-regulated in the distal ileum, but not in the liver (Sayin et al. 2013). Taken together, results of these studies, done in mice, indicate that the microbiologically-mediated formation of hydrophobic secondary bile acids diminishes concentrations of hydrophilic muricholic acid. Thus, removal of the muricholic FXR-antagonists subsequently results in activation of this receptor in the intestine to regulate bile acid homeostasis (Sinha et al. 2008; Sayin et al. 2013; Hu et al. 2014; Ridlon et al. 2014).

In addition to regulation of cholesterol and bile acid homeostasis, bile acids also regulate important metabolic pathways via FXRα and the cell membrane receptor G-protein coupled receptor 5 (TGR5). Altered pathways include those involved in drug, lipoprotein, glucose, and energy metabolism and transport (Nguyen and Bouscarel 2008; Hylemon et al. 2009). A subset of the processes and effects known to be regulated and controlled by bile acids is shown in Table 1, and these processes have been thoroughly reviewed (Nguyen and Bouscarel 2008; Hylemon et al. 2009).

The gut microbial environment

The human colon is colonized by approximately $10^{11\text{-}12}$ bacteria, with greater than 90% belonging to the phyla *Bacteroidetes* and *Firmicutes* (Dethlefsen et al. 2007; The Human Microbiome Consortium 2012). Bacterial diversity in the gut is relatively low, as compared to microbial communities found in water and soil environments, and is comprised of mainly four other phyla, including the *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, and *Verrucomicrobia* (Dethlefsen et al. 2007). Prevalent genera identified thus far include *Bacteroides*, *Eubacterium*, *Bifidobacterium*, *Ruminococcus*, *Peptostreptococcus*, *Propionibacterium*, *Clostridium*, *Lactobacillus*, *Escherichia*, *Streptococcus*, and the archaeal genus *Methanobrevibacter* (Dethlefsen et al. 2007). Many of these bacteria, especially some of the clostridia, have been shown to be active in transformation of primary to secondary bile acids (Hofmann 1999; Ridlon et al. 2006).

In the colon, nearly all bile acids are biotransformed into secondary bile acids (LCA from CDCA and DCA from CA) by 7α-dehydroxylation, and are either reabsorbed via portal circulation or excreted in the stool (Hofmann 1999; Ridlon et al. 2006). The 7α-dehydroxylation reaction has been described as the most quantitatively important process performed by colonic microflora, resulting in the formation of secondary bile acids that are predominantly found in feces (Ridlon et al. 2006). However, current estimates indicate that

approximately only 0.0001% of colonic bacteria are capable of performing this reaction, and 16S rRNA sequence analyses performed to date have indicated that these bacteria only belong to the genus *Clostridium* (Wells et al. 2000; Wells et al. 2003; Ridlon et al. 2006).

The pathway for this reaction is encoded by the bile acid inducible (*bai*) operon, which has been characterized and is highly conserved in both *Cl. scindens* and *Cl. hylemonae* strains, although the efficiency of transformation among these strains differ (Ridlon et al. 2010). The ability to perform 7α-hydroxylation is hypothesized to result in net energy gain and supports a unique niche for these bacteria, possibly by also excluding microbes that are more sensitive to the secondary bile acid end products (Ridlon et al. 2006).

The roles of bile acids in control of microbiota composition in the gut

Bile acids can exert direct influences, both positive and negative, on gut bacteria (Begley et al. 2005a). Several studies have demonstrated that bile acids have an overall negative impact on membrane integrity, likely due to an increase in membrane permeability leading to cell death (Albalak et al. 1996; De Boever et al. 2000). The extent of cell damage is related to the hydrophobicity and structure of bile acids, where more hydrophobic bile acids, and those with two rather than three hydroxy groups, are more detrimental to membrane integrity than other bile acids (Hofmann et al. 2001; Begley et al. 2005a). Bile acids also damage DNA and promote increases in enzymes involved in DNA repair (Kandell and Bernstein 1991; Begley et al. 2005a). Moreover, there is further evidence that bile acids may also cause oxidative and pH stress, and may chelate important cellular ions, such as calcium (Bernstein et al. 1999; Begley et al. 2005a). Gram-negative bacteria are currently thought to be more resistant to bile acids than Gram-positive microorganisms, although few studies have characterized bile acid resistance among Gram-negative species (Begley et al. 2005a). Importantly, bile resistance, tolerance, and susceptibility is strain-specific, with large variations in the degree of tolerance within a single bacterial species (Chateau et al. 1994; Záratea et al. 2000).

Bile acids can also have indirect antimicrobial effects mediated by FXRα, the activation of which results in up-regulation of genes involved in mucosal defense in the ileum of mice (Inagaki et al. 2006). In a mouse model, biliary duct ligation resulted in overgrowth of aerobic and anaerobic bacteria in the ileum and cecum, but this overgrowth could be blocked by activation of FXRα with the agonist GW4064 (Inagaki et al. 2006). This study further demonstrated that FXRα activation prevented translocation of bacteria across the intestinal mucosal barrier by impeding damage and inflammation that typically results from biliary duct ligation. A subsequent review of the effects of bile acids on mucosal defense suggested that antimicrobial effects are direct when conjugated bile acids are at high concentrations. In contrast, regulation of bacterial densities via signaling occurs when conjugated bile acid concentrations are depleted in the distal ileum (Hofmann and Eckmann 2006).

Several studies have evaluated bacterial community dynamics that are potentially regulated by, or affect the distribution of, bile acids in the small and large intestines (Islam et al. 2011; Ridlon et al. 2013; Kakiyama et al. 2013). Primary bile acids such as taurocholate can provide homing signals to gut bacteria and promote germination of spores, which may be

present in dormant and non-toxic forms, and may facilitate recovery of microbiota after dysbiosis induced by antibiotics or toxins (Browne et al. 2016). This mechanism can be exploited by pathogens that have arisen from indigenous gut microbiota, like *Clostridium difficile* (see below), that germinates into a vegetative, potentially toxigenic form. Primary bile acids may also serve as a control mechanism to prevent outgrowth of pathogenic Gram-negative bacteria in the small intestine (Kakiyama et al. 2013). A recent study in cirrhotic patients with synthetic deficits in production of bile acids found that increases in potentially pathogenic *Enterobacteriaceae* were positively associated with CDCA concentrations and lower concentrations of secondary bile acids in stool. This likely resulted from a concomitant reduction in typical resident groups of clostridia able to perform 7α-dehydroxylation (*i.e. Clostridium* cluster XVIa) (Kakiyama et al. 2013).

Separate studies done in rats and mice found that the concentration of CA can regulate gut community structure. Specifically, increased CA concentrations resulted in increases in *Firmicutes*, especially groups capable of 7α-dehydroxylation, and a decrease of *Bacteroidetes* (Islam et al. 2011; Ridlon et al. 2013). As suggested by Ridlon, *et al.* (2014), it is still unclear whether the increase in 7α-dehydroxylating bacteria, such as *Clostridium* cluster XVIa, in response to increases in primary bile acids, reflects: 1) an antagonistic effect of bile acid on other community members, 2) increases in production of an antimicrobial compound by these members, or 3) use of these bile acids as an electron acceptor in a fermentative pathway.

Microbial influence on intestinal bile acids

As bile acids move through the small intestine, they are subjected to biotransformation by the resident microbial community. The gut microbiota has been reported to consist primarily of members of the bacterial genera *Lactobacillus*, *Streptococcus*, *Staphylococcus*, and *Veillonella*, typically at concentrations of 10^3 to 10^4 bacteria ml^{-1} in the duodenum and jejunum (Ridlon et al. 2006). Bacteria in the ileum are mainly comprised of *Enterobacteria*, *Enterococcus*, *Bacteroides*, *Clostridium*, *Lactobacillus*, and *Veillonella*, at much larger concentrations of ~ 10^6 to 10^8 bacteria ml^{-1} (Ridlon et al. 2006). Together these bacteria are responsible for deconjugation of bile acids from glycine or taurine by hydrolases and the oxidation of hydroxy groups (Ridlon et al. 2006). The majority of bile acids (95%) are reabsorbed in the distal ileum and recirculated through the liver (Ridlon et al. 2006; Chiang 2009); however, a small amount (400-800 mg day^{-1}) continues through to the colon where they serve as substrates for a variety of biotransformation reactions by gut bacteria (Ridlon et al. 2006).

Transformation of bile acids

The transformation of bile acids involves several processes including deconjugation to liberate free bile acids, reversible epimerization between α and β orientations, and the oxidation of the 3-, 7-, and 12-hydroxy groups. Hydroxy groups of bile acids, synthesized in the liver, are all in the α orientation resulting in amphipathicity and allowing efficient solubilization of lipid molecules (Ridlon et al. 2006). Deconjugation and epimerization of bile acids results in alteration of the hydrophilicity of bile acids (Armstrong and Carey

1982), and may affect the efficiency of lipid solubilization or affinity of hydroxysteroid dehydrogenases (Ridlon et al. 2006). Furthermore, epimerization of bile acids by gut microbiota, and the accumulation of bile acids with β-oriented hydroxy groups in the bile acid pool, confers a protective effect on the liver against more toxic, hydrophobic, bile acids (Heuman et al. 1991; Hofmann 1995). Therefore, the composition of the microbial community may have significant effects on nutrient absorption and bile acid toxicity, affecting host metabolic responses based on its ability to perform specific bile acid transformations.

The variety of isoforms of the enzymes responsible for these reactions and their distributions among gut microbiota are relatively understudied, and current knowledge is primarily based on biochemical studies in which these enzymes are characterized from single, cultivatable species. However, due to the potentially great variety of isoforms and the potential for limited distribution of these enzymes among only a few microbial species, it is possible that specific community structures have discrete effects on host health via modification of primary and secondary bile acid pools. Furthermore, expression of particular enzymes by certain microbial species may influence community composition by conferring selective advantages to these species *in situ*.

Results of more recent bioinformatic and metagenomic studies highlight a considerably greater diversity of microorganisms that are capable of performing bile acid transformations, , including members of the *Archaea*, than were previously recognized by biochemical characterization (Jones et al. 2008; Kisiela et al. 2012). Further study of these previously unknown, and likely unculturable, members of the gut microbiota will be important in order to better understand how these groups may influence disease states and community-level processes important to host metabolic processes.

Bile salt hydrolases

The first step in bile acid modification by the microbial community involves the deconjugation of bile acids by bile salt hydrolases (BSHs) (Ridlon et al. 2006). These enzymes share a high degree of structural similarity to penicillin V acylase from *Bacillus sphaericus* (and other species) and are relatively widely distributed among both Gram-negative and -positive members of the gut microbiota (Ridlon et al. 2006; Kumar et al. 2006; Jones et al. 2008). There are at least nine types of BSHs (Jones et al. 2008), and these differ in size, pH optima, substrate specificity, and genetic organization and regulation (Ridlon et al. 2006). Several studies have suggested that BSHs play a role in human gut colonization, especially by pathogens such as *Listeria monocytogenes* and *Brucella abortus* (Begley et al. 2005b; Delpino et al. 2007), and detoxification of bile acids (De Smet et al. 1995). Other studies have suggested that BSHs facilitate scavenging of carbon, nitrogen, and sulfur (Van Eldere et al. 1996; Tanaka et al. 2000).

Bile salt hydrolases have been characterized from species of *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium*, and *Listeria* (Ridlon et al. 2006). A more recent functional metagenomic study of approximately 90,000 clones containing DNA fragments from the gut microbiome revealed that 142 clones demonstrated BSH activity and, among the 90 that could be taxonomically assigned, ~ 30, 14 and 9% (corresponding to 27, 13, and 8 clones)

belonged to the *Firmicutes*, *Bacteroidetes*, and *Actinobacteria*, respectively. Most of the remaining (43%) positive clones could not be assigned (Jones et al. 2008)., although updated databases may now allow for classification of many of these. In this study, clones assigned to *Firmicutes* and *Actinobacteria* were capable of deconjugating all the glyco- and tauro-conjugated bile acids tested, while *Bacteroidetes* had specific activity against tauro- but not glyco-conjugated bile acids. Furthermore, one BSH showed a high degree of similarity (56%) to that found in *Methanobrevibacter smithii*, and the authors demonstrated that this enzyme deconjugated both glyco- and tauro-conjugated bile acids and suggested a role of horizontal gene transfer (transfer of genes between species) in the dissemination of BSHs (Jones et al. 2008).

Hydroxysteroid dehydrogenases

Hydroxysteroid dehydrogenases (HSDs) are bacterial enzymes that act on the 3-, 7-, and 12-position hydroxy groups of bile acids to catalyze epimerization and oxidation/reduction. This alters the hydrophobicity and toxicity of bile acids and provides potential energy sources for cellular processes (Sherrod and Hylemon 1977; Heuman et al. 1991; Ridlon et al. 2006). Epimerization of hydroxy groups requires the activity of two stereochemically-distinct (α or β), position-specific enzymes to form stable oxo-bile acids. The process may be carried out by a single species possessing both enzymes (e.g. *Cl. absonum*) (Sutherland and Macdonald 1982) or via interspecies interaction (a synergism) in which one species possesses the α-HSD and another the β-HSD (MacDonald et al. 1982). Oxidation and reduction of oxo-bile acids is largely dependent on the redox potential (oxygen state) of the microenvironment in the intestinal tract, such that reduction is favored in the intestinal lumen where redox potential is low (anoxic), and oxidation may be favored closer to the mucosal surface where redox potential is more positive (oxic) (Ridlon et al. 2006).

Bioinformatic analysis has revealed two different 3α-HSDs found in either *Cl. scindens* or *Comomonas testosteroni* that have low (22%) amino acid sequence similarity with each other (Kisiela et al. 2012). Homologs to the former 3α-HSD were found primarily among the *Firmicutes*, but also in more extremophilic groups (those that survive in extreme environments) including *Thermotogae* and *Aquificae* (Kisiela et al. 2012), which is in agreement with prior studies that have characterized these enzymes from several species of abundant, and some less abundant, intestinal bacteria including *Cl. hiranosis* (Wells and Hylemon 2000), *Cl. perfringens* (Macdonald et al. 1976), *Cl. scindens* (Mallonee et al. 1995), *Eggerthella lenta* (Macdonald et al. 1979), and *Peptostreptococcus productus* (Edenharder et al. 1989a). Homologs to the latter 3α-HSD in *C. testosteroni* were identified among several potentially pathogenic groups, primarily within the phylum *Actinobacteria* (Kisiela et al. 2012). A convergent evolutionary process has been suggested to explain the emergence of these two 3α-HSDs from different ancestors (Kisiela et al. 2012). 3β-HSDs have also been identified within the genera *Clostridium* (Edenharder et al. 1989b) and *Ruminococcus* (Akao et al. 1987).

Based on biochemical characterization, 7α- and 7β-HSDs have been found in several of the *Bacteroides* and *Clostridia*, and also in *E. coli*, *C. testosteroni*, and *Ruminococcus* spp. (Macdonald et al. 1976; Akao et al. 1987; Yoshimoto et al. 1991; Baron et al. 1991;

Coleman et al. 1994; Ferrandi et al. 2012; Ji et al. 2014). Three distinct types of 7α-HSDs were found by bioinformatic analysis that were similar to proteins identified in either: 1) *E. coli*/*Br. melitensis* (merged model), 2) *B. fragilis*, or 3) *Cl. scindens* (Kisiela et al. 2012). Homologs to the *Escherichia/Brucella* 7α-HSD were found in a variety of species from natural environments as well as gut-associated members of *Enterobacteriaceae* (*i.e. Pseudomonas* spp., *Cyanothece* spp., *Comamonas* spp., *Psychrobacter* spp., *Acinetobacter* spp., *Rhodobacter* spp., *Pseudoalteromonas* spp., *Brevundimonas* spp., *Nitrosomonas* spp., *Fusobacterium* spp.), including several potentially pathogenic species. The second 7α-HSD of *B. fragilis* had homologs primarily among other *Bacteroides* spp., but also among *Rhodococcus* spp., *Strepytomyces* spp., *Lysinsibacillus* spp., and *Bacillus* spp. The homologs to the *Cl. scindens* 7α-HSD were restricted to *Firmicutes* (primarily *Clostridium* spp.), with some detected in *Spirochaetes*.

The mechanism of dehydroxylation by *Cl. scindens* is of particular importance due to the predominance of secondary bile acids in feces and the presumed sequestration of these genes within a limited number of *Firmicutes*(Ridlon et al. 2006).The reaction is catalyzed by enzymes encoded on the bile acid induced *bai* operon, described in detail elsewhere (Ridlon et al. 2006; Ridlon and Hylemon 2012). Importantly, the process acts only on unconjugated, primary bile acids (Batta et al. 1990), and may provide function in niche restriction via the production of hydrophobic secondary bile acids. This latter supposition is of particular important to the host since the inhibitory effects of secondary bile acids can serve a protective effect, for example, by inhibiting germination of toxigenic *Cl. difficile* (described in detail below) (Buffie et al. 2015).

The *Firmicutes*, especially *Clostridium* spp., are the predominant group from which 12α/β-HSDs have been identified and characterized both biochemically and bioinformatically (Ridlon et al. 2006; Kisiela et al. 2012). Bioinformatic analyses also revealed homologs to the *Clostridium* 12α-HSD among the *Actinobacteria*, *Bacteroidetes*, and *Tenericutes* as well as the *Methanobacteria* within *Euryarchaeota* (Kisiela et al. 2012). Among the clostridia, 12α- and 12β-HSDs have been characterized from *Cl. difficile* (Edenharder and Schneider 1985), *Cl. leptum* (Harris and Hylemon 1978), *Cl. paraputrificum* (Edenharder and Schneider 1985), *Cl. perfringens* (Macdonald et al. 1976), and *Cl. tertium* (Edenharder and Schneider 1985), and are all constitutively expressed, except for the 12β-HSD in *Cl. paraputrificum* (Edenharder and Pfützner 1988). Similar to other HSDs, the 12α/β-HSDs have been suggested to play a role in host colonization (Macdonald et al. 1976).

Bile-acid-associated diseases states

Altered bile acid pool composition has been associated with several disease states, including recurrent *Cl. difficile* infection (Wilson 1983), inflammatory bowel diseases (Cima and Pemberton 2001), metabolic syndrome (Mudaliar et al. 2013), and cancers (Nagengast et al. 1995). Recently, the role of the gut microbial community in causing or abrogating disease states has also received considerable attention (Hamilton et al. 2013; Ridaura et al. 2013). Based on current knowledge, it is apparent that both the influence of the bile acid pool on the composition of the bacterial communities, as well as the effect of bacterial metabolism of bile acids, on host signaling may play important roles in these conditions. As such, the

elucidation of these interactions has become a contemporary area of research both to better understand the etiology of these diseases, as well as identify efficacious treatment strategies. In this section, we summarize the current state of research as it relates to the gut microbiome, bile acid metabolism, and associated disease states.

Clostridium difficile infection

Clostridium difficile infection (CDI) is a common nosocomial disease of the colon, generally characterized by diarrhea and abdominal pain. In a typical case of CDI, a patient receives antibiotics, within a healthcare setting, and subsequently ingests spores of *Cl. difficile*, which can germinate in the newly permissive colonic environment and produce the toxins that cause disease (Kachrimanidou and Malisiovas 2011). The relationship between antibiotics and CDI has been well established in epidemiological and other studies, and antibiotic exposure is far and away the strongest risk factor for CDI (Fashner et al. 2011).

The link between antibiotic usage and risk for CDI is most likely to be the effect of the antibiotic(s) on the native colonic microbiota. In mouse models (Robinson and Young 2010; Buffie et al. 2012; Pérez-Cobas et al. 2013) and humans (Dethlefsen and Relman 2011), antibiotics radically alter the composition of the colonic microbiota as well as its metabolic state. Furthermore, restoration of native microbiota, via fecal microbiota transplantation, results in effective treatment of CDI (Khoruts et al. 2010; Shahinas et al. 2012; Hamilton et al. 2013; van Nood et al. 2013). Originally, it was hypothesized that the increase in space and resources for colonization (allowing niche placement) following antibiotic therapy allowed *Cl. difficile* to flourish in the colon, providing loss of “colonization resistance” (Brandt and Reddy 2011; Britton and Young 2012). In recent studies, however, it has been suggest that a shift in colonic bile acid composition contributes to a permissive environment in which the bacterium may thrive (Weingarden et al. 2014; Buffie et al. 2015; Weingarden et al. 2016b; Weingarden et al. 2016a). Thus restoration of gut microbial ecology is due to colonization and chemical signal exchange.

Certain bile acids have long been known to play a role in the growth of *Cl. difficile*. The primary bile acid cholic acid and its taurine-conjugated derivative, taurocholic acid, have been known for decades to stimulate germination of *Cl. difficile* spores (Wilson 1983), and sodium taurocholate is a typical reagent used for growing *Cl. difficile in vitro* (Wilson et al. 1982; Buggy et al. 1985). More recent work has shown that both CA and TCA stimulate the first step of germination of *Cl. difficile* spores and allow for outgrowth of colonies (Sorg and Sonenshein 2008). In contrast, other bile acids including CDCA, LCA, and UDCA, inhibit germination by taurocholate (Sorg and Sonenshein 2009; Sorg and Sonenshein 2010). More significantly, recent studies have shown that there are major shifts in fecal bile acids after antibiotic treatment, characterized by an increase in primary bile acids and a decrease in secondary bile acids, that may promote infection with *Cl. difficile* (Giel et al. 2010; Theriot et al. 2014). Opposite shifts in fecal bile acids are observed in human patients after fecal microbiota transplantation (Figure 4) (Weingarden et al. 2014). Thus, the composition of bile acids in the colon may have a significant impact on CDI, with primary bile acids, dominant after antibiotic treatment, promoting the germination of *Cl. difficile* spores, and secondary bile acids, produced by specific colon bacteria, that are restored after fecal

transplantation, inhibiting germination (Weingarden et al. 2016b; Khoruts and Sadowsky 2016; Weingarden et al. 2016a).

Although CDI is primarily a colonic disease, it is thought to be responsible for 10% of diarrhea in patients with a surgically created ileal pouch reservoir after colectomy (Seril and Shen 2014). It has been noted these patients frequently do not respond to FMT with the same success as patients with intact colons (Hamilton et al. 2012; Borody et al. 2014; Patel et al. 2014). However, we have successfully treated a patient with recurrent CDI of the ileal pouch with oral UDCA, suggesting that even without a normal, intact colonic microbiota, direct replacement of secondary bile acids may be a viable strategy for treatment of CDI pouchitis (Weingarden et al. 2016a). Notably, germination of spores from the strain of *Cl. difficile* isolated from this patient was found to be inhibited by UDCA, again supporting the idea that bile acid supplements may represent an alternative treatment for CDI in select patients.

Inflammatory bowel disease

Inflammatory bowel disease (IBD) describes a variety of clinical conditions, including Crohn's disease, ulcerative colitis, and microscopic colitis, which are characterized by chronic inflammation of the gastrointestinal tract (Israel and Kleinman 1994; Maxson et al. 1994). The etiology of each of these conditions is obscure, though it is likely caused by several factors, including host genetics, diet, and gut microbiota (Ek et al. 2014; Huang et al. 2014; Sobczak et al. 2014).

That gut microbiota may contribute to IBD is an attractive hypothesis, given the intimate relationship between the gut and its commensal microflora. It is well established that the fecal and intestinal mucosal microbiota of human patients with IBD, particularly ileal Crohn's disease, are highly altered compared to healthy individuals (Swidsinski et al. 2005; Frank et al. 2007; Sokol et al. 2008; Willing et al. 2009). Furthermore, intestinal inflammation in animal models of IBD is highly attenuated in germ-free animals, which lack intestinal microbiota (Sellon et al. 1998; Schultz et al. 1999; Garrett et al. 2010). Patients with underlying IBD also have a lower success rate following FMT for CDI compared to patients without IBD (Khoruts et al. 2016). Despite this relationship, as well as the extensive body of knowledge of intestinal bacterial bile acid metabolism, little work has examined how bile acid metabolism might affect IBD.

Most research on the relationship between bile acids and IBD has focused on how the disease affects both intestinal and serum bile acids. One common complication of IBD is resection of the terminal ileum. Because the terminal ileum is the site of reabsorption of bile acids by the GI tract, the removal of this section of the small intestine can lead to bile acid malabsorption, typically characterized by bile acid-mediated diarrhea or, in large resections of the terminal ileum, a loss of fat digestion (Cima and Pemberton 2001; Domènech et al. 2014; Gothe et al. 2014). Several studies have also noted alterations in the composition of bile acids in the colon, characterized by an increase in conjugated bile acids and decreased secondary bile acids in the feces of patients (Duboc et al. 2013). There are also significant

alterations to bile acid concentrations in both portal vein (Holzbach et al. 1980) and systemic serum (Gnewuch et al. 2009) with the disease, even in the absence of small bowel resection.

Although these investigations focused on the effects of IBD on bile acid composition in the colon and blood, recent studies have examined whether bile acids may play a role in the etiology of the disease. In a model of IBD, which uses mice lacking interleukin (IL)-10 an anti-inflammatory cytokine, feeding these mice a diet high in milk fat led to an increase in the concentration of taurocholic acid in the gut. This in turn led to an increase in the abundance of sulfite-producing bacteria and an increase in disease symptoms (Figure 5) (Devkota et al. 2012). These results suggest that the effects of particular bile acids on IBD is an area worth exploring, even though little work has so far investigated this relationship.

Another area of potential interest in the relationship between bile acids and IBD is the mediation of inflammation by FXR. In addition to its role in regulating bile acid metabolism by the host, FXR is thought to be involved in the regulation of intestinal innate immunity and permeability (Vavassori et al. 2009). A recent study revealed that treatment with an FXR agonist is protective in mouse models of chemically-induced colitis (Gadaleta et al. 2011). These findings suggest that the observed alterations in colonic bile acid composition in IBD not only reflect underlying changes to microbial bile acid metabolism but may also have a key role in mediation of inflammation in these diseases (Ogilvie and Jones 2012). Furthermore, FXR agonists may represent a novel therapeutic option for IBD.

Primary Sclerosing Cholangitis

Primary sclerosing cholangitis (PSC) is strongly associated with IBD (Boonstra et al. 2012) and is a chronic cholestatic liver disease characterized by bile duct destruction leading to cirrhosis and liver failure (Hirschfield et al. 2013). PSC may also occur without IBD and, while the etiology is not well understood, is thought to be due to genetic and environmental risk factors (Hirschfield et al. 2013). Incidence of PSC with IBD has been indicated as a risk factor for the development of colorectal cancers (Soetikno et al. 2002), but there is no indication of increased risk in the absence of IBD (Claessen et al. 2009).

Inflammation and obstruction of biliary ducts results in alteration of bile flow and a decrease in resistance to bile acid toxicity (Hohenester et al. 2012; Hirschfield et al. 2013). Several placebo-controlled studies have indicated that administration of UDCA is effective at improving serum liver biochemistry (Chazouillères et al. 1990; Beuers et al. 1992). A more recent study, however, showed that a high dose (~30 mg kg^{-1}) of UDCA presented significantly higher risk of adverse effects in the group receiving UDCA versus a placebo control (Lindor et al. 2009). Furthermore, the role of the gut microbiota on disease progression was investigated in a study that used a treatment of UDCA alone, or UDCA with metronidazole, and found that while combination therapy improved biochemistry, the use of the antibiotic did not affect overall clinical outcomes (Färkkilä et al. 2004). In addition, high-dose UDCA treatment was suggested to increase the risk of colorectal cancers (Eaton et al. 2011). Currently, the interaction of the gut microbiota and bile acid pool composition as it relates to PSC is enigmatic and will require further study to elucidate how these factors contribute to and may be used to treat this condition. Whether FMT can

positively impact PSC is currently unknown, although a clinical trial is currently in progress to address this [https://clinicaltrials.gov/ct2/show/NCT02424175].

Metabolic syndrome

Metabolic syndrome is one of the most common diseases in the United States. According to the Centers for Disease Control and Prevention, the prevalence of one component of metabolic syndrome, obesity, has dramatically increased over the past several decades. It is currently estimated that more than 1/3 (34.9%) of individuals in the U.S. are obese [http://www.cdc.gov/obesity/data/adult.html]. Metabolic syndrome increases the risk of several diseases, particularly type 2 diabetes and cardiovascular disease (Alberti et al. 2005).

While metabolic syndrome is generally thought to be due to multiple factors, including genetics, the environment, and behavior (Alberti et al. 2005), emerging evidence suggests the gut microbiota may also contribute to the disease. Early studies demonstrated an increase in the relative proportion of the *Firmicutes* phylum and a concomitant decrease in *Bacteroidetes* in genetically obese versus lean mice (Ley et al. 2005). Initially, this trend was thought to be similar in obese versus lean humans(Ley et al. 2006), but these findings have been recently challenged (Walters et al. 2014; Sze and Schloss 2016). Nonetheless, transplantation of fecal or cecal microbiota from several mouse models of obesity into germ-free animals frequently recapitulates the disease phenotype, including weight and fat gain as well as glucose intolerance and triglyceride abnormalities (Turnbaugh et al. 2006; Vijay-Kumar et al. 2010; Cox et al. 2014; Suez et al. 2014). Transplantation of fecal microbiota from obese humans also causes increased weight gain in germ-free mice compared to transplantation with microbiota from lean individuals, suggesting that while differences in the microbiota may exist between lean and obese humans and mice, the effects of the microbiota on phenotype can be recapitulated using human-derived populations (Ridaura et al. 2013). Several mechanisms for these observations have been proposed, including increased caloric extraction from food by the gut microbiota (Turnbaugh et al. 2006) and increased host appetite (Vijay-Kumar et al. 2010; Cox et al. 2014).

Bacterial bile acid metabolism may provide an alternative mechanism underlying the relationship between the gut microbiota and metabolic syndrome. The role of bile acids and their nuclear receptor, FXRα, in metabolic syndrome has been extensively reviewed (Duran-Sandoval et al. 2005; Claudel et al. 2005; Cariou and Staels 2007; Kuipers et al. 2007; Lefebvre et al. 2009; Porez et al. 2012). A therapeutic option for dyslipidemia and other aspects of metabolic syndrome, in fact, is bile acid-binding resins such as cholestyramine, which decrease the concentration of bile acids returned to the liver via enterohepatic cycling and therefore stimulate the conversion of cholesterol to bile acids (Ginsberg and Stalenhoef 2003; Claudel et al. 2005; Turnbaugh et al. 2006; Yamaoka-Tojo et al. 2008). Furthermore, it appears that activation of FXRα can also improve aspects of metabolic syndrome in animal models, including glucose homeostasis (Duran-Sandoval et al. 2005) and triglyceride control (Bilz et al. 2006; Del Bas et al. 2009). A recent Phase II trial in human patients demonstrated that increased insulin sensitivity following treatment with an FXRα agonist (Mudaliar et al. 2013). Activation of the bile acid cell membrane receptor TGR5 may also affect metabolic syndrome. TGR5 can stimulate glucagon-like protein 1 production in

enteroendocrine cells, leading to improved glucose homeostasis and can activate thyroid hormone in brown adipose tissue and muscle, which increases energy expenditure (Thomas et al. 2008; Zhong 2010; Pols et al. 2011).

Recent work has indicated that bacterial bile acid metabolism can affect FXRα signaling, and therefore metabolic syndrome. Inoculation of mice with *E. coli* overexpressing a highly active allele of BSH significantly increased FXRα signaling and led to a significant decrease in weight gain and liver triglycerides (Figure 6) (Joyce et al. 2014).

The relationship between bacterial bile acid metabolism and metabolic syndrome in humans has also been explored. One study has shown that fecal transplantation with material from lean donors improved insulin sensitivity in individuals with metabolic syndrome (Vrieze et al. 2012). In contrast, administration of oral vancomycin to patients with metabolic syndrome results in substantial changes to the fecal microbiota, along with a significant decrease in fecal secondary bile acids and decreased peripheral insulin sensitivity (Vrieze et al. 2014). Taken together, this work suggests that bacterial bile acid metabolism in the gut can significantly impact metabolic syndrome in the host.

Colorectal Cancers

Secondary bile acids, especially DCA, have long been known to accumulate at high levels in the bile acid pool of certain individuals on high fat, ‘Western’ diets (Ridlon et al. 2014). It has been suspected that bacterial metabolism of these bile acids produces carcinogenic or co-carcinogenic compounds (Hill et al. 1975; Nagengast et al. 1995; Bernstein et al. 2005). The mechanisms of action have not been clearly elucidated, but DNA damage and induction of apoptosis are among those most commonly suggested (Bernstein et al. 2005). The majority of studies have investigated the role of bile acids in colon cancer, where patients with cancer had higher levels of fecal bile acids relative to healthy controls or those with other diseases (Hill et al. 1975; Nagengast et al. 1995). In addition, patients with colon cancer also had higher concentrations of 7α-dehydroxylating clostridia in their stool, suggesting a link between these bacteria and the disease state (Hill et al. 1975). On the other hand, epidemiological data did not find an increase in gastrointestinal cancer in patients who had undergone partial ileal resection, a group of patients which may have had increased bile acid load to the colon (Buchwald et al. 1990). A relationship between the gut microbiome and colorectal cancer has been reviewed (Dulal and Keku 2014; Nistal et al. 2015) and Burns and colleagues have identified specific bacterial species that are correlated with colorectal cancer and that the gut microbiota play a pivotal role in cancer development or progression (Burns et al. 2015). As pointed out by Ridlon and co-workers, control of the gut microflora and bile acid metabolism may be key to preventing a large number of human diseases (Ridlon et al. 2016).

In contrast to the highly hydrophobic DCA, UDCA has been shown in several studies to suppress tumor development, especially in colon cancers (Pardi et al. 2003; Alberts et al. 2005; Centuori and Martinez 2014). However, a more recent analysis found the effects of UDCA to be gender dependent. While men treated with UDCA showed a reduced risk for developing advanced lesions, some women showed a significantly higher risk (Thompson et

al. 2009). These results, as well as the dose-dependent outcomes discussed above (Eaton et al. 2011) and the examination of UDCA in patients with other underlying disorders such as ulcerative colitis and primary sclerosing cholangitis (Pardi et al. 2003), suggest that the mechanism(s) by which DCA and UDCA effect the progression of colorectal cancers is complex and is currently not well understood. Research efforts are currently shifting to examine the role of these bile acids as signaling molecules that regulate pathways involved in the progression of colon cancers to better understand the mechanistic relationships between these molecules and disease progression (Centuori and Martinez 2014). It should be noted, however, that the influence of bile acids on cancer may not be direct. For example, Ridlon et al. (2016) argue that the genotoxic effects of taurocholic acid may be due to bacterial transformation of this bile acid to H_2S and cholic acid.

Other Cancers

In addition to a probable role in colorectal cancers, secondary bile acids have also been suggested as potential causative agents of cancers in several other body sites (Bernstein et al. 2005). Bile acid exposure resulting from laryngopharyngeal reflux has been suggested to result in carcinoma of the upper aerodigestive tract (Lewin et al. 2003). Similarly, bile acid exposure resulting from gastroesophageal reflux has been hypothesized to eventually lead to esophageal adenocarcinoma (Stamp 2002) and exposure from duodenogastric reflux to gastric stump carcinoma (Kondo 2002). Due to the association of pancreatic cancer with the Western diet, bile acids have also been hypothesized to be associated with pancreatic cancer (Tucker et al. 2004). Similarly, based on the formation of adenocarcinomas near the point of bile acid entry to the small intestine, a role for bile acids in carcinogenesis in the small intestine has also been suggested (Ross et al. 1991).

More recently, alterations of the gut bacterial community associated with obesity and the increased production of DCA have been associated with formation of hepatocellular carcinomas (Yoshimoto et al. 2013). In a mouse model, recirculation of DCA resulted in secretion of pro-inflammatory and pro-tumorigenic factors in the liver, but blocking DCA or reducing the gut microbiota prevented carcinoma development (Yoshimoto et al. 2013). Analysis of the microbiome of mice fed a high fat versus a normal fat diet revealed that the former mice had significantly higher relative abundances of *Clostridium* clusters XI and XIVa (Yoshimoto et al. 2013) than normal diet mice, which are able to perform 7α-dehydroxylation of primary bile acids. Further analysis revealed that, when fed the high fat diet, *Clostridium* cluster XI was comprised of a single strain of *Cl. sordellii* and that there was an increase in the abundance of the *baiJ* gene which is involved in 7α-dehydroxylation. This strongly suggested that this strain was responsible for the increase in DCA (Yoshimoto et al. 2013).

Conclusions

Bile acids are vital components of cholesterol metabolism, lipid digestion, and other regulatory pathways in the human host. Diet and the gut microbial community interact with the bile acid pool, via biotransformation reactions, that affect the hydrophobicity, toxicity, and regulatory effects of bile acids. Disturbance of the bile acid pool, by disease or

temporary antibiotic-induced dysbiosis, may result in a variety of disease states. Consequently, increasing attention is being paid to how microbial community structure, especially that in a dysbiotic state, and microbial processes act on these molecules to cause disturbances. Recent metagenomic and bioinformatic analyses are revealing novel bacterial species and enzymes capable of bile acid transformation, as well as revealing potentially meaningful shifts in community composition associated with these disease states. This knowledge will better inform therapeutic practices to alleviate bile-acid-mediated illness. Future research must focus on elucidating the casual mechanisms by which gut microbiota and bile acids interact, including addressing previously unculturable, unidentified, species that may be critically involved in these processes at the single-species or community level.

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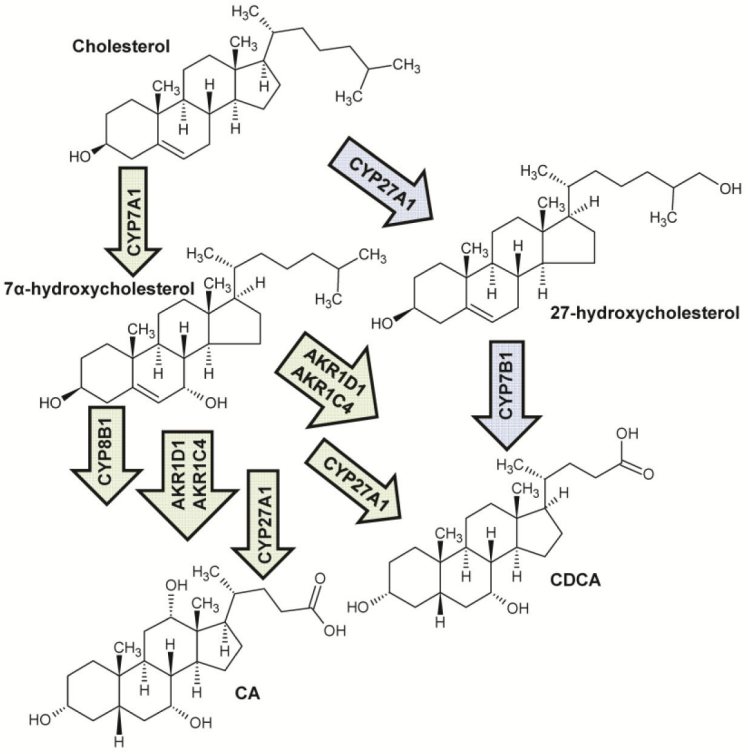

Figure 1.

Bile acid synthesis pathway in humans. Green arrows reflect the primary process of bile acid synthesis while blue arrows show an alternative pathway.

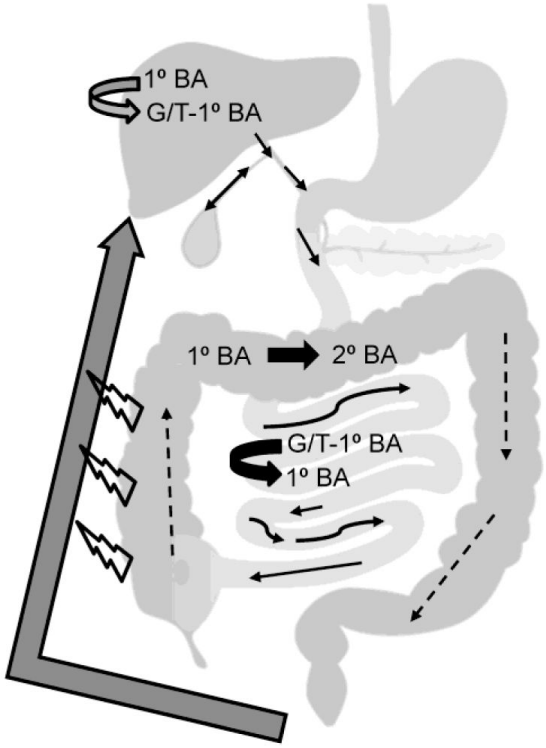

Figure 2.
Overview of enterohepatic circulation and microbial processing of bile acids. Gray arrows indicate host processes, and thick black arrows reflect processes performed by the microbiome. Thin, solid arrows reflect the direction of primary acid flow while dashed arrows reflect secondary bile acids. Open symbols reflect passive transport. BA: bile acid, G: glycine, T: taurine.

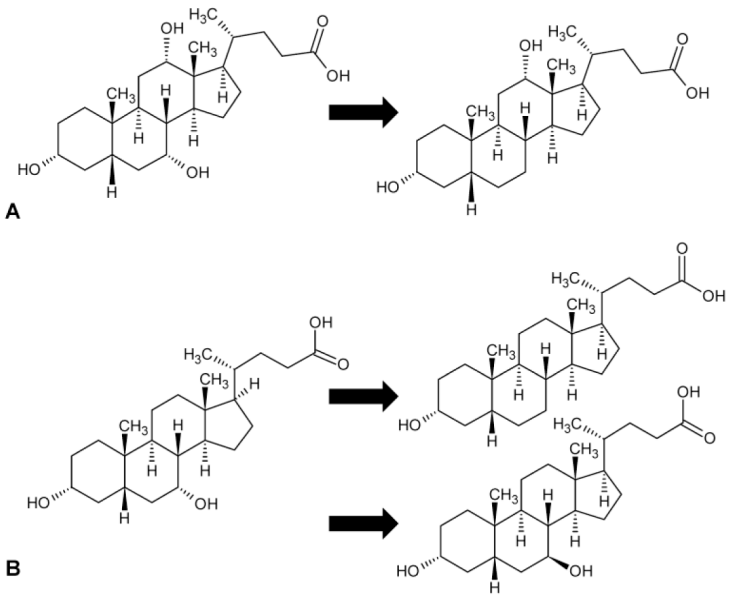

Figure 3.
Chemical structures of secondary bile acids derived from (A) cholic acid and (B) chenodeoxycholic acid.

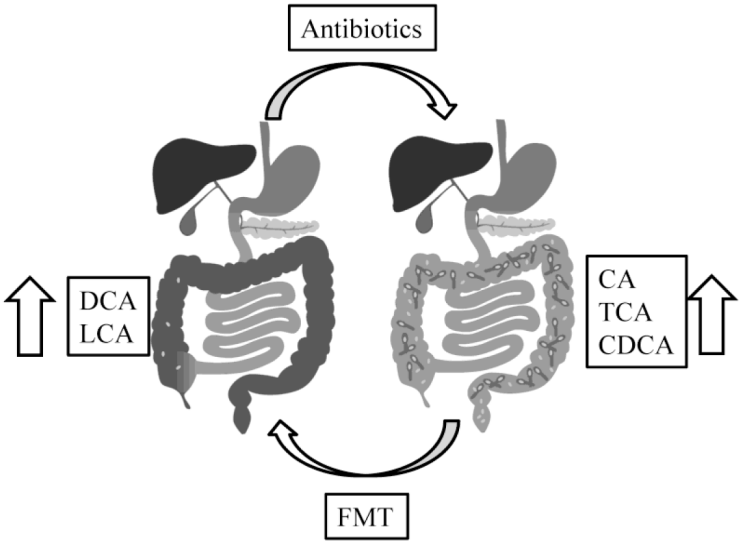

Figure 4.

Bile acid composition in the colon and *Cl. difficile* infection. Right panel: Following antibiotic treatment, the proportion of primary bile acids, including CA, TCA, and CDCA, increases in the colon and feces, along with susceptibility to CDI, possibly by allowing *Cl. difficile* spores to germinate into vegetative cells (dark blue). (Left panel: An FMT can reverse these effects, increasing the proportion of secondary bile acids, such as DCA and LCA, in the feces, and prevent recurrence of CDI, possibly by inhibiting germination of *Cl. difficile* spores (light blue). CA: cholic acid; TCA: taurocholic acid; CDCA: chenodeoxycholic acid; DCA: deoxycholic acid; LCA: lithocholic acid; FMT: fecal microbiota transplantation. Modified from[93].

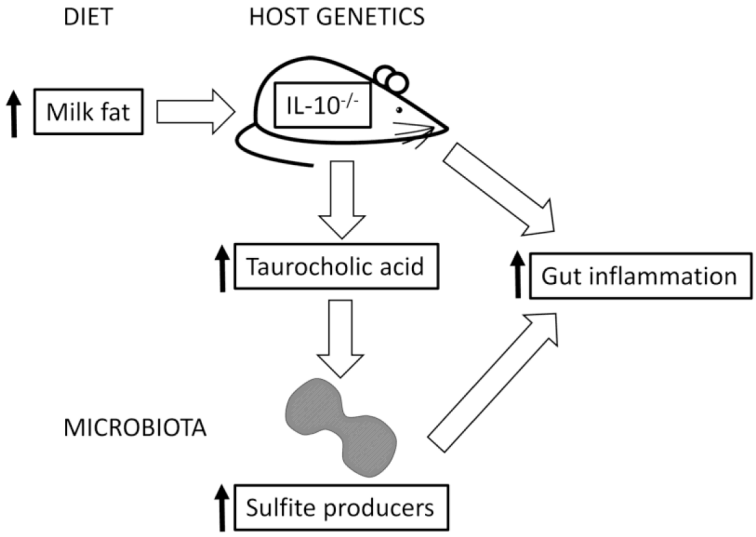

Figure 5.

Effects of diet, bile acids, host genetics, and intestinal bacteria on colitis. In genetically susceptible mice (IL-10-/-), a diet rich in milk fat increases taurocholic acid production, which in turn increases the abundance of sulfite-producing bacteria in the colon. Together these factors increase intestinal inflammation.

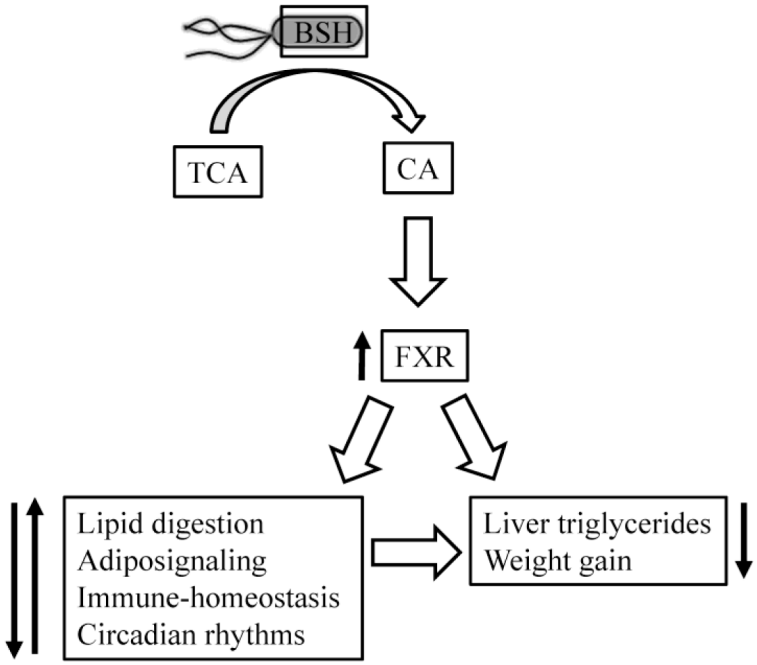

Figure 6.

Bacterial bile acid metabolism can influence metabolic syndrome. Expression of bile salt hydrolase (BSH) by *E. coli* increases deconjugation of taurocholic acid (TCA) to cholic acid (CA), which increases FXR activation to alter gene expression associated with lipid digestion, adiposignaling, immune-homeostasis, and circadian rhythms, ultimately leading to decreased liver triglycerides and decreased weight gain in the host.

Table 1

Regulatory effects of bile acids.

Effect	Bile acids*	Human Receptor(s)/Mechanism
Detoxification/excretion of xenobiotic compounds and LCA (Xie et al. 2001; Staudinger et al. 2001; Sonoda et al. 2002)	LCA	steroid and xenobiotic receptor (SXR) pregnane X receptor (PXR)
Detoxify LCA (Makishima et al. 2002; Ishizawa et al. 2008)	LCA & derivatives	vitamin D receptor (VDR)
Mobilize intracellular calcium (Voronina et al. 2002; Fischer et al. 2007; Nguyen and Bouscarel 2008)	(T)CDCA TCA (T)DCA TLCA TLCA-S TUDCA	phosphatidylinositol 3-kinase (PI3K) inositol triphosphate (IP_3) ryanodine receptors (RyRs)
Stimulatation/inhibition of cAMP synthesis (tissue-specific effects) (Bouscarel et al. 1995; Bouscarel et al. 1999; Chignard et al. 2003; Keitel et al. 2007; Nguyen and Bouscarel 2008)	(T)CDCA TCA (T)DCA TLCA (T)UDCA UCA	glucagon protein kinase C (PKC) G-protein coupled receptor (TGR5)
PKC activation and translocation (Beuers et al. 1999; Lau et al. 2005; Looby et al. 2005; Shah et al. 2005; Le et al. 2006; Nguyen and Bouscarel 2008)	(G)CDCA (T)CA DCA TLCA (T)UDCA	unknown†
Induction of expression of interleukin 1 (IL-1) and tumor necrosis factor alpha (TNFα) (Miyake et al. 2000)	CDCA	interaction with Kupffer cells
Production of reactive oxygen species (ROS)(Fang et al. 2004)	(T)DCA	induction of mitochondria

*Bile acids known to be associated with the effect. Conjugates in parenthesis indicate both the conjugated and deconjugated bile acid have effect.

†Unknown if reaction is direct or receptor-mediated.