Bile Acid and Gut Microbiota in Irritable Bowel Syndrome

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Gut microbiota and their metabolites like bile acid (BA) have been investigated as causes of irritable bowel syndrome (IBS) symptoms. Primary BAs are synthesized and conjugated in the liver and released into the duodenum. BA biotransformation by gut microbiota begins in the intestine and results in production of a broad range of secondary BAs. Deconjugation is considered the gateway reaction for further modification and is mediated by bile salt hydrolase, which is widely expressed by the gut microbiota. However, gut bacteria that convert primary BAs to secondary BAs belong to a limited number of species, mainly Clostridiales. Like gut microbiota modify BA profile, BAs can shape gut microbiota via direct and indirect actions. BAs have prosecretory effects and regulates gut motility. BAs can also affect gut sensitivity. Because of the vital role of the gut microbiota and BAs in gut function, their bidirectional relationship may contribute to the pathophysiology of IBS. Individuals with IBS have been reported to have altered microbial profiles and modified BA profiles. A significant increase in fecal primary BA and a corresponding decrease in secondary BA have been observed in IBS with predominant diarrhea. In addition, primary BA was positively correlated with IBS symptoms. In IBS with predominant diarrhea, bacteria with reduced abundance mainly belonged to the genera in Ruminococcaceae and exhibited a negative correlation with primary BAs. Integrating the analysis of the gut microbiota and BAs could better understanding of IBS pathophysiology. The gap in this field needs to be further filled in the future. (J Neurogastroenterol Motil 2022;28:549-561)

Key Words
Bile acids and salts; Feces; Irritable bowel syndrome; Intestines; Microbiota

Introduction

Irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder characterized by recurrent abdominal pain related to defeation and/or changes in the frequency or form of stool.1 According to the predominant stool form of the patients, IBS is classified as IBS with predominant diarrhea (IBS-D), IBS with predominant constipation (IBS-C), IBS with mixed bowel habits, and IBS unclassified.2 The mechanism of symptom generation is multifactorial, including altered motility of the gut, visceral hypersensitivity, central dysfunction, low-grade inflammation, increased intestinal permeability, disorders of the brain-gut axis, and altered gut microbiota.3,4 Over the last decade, microbiota and their metabolites have been paid attention to as the cause of IBS symptoms.5,6

Alteration of the gut microbiota has been reported in patients...
with IBS. The impact of the microbiome on disease etiology could occur via the actions of microbiota-derived metabolites, including bile acids (BAs). BAs are amphipathic molecules produced in the liver, which solubilize lipids into micelles for digestion and absorption. Approximately 95% of secreted BAs are reabsorbed in the terminal ileum, and the remaining BAs reach the colon, where they are metabolized by gut microbiota, forming a plethora of microbially modified secondary BAs. According to the characteristics of BA profiles, BAs can exert their variable effects on gut function, including fluid secretion, mucosal permeability, and bowel motility. BAs can also modify gut microbiota. The vital role of gut microbiota and BAs in regulating gut function, their bidirectional relationship may contribute to the pathophysiology of IBS. Indeed, individuals with IBS have been reported to have altered microbial profiles and modified BA profiles.

In this review, we describe BA synthesis and enterohepatic circulation (EHC), transformations of BAs, BA signaling mechanisms, and influences of BA on gut microbiota and functions, and summarize the clinical trials investigating alterations of gut microbiota and BA profiles in patients with IBS.

Bile Acid Synthesis and Enterohepatic Circulation

BAs are hydroxylated, amphipathic molecules synthesized in the peroxisomes of the liver from cholesterol through 2 major pathways. The newly synthesized BAs are termed primary BAs, including chenodeoxycholic acid (CDCA) and cholic acid (CA), to distinguish them from the products of microbial transformation, termed secondary BAs. Of the 2 major pathways, the classical pathway is more important in adult humans and produces both primary BAs favoring CA biosynthesis. The alternative pathway results in CDCA biosynthesis and involves less than 10% of BA synthesis. The enzyme cholesterol 7α-hydroxylase (CYP7A1) is the rate-limiting step in the classical pathway. 7α-hydroxy-4-cholesten-3-one (C4) is a downstream product of CYP7A1, reflecting the enzymatic activity of hepatic CYP7A1. Thus, measuring serum C4 is a simple test for analyzing hepatic BA synthesis, although it requires a standardized specimen collection time because of diurnal variability. The primary BAs (CDCA and CA) are conjugated to the hydrophilic amino acids, either glycine or taurine (GCDC/TCDC and GCA/TCA) in the liver. Humans preferentially use glycine for conjugation. The conjugation of BAs permits complete ionization of BAs, which increases their solubility and decreases their passive diffusion across the intestinal epithelial barrier, leading to high intraluminal concentrations that facilitate micellar solubilization of dietary lipids. These primary BAs are secreted into the gallbladder, where they are stored until the consumption of food. Ingestion of food triggers the release of cholecystokinin by enteroendocrine cells, which causes gallbladder contraction and the release of BAs into the duodenum. There, BAs facilitate the digestion and absorption of dietary lipids, fatty acids, cholesterol, fat-soluble vitamins, and other hydrophobic components of the diet via their surfactant properties, which emulsify fats into micelles. Approximately 95% of secreted BAs are reabsorbed in the terminal ileum and transported back into the liver via the EHC. The ileal apical Na+-dependent bile salt transporter (ASBT), which has a greater affinity for conjugated than non-conjugated BAs, actively reuptakes conjugated BAs. After the uptake of BAs by ASBT, ileal lipid-binding proteins bind to intracellular BAs, shutting them to the heterodimeric protein, organic solute transporter alpha-beta, which efficiently exports them to the portal circulation. Some passive diffusion across the gut epithelium can also occur for both conjugated and non-conjugated BAs. The remaining 5% of BAs that reach the colon are either reabsorbed via passive diffusion or lost in the feces (Figure).

In ileal enterocytes, BAs activate the nuclear receptor farnesoid X receptor (FXR), with CDCA being the most potent agonist (Table 1). FXR then induces the expression of fibroblast growth factor 19 (FGF19; rodent ortholog is FGF15). FGF19 is secreted from enterocytes into the portal circulation and activates the cell surface receptor, a complex of the β-klotho protein and FGF receptor 4 in hepatocytes, resulting in the downregulation of CYP7A1 and thereby reducing BA synthesis. As serum FGF19 decreases and is inversely related to serum C4 during BA malabsorption, it could be used for screening tests for malabsorption.

Microbial Transformations of Bile Acids

Small quantities of primary BAs that escape EHC reach the colon and undergo extensive microbial biotransformations, including deconjugation, 7α-dehydroxylation, oxidation/epimerization, and sulfation by the gut microbiota, to produce a broad range of secondary BAs. In fact, BA biotransformations begin in the small intestine and continue in the colon. Deconjugation of BAs changes their physiochemical properties, making them more lipophilic and susceptible to microbial biotransformation; thus, this is considered the gateway reaction for further modification. Cleavage of amino acid side chains on conjugated BAs is mediated by bile salt hydrolase (BSH) enzymes that are widely expressed by the gut micro-
The diversity of intestinal gram-positive bacteria, including *Clostridium*, *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Listeria*, contributes to amino acid hydrolysis. Some gram-negative bacteria such as *Bacteroides*, *Stenotrophomonas*, and *Brucella* are also capable of amino acid hydrolysis. Using metagenomic analysis, Jones et al. identified functional BSH among all major bacterial divisions and archaeal species in the gut. Most metagenomic BSH-active clones belonged to the phyla Firmicutes, *Bacteroidetes*, and *Actinobacteria*. In addition, *Methanobrevibacter smithii* and *Methanospira stadtmanae* encode proteins with high identity to bacterial BSH enzymes. This widespread distribution indicates that BSH is enriched in the human gut community. However, BSHs display different catalytic efficiencies and substrate specificities. The organization and regulation of genes encoding BSH differ between species and genera, and conjugations are important in substrate specificity. In a taxonomic analysis of BSHs among 11 different populations from 6 continents, 591 BSHs were identified over 117 genera from 12 phyla. Among the bacteria positive for BSH activity, more than half of the bacteria belonged to Firmicutes. Notably, significant variations in BSH distribution patterns were observed based on the geographic region but not sex, age, or body mass index. In addition, BSHs within genera showed a broad range of sequence dissimilarities, owing to the paralogs of BSHs in many strains. Thus, the genus-level patterns of BSH abundance did not reflect the functional variations, necessitating the reclas-
sification of BSHs. BSH activity and subsequent BA modification could significantly affect host physiology, including the regulation of cholesterol metabolism, energy, and inflammation homeostasis.\textsuperscript{3,7} When treating recurrent Clostridioides difficile infection with fecal microbiota transplant, the restoration of gut BSH functionality contributes to the efficacy of transplant.\textsuperscript{18} Because of the important roles of BSHs, gaps in the understanding of these enzymes require further research.

Once deconjugated, free primary BAs are metabolized by the resident microbiota into free secondary BAs, such as deoxycholic acid (DCA), lithocholic acid (LCA), and ursodeoxycholic acid (UDCA) via 7α-dehydroxylation and oxidation/epimerization.\textsuperscript{3,9,61} CDCA is transformed into DCA and CA into LCA by 7α-dehydroxylation. Because DCA and LCA predominate in human feces, 7α-dehydroxylation is the most quantitatively important microbial transformation.\textsuperscript{41} Human intestinal bacteria capable of 7α-dehydroxylation belong to the genus Clostridium.\textsuperscript{52-64} Multiple \textit{bai} genes encoding proteins required for 7α-dehydroxylation have been characterized from \textit{Clostridium scindens}.\textsuperscript{41}

Epimerization of the 3-, 7-, and 12-hydroxy groups of BAs is carried out by hydroxysteroid dehydrogenase (HSDH) expressed by intestinal bacteria, which diversifies the chemistry of secondary BAs. Epimerization is a reversible change in stereochemistry from the α to β configuration (or vice versa) with the generation of a stable oxo-BA intermediate. While α-hydroxy BAs are amphipathic, both faces of BA are hydrophilic in β orientation.\textsuperscript{41} Epimerization requires 2 distinct steps: oxidation of the hydroxyl group by a position-specific HSDH, followed by the reduction of the hydroxyl group by another position-specific HSDH.\textsuperscript{3,9,61} CA can be epimerized to form ursoCA, 12-epiCA, or isoCA, and CDCA can be epimerized to form either UDCA (7β-hydroxy) or isoCDCA. Epimerization of UDCA to CDCA can also be carried out by 7β-HSDH.\textsuperscript{41} 3-oxoLCA and isoLCA produced by 3α-HSDH are known to suppress differentiation of T helper cells expressing IL-17A and may contribute to gut immune homeostasis.\textsuperscript{65} Although their enzyme characteristics vary, several intestinal microbes have been observed to produce HSDHs, including a small number of species of \textit{Clostridium}, \textit{Ruminococcus}, \textit{Bacteroides}, and \textit{Escherichia coli}.\textsuperscript{66-77}

Secondary BAs may undergo further modification including sulfation, imparting changes in their solubility, metabolism, excretion, and toxicity.\textsuperscript{3,7} Specifically, sulfated BAs are more rapidly excreted in the urine, and sulfated LCA is less efficiently reabsorbed in the intestine than non-sulfated.\textsuperscript{79} Sulfation of BAs may be associated with constipation.\textsuperscript{80} In a subset of children with functional constipation, dominant fecal BA was the 3-sulfate of CDCA. Such sulfation may abolish the secretory activity of CDCA and contribute to constipation. In an animal experiment, sulfation prevented secretion caused by di-α-hydroxy BAs (DCA and CDCA) in the colon.\textsuperscript{81} Notably, increased fecal sulfated BAs were also observed in patients with IBS-D compared with that in IBS-C.\textsuperscript{82} Further research that includes measurements of sulfotransferase and sulfatase activity is necessary.

### Bile Acids in Small Intestinal Bacterial Overgrowth

Small intestinal bacterial overgrowth (SIBO) is one manifestation of gut microbiome dysbiosis and is highly prevalent in IBS.\textsuperscript{82} Many bacteria in the small intestine have the capacity to metabolize BAs. Shindo et al\textsuperscript{83} isolated bacterial species from the jejunal fluid obtained from patients with progressive systemic sclerosis and positive \textsuperscript{14}CO₂ breath test. The isolated \textit{Bacteroides vulgatus}, \textit{Eubacterium lentum}, \textit{Enterococcus}, and \textit{Lactobacillus bifi dus} (except for \textit{E. coli} and \textit{Aerobacter aerogenes}) were capable of hydrolyzing conjugated BAs in ox gall. Similarly, higher unconjugated BAs from the upper gut aspirate have been observed in malabsorption syndrome patients with SIBO than those without SIBO.\textsuperscript{84} However, the amount of unconjugated BAs did not correlate with colony counts of isolated bacteria. This observation suggests the different BSH activity among the isolated bacteria. As unconjugated BAs are absorbed from the small intestine into the portal blood, elevated unconjugated serum BA levels have also been found in patients with SIBO.\textsuperscript{85} Because BA profiles in the small intestine are less affected by gut transit and ileal absorption, investigating the associations between the BA profile and gut microbiome using small bowel aspiration samples of individuals with SIBO could offer us opportunities to further fill the gap of knowledge (Figure).

### Bile Acid Signaling Mechanisms

Because of the variety of levels and types of BAs in the intestine, biliary tract, and liver, BAs have emerged as important regulators of epithelial physiology and pathophysiology.\textsuperscript{11,86} Since the discovery of BA receptors, there have been advances in understanding how BAs exert their effects.

G protein-coupled BA receptor 1, also called Takeda G protein-coupled receptor 5 (TGR5), is responsive to BAs as a cell surface receptor.\textsuperscript{87,84} TGR5 is a member of the G protein-coupled receptor family, which stimulates cAMP synthesis and activates protein kinase-A, leading to the expression of target genes.\textsuperscript{85} TGR5
is widely expressed throughout the body, including in the intestine, liver, biliary tract, and gallbladder. The functions of TGR5 are thought to be broader than just being a metabolic regulator of energy homeostasis, BA homeostasis, and glucose metabolism. Particularly notable is that the activation of TGR5 on the intestinal motor neurons by BAs regulates intestinal motility. Conjugated and unconjugated BAs bind to TGR5, with the secondary BAs LCA and DCA being most potent, followed by CDCA and CA.

Physiological concentrations of free and conjugated BAs activate the nuclear receptor FXR as ligands. The structure–activity relationship of BAs in activating FXR shows the order of potency of CDCA > DCA > LCA > CA. CDCA is an extremely effective activator of FXR, whereas CA is inactive. CA and conjugated BAs are hydrophilic compounds that do not readily cross cell membranes; instead, they are passively diffused or facilitated by BA transport proteins. FXR is widely expressed throughout the body and highly expressed in the liver, intestine, and kidneys. As aforementioned, the primary function of FXR activation by BAs is the feedback inhibition of BA synthesis through the downregulation of CYP7A1. In addition, FXR is important in metabolic homeostasis.

Pregnane X receptor (PXR) is another nuclear receptor that can be activated by BAs and is highly expressed in the liver and intestine. The role of PXR in the detoxication of xenobiotics and LCA is well known. PXR also contributes to maintaining intestinal immune homeostasis. In rats, CA feeding induced the significant expansion of proinflammatory microbial taxa to expand, and increased levels of BAs in the gut favor gram-negative bacteria, bile tolerance is a strain-specific trait, and tolerance of species cannot be generalized. For example, Listeria monocytogenes cholecystitis has been reported suggesting a very high level of bile resistance. However, decreased levels of BAs in the gut favor gram-negative bacteria allowing proinflammatory microbial taxa to expand, and increased BAs levels favor gram-positive bacteria of the Firmicutes, including bacteria that 7α-dehydroxylate primary BAs to toxic secondary BAs. In rats, CA feeding induced the significant expansion of DCA-producing bacteria, expanding phylum Firmicutes, class Clostridia, and genus Blautia.

Influence of Bile Acids on Gut Microbiota —

Like bacterial enzymes chemically modify BA profile, BAs can modify the gut microbiota. BAs are potent antimicrobials and play an important role in the innate immune defense within the intestine. As BAs act as detergents in the gut, they allow for the disruption of bacterial membranes, leading to the leakage of proton, potassium ion, and other cellular components and eventually cell death. The antimicrobial potency of DCA is greater than that of CA due to its hydrophobicity and detergent properties on bacterial membranes. When tested against Staphylococcus aureus, unconjugated BAs exhibit more potent antibacterial action than conjugated BAs. Because deconjugation by BSH makes the BAs more lipophilic, unconjugated BAs are likely to disrupt membranes and cause intracellular damage. On the other hand, conjugated BAs can have a more indirect action on the gut microbiota. Activation of FXR induces genes involved in enteroprotection and inhibits bacterial overgrowth and mucosal injury, resulting in the protection of the small intestine from bacterial invasion.

Gram-negative bacteria are thought to have a higher BA tolerance than Gram-positives. Salmonella, E. coli, and Campylobacter are very bile resistant and have been isolated from the gallbladder. Although gram-positive bacteria are more sensitive to the deleterious effects of bile than gram-negative bacteria, bile tolerance is a strain-specific trait, and tolerance of species cannot be generalized.

Influence of Bile Acids on Gut Functions —

Generally, BAs induce colonic fluid secretion at high levels. However, there is a marked structural specificity for BA-induced secretion, and the α-dihydroxy BAs CDCA and DCA have prosecretory effects. The trihydroxy BA (CA) does not have prosecretory effects, and UDCA (7β-OH epimer of CDCA) has antisecretory effects. The conjugation status of BAs is also an important determining factor of their secretory effects. Because conjugated BAs are hydrophilic and need to be more lipophilic to cross the cell membrane, they do not have secretory effects. However, conjugated BAs can increase epithelial permeability at a relatively high concentration, which allows them to gain access to regions where they can exert their secretory effects. In contrast to prosecretory effects at pathophysiological concentrations, lower
In another human infusion study, CDCA was more strongly associated with gut motility. In humans, rectal CDCA infusion induces propagating pressure waves arising in the proximal colon. In another human infusion study, CDCA was more strongly associated with a higher colonic motility index than TCA, which contrasts with the animal (rabbit) results obtained in the same study. In an in vitro study, DCA increased isolated human colon motility, whereas CDCA and CA did not. The mechanism of action of DCA on smooth muscle activity was revealed as a local neuronal phenomenon in the rabbit colon in vitro. However, inhibitory actions of BAs on colon motility have also been shown in animal studies. Luminal bile from the gallbladder and conjugated primary BA (TCA and TCDCA) inhibit contractions of the intestine (isolated rabbit terminal ileal segment and isolated guinea pig ileum smooth muscle strips). A mouse intestine study demonstrated that DCA inhibits intestinal motility by activating TGR5 on inhibitory motor neurons to release nitric oxide, whereas the effects of UDCA and TDCA were not significant. However, contractile inhibition of in vitro colon tissue in particular muscle strips does not indicate decreased gut motility, because peristalsis requires the both contraction and relaxation of gut muscles. In a mouse study, DCA reduced the contractility of colonic longitudinal muscles but could stimulate the ascending contraction and descending relaxation components of the peristaltic reflex of the flat sheet preparation of the proximal colon. Furthermore, oral administration of CDCA improved bowel function in patients with either IBS-C or chronic constipation. BAs can also affect gut sensitivity. Rectal infusion of DCA and CDCA at physiological concentrations reduces rectal sensory thresholds. The mechanism of visceral hypersensitivity induced by BAs has been investigated in animal studies. BAs stimulate the release of nerve growth factor from mucosal mast cells through the activation of FXR, resulting in the activation of transient receptor potential vanilloid 1. TGR5 agonists, including DCA, also activate subsets of colonic sensory neurons and evoke colonic different mechanical hypersensitivity via a transient receptor potential ankyrin A1-dependent mechanism.

### Altered Bile Acid Profile and Gut Microbiota in Patients With Irritable Bowel Syndrome

In several clinical studies, the BA profiles of patients with IBS and those of healthy controls (HC) differ (Table 2). The level of total fecal BA was higher in IBS-D patients than that in HC, according to 3 studies of Asian groups. On the other hand, Western studies demonstrated no differences in the level of total fecal BA between IBS-D groups and HC. Most studies had measured total BA excretion in a single stool, which might be acceptable but is not ideal. When stool samples were collected over 48 hours, the level of total fecal BA was higher in IBS-D group than in IBS-C group, but not in HC. In addition, total fecal BA correlated with stool weight. However, because total fecal BA was determined from total 3g hydroxy BAs, some subgroups of BAs could have been missed. Taken together, the levels of fecal total BAs in the IBS-D group showed an increasing tendency compared with the levels in IBS-C group or HC. Notably, a systematic review showed that 32% of patients with symptoms consistent with IBS-D had moderate BA malabsorption. These observations suggest that BAs play an important role in regulating colonic fluid levels. In addition to the regulation of fluid transport, BAs can exert their effects on gut motility. In humans, rectal CDCA infusion induces propagating pressure waves arising in the proximal colon. In another human infusion study, CDCA was more strongly associated with a higher colonic motility index than TCA, which contrasts with the animal (rabbit) results obtained in the same study. In an in vitro study, DCA increased isolated human colon motility, whereas CDCA and CA did not. The mechanism of action of DCA on smooth muscle activity was revealed as a local neuronal phenomenon in the rabbit colon in vitro. However, inhibitory actions of BAs on colon motility have also been shown in animal studies. Luminal bile from the gallbladder and conjugated primary BA (TCA and TCDCA) inhibit contractions of the intestine (isolated rabbit terminal ileal segment and isolated guinea pig ileum smooth muscle strips). A mouse intestine study demonstrated that DCA inhibits intestinal motility by activating TGR5 on inhibitory motor neurons to release nitric oxide, whereas the effects of UDCA and TDCA were not significant. However, contractile inhibition of in vitro colon tissue in particular muscle strips does not indicate decreased gut motility, because peristalsis requires the both contraction and relaxation of gut muscles. In a mouse study, DCA reduced the contractility of colonic longitudinal muscles but could stimulate the ascending contraction and descending relaxation components of the peristaltic reflex of the flat sheet preparation of the proximal colon. Furthermore, oral administration of CDCA improved bowel function in patients with either IBS-C or chronic constipation. BAs can also affect gut sensitivity. Rectal infusion of DCA and CDCA at physiological concentrations reduces rectal sensory thresholds. The mechanism of visceral hypersensitivity induced by BAs has been investigated in animal studies. BAs stimulate the release of nerve growth factor from mucosal mast cells through the activation of FXR, resulting in the activation of transient receptor potential vanilloid 1. TGR5 agonists, including DCA, also activate subsets of colonic sensory neurons and evoke colonic different mechanical hypersensitivity via a transient receptor potential ankyrin A1-dependent mechanism.

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Moreover, fecal primary BA percentage was positively correlated with the Bristol stool score and stool frequency, whereas secondary BA was negatively correlated with these parameters. Similarly, in other studies, primary BA was positively correlated with the Bristol stool score and defecation frequency, and secondary BA was negatively correlated with defecation frequency.

**Table 2. Summary of Clinical Studies Investigating the Bile Acid Profiles and Fecal Microbiota in Irritable Bowel Syndrome**

| Study               | Participants (n) | Fecal BA profile | Fecal microbiota |
|---------------------|------------------|-------------------|------------------|
| Wong et al (2012)   | HC (26), IBS-C (26), and IBS-D (26) | Higher total BA in IBS-D than in IBS-C but not than in HC | Total BA correlated with stool weight and fat |
| Duboc et al (2012)  | HC (18) and IBS-D (14) | Similar total BA in IBS-D and HC | Decreased the leptum and Bifidobacterium groups in IBS-D than in HC |
| Shin et al (2013)   | HC (30), IBS-C (30), and IBS-D or FD (31) | Increased total UBA in IBS-D than in IBS-C but not than in HC | Not investigated |
| Dior et al (2016)   | HC (15), IBS-C (15), and IBS-D (16) | No differences of total BAs among the three groups | No differences in the total fecal bacteria counts in the 3 groups |
| Zhao et al (2020)   | HC (89) and IBS-D (290) | Increased total BA in IBS-D than in HC | Increased relative counts of E. coli in IBS-D compared to HC |
| Wei et al (2020)    | HC (28) and IBS-D (53) | Increased total fecal BA in IBS-D than in HC | Increased relative counts of Bacteroides and Bifidobacterium in IBS-C compared to IBS-D and HC |
| Wei et al (2021)    | HC (32) and IBS-D (52) | Increased total fecal BA in IBS-D than in HC | Increased abundances of Clostridia bacteria, Bifidobacterium, Escherichia, and Bilophila and decreased abundances of Alstipes and Bacteroides in IBS-D with high fecal BA excretion |
| James et al (2021)  | HC (97), IBS-D (52), and IBS-C (24) | Increased CA in IBS-D than in HC but not in IBS-C | Positive correlation of the abundances of Clostridia genera and C. scindens species with total BAs and serum C4 |

BA, bile acid; HC, healthy control; IBS-C, constipation-predominant irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; PBA, primary bile acid; SBA, secondary bile acid; E. coli, Escherichia coli; FD, functional diarrhea; UBA, unconjugated bile acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; CA, cholic acid; 7-KDCA, 7-ketodeoxycholic acid; 12-KLCA, 12-ketolithocholic acid; C. scindens, Clostridium scindens; C4, 7α-hydroxy-4-cholesten-3-one; CBA, conjugated bile acid; GCA, glyco-CA.
cy. Two \( \alpha \)-dihydroxy BAs, CDCA and DCA, have prosecretory effects. Indeed, CDCA and DCA correlate with stool frequency and the score in IBS-D,140 and percentages of CDCA and DCA were lower in IBS-C than that in HC. 140,141 However, decreased secondary DCA in IBS-D was also observed.16 From other studies providing the absolute amounts of individual BAs, differences in DCA were not significant, but CDCA was 20 times to 30 times higher in IBS-D than in HC.136,138 Thus, the change in CDCA amount seems to predominantly exert an effect on the stool score and frequency in IBS-D. The relationship between fecal BA excretion, fecal BA profile, and colon transit appears to be very complex as being a cause and/or consequence. First, clarification is necessary regarding whether the altered BA profile is due to a dysfunction of microbial biotransformation related to gut dysbiosis or due to rapid gut transit, decreasing the time for gut microbiota to metabolize BAs in patients with IBS-D.

In addition, primary BA showed a positive correlation with abdominal pain in IBS-D.36 Wei et al.138 investigated the relationship between BAs and their receptors (TGR5 and VDR) in patients with IBS-D. They observed that the level of TGR5 immunoreactivity in rectosigmoid mucosal biopsies was higher in IBS-D than that in HC. Furthermore, the level of TGR5 was higher in patients with more severe or frequent abdominal pain and was positively associated with primary BAs and negatively associated with secondary BAs. Although no direct link between fecal BAs and abdominal pain has been demonstrated, BAs might contribute to the hypersensitivity of patients with IBS-D via increased TGR5 level in the colon. Increased TGR5 expression is thought to be a compensatory response to decreased levels of potent agonists, secondary BAs (LCA and DCA).

Importantly, an imbalance between primary and secondary BAs in IBS-D has been consistently reported. Because BAs are metabolized by the gut microbiota, dysbiosis could be a critical factor in the altered BA profiles observed in IBS-D patients. Duboc et al16 demonstrated the presence of gut dysbiosis and altered BA profiles in patients with IBS. They observed an increase in \( E. \) coli and a decrease in \( \text{Bifidobacterium} \) and \( \text{Clostridium leptum} \) in patients with IBS-D compared with that in HC. Although a decrease in the number of bacteria involved in BA transformation could lower the biotransformation of BAs, the results did not show a direct causal link. Thus, they performed an in vivo test to determine the ability of feces to deconjugate BAs in the following study.16 Deconjugation activity was decreased in IBS compared with that in HS and did not differ between IBS-D and IBS-C groups. The BA profiles in stool and blood were also similar between IBS-D and IBS-C. However, the IBS-D group showed an increase in \( E. \) coli compared with that in the HC group, and IBS-C showed an increase in \( \text{Bifidobacterium} \) and \( \text{Bacteroides} \) compared with that in the HC and IBS-D groups.

To determine whether the alteration of BA profiles is due to a dysfunction in biotransformation related to gut dysbiosis, establishing a direct link between the BA profile alteration and microbial variations is necessary. Wei et al.136 assessed the correlation between fecal BAs (CA, CDCA, DCA, LCA, and UDCA) and the gut microbiome in Chinese patients with IBS-D. At the genus level, nine genera were significantly less abundant, including the genera in Ruminococcaceae (\( \text{Anaerofilum} \), \( \text{Anaerotruncus} \), \( \text{Faecalibacterium} \), \( \text{Gemmiger} \), and \( \text{Oscillibacter} \)), in Lachnospiraceae (\( \text{Coprococcus} \)), in Porphyromonadaceae (\( \text{Odoribacter} \)), in Rikenellaceae (\( \text{Alitipes} \)), and in Synergistaceae (\( \text{Cloacibacillus} \)); 8 genera were more abundant, including \( \text{Escherichia/Shigella} \), \( \text{Enterococcus} \), \( \text{Streptococcus} \), \( \text{Rotthia} \), \( \text{Klebsiella} \), \( \text{Saccharibacteria genera incertae sedis} \), \( \text{ Fusobacterium} \), and \( \text{Veillonella} \). The 8 genera that were reduced in IBS-D, except for \( \text{Cloacibacillus} \), exhibited a negative correlation with primary BAs (CA and CDCA) and a positive correlation with secondary BAs (DCA, LCA, and UDCA). However, correlations cannot be equated to causal associations, and a longitudinal study is required to confirm these results.

### Other Conditions That Affect the Luminal Bile Acid Profile

Conditions other than microbial biotransformation are linked to changes in luminal BA characteristics. The BA pool in patients with cirrhosis is depleted due to decreased synthesis.144 The decrease in fecal BAs promotes the depletion of Firmicutes and expansion of proinflammatory pathogenic bacteria of Proteobacteria.111 Depletion of \( \alpha \)-dehydroxylation bacteria leads to an increased primary to secondary BA ratio in patients with cirrhosis. Because the gallbladder stores and releases primary BAs, cholecystectomy may affect BA homeostasis. Gallbladder removal may increase the formation and pool size of secondary BAs due to the increased exposure of primary BAs to bacterial biotransformation in the intestine. However, cholecystectomy does not lead to significant changes in the BA profile in the long term.145,146 Defects in the formation and transport of BA could affect the BA pool.147 Several inherited defects in enzymes, including CYP7A1, could be involved in BA synthesis. In multiple biosynthetic pathways, a single enzyme defect is usually not sufficient to block the production of all BAs. These rare genetic diseases are characterized by cholestasis, neurological disorders, and...
fat-soluble vitamin deficiency.\textsuperscript{148} Inherited transporter defects are also rare, and the spectrum ranges from benign conditions such as benign recurrent intrahepatic cholestasis to progressive familial intrahepatic cholestasis.\textsuperscript{149} Typically, the first presentation of progressive familial intrahepatic cholestasis is in early childhood, frequently followed by a severe course requiring liver transplantation before adulthood.\textsuperscript{150}

**Conclusion**

Diverse BA profiles can regulate gut functions in terms of fluid absorption and secretion, motility, and sensitivity. Elucidating the underlying mechanisms of action help clarify their contributions to the pathophysiology of IBS, especially in IBS-D. Because of the reciprocal relationship between altered BA profiles and dysbiosis in IBS, integrating their analysis seems necessary and could provide insights into the pathophysiology and treatment of IBS. However, few relevant studies have been conducted, and they involved only a small number of subjects, mostly patients with IBS-D. Furthermore, the observational and cross-sectional study designs did not show causal associations among altered BA profiles, gut dysbiosis, and bowel symptoms. Therefore, associations between the BA profile and gut microbiome require further investigation using several conditions and samples, for example, small bowel aspiration samples of patients with SIBO. In addition, large longitudinal and interventional studies are warranted to verify previous observations.

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