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Journal

JHEP Reports, 6(12)

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Publication Date

2024-12-01

DOI

10.1016/j.jhepr.2024.101188

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JHEP|Reports

Fecal deoxycholic acid associates with diet, intestinal microbes, and total bilirubin in primary sclerosing cholangitis

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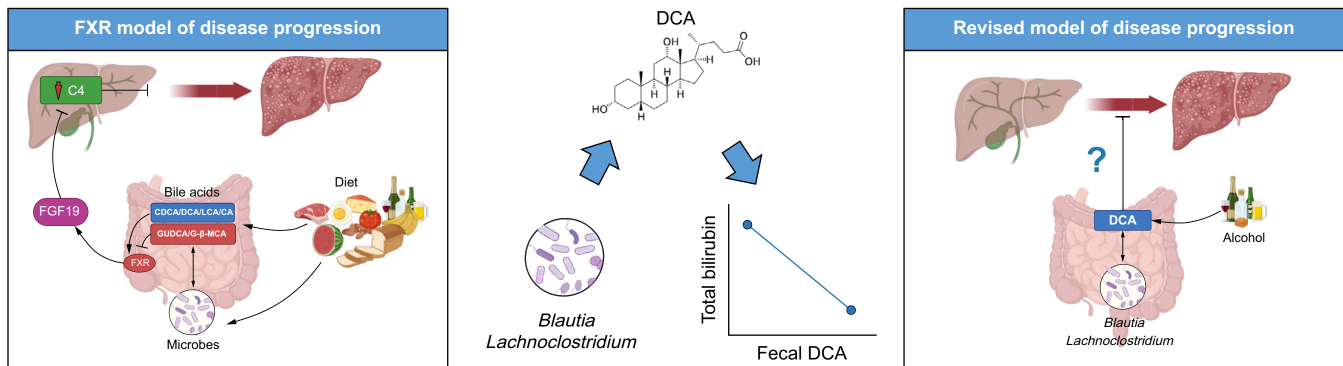
Graphical abstract



Highlights

- Primary sclerosing cholangitis is associated with intestinal dysbiosis.
- Fecal deoxycholic acid is decreased in primary sclerosing cholangitis.
- Increasing fecal deoxycholic acid associates with lower total bilirubin.
- *Blautia* and *Lachnoclostridium* associate with fecal deoxycholic acid.

Impact and implications

Primary sclerosing cholangitis (PSC) is a cholestatic liver disease with a direct interaction between the gut and the liver. In this study of patients with early-stage PSC, levels of fecal deoxycholic acid correlated with serum total bilirubin, a marker of clinical outcomes. In addition, *Blautia* and *Lachnoclostridium* were associated with fecal deoxycholic acid suggesting an interaction between these gut bacteria, fecal bile acids, and disease progression. Future research to determine the underlying mechanisms of these associations may lead to novel targets to prevent PSC disease progression.

https://doi.org/10.1016/j.jhepr.2024.101188
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JHEP|Reports

Fecal deoxycholic acid associates with diet, intestinal microbes, and total bilirubin in primary sclerosing cholangitis

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JHEP Reports **2024**. vol. 6 | 1–10

Background & Aims: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease with a strong association with inflammatory bowel disease and variable disease progression. We aimed to gain insights into the role of fecal bile acids (BA) on disease progression by determining the relationships between fecal BA, diet, and gut microbes, with markers of disease progression, BA synthesis, and farnesoid X receptor (FXR) activity.

Methods: BA levels in serum and stool, dietary intake, and markers of BA synthesis, and FXR activity were measured in 26 patients with early stage, large duct PSC. Fecal microbiota were quantified by 16S rRNA gene sequencing.

Results: Compared with controls, fecal unconjugated deoxycholic acid (DCA) levels were lower in patients with PSC (p_{adj} = 0.04). Alcohol intake and the abundance of *Blautia* and *Lachnoclostridium* were associated with greater fecal DCA levels in patients with PSC after adjusting for inflammatory bowel disease and treatment with ursodeoxycholic acid. Fecal DCA levels were negatively associated with total bilirubin levels in patients with PSC (p = 0.006) suggesting a protective role. However, fecal DCA was associated with greater serum levels of 7α-hydroxy-4-cholesten-3-one, a marker of BA synthesis, and was not associated with fibroblast growth factor 19, a marker of intestinal FXR activity.

Conclusions: Alcohol intake, *Blautia* and *Lachnoclostridium* abundance was associated with increased fecal DCA levels, which in turn seemed to have had a protective effect in patients with early-stage PSC. However, this effect was not mediated by BA synthesis or FXR activation.

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Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease notable for its progressive biliary inflammation and fibrosis and its association with inflammatory bowel diseases (IBD).[1] PSC can lead to cirrhosis, liver failure, and the need for liver transplantation, but disease progression is variable among individuals, with a median transplant-free survival from time of diagnosis reported to be from 9.7 to 20.6 years.[2] The underlying causes of this variable disease progression are not understood. Although genetic susceptibility to PSC has largely been linked to autoimmune-related loci, in a genome wide search for modifying genes, only a single genetic locus was associated with risk of liver transplant or death.[3] Similarly, environmental factors, including diet have been linked to susceptibility to PSC, but their contribution to disease progression has not been elucidated.[4]

Like other cholestatic liver diseases, bile acids (BA) play a critical role in secondary liver injury and progression of PSC,[5,6] making them a target for PSC therapies. The two primary BA in humans, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized and conjugated in the liver with glycine, taurine, and to a lesser degree glucuronic acid.[7–9] Within the intestines, BA can alter the microbial community by farnesoid X receptor (FXR)-induced expression of genes such as *iNOS* and *IL18*[10,11] and by direct detergent activities of BA limiting the growth of specific microbes. Conversely, bacteria that express bile salt hydrolase (BSH) deconjugate BA, weakening the BA detergent properties, limiting BA intestinal absorption, and allowing deconjugated BA to undergo biotransformation by other bacteria into secondary BA, including deoxycholic acid (DCA), ursodeoxycholic acid (UDCA), and lithocholic acid (LCA).[12] In addition, the fecal BA pool may be shaped by diet with dietary intake of fat, animal protein, and alcohol leading to increases in fecal secondary BA.[13–15] Further, IBD is associated with increases in primary and decreases in secondary BA.[16,17]

Fecal BA act as important molecules that are central to BA homeostasis through FXR signaling in the ileum and also affect immune responses,[18,19] and metabolic processes.[20] The two most potent FXR agonists are CDCA and DCA.[21] Activation of FXR in the ileum induces expression of fibroblast growth factor 19 (FGF19), which downregulates CYP7A1, leading to reduced conversion of cholesterol to CA and CDCA.[22]

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https://doi.org/10.1016/j.jhepr.2024.101188

The objective of the current study was to elucidate the complex relationships between fecal BA, intestinal microbes, and diet using alkaline phosphatase (ALP) and total bilirubin as markers of PSC disease progression. We measured fecal microbes and BA and assessed dietary intake in a well-defined cohort of patients with early-stage PSC and identified associations of markers of clinical outcomes with fecal DCA linked to intestinal microbes and dietary factors.

Patients and methods

Patients

Patients with a diagnosis of large duct PSC in accordance with AASLD and EASL guidelines were enrolled.[1,23] Exclusion criteria included other concomitant liver disease, hepatic decompensation, current smoking, history of liver transplant or current listing for liver transplantation, history of colectomy, use of antibiotics within 6 months of stool collection, and current use of immunomodulators including corticosteroids, azathioprine, 6-mercaptopurine, mycophenolate mofetil, tacrolimus, or biologics including anti-TNF and anti-integrin therapies. Fecal samples for controls were obtained from overweight but otherwise healthy individuals who had participated in a previous clinical trial and had received placebo.[24] All patients provided written informed consent. The study was approved by the institutional review board and was conducted in accordance with the principles of the Declarations of Helsinki and Istanbul.

Food frequency questionnaires

All patients completed a self-administered Harvard Willett Food Frequency Questionnaire (FFQ) (2007 version), which is a validated semi-quantitative tool to estimate the daily intake of macro- and micronutrients.[25] Sulfur microbial diet scores were calculated as previously published based upon the FFQ findings.

Serum measurements

Blood samples were collected at the time of stool collection. Serum was sent for standard liver biochemistries, 7α-hydroxy-4-cholesten-3-one (C4) by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) (Metabolon, Morrisville, NC) and FGF19 was measured by a single-plex bead assay (Eve Technologies, Calgary, Canada).

Stool collection

Whole stool samples were collected in sterile test tubes and stored at -80 °C.

Bile acid measurements

BA measurements of stool and serum specimens from patients with PSC were performed by LCMS with a liquid-liquid extraction with appropriate internal standards at the UC Davis West Coast Metabolomics Center. Fecal BA from the patient samples and from controls were measured by similar methods as previously reported.[27] There was strong correlation (–r = 0.91, p <0.001) between measurements by site. Details are provided in the Supplementary Materials.

Bile salt hydrolase assay

The BSH assay was adapted from a previously reported method.[28] Briefly, fecal homogenates (5 μg proteins/reaction) were suspended in 20 mM HEPES-50 mM NaCl assay buffer pH 6.0 and incubated (5 μg proteins/reaction) at 37 °C for 4 h with 100 μM of glycol-DCA (GDCA). All analytes were quantified by LC-MS/MS using an API3200 instrument (Applied Biosystems, Concord, ON, Canada). The limit of quantification of the LC-MS/MS method used for these assays was 50 nM, meaning that the BSH assays was able to detect as low as 0.05% of conjugated BA conversion into unconjugated ones. The percentages of conversion were calculated using molar amount of unconjugated-DCA produced by the enzymatic reaction (endogenous values are subtracted) divided by the initial amount of substrate. Details are provided in the Supplementary Materials.

DNA extraction and 16S rRNA gene amplicon DNA sequencing

DNA was extracted from 200 mg frozen stool and 16S rRNA amplified as described previously.[29,30] PCR products were pooled for library construction and sequencing on an Ion Chef/S5 system (Thermo Fisher Scientific, Waltham, MA, USA) as previously reported.[29] Quantitative Insights Into Microbial Ecology 2 (QIIME2) v.2022.2 was used for 16S rRNA gene bacterial community diversity analysis. Amplicon sequence variants (ASVs) were generated using DADA2. Shannon Evenness, Observed Features, Simpson and Fisher alpha diversity indices were calculated using the phyloseq package v.1.40.[31] Principal coordinates analyses (PCoA) were used to visualize dissimilarities and to examine the distance matrices corresponding to Bray-Curtis, Unweighted and Weighted Unifrac metrics for beta diversity using the vegan package v.2.6.[32] For taxonomy assignment, the QIIME2 Naïve Bayes trained classifier on the SILVA 138 database clustered at 99% for the V3-V4 regions was used. Core ASVs, defined as ASVs shared in at least 50% of all samples, were then classified using The Basic Local Alignment Search Tool (BLAST).[33] Further details are provided in the Supplementary Materials. 16S rRNA sequence data are available in the European Nucleotide Archive (ENA) under Accession No. PRJEB60105.

PRISM data

Publicly available data from a prior study of the Prospective Registry in IBD Study at MGH (PRISM) including 53 patients with ulcerative colitis (UC), 68 with Crohn's disease (CD), and 34 controls without IBD, and an independent cohort of 23 patients with UC, 20 with CD, and 22 population controls from the Netherlands were used for validation.[17] Fecal BA were quantified by non-targeted metabolomics. Metagenomic shotgun sequencing was used to measure microbial taxa abundance.

Statistical analysis

Continuous variables were expressed as median and IQR. Comparisons between groups were performed using the Kruskal–Wallis rank sum test or Mann–Whitney where appropriate. Bonferroni correction for multiple comparisons was employed where appropriate. Spearman's correlation coefficient was used to measure the correlation between fecal BA

with serum alkaline phosphatase (ALP) and total bilirubin and between the most abundant taxa with fecal and serum BA concentrations. Generalized linear models were constructed from patient-level clinical and laboratory measures and fecal and serum bile acid measurements. All analyses were performed using SAS v.9.4 (SAS Institute, Cary, NC, USA), SPSS v.28 (IBM; Armonk, NY, USA), or Graphpad® v.9.3 (GraphPad Software, San Diego, CA, USA).

For 16S rRNA analysis, Permutational Multivariate Analysis of Variance (PERMANOVA) was used to determine the significance of the PCoA results. Analysis of the Composition of Microbiomes (ANCOM) was used to assess for differentially abundant taxa among treatments at the genus level. Diversity analyses and the correlation plots were visualized using R v. 4.2.0 packages phyloseq v. 1.40.0 and ggplot2 v. 3.3.6 (R Foundation for Statistical Computing, Vienna, Austria).[34] Significance level of p <0.05 was set for all analyses.

Results

Patient characteristics

A total of 26 patients with PSC were enrolled; median age was 53.8 years (Table 1). Fourteen (58.3%) patients were male, 17 (70.8%) had IBD (14 with UC and three with CD), and 11 (45.8%) were taking UDCA at a median dose of 12.6 mg/kg/day. Laboratory tests were consistent with a cohort of patients with early-stage PSC as reflected by median total bilirubin levels of 1.0 mg/dl and median albumin of 3.9 g/dl. Controls consisted of patients with metabolic syndrome without diabetes. Nineteen patients had a liver stiffness measurement or liver biopsy within 12 months of sample collection among which 17 were consistent with F3 or less fibrosis and only two were consistent with cirrhosis. The control group had a median age of 37.9 (32.0–42.0) and 50.0% were male; controls were only included in the fecal BA and BSH activity measurements.

Table 1. Demographic and clinical characteristics of the study population.

Characteristic	Value
Age, years	53.8 (43.2–65.4)
Sex, male	14 (58.3%)
PSC duration, years	4.5 (1.5–8.0)
IBD status	
Ulcerative colitis, n (%)	14 (58.3%)
Crohn's disease, n (%)	3 (12.5%)
None, n (%)	7 (29.2%)
IBD duration, years (n = 11)	8.0 (3.0–17.0)
UDCA, current use	11 (45.8%)
ALP, IU	174.5 (109.5–359.5)
ALP ≥1.5 × ULN	54.2
AST, IU	42.0 (34.0–61.0)
ALT, IU	63.0 (34.0–72.0)
Total bilirubin, mg/dl	1.0 (0.7–1.2)
Total bilirubin ≥1.0 mg/dl	14 (58.3%)
Albumin, g/dl	3.9 (3.8–4.1)
Saturated fat, g/day	25.3 (18.2–31.6)
Monosaturated fat, g/day	25.7 (20.2–40.3)
Polyunsaturated fat, g/day	13.6 (10.1–22.5)
Protein, g/day	80.5 (59.3–97.8)
Fiber, g/day	21.2 (17.1–36.1)
Alcohol, g/day	0.56 (0–6.9)

Categorical values are expressed as n (%); Continuous values are expressed as median (IQR). Saturated fat, monosaturated fat, and polyunsaturated fat median values were calculated based on a sample size of N = 22. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IBD, inflammatory bowel disease; PSC, primary sclerosing cholangitis; UDCA, ursodeoxycholic acid.

Fecal DCA was decreased in PSC compared with controls

To determine the impact of PSC on fecal BA level, we compared fecal BA profiles from patients with PSC and controls. Unconjugated fecal DCA but not tauro- or glyco-conjugated DCA was lower in patients with PSC compared with controls (3,684 ± 2,845 *vs.* M7,512 ± 3,684 pmol/mg, p = 0.009, p_{adj} = 0.04) (Fig. 1). No differences were found in fecal CA, CDCA, LCA, hyocholic acid, UDCA, or muricholic acids levels (Fig. 1 and Fig. S1 and S2). Among patients with PSC, there were no differences in fecal DCA levels between those with and without IBD (p = 0.10).

Fecal DCA is negatively associated with total bilirubin levels in PSC

To explore the relationship between BA with markers of PSC disease progression, we examined the correlations between fecal and serum BA with serum levels of ALP and total bilirubin (Fig. 2A). Several serum levels of primary BA including taurine and glycine conjugated CA, CDCA, DCA, as well as taurine conjugated LCA positively correlated with serum levels of ALP and total bilirubin, whereas serum levels of unconjugated DCA were negatively correlated with serum total bilirubin levels. Similarly, fecal unconjugated DCA was negatively associated with serum total bilirubin (Fig. 2B). No other fecal BA, including conjugated forms of DCA, correlated with serum levels of ALP or total bilirubin. Moreover, no correlation was found between any fecal BA and Mayo Risk score.

We further examined the relationship between fecal BA known to be FXR agonists or antagonists with serum ALP and total bilirubin adjusting for IBD status and UDCA use. Only fecal DCA was associated with serum total bilirubin with increasing fecal DCA associated with lower serum total bilirubin (p = 0.006) (Table 2). Fecal CA, CDCA, LCA, TUDCA, TαMCA, and TβMCA were not associated with serum total bilirubin. There were no associations between ALP and any of the fecal bile acid FXR ligands.

Effect of diet on BA and total bilirubin

Dietary constituents impact on fecal levels of DCA including dietary fat, protein, fiber, and alcohol were examined for associations with fecal DCA. The intake of dietary fat, protein, and fiber was consistent with a low-fat diet (Table 1). Twelve of the 24 participants reported not consuming alcohol, whereas 11 participants reported consuming 1.1–14.3 g/day and one participant reported consuming greater than 50 g/day. Alcohol intake, but not fats, protein, or fiber, was associated with higher fecal DCA levels (Table 3). In addition, alcohol intake was significantly associated with lower serum total bilirubin in patients with PSC. There was also a negative association between dietary fiber intake and serum total bilirubin, but not with fecal DCA. In addition, alcohol and fiber were negatively associated with total serum taurine conjugated BA (Table 3), but not with total fecal taurine conjugated BA (Table S1). No significant associations were found with the sulfur microbial diet score (Table 3), which incorporates food groups linked to

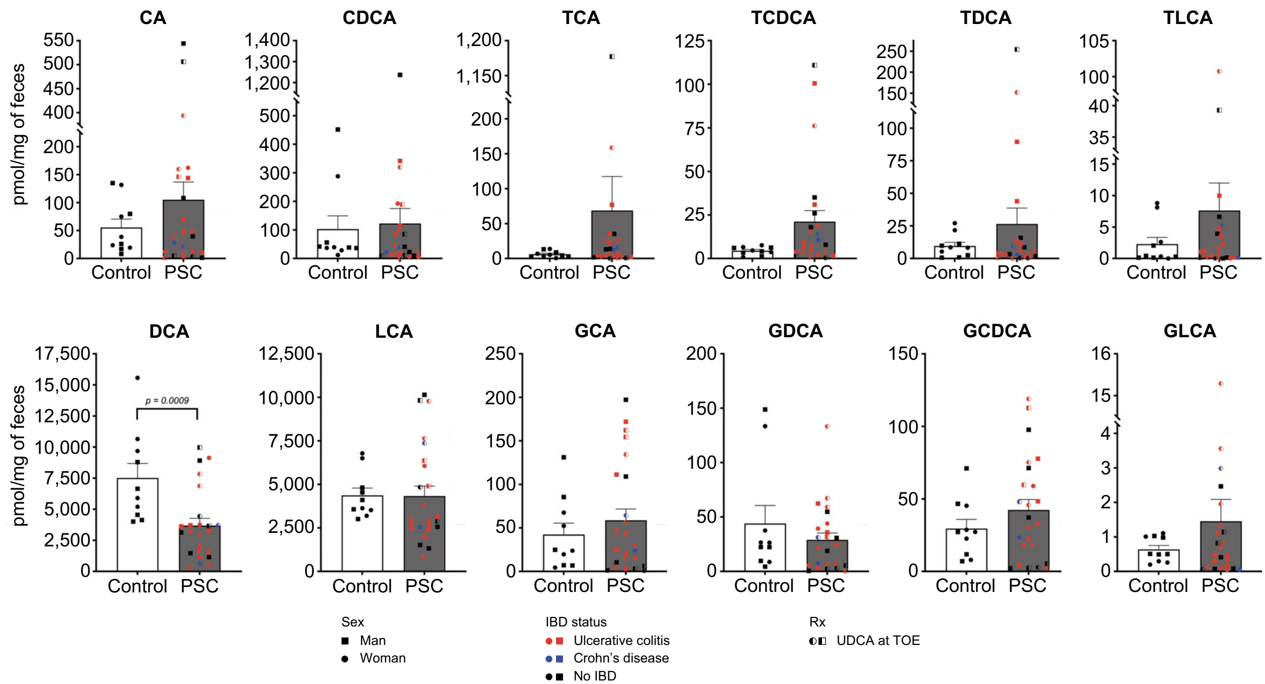

Fig. 1. Fecal bile acid levels in patients with primary sclerosing cholangitis compared with controls. Comparisons of individual fecal bile acid levels using the Mann–Whitney test with Bonferroni correction for multiple comparisons. Bars represent mean and standard deviations with individual values represented at circles (females) and squares (males). CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid, LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; TLCA, taurolithocholic acid; GCA, glycocholic acid; GDCA, glycochenodeoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; UDCA, ursodeoxycholic acid; TOE, time of enrollment.

	Fecal bile acid		Serum bile acid	
Bile acid	ALP	Total bilirubin	ALP	Total bilirubin
CA	-0.115	-0.013	0.199	-0.019
TCA	0.122	-0.149	0.614	0.644
GCA	0.042	0.044	0.665	0.61
CDCA	-0.119	0.147	0.133	0.022
TCDCA	0.264	0.317	0.594	0.602
GCDCA	-0.049	0.005	0.535	0.534
DCA	-0.118	-0.669	-0.067	-0.422
TDCA	0.056	-0.107	0.527	0.478
GDCA	-0.004	-0.128	0.403	0.192
LCA	-0.002	-0.303	0.043	0.022
TLCA	0.169	0.146	0.566	0.565
GLCA	0.071	0.111	0.291	0.076
TUDCA	-0.003	0.055	0.333	0.261
GUDCA	0.017	0.02	0.242	0.17
α-MCA	0.178	0.145	0.065	-0.09
T-α-MCA	0.04	-0.3	0.308	0.142
β-MCA	0.12	-0.106	-0.06	-0.001
T-β-MCA	0.037	-0.142	0.203	0.27
ω-MCA	0.056	-0.167	0.226	0.073
T-ω-MCA	-0.102	-0.082	0.32	0.341
TDHCA	-0.051	-0.149	0.458	0.574
GHDCA	-0.09	-0.2	0.202	0.079

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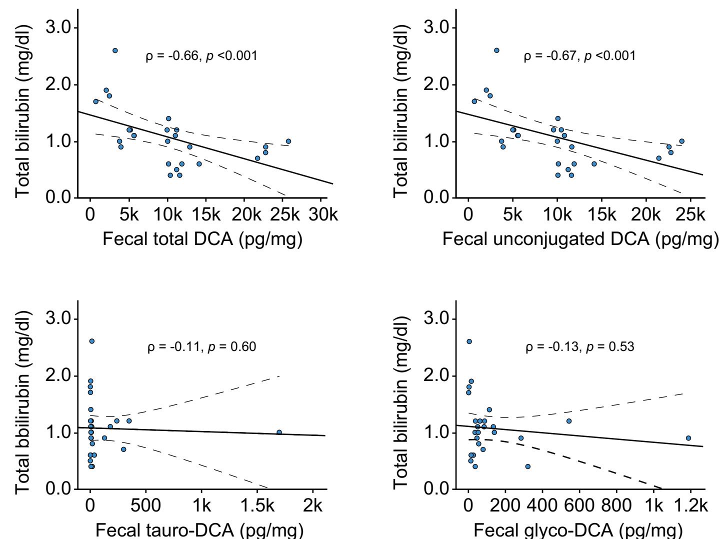


Fig. 2. Correlations between fecal and serum bile acids with serum levels of alkaline phosphatase (ALP) and total bilirubin. (A) Heat map of Spearman's rank correlation coefficients of fecal and serum bile acids with serum alkaline phosphatase and total bilirubin. Correlations with a p <0.01 are in bold and p <0.05 are underlined. (B) Scatter plots of fecal total, unconjugated, taurine conjugated, and glycine conjugated deoxycholic acid with serum total bilirubin. Each circle represents an individual with the solid line representing the linear regression and dotted lines the 95% confidence interval. Spearman rank correlation coefficients and p values are also shown. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycol-cholic acid; GCDCA, glyco-chenodeoxycholic acid; GDCA, glycol-deoxycholic acid; GHDCA, glyco-dehydrocholic acid; GLCA, glycol-lithocholic acid; GUDCA, glycol-ursodeoxycholic acid; LCA, lithocholic acid; T-α-MCA, tauro-α-muricholic acid; T-β-MCA, tauro-β-muricholic acid; T-ω-MCA, tauro-ω-muricholic acid; TCA, tauro-cholic acid; TCDCA, tauro-chenodeoxycholic acid; TDCA, tauro-deoxycholic acid; TDHCA, tauro-dehydrocholic acid; TLCA, tauro-lithocholic acid; TUDCA, tauro-ursodeoxycholic acid; α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid.

Table 2. Association of fecal bile acids with serum alkaline phosphatase and total bilirubin adjusted for IBD status and UDCA use.

	Alkaline phosphatase		Total bilirubin		Mayo risk score	
	Standardized β (95% CI)	p value	Standardized β (95% CI)	p value	Standardized β (95% CI)	p value
DCA	74.3 (-55.3, 203.8)	0.26	-0.45 (-0.76, -0.13)	0.006	-0.19 (-0.67, 0.29)	0.43
CA	65.7 (-73.6, 205.0)	0.36	0.24 (-0.10, 0.58)	0.16	-0.02 (-0.54, 0.51)	0.94
CDCA	-44.5 (-183.6, 94.6)	0.53	0.08 (-0.26, 0.42)	0.65	0.03 (-0.52, 0.58)	0.93
LCA	-27.5 (-218.7, 163.7)	0.78	-0.02 (-0.49, 0.44)	0.92	0.05 (-0.7345, 0.8359)	0.90
TUDCA	35.8 (-48.5, 120.2)	0.40	-0.00 (-0.21, 0.20)	0.96	0.06 (-0.2502, 0.3742)	0.70
TαMCA	-65.3 (-143.1, 12.5)	0.10	0.02 (-0.17, 0.21)	0.84	0.18 (-0.10, 0.47)	0.21
TβMCA	-58.8 (-135.0, 17.5)	0.13	-0.06 (-0.24, 0.13)	0.55	-0.0002 (-0.28, 0.28)	1.00

Generalized linear models were constructed from patient-level clinical and laboratory measures and fecal and serum bile acid measurements and adjusted for IBD status and ursodeoxycholic acid use. CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; TUDCA, tauro-ursodeoxycholic acid; TαMCA, tauro-α-muricholic acid; TβMCA, tauro-β-muricholic acid.

bacterial species involved in sulfur metabolism and has been associated with the risk of colorectal cancer.[26,35]

Gut microbiota associated with fecal BA

Bacterial composition in stool samples was assessed using 16S rRNA gene amplicon DNA sequencing and the observed bacterial alpha and beta diversity analyses were examined relative to sex, IBD status, serum ALP (<1.5 times upper limit of normal [ULN] *vs*. ≥1.5 × ULN), and serum total bilirubin (<1.0 mg/dl and ≥1.0 mg/dl). Bacterial alpha (Fig. S3) and beta (Fig. S4) diversity did not differ by sex, IBD status, ALP, or total bilirubin. Only the Simpson index for alpha diversity was significantly different by sex and ALP (p <0.05).

Consistent with these findings, the bacterial taxonomic distribution was similar between patients and was dominated by the phyla Bacillota (Firmicutes) and Bacteroidota (Bacteroidetes) (Fig. S5). *Lachnospiraceae*, *Bacteroidaceae*, *Ruminococcaceae* and *Prevotellaceae* were the four most prevalent families (Fig. S6). Among the genera, *Bacteroides*, *Blautia*, *Faecalibacterium*, and *Roseburia* were present in more than 50% of the samples (Fig. 3A). *Klebsiella* was present at a very small abundance (0.056% of the total features identified in the dataset). There were no differentially abundant taxa when the taxa were categorized based on sex, IBD status, ALP activity, or total bilirubin (p >0.05).

To investigate potential relationships between bacterial abundance and BA, we examined correlations between fecal DCA and total BA (TBA) with the predominant 16 genera (*Bacteroides*, *Blautia, Prevotella, Faecalibacterium, Roseburia, Parabacteroides, Agathobacter, Subdoligranulum, Streptococcus, Lachnoclostridium, Dorea, Alistipes, Eubacterium hallii* group, *Lachnospiraceae* NK4A136 group, *Bifidobacterium, and Ruminococcus torques* group). *Blautia* was positively correlated with both fecal DCA and TBA (p <0.05) (Fig. 3B and Figs S7 and S8). Additionally, the proportions of *Lachnoclostridium* and *Streptococcus* were positively and negatively correlated with fecal DCA (p <0.01), respectively (Fig. 3B). Furthermore, we examined the relationships between these microbes and biomarkers for clinical outcomes including serum ALP, total bilirubin, tauro-cholic acid (TCA), and tauro-chenodeoxycholic acid (TCDCA) but no significant associations were found (Figs S9–S12).

To determine whether the associations between *Blautia, Lachnoclostridium,* and *Streptococcus* were consistent with changes observed in patients with IBD, we analyzed publicly available data from a cohort of patients with IBD and non-IBD controls.[17] As previously reported, fecal DCA as a fraction of total fecal BA was significantly lower in patients with UC and CD compared with non-IBD controls (Fig. S13). In addition, among patients with IBD, fecal DCA was strongly correlated with *Lachnoclostridium* and to a lesser extent with *Blautia* and negatively correlated with *Streptococcus* whereas no correlations were found among non-IBD controls (Table 4 and Fig. 3C). Notably, among patients with IBD, these associations were most pronounced in patients with CD.

Fecal BSH activity

Blautia is one of several genera that express BSH, which is required for the transformation of primary BA to secondary BA including DCA. To investigate the potential role of BSH in our findings, we measured the BSH activity of fecal extracts of patients with PSC and controls. BSH activity did not differ between patients with PSC and controls (Fig. S14). In addition,

Table 3. Associations of dietary intake with fecal DCA and serum total bilirubin.

	Fecal DCA		Serum total bilirubin		Total serum taurine conjugates	
	Unstandardized β	p value	Unstandardized β	p value	Unstandardized β	p value
Multivariate model of dietary constituents						
Saturated fat	-108.8 (-551.2, 333.6)	0.63	0.03 (0.00, 0.06)	0.062	79.0 (-20.1, 178.2)	0.12
Monounsaturated fat	90.1 (-435.4, 615.6)	0.74	0.01 (0.03, 0.04)	0.69	-61.0 (-178.8, 56.7)	0.31
Polyunsaturated fat	-204.9 (-1,021.2, 611.3)	0.62	0.00 (-0.06, 0.05)	0.87	104.1 (-78.8, 287.0)	0.26
Protein	-90.4 (-283.5, 102.7)	0.36	0.01 (0.00, 0.02)	0.19	30.8 (-12.5, 74.0)	0.16
Fiber	73.5 (-261.1, 408.2)	0.67	-0.02 (-0.05, 0.00)	0.037	-88.4 (-163.4, -13.5)	0.02
Alcohol	449.6 (28.3, 870.8)	0.04	-0.07 (-0.10, -0.04)	<0.0001	-163.2 (-257.5, -68.8)	0.0007
Univariate model of sulfur microbial diet						
Sulfur microbial diet score	-1,410.2 (-3,054.4, 234.0)	0.09	0.08 (-0.07, 0.22)	0.31	129.7 (-321.3, 580.7)	0.57

Generalized linear models were constructed from patient-level nutrient intake based upon food frequency questionnaires and measures of fecal DCA, serum total bilirubin, and total taurine conjugated serum bile acids. The sulfur microbial diet score was calculated as previously described.[35] All analyses were adjusted for IBD status and ursodeoxycholic acid use. DCA, deoxycholic acid.

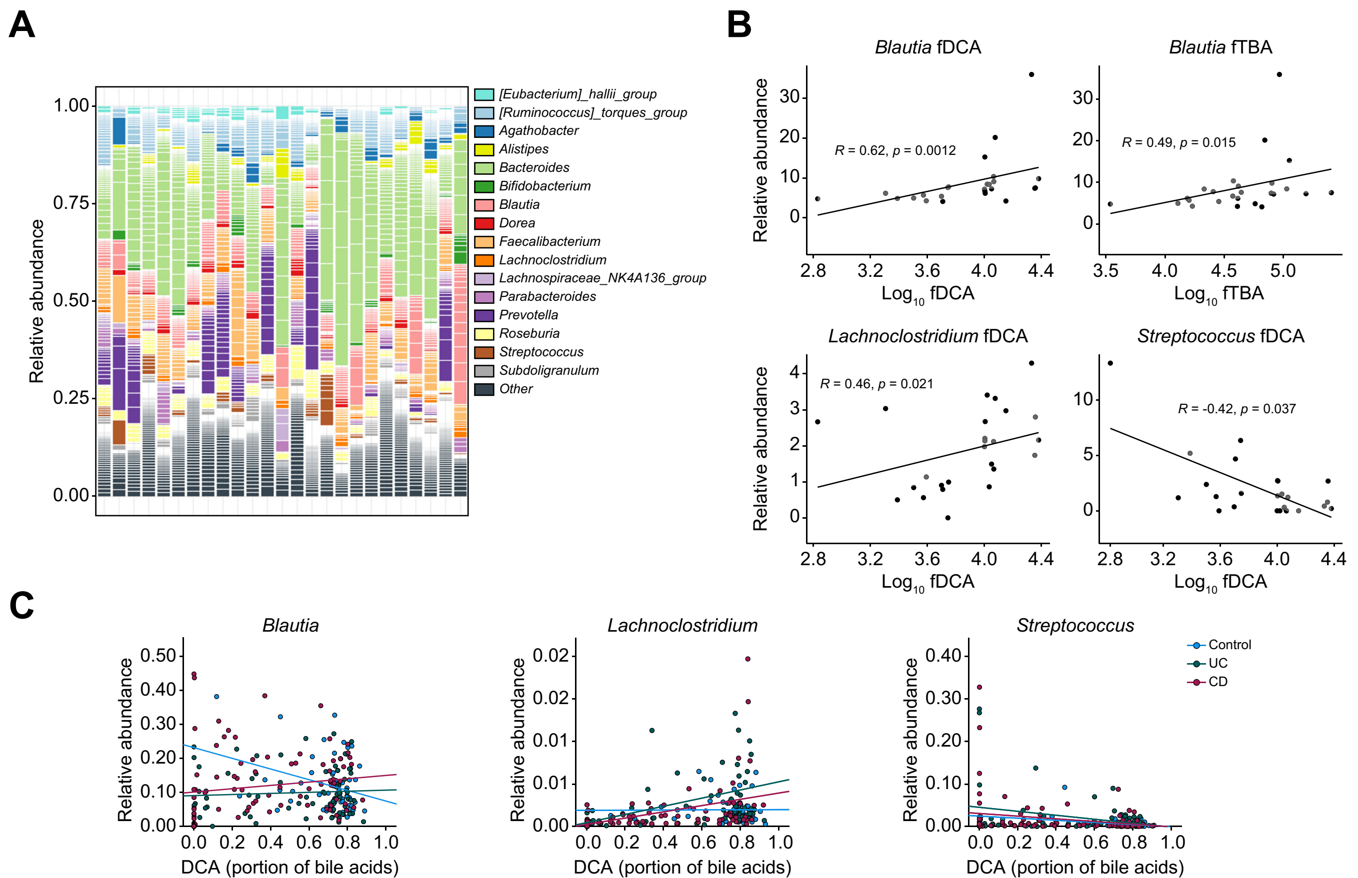


Fig. 3. Relative abundance at level of genera (A) and correlations (B and C) of abundance with fecal deoxycholic acid (fDCA) and total bile acids (fTBA) concentrations. Abundance (A) was calculated based on 16S rRNA sequencing of stool collected from patients with PSC and correlations fecal DCA and TBA (B). Lines represent liner regression with significant correlations identified using the Pearson test (p <0.05). (C) Correlations of relative abundance of *Blautia, Lachnoclostridium,* and *Streptococcus* with fecal deoxycholic acid from the PRISM study of patients with UC, CD, or no IBD.[17] Lines represent the linear regression for each group. CD, Crohn's disease; fDCA, fecal deoxycholic acid; fTBA, fecal total bile acids; IBD, inflammatory bowel disease; UC, ulcerative colitis.

BSH activity did not correlate with *Blautia* abundance or fecal DCA (Table S2). Further, we found no correlation of BSH activity with ratios of fecal GDCA/DCA, total glyco-BA/total BA, or primary BA/secondary BA.

Bile acids correlations with serum FGF19 and C4 levels

Finally, we examined correlations between BA with downstream FXR signaling, specifically FGF19 and C4 (Fig. 4). Serum levels of FGF19 correlated with serum levels of TCDCA and GUDCA. Serum C4 levels were strongly correlated with several serum BA including CDCA, DCA, and ω-MCA. Fecal levels of the FXR agonist CDCA correlated with serum FGF19 levels, but so did levels following treatment to FXR antagonist tauro-β-MCA. After adjusting for IBD and UDCA use, fecal DCA associated with increasing serum C4 (p = 0.02) but no association with FGF19 was found (p = 0.75).

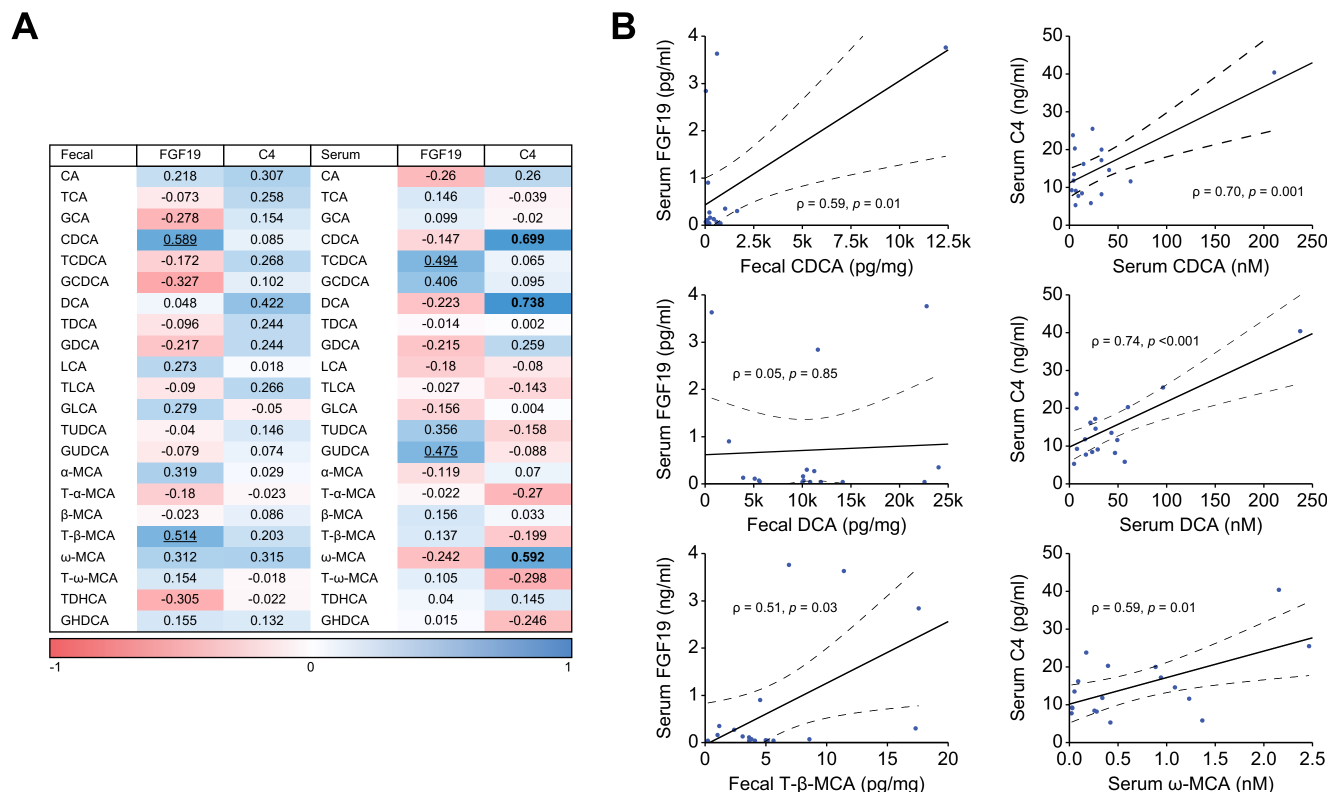
Discussion

In the present study, we demonstrated that fecal DCA was lower in patients with early stage PSC compared with controls and that fecal DCA was inversely associated with total bilirubin, a major predictor of clinical outcomes.[36–39] In addition, we found that the abundance of *Blautia* and *Lachnoclostridium* and dietary intake of alcohol were associated with increased fecal DCA. In contrast, *Streptococcus* abundance was associated with decreased fecal DCA levels. Similar associations with fecal

Table 4. Correlations of microbial abundance and fecal deoxycholic acid in patients with IBD and non-IBD controls from the PRISM cohort.

	DCA (fraction of total bile acids)			
	IBD (n = 164)	**UC (n = 76)**	**CD (n = 88)**	**Non-IBD (n = 56)**
Blautia	0.21 (0.007)	0.10 (0.39)	0.35 (<0.001)	-0.17 (0.21)
Lachnoclostridium	0.61 (<0.001)	0.51 (<0.001)	0.62 (<0.001)	-0.11 (0.42)
Streptococcus	-0.16 (0.03)	-0.15 (0.19)	-0.19 (0.07)	-0.13 (0.34)

Spearman correlation coefficients (p value) were calculated from data on microbial abundance of *Blautia, Lachnoclostridium,* and *Streptococcus* with fecal DCA levels from 164 patients with IBD (76 UC and 88 CD) and 56 people without IBD from the previously published PRISM cohort.[17] CD, Crohn's disease, DCA, deoxycholic acid; IBD, inflammatory bowel disease; UC, ulcerative colitis.



Fecal	FGF19	C4	Serum	FGF19	C4
CA	0.218	0.307	CA	-0.26	0.26
TCA	-0.073	0.258	TCA	0.146	-0.039
GCA	-0.278	0.154	GCA	0.099	-0.02
CDCA	<u>0.589</u>	0.085	CDCA	-0.147	**0.699**
TCDCA	-0.172	0.268	TCDCA	<u>0.494</u>	0.065
GCDCA	-0.327	0.102	GCDCA	0.406	0.095
DCA	0.048	0.422	DCA	-0.223	**0.738**
TDCA	-0.096	0.244	TDCA	-0.014	0.002
GDCA	-0.217	0.244	GDCA	-0.215	0.259
LCA	0.273	0.018	LCA	-0.18	-0.08
TLCA	-0.09	0.266	TLCA	-0.027	-0.143
GLCA	0.279	-0.05	GLCA	-0.156	0.004
TUDCA	-0.04	0.146	TUDCA	0.356	-0.158
GUDCA	-0.079	0.074	GUDCA	<u>0.475</u>	-0.088
α-MCA	0.319	0.029	α-MCA	-0.119	0.07
T-α-MCA	-0.18	-0.023	T-α-MCA	-0.022	-0.27
β-MCA	-0.023	0.086	β-MCA	0.156	0.033
T-β-MCA	<u>0.514</u>	0.203	T-β-MCA	0.137	-0.199
ω-MCA	0.312	0.315	ω-MCA	-0.242	**0.592**
T-ω-MCA	0.154	-0.018	T-ω-MCA	0.105	-0.298
TDHCA	-0.305	-0.022	TDHCA	0.04	0.145
GHDCA	0.155	0.132	GHDCA	0.015	-0.246

Fig. 4. Correlations between fecal and serum bile acids with serum levels FGF19 and C4. (A) Heat map of Spearman's rank correlation coefficients of fecal and serum bile acids with serum FGF19 and C4. Correlations with p <0.01 are in **bold** and p <0.05 are <u>underlined</u>. (B) Scatter plots of fecal and serum bile acids with serum FGF19 and C4. Each circle represents an individual with the solid line representing the linear regression and dotted lines the 95% confidence interval. Spearman rank correlation coefficients and p values are also shown. α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GCA, glycol-cholic acid; GCDCA, glyco-chenodeoxycholic acid; GDCA, glycol-deoxycholic acid; GHDCA, glyco-dehydrocholic acid; GLCA, glycol-lithocholic acid; GUDCA, glycol-ursodeoxycholic acid; LCA, lithocholic acid; T-α-MCA, tauro-α-muricholic acid; T-β-MCA, tauro-β-muricholic acid; T-ω-MCA, tauro-ω-muricholic acid; TCA, tauro-cholic acid; TCDCA, tauro-chenodeoxycholic acid; TDCA, tauro-deoxycholic acid; TDHCA, tauro-dehydrocholic acid; TLCA, tauro-lithocholic acid; TUDCA, tauro-ursodeoxycholic acid.

DCA with *Blautia and Lachnoclostridium* were found in an independent cohort of patients with IBD but not in non-IBD controls. Further, we found that fecal DCA was unexpectedly associated with increased serum C4 levels.

Prior studies have examined differences in the fecal microbiota, serum BA, and diet between patients with PSC and healthy individuals and patients with IBD.[4,40,41] Although these studies can provide insights into the potential role that these factors play in the initiation of PSC, they do not address how they relate to disease progression. In addition, these studies included patients with advanced liver disease which alters BA homeostasis and intestinal microbes. In contrast to these studies, we selected patients with early-stage disease and excluded potential cofounders such as use of antibiotics, immunosuppressants, or proton pump inhibitors. In addition, our focus on fecal BA and microbiota and disease progression is meant to provide insights into the mechanisms of disease progression rather than pathogenesis.

The lower fecal DCA levels in patients with PSC compared with a control group of patients with metabolic syndrome without diabetes is consistent with the increased fecal DCA observed in patients with metabolic syndrome[42,43] and the decreased secondary bile acids in patients with IBD.[16,17] In two prior studies comparing fecal BA in patients with PSC and IBD to patients with IBD, LCA was the only BA found to differ and this was in a study which included only seven patients with PSC.[44,45] The only other study evaluating fecal BA in PSC used an untargeted metabolomic approach and also found a decrease in DCA compared with controls despite that only 10 of the 37 patients with PSC also had IBD.[46]

Fecal DCA is the result of transformation of primary BA into secondary BA by specific microbes. This process includes BSH followed by transformation by *bai* operon encoded genes present in a limited number of microbes, including *Blautia spp*.[47,48] Bacteria from the *Blautia* genus are also able to perform 7α-dehydroxylation, a necessary step in the biotransformation of BA.[47] Compared with patients with IBD without PSC or controls, the mucosa associated microbiota of PSC patients have been shown to be enriched with *Blautia*.[49] Although we were unable to demonstrate a direct association of fecal BSH activity with fecal DCA, the consistent association between *Blautia* and *Lachnoclostridium* with fecal DCA in IBD add further support to the important role of these microbes in the production of fecal DCA in PSC.

Diet also shapes the bile acid pool of Western diets, which are characterized by high-fat and animal proteins, leading to increases in fecal secondary BA, particularly fecal DCA,[50] whereas a diet with low fat and high fiber results in

decreased total fecal BA, including fecal DCA.[13] The lack of significant associations between fecal DCA and fats in our study may be attributed to the relatively low levels of dietary fat within our cohort. However, we found that even low to moderate levels of alcohol use in patients with PSC was associated with increases in fecal DCA. This is consistent with the increase in fecal DCA seen with alcohol use in the absence of cirrhosis among people with alcohol use disorder as well as modest alcohol intake in people with metabolic dysfunction-associated steatotic liver disease (MASLD) and even people without liver disease.[14,15,42] Taken together, these results indicate that fecal DCA in patients with PSC is controlled by the same dietary and microbial factors observed in other conditions.

The most significant finding of our study was the relationship between greater fecal DCA with lower serum total bilirubin. A similar finding was observed in a cohort of patients with PSC in China, which unlike our study, also found a strong negative association with serum alkaline phosphatase.[46] In contrast, in patients with MASLD and advanced fibrosis stage, fecal DCA is greater compared with those with early fibrosis.[42,43,51] The mechanisms by which fecal DCA might ameliorate or exacerbate PSC or MASLD are not clear. One potential mechanism fecal DCA might protect against disease progression is through FXR activation, a therapeutic target for both MASLD and PSC. In patients with advanced PSC when serum BA are elevated, serum C4 levels are inversely correlated with serum bile acid levels consistent with an intact FXR-FGF19 signaling axis.[52] However, C4 appeared to be fully suppressed in late stage disease and associated with increased risk of liver transplantation or death. Further, in a study of oral CDCA challenge in patients with advanced PSC, C4 was not reduced, indicating that FXR signaling was maximally suppressed at end-stage disease.[53]

Surprisingly, in our study as well as in studies involving obesity and MASLD,[43,54] increasing fecal DCA was paradoxically associated with increasing C4 levels. Although DCA directly and effectively activates FXR and suppresses CYP7A1 in hepatocytes, it is not effective for co-activator recruitment considered essential for full FXR signaling.[55] Relevant to our study, the induction of FGF19 by DCA in the ileum is only 20–40% of the levels induced by CDCA.[56,57] Therefore, at physiologic concentrations in the presence of CDCA, DCA may competitively bind to FXR and effectively inhibit full activation by CDCA. This is supported by studies in mice in which feeding DCA increases fecal DCA but reduces ileal expression of FXR and FGF15, the mouse equivalent of FGF19, and increases hepatic expression of *Cyp7a1* consistent with DCA inhibition of FXR activity.[58] Further complicating our understanding of the potential effects of fecal DCA on regulation of BA are prior reports demonstrating that hepatic expression of FGF19 is induced in patients with cholestasis, including those with PSC.[59,60]

DCA and other BA also shape the intestinal microbial community either by acting as a carbon source or through cellular toxicity.[61] Compared with other BA, DCA has shown the highest level of bacterial toxicity to *Bifidobacterium breve*[62] and several other intestinal microbes.[63] In addition, DCA inhibits *Clostridioides difficile* germination and vegetative cell outgrowth[64] and mice fed DCA have reduced abundance of *Lactobacillus*, *Clostridium XI*, and *Clostridium XIV*.[58] Changes in these and/or other microbes along with their metabolites provide another potential mechanism by which DCA may alter the progression of PSC.

The limitations of the study include the correlative cross-sectional design of analyzing BA levels, clinical data, and dietary intake estimated from FFQs. As such, these findings require validation in additional study populations as well as investigations to establish causality of these associations. Similarly, small but significant effects may not have been identified. Additionally, lack of metagenomic data prevented further analysis at the species level and did not allow to determine associations with BA transforming enzymes, which may or not be encoded by *Blautia*. Fecal DCA is associated with colon cancer. Patients with PSC have an increased risk of colon cancer and thus understanding the balance of risks related to fecal DCA with liver disease progression and colon cancer will need to be considered in future studies. Lastly, our choice of control group composed of patients with obesity and metabolic syndrome is a limitation of our study since obesity is associated with increased fecal DCA.

In conclusion, diet and intestinal microbes were found to be associated with increased fecal DCA, which in turn was associated with lower serum total bilirubin. Further research into the relationship between diet and the microbiome along with its effect on BA composition and PSC disease progression may lead to a better understanding of the variable progression of PSC and potential novel therapies.

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Abbreviations

α-MCA, α-muricholic acid; β-MCA, β-muricholic acid; ω-MCA, ω-muricholic acid; ALP, alkaline phosphatase; ASVs, amplicon sequence variants; BA, bile acids; BSH, bile salt hydrolase; C4, 7α-hydroxy-4-cholesten-3-one; CA, cholic acid; CD, Crohn's disease; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; FFQ, food frequency questionnaire; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; GCA, glycol-cholic acid; GCDCA, glyco-chenodeoxycholic acid; GDCA, glyco-deoxycholic acid; GHDCA, glyco-dehydrocholic acid; GLCA, glycol-lithocholic acid; GUDCA, glyco-ursodeoxycholic acid; IBD, inflammatory bowel disease; LCA, lithocholic acid; LC-MS/MS, liquid chromatography coupled to tandem mass spectrometry; MASLD, metabolic dysfunction associated steatotic liver disease; MCA, muricholic acid; PSC, primary sclerosing cholangitis; TCA, tauro-cholic acid; T-α-MCA, tauro-α-muricholic acid; T-β-MCA, tauro-β-muricholic acid; T-ω-MCA, tauro-ω-muricholic acid; TCA, tauro-cholic acid; TCDCA, tauro-chenodeoxycholic acid; TDCA, tauro-deoxycholic acid; TDHCA, tauro-dehydrocholic acid; TLCA, tauro-lithocholic acid; TUDCA, tauro-ursodeoxycholic acid; UC, ulcerative colitis; UDCA, ursodeoxycholic acid.

Financial support

The Barbier's lab is supported by a grant from the Canadian Institute for Health Research (CIHR; # PJT-175310). The authors declare that the research was conducted in the absence of any commercial or financial relationship.

Conflicts of interest

CC, ML, PF, WG, RD, MY, JZ, M-CV, OB, MT declare no conflicts of interest related to this manuscript. MT received grant support from Albireo, Alnylam, Cymabay, Falk, Gilead, Intercept, MSD, Takeda and UltraGenyx; honoraria for

consulting from Abbvie, Albireo, Boehringer Ingelheim, BiomX, Falk, Genfit, Gilead, Hightide, Intercept, Jannsen, MSD, Novartis, Phenex, Pliant, Regulus, Siemens and Shire; speaker fees from Albireo, Bristol-Myers Squibb, Falk, Gilead, Intercept, MSD and Madrigal, as well as travel support from AbbVie, Falk, Gilead and Intercept. He is also co-inventor on patents on the medical use of norUDCA/norucholic acid filed by the Medical University of Vienna. JRK is on the advisory board of Corevitas, Promakhos, Thetis, ClostraBio and Founder of Colonary Concepts, and Bilayer Therapeutics. MLM is on the scientific advisory board for Nura, USA. CLB has received grant/research support from Calliditas, Gilead, Intercept, Bristol Myers Squibb, GSK, Cour, Novo Nordisk, CymaBay, Genfit, Pliant, Boston Scientific, Viking, and Cara and has consulted for Ipsen, Pliant, Cymabay, and GSK.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

Conceptualized and designed the study: CLB, MM, and OB. Analyzed data, interpreted results, and drafted the manuscript: CLB, MLM, ML, PF, OB, WG, CC, MT, and MY. Enrolled patients, collected data, and critically revised the manuscript: CLB, JZ, JRK, M-CV, and RD. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

Data availability statement

All relevant data that support the findings are presented in the manuscript.

Acknowledgements

Authors are grateful to Dr Jocelyn Trottier and Mélanie Verreault for their helpful technical contribution in fecal bile acid profiling and BSH assays, respectively, and Wannes van Beeck for assistance with 16S rRNA data submission.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2024.101188.

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Keywords: Cholestatic liver disease; Disease progression; Bile acids; Microbes; Diet.
Received 27 January 2024; received in revised form 1 August 2024; accepted 14 August 2024; Available online 22 August 2024