Associations of Fecal Short Chain Fatty Acids With Colonic Transit, Fecal Bile Acid, and Food Intake in Irritable Bowel Syndrome

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INTRODUCTION: **Short-chain fatty acids (SCFAs) correlate with colonic transit time (CTT) and may influence irritable bowel syndrome (IBS) pathophysiology. However, the clinical significance of fecal SCFAs, relationships between SCFAs and other metabolites (bile acids [BAs]), and real-time diet effects on SCFAs in IBS are uncertain. The aim was to evaluate fecal SCFA associations with IBS phenotype and mechanisms and explore effects of real-time diet.**

METHODS: **We conducted a prospective observational study of fecal SCFA, BAs, and CTT in healthy controls (HCs) and participants with IBS. We compared study end points across groups, analyzed relationships between end points, and evaluated the discriminative ability of SCFAs. Diet effects were explored in participants with dietary data.**

RESULTS: **Among 21 HCs and 43 participants with IBS, fecal SCFAs (total, individual) were inversely correlated with overall (all $P < 0.01$) and segmental (all $P < 0.05$) CTT; similar associations were observed within HC and IBS groups. The acetate-to-butyrate ratio correlated with slower overall and left CTT in all and in HCs (both $P < 0.01$). SCFAs (total, acetate) correlated with BAs (total, % primary) in all participants and in those with IBS with diarrhea. Logistic regression analyses demonstrated associations of acetate with slower transit (odds ratio = 0.988, $P = 0.002$) and BA diarrhea (BAD; odds ratio = 1.014, $P = 0.001$). Acetate accurately predicted delayed CTT (area under the receiving operating characteristic curve = 0.84) and BAD (area under the receiver operating characteristic curve = 0.79). Adjusting for diet strengthened correlations of total SCFAs with overall CTT ($R = [-0.46]$, $P = 0.04$) and SCFAs with transverse CTT (all $P < 0.05$).**

DISCUSSION: **Fecal SCFAs correlate with CTT and fecal BAs and reliably exclude delayed CTT and BAD. Accounting for diet strengthens SCFA associations with transit.**

SUPPLEMENTARY MATERIAL accompanies this paper at http://links.lww.com/CTG/A886

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INTRODUCTION

Short chain fatty acids (SCFAs) are key microbial metabolites produced by bacterial fermentation of complex carbohydrates within the colon. SCFAs regulate multiple aspects of gastrointestinal physiology including motility (1–3), fluid secretion (4), visceral sensation (5), mucosal immunity, and barrier function (6,7). However, the role of luminal SCFAs in common bowel disorders such as irritable bowel syndrome (IBS) is poorly characterized. Data on the nature and direction of the SCFA profiles in IBS are conflicting. For example, Tana et al. (8) previously reported higher fecal SCFAs in patients with IBS and a positive correlation between fecal acetate and gastrointestinal symptoms. Others have reported no differences (9) in major SCFAs (acetate, propionate, butyrate) between patients with IBS and controls or have described (10) decreased fecal SCFAs in patients with IBS with constipation (IBS-C) compared with those with IBS with diarrhea (IBS-D) and with controls, but no correlation with symptom severity or quality of life. Clear relationships of fecal SCFA profiles with IBS symptoms or

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phenotypes may be difficult to ascertain because of the pathobiological heterogeneity of the disorder.

Despite these challenges, studies evaluating relationships between fecal SCFAs and quantitative features in IBS, rather than clinical phenotypes, have yielded more consistent findings. Prior research has shown an inverse correlation between fecal SCFA levels and colonic transit time (CTT) (10–12) and associations of SCFAs with stool form and frequency (13,14), suggesting that SCFAs are linked to physiologic features in IBS. However, it is unclear whether excreted SCFAs are stable physiological traits in IBS or whether measurements are determined by modifiable factors such as diet. Although some studies have failed to show clear changes in SCFAs after dietary interventions in IBS (15,16) and no clear association between SCFAs and habitual diet based on dietary recall (13), data on the effects of actual food intake during stool collection are lacking.

SCFA effects within the gastrointestinal tract may also be determined by the local microenvironment and other major luminal metabolites such as fecal bile acids (BAs), which have been validated as diagnostic biomarkers in IBS (17). Investigating associations of fecal SCFAs with mechanistic IBS subtypes (i.e., abnormal transit or BAs) is necessary to interpret the physiological and clinical effects of excreted SCFAs in IBS and their role as putative IBS biomarkers. Therefore, we aimed to (1) evaluate associations of fecal SCFAs with clinical and mechanistic IBS subtypes, (2) assess the diagnostic accuracy of fecal SCFAs for detecting abnormal CTT and BAs, and (3) explore the effects of actual food intake on fecal SCFA measurements and SCFA associations with CTT in patients with IBS and healthy controls (HCs).

METHODS

Study participants and design

Adults aged 18–65 years were recruited from the local community, Indiana University Gastroenterology clinics, and Indiana Clinical and Translational Research Registry for a prospective observational study. Patients with IBS-D or IBS-C based on Rome IV criteria and HCs were eligible for participation (18). Detailed eligibility criteria are provided in the Appendix (see Supplementary Digital Content 1, http://links.lww.com/CTG/A886). Study eligibility, medications, medical history, and bowel symptoms were assessed during a screening visit by a study physician. Over a 7-day period, participants underwent CTT assessments using a validated radiopaque marker method (19), submitted stool samples for SCFA and BA quantification by high-performance liquid chromatography, and completed 4-day food diaries (see Appendix, Supplementary Digital Content 1, http://links.lww.com/CTG/A886 for detailed study procedures). All stool passed within the last 48 hours of a 4-day 100-g/d fat diet were collected and pooled, refrigerated during collection, and returned on ice within 4 days of collection to be immediately aliquoted and frozen at −80 °C for later homogenization and analysis. All participants provided written informed consent before study participation. The study protocol was approved by the Indiana University Institutional Review Board and registered within ClinicalTrials.gov (NCT02981888).

Statistical analysis

Data were summarized as mean (SD) or median (interquartile range [IQR]) values. Primary study end points included fecal SCFA (total and individual), fecal BAs (total and primary), and overall CTT. Secondary end points included fecal acetate-to-butyrate ratio and segmental CTT. Four-day food intakes (total energy, macronutrients, fiber) were included as exploratory end points. Based on data from a preliminary cohort, a sample size of 19 participants per group was anticipated to detect an effect size of 0.24 (Cohen f) for fecal SCFAs with 80% power at the 5% significance level using analysis of variance among 3 groups.

The study end points were compared across groups using the analysis of variance F-test or Kruskal-Wallis test for continuous variables and the Pearson χ^2 test or Fisher exact test for categorical variables. Participants with missing data were excluded from the analysis for that end point. Pearson correlations (R) were assessed between SCFAs and CTT and between SCFAs and BAs for the overall cohort and within clinical phenotype groups. Associations of SCFA with transit (normal, rapid, delayed) and the presence or absence of BA diarrhea (BAD) based on established reference values (20,21) were analyzed by ordinal and binary logistic regression. Area under the receiver-operating characteristic (AUROC) curves were constructed to assess diagnostic accuracies of SCFAs; optimal cutoff values were determined by the Youden J statistic. Relationships of SCFA with food intake were explored in participants with food intake data using Pearson correlations. Effects of diet on associations of SCFAs and CTT in the overall cohort were examined using linear regression. A P value of < 0.05 was denoted as significant. All analyses were conducted using SAS version 9.4 (SAS Institute, Cary, NC).

RESULTS

Study participants characteristics

The final study cohort included 21 HCs and 43 participants with IBS (26 IBS-D, 17 IBS-C, 76.6% female, mean [SD] age 35 [12.3] years, mean [SD] body mass index 26.4 [7.4] kg/m^2). There were no significant differences in age, sex, or body mass index across groups (Figure 1).

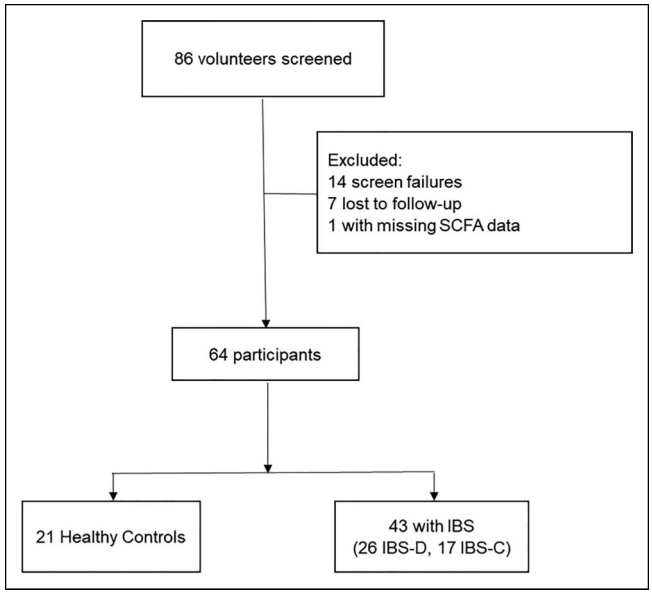

Figure 1. Flow diagram of study participants. IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; SCFA, short chain fatty acid.

Fecal SCFAs

In the overall cohort, total fecal SCFA (median [IQR]) concentration according to wet stool weight was 173.9 (115.3–273.6) mmol/kg; concentrations were highest for fecal acetate (130.6 [85.4–190.5] mmol/kg), followed by fecal propionate (29.7 [19.2–44.3] mmol/kg) and then butyrate (21.5 [10.9–40.4] mmol/kg). Total fecal SCFA concentrations were numerically highest in IBS-D, but differences across groups were not statistically significant. Differences in individual SCFAs and acetate-to-butyrate ratios were not significant across groups, although the highest levels of acetate and higher acetate-to-butyrate ratios were observed in IBS-D (Table 1).

Fecal BAs

Among 61 participants with fecal bile acid data, median (IQR) values for total fecal BAs and % primary BAs were 479 (198.0–671.0) μmol/48 hr and 2.5 (0.8–7.7)%, respectively, for the overall cohort. Comparisons of BAs across clinical groups revealed the highest total BAs ($P = 0.006$) in IBS-D (Table 1).

Colonic transit time

In the overall cohort, median (IQR) values for CTT in days were 1.1 (0.7–2.1) for overall CTT, 0.4 (0.2–0.7) for right CTT, 0.1 (0.0–0.5) for transverse CTT, and 0.5 (0.2–1.0) for left CTT. Left CTT was fastest in the IBS-D group ($P = 0.04$). The fastest overall CTT in IBS-D did not achieve significance ($P = 0.052$). There were no other significant differences in overall or segmental CTT across groups (Table 1).

Food intake

Diet diaries were collected from 28 participants (n = 16 IBS-D, n = 8 IBS-C, and n = 4 HC). Total energy, macronutrients, or fiber intake did not differ across groups (Table 1).

Table 1. Demographics and clinical characteristics of study participants by group

	Healthy controls (n = 21)	IBS-C (n = 17)	IBS-D (n = 26)
Age (yr), mean ± SD	32.1 ± 12.8	34.9 ± 10.2	37.3 ± 13.0
Females, n (%)	16 (76.2)	15 (88.2)	18 (69.2)
BMI (kg/m^2), mean ± SD	26.3 ± 5.8	25.9 ± 5.8	26.8 ± 9.4
SCFAs, median (IQR)			
Acetate (mmol/kg)	107.5 (57.9–141.1)	117.8 (86.4–188.1)	156.8 (95.3–241.6)
Propionate (mmol/kg)	23.5 (12.2–36.1)	29.5 (19.7–37.7)	32.7 (21.0–56.2)
Butyrate (mmol/kg)	20.4 (10.7–35.1)	25.1 (10.4–46.3)	21.5 (11.1–40.7)
Total SCFA (mmol/kg)	148 (76.2–226.9)	172.7 (115.0–265.8)	222.0 (133.8–333.5)
Acetate-to-butyrate ratio	5.5 (4.8–9.8)	5.5 (4.1–7.5)	6.7 (4.9–9.9)
BAs, median (IQR)[a]			
Total BAs (μmol/48 hr)*	405.0 (142.0–603.0)	250.5 (116.5–497.5)	605.5 (403.0–1,234.5)
BAs, %CDCA + CA	1.4 (0.7–8.2)	2.1 (0.8–5.4)	3.1 (0.8–12.4)
CTT (d), median (IQR)			
Total CTT**	1.4 (0.9–2.5)	1.5 (1.0–2.1)	0.9 (0.5–1.5)
Right CTT	0.6 (0.2–0.7)	0.4 (0.3–0.7)	0.4 (0.2–0.6)
Transverse CTT	0.1 (0.0–0.4)	0.3 (0.0–0.8)	0.1 (0.0–0.4)
Left colon transit time***	0.8 (0.3–1.3)	0.5 (0.2–1.1)	0.3 (0.1–0.5)
Transit category, n (%)			
Rapid	2 (9.5)	2 (11.8)	7 (26.9)
Normal	16 (76.2)	14 (82.4)	17 (6.5)
Delayed	3 (14.3)	1 (5.9)	2 (7.7)
4-day dietary intake, mean ± SD	n = 4	n = 8	n = 16
Calorie (kilocalories)	8,397.0 ± 1,419.1	9,160.0 ± 2042.6	8,947.2 ± 2,152.9
Fat (g)	355.7 ± 118.9	469.3 ± 130.8	420.8 ± 101.1
Protein (g)	357.8 ± 103.8	397.5 ± 82.8	381.3 ± 89.7
Carbohydrate (g)	820.0 ± 144.2	942.6 ± 508.5	848.1 ± 286.5
Fiber (g)	85.0 ± 31.8	67.9 ± 27.6	64.3 ± 34.3
Saturated fat (g)	131.0 ± 19.8	161.0 ± 37.7	149.7 ± 27.9

BA, bile acid; BMI, body mass index; CA, cholate; CDCA, chenodeoxycholate; CTT, colonic transit time; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IQR, interquartile range; SCFA, short chain fatty acid.

[a]n = 3 participants with missing bile acid data.

*$P = 0.006$; **$P = 0.052$; ***$P = -0.04$.

Table 2. Pearson correlations (*R*) of fecal SCFA concentrations with CTT and fecal bile acid excretion

	Acetate		Propionate		Butyrate		Total SCFA		Acetate-to-butyrate ratio	
	R	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value	*R*	*P* value
Total CTT	−0.467	<0.001	−0.428	<0.001	−0.368	0.003	−0.473	<0.001	0.363	0.003
Right CTT	−0.429	<0.001	−0.313	0.01	−0.360	0.003	−0.424	<0.001	0.245	0.05
Transverse CTT	−0.328	0.008	−0.301	0.02	−0.256	0.04	−0.332	0.007	−0.071	0.58
Left CTT	−0.294	0.02	−0.303	0.01	−0.223	0.08	−0.302	0.02	0.405	<0.001
Total BAs	0.391	0.002	0.249	0.05	0.259	0.04	0.368	0.004	−0.091	0.48
%CDCA + CA	0.497	<0.001	0.280	0.03	0.293	0.02	0.446	<0.001	−0.058	0.66
Total BAs (*outlier excluded*)	0.420	<0.001	0.416	<0.001	0.303	0.02	0.423	<0.001	−0.127	0.33

BA, bile acid; CA, cholate; CDCA, chenodeoxycholate; CTT, colonic transit time; SCFA, short chain fatty acid.

Associations of SCFAs with BAs and CTT. Higher levels of total and individual fecal SCFAs were significantly associated with faster overall (all $P < 0.01$) and segmental (all $P < 0.05$) CTT in the overall cohort, except for butyrate and left CTT (Table 2). Higher acetate-to-butyrate ratios were associated with slower overall and left CTT (both $P < 0.01$). Similar inverse relationships between total or individual SCFAs with overall and segmental CTT were observed within subgroups; however, not all associations were statistically significant (Figure 2). Among HCs, acetate-to-butyrate ratios were positively associated with total and left CTT (both $R = 0.66$, $P = 0.001$).

Higher total SCFAs (both $P < 0.01$), acetate (both $P < 0.01$), and butyrate (both $P < 0.05$) were significantly associated with higher total BAs and a higher % primary BAs in the overall cohort (Table 2). Fecal propionate was significantly associated with % primary BAs ($P = 0.03$). A positive association between propionate and total BAs was of borderline significance ($P = 0.05$). There were no significant associations between acetate-to-butyrate ratios and total or % primary BAs in the overall cohort. Within each clinical group, similar significant associations of higher SCFAs (total and acetate) with higher BAs (total and % primary) were observed in IBS-D, but not in HCs or participants with IBS-C (Figure 3). Acetate-to-butyrate ratios were not significantly associated with total or % primary BAs within individual groups. Visual inspection of scatterplots revealed 1 extreme outlier for total fecal BAs in the IBS-D group; removal strengthened all associations of total and individual SCFAs with total BAs in the overall cohort and in the IBS-D group (Figure 4).

Diagnostic accuracy of fecal SCFAs

In the overall cohort, transit (rapid, normal, delayed) and abnormal BA (BAD) phenotypes were significantly associated with SCFAs, but not with the clinical group (Table 3). A higher proportion (both overall $P < 0.05$) of men had rapid overall CTT (40.0%) and BAD (50.0%) than women (10.2% rapid overall CTT, 20% BAD).

Ordinal logistic regression with stepwise variable selection revealed a significant negative association between fecal acetate and CTT (odds ratio = 0.988, 95% confidence interval: 0.981–0.996). The AUROC estimate associated with acetate was 0.84 for predicting delayed CTT (Figure 5). The optimal acetate cutoff for detecting delayed CTT was ≤94.36 mmol/kg with 83% sensitivity, 72.4% specificity, 23.8% positive predictive value, and 97.7% negative predictive value. Logistic regression with stepwise variable selection further revealed a significant positive association between fecal acetate and abnormal BAs (odds ratio = 1.014, 95% confidence interval: 1.005–1.022). The AUROC estimate associated with fecal acetate was 0.79 for predicting abnormal BAs (Figure 5). The optimal acetate cutoff for detecting elevated abnormal BAs was 187 mmol/kg with 63% sensitivity, 87% specificity, 62.5% positive predictive value, and 86.7% negative predictive value.

Effects of food intake on SCFAs and SCFA associations with CTT

Fecal butyrate was negatively correlated with total calories ($R = [-0.44]$; $P = 0.02$) in the overall cohort (n = 28) and with total calories ($R = [-0.54]$; $P = 0.04$) and saturated fat ($R = [-0.57]$; $P = 0.03$) in the IBS-D group (n = 16). There were no significant associations between total SFCA, acetate, or propionate with food intake. Total and segmental CTT values were not significantly associated with food intake in all 28 participants or in the IBS-D group. A significant negative correlation was observed between butyrate and right CTT ($R = [-0.43]$; $P = 0.03$) before, but not after (P = non-significant), adjusting for all food intake. Inverse relationships between fecal SCFAs (total, acetate, propionate) and CTT (total, right, transverse) were of borderline significance in unadjusted analyses. After adjusting for diet, correlations between total SCFAs and total CTT ($R = [-0.46]$; $P = 0.04$) and between SCFAs (total, butyrate, acetate, propionate) and transverse CTT (all $P < 0.05$) were strengthened.

DISCUSSION

In this study, we describe associations of fecal SCFA concentrations with quantitative IBS biomarkers, colonic transit, and fecal BAs and further examined the effect of real-time food intake on the relationships of SCFAs with transit. Our results demonstrate that fecal SCFAs are significantly associated with both CTT and fecal BAs and reliably exclude delayed CTT or abnormal BAs. Accounting for food intake further strengthens relationships of SCFAs and transit.

In our cohort, participants with IBS-D exhibited higher levels of total fecal BA and faster left-sided CTT compared with participants with IBS-C or HCs. Our findings are consistent with the known literature. BA malabsorption is reported in up to half of the patients with IBS-D or functional diarrhea (22,23) while

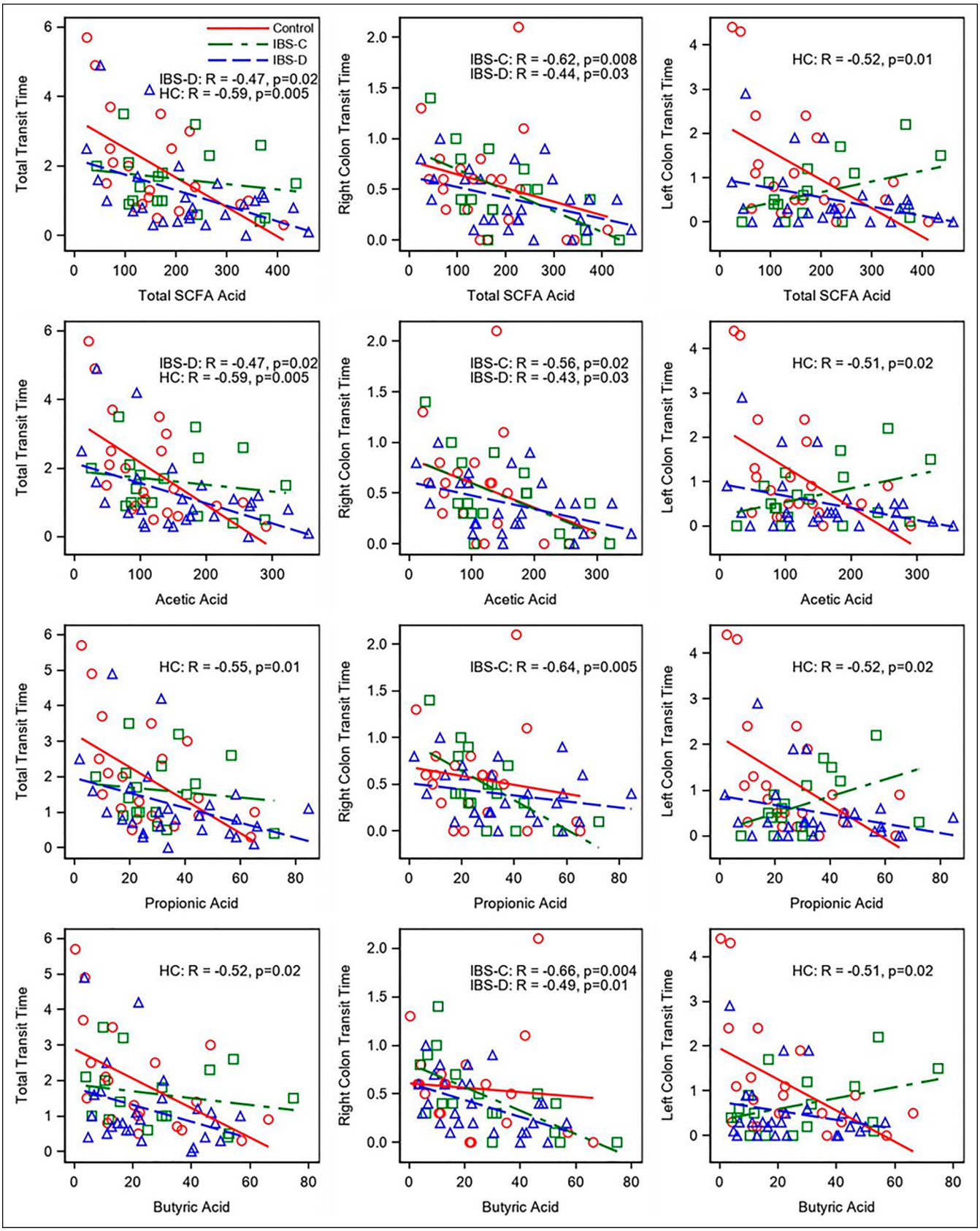

Figure 2. Pearson correlations (*R*) of total or individual fecal SCFA concentrations with overall and segmental (right, left) colonic transit time in HCs and IBS groups (IBS-D and IBS-C). *P* values shown for significant associations only. HC, healthy control; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; SCFA, short chain fatty acid.

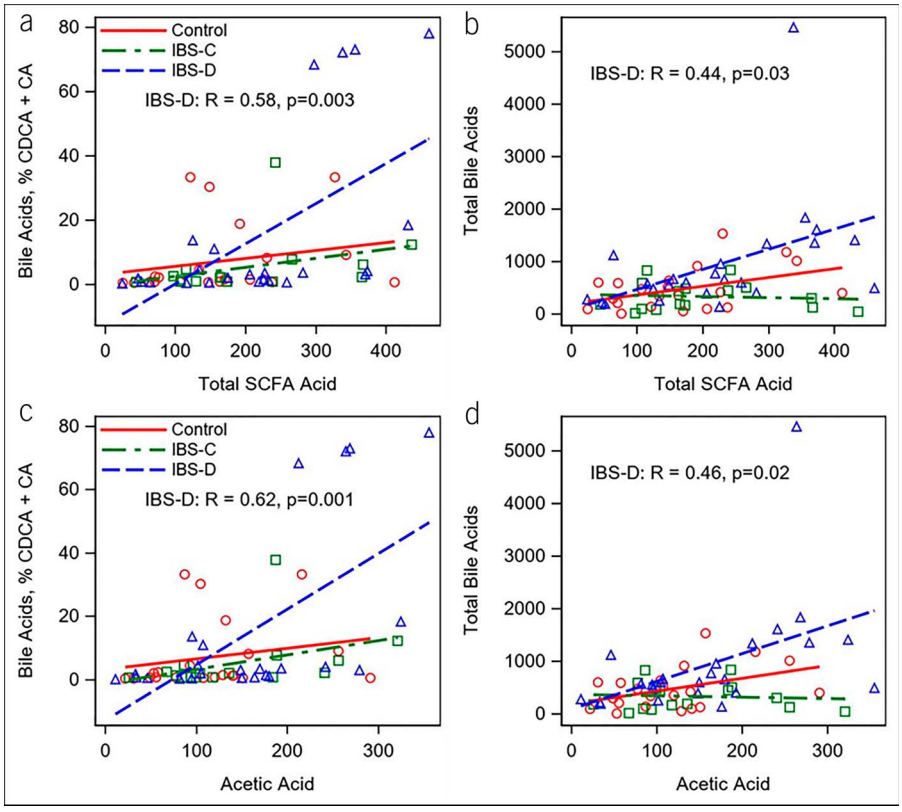

Figure 3. Pearson correlations (*R*) of total fecal SCFA and fecal acetate concentrations with percent primary (CDCA + CA) and total fecal bile acids in healthy controls and IBS groups (IBS-D and IBS-C). *P* values shown for significant associations only. CA, cholate; CDCA, chenodeoxycholate; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; SCFA, short chain fatty acid.

accelerated transit may be detected in up to 30% of patients with IBS-D (24,25). By contrast, higher total SCFAs, acetate, and acetate-to-butyrate ratios in patients with IBS-D compared with those with IBS-C and HCs were not statistically significant.

The lack of measurable differences in fecal SCFA concentrations or ratios across clinical phenotype groups in our study is not surprising. Although earlier reports suggested altered SCFA profiles in IBS (8,26), the strength and direction of these reported associations have differed (9). Recent analysis of SCFA profiles by IBS subtype and examination of SCFA associations with quantitative traits have demonstrated fecal SCFAs to be lower in patients with IBS-C (10,27) and associated with stool form (14) and CTT (10). Findings suggest that although luminal SCFA profiles do not discriminate IBS from health, they may correlate with measurable traits. We observed significant associations of fecal SCFAs with CTT and fecal BAs. Total and individual SCFAs were associated with faster CTT in both HCs and participants with IBS. Analysis by clinical phenotype group revealed significant associations of total and individual SCFAs with right-sided CTT in IBS suggesting that right-sided transit may exert the greatest degree of effect on SCFA excretion in IBS, perhaps through increased nutrient load to the distal colon. Overall, our findings demonstrate a relationship between fecal SCFAs and mechanistic IBS subtypes. The relative contributions of various mechanistic disturbances to symptoms may differ between individuals with IBS. Therefore, SCFA profiles should be studied as physiologically informative, rather than confirmatory IBS biomarkers.

To examine fecal SCFA as a physiologic IBS biomarker, we assessed the diagnostic accuracy of fecal SCFAs for detecting abnormal CTT and fecal BAs. Findings are important for several reasons. First, the validity of measuring excreted fecal SCFAs has been questioned because of concerns for *ex vivo* fermentation and SCFA volatility. In our study, fecal SCFAs demonstrated good discriminatory power for detecting delayed CTT and abnormal BAs, suggesting that the methods for SCFA quantification in our study yield biologically valid measurements. Second, the ability to detect abnormal transit using fecal SCFAs may be clinically useful. Quantitative assessment of CTT by whole-gut scintigraphy, wireless motility capsule, or modified radiopaque marker methods is not consistently available outside tertiary referral centers. Although some studies suggest that stool form and frequency represent surrogates for transit, reported correlations are moderate (28,29) in strength. In addition, CTT is commonly pursued to aid in clinical decision making. Although bowel functions may guide management, identifying abnormal CTT through objective testing may be important for patients in whom prokinetics or surgery is being considered. In settings where access to specialized motility or radiographic studies is limited, fecal SCFAs could decrease the need for formal transit testing.

It has been proposed that fecal SCFAs are largely controlled by CTT to a greater extent than microbial fermentation and that rapid CTT may lead to decreased SCFA absorption (10). We observed inverse correlations of both total and individual fecal SCFAs with CTT. Although the positive effects of SCFAs on stimulating colonic motility and transit have been demonstrated

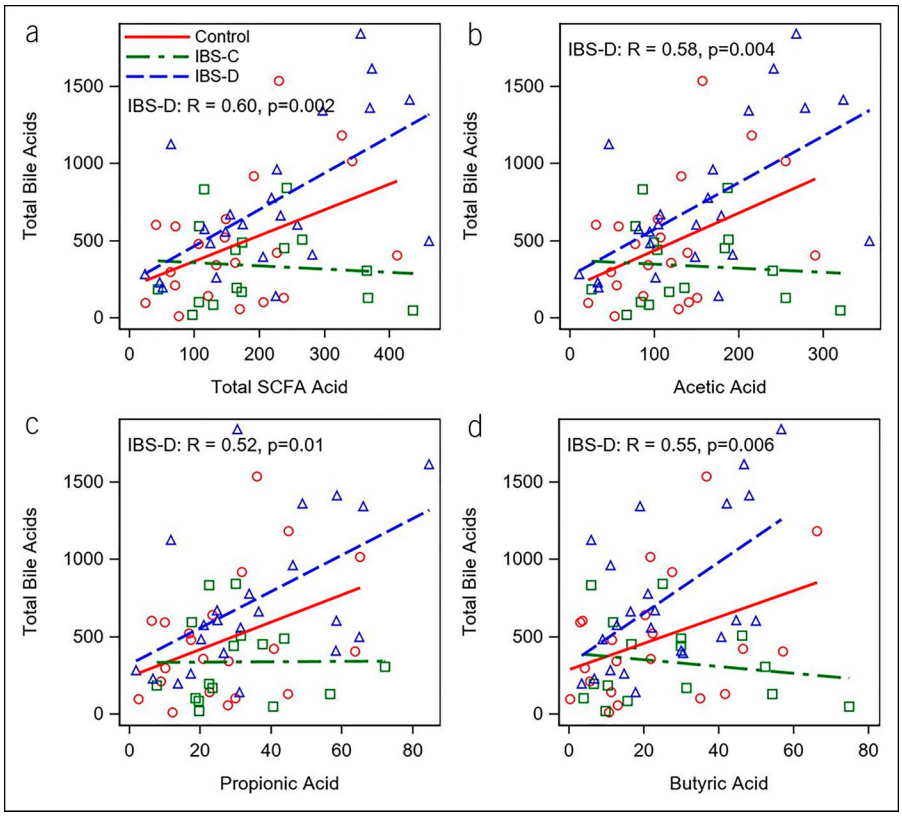

Figure 4. Pearson correlations (*R*) of total and individual fecal SCFAs with total fecal bile acids in healthy controls and IBS groups (IBS-D and IBS-C) after the removal of outlier. *P* values shown for significant associations only. IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; SCFA, short chain fatty acid.

in experimental models, studies have suggested that effects of individual SCFAs may differ. Mitsui et al. (30) previously showed that propionate and butyrate, but not acetate, induce high-amplitude phasic contractions, followed by low-amplitude tonic contractions, in the distal rat colon. Separately, Hurst et al. (31) demonstrated increased pellet velocity in guinea pig colon with butyrate infusion and decreased pellet velocity with propionate. Our observations of consistently inverse, rather than differential, associations of SCFAs with CTT suggest that excreted SCFAs are largely determined by CTT effects on SCFA absorption rather than SCFA effects on transit.

Although microbial composition or activity, the effects on high fat intake on microbiome structure, and SCFA production were not directly evaluated in our study, we attempted to assess potential effects of microbial fermentation by analyzing associations of acetate-to-butyrate ratios with CTT. It would be expected that change in SCFA excretion driven by transit alone would yield uniform or similar degrees of change across individual SCFAs. Meanwhile, differential changes in individual SCFA excretion (altered acetate-to-butyrate ratios) could indicate changes in microbial metabolism resulting from net acetate utilization for butyrate production (32). We observed positive correlations between acetate-to-butyrate ratios and slower transit in the overall cohort and in HCs. Positive associations could suggest that decreased microbial butyrate production slows transit through a reduction in motility-stimulating effects of butyrate. Alternatively, altered SCFA ratios may reflect microbial composition and direct microbial effects on CTT. Cross-feeding between bacterial groups (33) such as

Table 3. Associations of SCFA with colonic transit (rapid, normal, delayed) and bile acid (abnormal or normal bile acid excretion) phenotypes

	Transit phenotype				Bile acid phenotype		
Data show median (IQR)	Rapid	Normal	Delayed	*P* value	Normal	Abnormal	*P* value
Acetate (mmol/kg)	169.6 (120.1–288.9)	129.0 (84.3–188.1)	50.8 (31.1–94.7)	0.003	103.8 (67.5–150.9)	213.8 (119.7–266.1)	0.001
Propionate (mmol/kg)	46.2 (24.9–63.8)	28.0 (17.7–40.9)	16.7 (6.3–31.4)	0.01	23.5 (16.9–36.5)	35.0 (27.4–61.8)	0.006
Butyrate (mmol/kg)	40.7 (21.9–52.4)	19.0 (11.1–35.1)	6.8 (3.4–21.9)	0.013	15.7 (9.8–30.5)	32.2 (21.1–47.5)	0.011
Total SCFA (mmol/kg)	169.6 (120.1–288.9)	129.0 (84.3–188.1)	50.8 (31.1–94.7)	0.003	103.8 (67.5–150.9)	213.8 (119.7–266.1)	0.002

IQR, interquartile range; SCFA, short chain fatty acid.

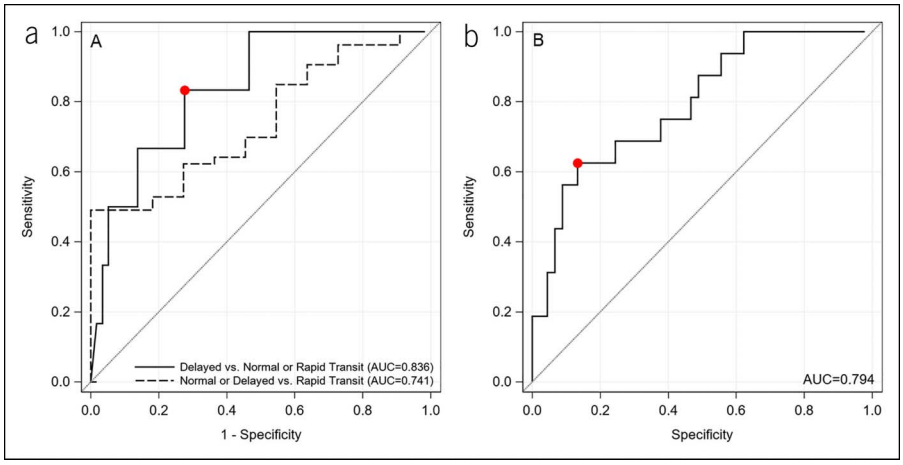

Figure 5. Cumulative receiver-operating characteristic curve indicating the discriminative ability of fecal acetate for the detection of (**a**) abnormal colonic transit time and (**b**) abnormal BA. Maximization of the Youden J statistic results in acetate threshold ≤94.36 mmol/kg and ≥187 mmol/kg for the detection of delayed transit and abnormal BA, respectively (denoted by the red dot in the plot). AUC, area under the curve; BA, bile acid.

Faecalibacterium prausnitzii and *Bifidobacterium adolescentis* can increase acetate-to-butyrate conversion. Parthasarathy et al. (32) demonstrated *Firmicutes*-associated taxa (e.g., *Faecalibacterium*) to be positively associated with faster CTT in adults with and without constipation. Others have demonstrated associations of fecal microbiota profiles with transit. Muller et al. (34) reported associations of higher alpha diversity with longer distal colon transit in healthy adults. Future studies should expand on these data by investigating relative SCFA profiles in the context of microbial composition and metabolic function in both healthy controls and participants with IBS.

To explore whether excreted SCFAs and their associations with CTT represent stable traits, we examined the effects of food intake to observe negative correlations of butyrate with total calories and saturated fat. Accounting for diet strengthened relationships between SCFAs and total or transverse CTT. The results imply that while food intake and SCFA excretion are correlated, the relationship between SCFAs and CTT is not explained by food intake alone. Associations of SCFAs with total caloric and saturated fat rather than fiber or total carbohydrate intake could further suggest that the physiologic effect of meal intake exerts a larger effect on SCFA excretion and SCFA relationships with CTT than ingestion of fermentable carbohydrates during stool collection. High fat diet has been shown to delay colonic transit (35,36) in animal studies, and in one controlled feeding trial, high fat intake was associated with decreased fecal SCFAs (37) in healthy adults despite no differences in fiber intake, possibly related to differences in unmeasured resistant starches. Overall, the lack of direct effects of carbohydrate and fiber intake on SCFAs in our cohort suggest that while dietary factors are important, modest variations in the intake of dietary polysaccharides do not significantly affect SCFA excretion in IBS-D. Others have reported no change in fecal SCFAs with psyllium, a poorly fermentable fiber supplement, in HCs (11,38) or with altered intake of fermentable short-chain carbohydrates (16). SCFA production from fermentable fibers may also vary according to fiber type, which was not examined in our cohort, and the resident microbiome (39). The 100-g fat diet procedures and small range of dietary fiber intakes in our cohort could have concealed significant associations of SCFA levels with fiber and other aspects of diet, which have been reported by other study populations (40). Further development of methods for SCFA measurement and interpretation should account for diet through standardized intake and quantification of resistant starches and nondietary fiber supplementation during periods with undefined dietary requirements.

Study strengths include prospective enrollment of HC and IBS participants, the use of the validated Rome IV criteria, collection of real-time dietary data, and the use of validated methods for CTT and fecal BA assessments. Our study has some limitations. The relatively small sample size may have reduced our ability to detect significant associations within subgroups. The sample size was calculated using preliminary data to detect an effect size of 0.24 using Cohen f, but may not have provided adequate power to detect smaller differences or differences in all end points. This study was conducted at a tertiary referral center and some patients may find stool collections to be burdensome or unappealing, which may limit generalizability and translatability. However, participants were recruited from the community through public advertising and collaboration with a state-wide research registry, and several stool-based biomarkers are already used in clinical practice. Diet diaries were available from a subset of participants, but not all. Therefore, the results pertaining to food intake effects should be interpreted cautiously. Stool samples were collected only once to measure SCFA concentrations, which does not directly assess SCFA production. However, samples were collected over a 2-day period rather than as a single-spot sample in attempts to capture intraindividual variations in SCFA excretion. Furthermore, this study was designed to assess the physiologic significance of excreted SCFAs rather than quantify the overall SCFA pool. Microbial composition and metabolic activity were not directly examined; however, we explored the potential effects of microbial metabolism by analyzing SCFA ratios and examining the effects of food intake.

In summary, our study shows that excreted fecal SCFAs correlate with mechanistic IBS subtypes and accurately exclude delayed CTT and abnormal BAs. Real-time calorie and saturated fat intakes are correlated with fecal butyrate in IBS-D. Accounting for diet strengthens the association between fecal SCFAs and CTT. Although further validation and studies examining contributions from and changes in gastrointestinal microbiota will be necessary, our findings suggest that fecal SCFAs represent

physiologically informative or investigational biomarkers that may identify mechanistic perturbations in IBS.

CONFLICTS OF INTEREST

Guarantor of the article: Andrea Shin, MD, MSc.

Specific author contributions: A.S.: developing the study concept. A.S.: serves on Ardelyx Scientific Communications Advisory Board for irritable bowel syndrome with constipation. A.S. and H.X.: planning the study design. R.S., T.J.-S., M.B., N.R., J.W., A.G., M.J., J.K., and A.S.: participant recruitment. A.G., M.J., J.K., and A.S.: data collection and study procedures. M.R.W., L.W., J.K., and A.S.: data management. H.X. and A.S.: data analysis and interpretation. H.X. and A.S.: drafting the manuscript. M.R.W., R.S., T.J.-S., M.B., N.R., J.W., and L.W.: critically revising the manuscript.

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IRB approval statement: The study protocol was approved by the Indiana University Institutional Review Board.

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Study Highlights

WHAT IS KNOWN

- ✓ Short chain fatty acids (SCFAs) are microbial metabolites that modulate gastrointestinal physiology.
- ✓ The clinical importance of SCFAs in irritable bowel syndrome (IBS) is poorly understood.

WHAT IS NEW HERE

- ✓ In adults with IBS, fecal SCFAs correlate with a mechanistic phenotype.
- ✓ Fecal SCFAs reliably exclude delayed colonic transit and bile acid diarrhea.
- ✓ Accounting for food intake strengthens relationships of SCFAs with transit.

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