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APPENDIX

Daily Stool Diaries

All participants completed bowel function diaries that documented the time of each bowel movement, stool consistency (Bristol Stool Form Scale [18]), the ease of passage (ranging from 1 = “manual disimpaction” to 7 = “incontinence”), and completeness of evacuation (1 = “yes” and 0 = “no”). In the first study, patients recorded diaries during at least 3 days of the baseline period and the 4 days of treatment. The diaries were recorded for at least 3 days of the baseline period and during the entire treatment period of the second study.

Gastrointestinal Transit Measurements

An adaptation of our established scintigraphic method was used to measure gastrointestinal and colonic transit [19]. Patients with IBS-D had baseline measurement of colonic transit since this is known to be a significant covariate in treatment responses.

¹¹¹In was adsorbed on to activated charcoal particles and delivered to the colon by means of a methacrylate-coated, delayed-release, oral capsule. The capsule was ingested following an overnight fast. After the capsule emptied from the stomach, a ^{99m}Tc-sulfur colloid radiolabeled meal was ingested. It consisted of two scrambled eggs, one slice of whole wheat bread and one glass of whole milk. This meal allowed measurement of gastric and small bowel transit. Subjects ingested standardized meals for lunch and dinner at 4 and 8 hours after the radiolabeled meal, respectively. Abdominal scans were obtained every hour for the first 6 hours (the first 4 hours for the assessment of gastric emptying) and at 8, 24, and 48 hours after ingestion of the ¹¹¹In capsule. The performance characteristics of this test are summarized elsewhere [19].

Transit Data Analysis

We quantitated the counts in the stomach and each of four colonic regions, ascending, transverse, descending, and combined sigmoid and rectum, with a variable region of interest program. Counts were corrected for isotope decay, tissue attenuation, and downscatter of ^{111}In counts in the $^{99\text{m}}\text{Tc}$ window [19].

Gastric emptying $t_{1/2}$ is a measure of the time for 50% of the radiolabeled meal (identifiable by radiolabeled tracer) to empty from the stomach. Colonic filling at 6 hours, or the proportion of the radiolabeled meal to have reached the colon at 6 hours, is an indirect measurement of small bowel transit time. Overall colonic transit was summarized as the colonic geometric center (GC) at specified times. The GC is the weighted average of counts in the different colonic regions [ascending (AC), transverse (TC), descending (DC), rectosigmoid (RS)] and stool, respectively 1 to 5. At any time, the proportion of counts in each colonic region is multiplied by its weighting factor as follows:

$$(\%AC \times 1 + \%TC \times 2 + \%DC \times 3 + \%RS \times 4 + \% \text{ stool} \times 5)/100 = GC$$

Thus, a higher GC reflects a faster colonic transit.

Ascending colon emptying was summarized by the $t_{1/2}$ calculated by linear interpolation of values on the AC emptying curve.

The primary endpoints were the colonic geometric center (GC) at 24 hours (GC 24) and ascending colon (AC) emptying $t_{1/2}$. Secondary transit endpoints were colonic GC at 48 hours, gastric emptying $t_{1/2}$, and colonic filling at 6 hours.

Colonic GC is an important endpoint which has been shown in previous pharmacodynamic studies using the same methods to be responsive to treatment with prokinetics and secretagogues [20] in patients with constipation-predominant IBS or functional constipation. It was also shown to be responsive to agents that retard colonic transit such as alosetron [21] in IBS-D.

Serum 7 α -C4 Measurements

The measurement of fasting serum 7 α -hydroxy-4-cholesten-3-one (7 α -C4) was conducted using high performance liquid chromatography with tandem mass spectrometry [23], which is a measurement of hepatic bile acid synthesis and is closely related to the fecal loss of bile acids. This is a valid screening method for identification of BAM [22].

Serum FGF19 Measurements

Serum FGF19 levels were measured by enzyme-linked immunosorbent assay [(ELISA) FGF19 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN). Data from 40 healthy controls were used for comparison with patients with IBS-D.

Colonic Permeability Measurement

In the second trial conducted in patients with IBS-D, we measured colonic permeability at baseline and at the end of the treatment period by the excretion of urine sugars measured by a liquid chromatography/mass spectrometry method [23]. After oral ingestion of the sugars (mannitol 1 g and lactulose 5 g) in a methacrylate-coated capsule, urine was collected in a 2-hour aliquot from 6 to 8 hours, and a 16-hour collection from 8 to 24 hours after ingestion of the sugars. We estimated the total content of each sugar in each aliquot, as well as the ratio of the excretion of the two sugars. The method has been described in detail elsewhere [24]. The primary permeability endpoint was the 8-24 hour urine mannitol

excretion. Secondary endpoints were urine mannitol at 6-8 hours, urine lactulose at 6-8 hours and 8-24 hours, and urine lactulose to mannitol ratio.

Statistical Analysis and Sample Size Considerations

Effect of Sodium Chenodeoxycholate

An analysis of covariance (ANCOVA) assessed the treatment effects of CDC dose on the primary endpoints, colonic GC 24 hours and AC emptying $t_{1/2}$, with age, gender and BMI as covariates. The ANCOVA analysis compared the responses overall among the three (randomly assigned) treatment groups. Specific pair-wise comparisons (e.g. each dose of CDC against placebo) were also examined.

Sample sizes selected (see Appendix Table IA) were based on the results of primary endpoints in healthy volunteers previously studied in our lab (data show mean \pm SD). The estimated effect sizes are based on a 2-sample t-test with N=20 per group, where effect size is the difference in group means as a percentage of the corresponding overall mean (shown in Appendix Table IA). Note that the effect size demonstrable for colonic GC24 hours and for AC $t_{1/2}$ was 34% and 50% respectively. Moreover, the observed variations (COV%) in these two primary endpoints in the subjects randomized to placebo in this study were 39% and 54%, which are very close to the *a priori* assumed variations (see Appendix Table IA).

Effect of Colesevelam Hydrochloride

An ANCOVA was used to compare treatment groups on the primary endpoints of transit (AC $t_{1/2}$ and geometric center at 24 h) including baseline transit GC24 values and serum 7 α C4 as covariates in the analysis (the latter after a rank transformation for skewness). The effects of colesevelam and placebo on urine mannitol excretion and the ratio of lactulose to mannitol excretion over 8-24 h was also assessed using an ANCOVA with the corresponding baseline values as covariates. In addition, separate ANCOVA models also examined the potential “interaction” of treatment and serum 7 α C4 by including a cross-

product term (rank transformation of serum $7\alpha C4$ values times treatment category, placebo versus colesevelam) in the model for each of the colonic transit endpoints. The test for a significant interaction effect assesses a potential “differential” treatment effect depending on the baseline level of serum $7\alpha C4$.

To assess the effects of colesevelam, with 12 subjects per group, there was approximately 80% power to detect a difference between colesevelam and placebo groups of 0.91 in mean colon GC 24 h values (corresponding to a difference of 37%) based on a two-sample t-test at an α level of 0.05 (two-sided) (see Appendix Table IB).

Appendix Tables IA and IB provide data regarding statistical power for the two trials.

Comment on Possible Explanation for Increased Colonic Bile Salt Concentrations

There are two possible considerations that may explain the higher colonic bile salt concentrations that led to the observed biological effects on colonic functions. Given the 4 days of administration of NaCDC in this study, we favor the simple explanation, that is, that the methacrylate coating of the delayed delivery capsule led to its dissolution in the ileocolonic region, and that enough NaCDC reached the colon to induce the clear effects on transit and stool consistency. An alternative hypothesis is that effects on colonic transit were the result of a newly established steady state concentration of colonic bile salts following repeated dosing with NaCDC and enterohepatic cycling with increased conjugated bile acid concentrations in the small intestine, leading to increased bile acid concentration in the colon. While the latter mechanism was certainly likely in patients participating in gallstone treatment trials which involved several months (rather than 4 days) of administration of CDC and did achieve steady state with increased colonic bile acid concentrations, we did not conduct kinetic studies to determine whether our patients had actually achieved this steady state. It is also worth noting that

the doses of 500 and 1000mg CDC were at the lower end of the dose range associated with diarrhea in gallstone-treated patients (13).

Appendix Table IA. Pooled Data Used to Determine Effect Size Demonstrable with 20 Healthy Participants per Treatment Group in CDC Study (data based on prior lab studies in healthy volunteers)

	Mean	SD	COV (%)	Effect size [†] (%) demonstrable with 80% power, $\alpha=0.05$
				n=20 per group
Ascending colon t $\frac{1}{2}$, h	15.4	8.5	55	50
Colon GC 24 h	2.05	0.77	38	34
Serum 7 α -C4 ng/ml	17	10	59	71

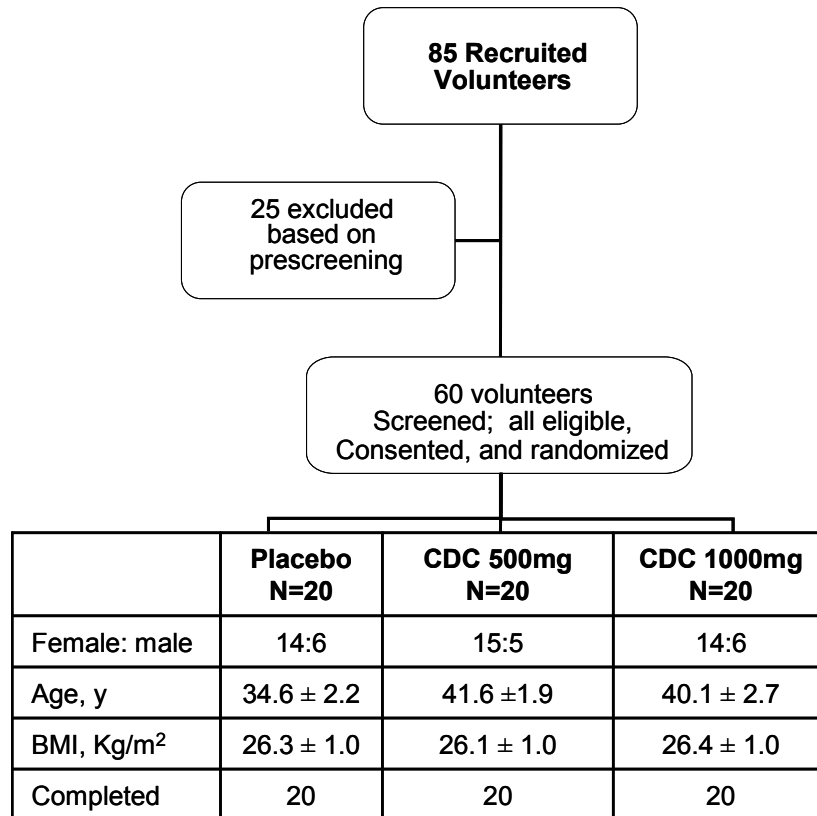
[†] Effect size is the difference in treatment means as a percentage of the overall mean (listed in the table).

Appendix Table IB. Pooled Data Used to Determine Effect Size Demonstrable with 12 Participants with IBS-D per Treatment Group in Colesevelam Study (data based on prior lab studies in patients with IBS-D)

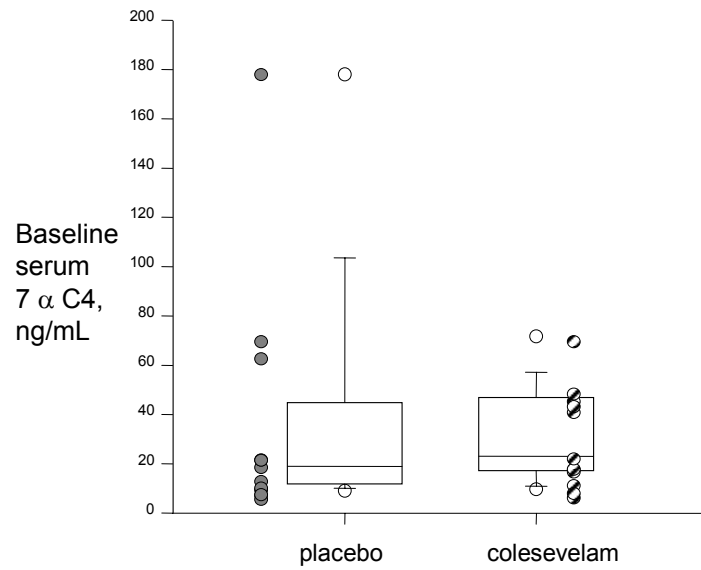
	Mean	SD	COV (%)	Effect size [†] (%) demonstrable with 80% power, $\alpha=0.05$
				n=12 per group
Ascending colon t $\frac{1}{2}$, h	14.9	9.2	62	74
Colon GC 24 h	3.53	0.87	25	30
Serum 7 α -C4 ng/ml	17	10	59	71
Urine 8-24hr Mannitol, mg/hr	8.7	8.6	99	115
Urine 8-24hr Lactulose, mg/hr	2.2	1.7	77	91

[†] Effect size is the difference in treatment means as a percentage of the overall mean (listed in the table).

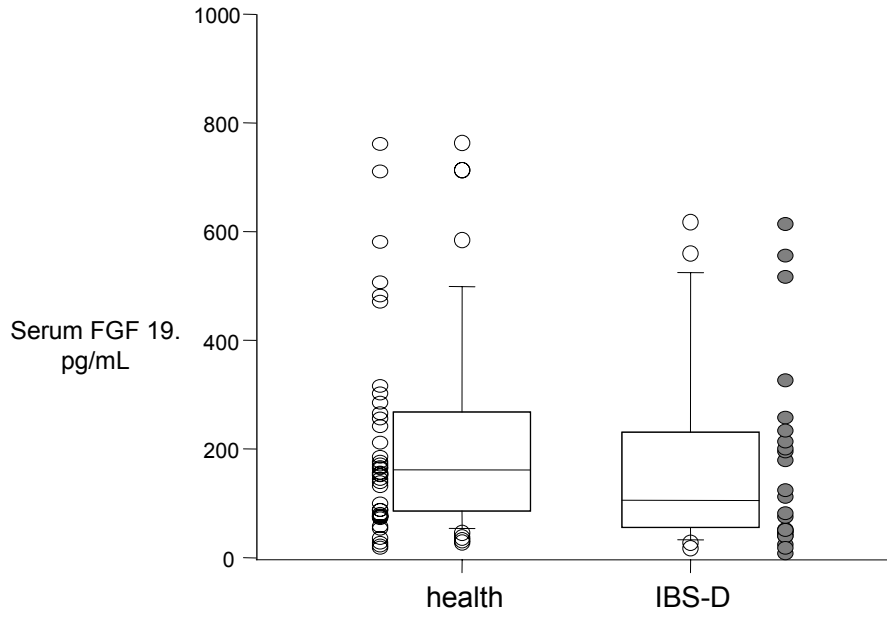
Appendix Figure 1 Study flow chart and demographics of participants in CDC study



Appendix Figure 2. Comparison of baseline serum 7 α C4 in the IBS-D patients randomized to placebo and colesevelam groups.



Appendix Figure 3. Comparison of serum FGF19 between healthy controls and IBS-D patients.



Appendix Figure 4. Relationship between serum 7 α C4 and FGF19. The right plot shows the ranks of 7 α C4 on the Y-axis and the regression line based on the ranks of 7 α C4 vs. serum FGF19. The Spearman rank correlation was used to estimate the strength of the association ($r_s = -0.414$, $p=0.044$).

