**Study Eligibility Criteria:** Exclusion criteria included a history of other chronic gastrointestinal diseases (microscopic colitis, inflammatory bowel disease, celiac disease), visceral cancer, chronic infectious disease or immunodeficiency, known liver disease, prior radiation therapy of the abdomen, uncontrolled thyroid disease, antibiotic use within three months prior to study initiation, pre- or probiotic use within two weeks prior to study initiation, or regular tobacco use. Participants who were pregnant or had undergone prior abdominal surgeries except for caesarean-section, tubal ligation, vaginal hysterectomy, appendectomy, or cholecystectomy > 6 months prior to study initiation were excluded. Participants were not allowed to take over-the-counter or prescription medications that could affect gastrointestinal transit or study interpretation (e.g., opioids, narcotics, anticholinergics, norepinephrine reuptake inhibitors, nonsteroidal anti-inflammatory agents, COX-2 inhibitors, bile acid sequestrants) within six months and two days of study initiation for HC and participants with IBS, respectively.

**Fecal SCFA and Bile Acids:** Frozen aliquots of stool were shipped to the Metabolite Profiling Facility at Purdue University to measure SCFA concentrations (acetate, propionate, butyrate) by liquid chromatography-mass spectrometry (LC-MS)1 and to the Mayo Clinic Department of Laboratory Medicine and Pathology for total and individual fecal BA by high-performance LC-MS.2, 3 Reference values for abnormal fecal BA have been previously reported by others and were used to define presence or absence of BA diarrhea.3, 4

**Colonic Transit Time Assessments:** Briefly, 10 markers were ingested daily for six consecutive days. On the final day, participants divided the daily dose of markers and consumed five markers in the morning and five markers in the evening to allow for identification of accelerated transit. An abdominal x-ray was performed on the subsequent morning and remaining markers counted to determine CTT expressed in days (calculated by dividing the number of retained markers by 10). Segmental transit time was assessed by counting the number of rings in each segment and dividing by 10.5 Previously published normal reference values6 were used to determine normal vs. abnormal (rapid or delayed) CTT.

**Food intake:**Throughout the four-day high-fat diet, participants were instructed to keep a food record using a food diary. Food intake was quantified by an automated dietary assessment tool in a subset of HC and IBS participants. Participants were instructed to maintain stable diet and avoid strict dietary interventions such as a gluten-free or diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols that could affect study interpretation.

**References:**

1. Park J, Kim M, Kang SG, et al. Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR-S6K pathway. Mucosal Immunol 2015;8:80-93.

2. Tagliacozzi D, Mozzi AF, Casetta B, et al. Quantitative analysis of bile acids in human plasma by liquid chromatography-electrospray tandem mass spectrometry: a simple and rapid one-step method. Clin Chem Lab Med 2003;41:1633-41.

3. Vijayvargiya P, Camilleri M, Shin A, et al. Methods for diagnosis of bile acid malabsorption in clinical practice. Clin Gastroenterol Hepatol 2013;11:1232-9.

4. Vijayvargiya P, Camilleri M, Chedid V, et al. Analysis of Fecal Primary Bile Acids Detects Increased Stool Weight and Colonic Transit in Patients With Chronic Functional Diarrhea. Clin Gastroenterol Hepatol 2019;17:922-929 e2.

5. Sadik R, Abrahamsson H, Ung KA, et al. Accelerated regional bowel transit and overweight shown in idiopathic bile acid malabsorption. Am J Gastroenterol 2004;99:711-8.

6. Sadik R, Abrahamsson H, Stotzer PO. Gender differences in gut transit shown with a newly developed radiological procedure. Scand J Gastroenterol 2003;38:36-42.