Effects of Low FODMAP Diet on Symptoms, Fecal Microbiome, and Markers of Inflammation in Patients With Quiescent Inflammatory Bowel Disease in a Randomized Trial

Selina Cox, James Lindsay, Sébastien Fromentin, Andrew Stagg, Neil Mccarthy, Nathalie Galleron, Samar Ibraim, Hugo Roume, Florence Levenez, Nicolas Pons, et al.

DOI:
10.1053/j.gastro.2019.09.024

Document Version
Peer reviewed version

Effects of Low-FODMAP Diet on Symptoms, Fecal Microbiome, and Markers of Inflammation in Patients With Quiescent Inflammatory Bowel Disease in a Randomized Trial

Selina R Cox[1], James O Lindsay[2,3], Sébastien Fromentin[4], Andrew J Stagg[3], Neil E McCarthy[3], Nathalie Galleron[4], Samar B. Ibraim[4], Hugo Roume[4], Florence Levenez[4], Nicolas Pons[4], Nicolas Maziers[4], Miranda C Lomer[1,5], S. Dusko Ehrlich[4], Peter M Irving[6], Kevin Whelan[1]

(1) King's College London, Department of Nutritional Sciences, London, United Kingdom
(2) Barts Health NHS Trust, Department of Gastroenterology, Royal London Hospital, London, United Kingdom
(3) Blizard Institute, Queen Mary University of London, Centre for Immunobiology, London, United Kingdom
(4) Metagénopolis, Institut National de la Recherche Agronomique, Université Paris-Saclay, France
(5) Guy's and St Thomas' NHS Foundation Trust, Department of Nutrition and Dietetics, London, United Kingdom
(6) Guy's and St Thomas' NHS Foundation Trust, Department of Gastroenterology, London, United Kingdom

Short title:

Low FODMAP diet in inflammatory bowel disease

Funding:

The study was funded by the Kenneth Rainin Foundation (Innovator and Breakthrough awards). The Kenneth Rainin Foundation had no role in the study design, data collection, data analysis, data

interpretation or writing of the manuscript. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Corresponding author: Professor Kevin Whelan

King's College London, Department of Nutritional Sciences, 150 Stamford Street, London, SE1 9NH, United Kingdom

kevin.whelan@kcl.ac.uk Phone: +44 (0)207 848 3858

Conflict of interest statement:

SRC, JOL, SF, AJS, NEM, NG, SBI, HR, FL, NP, NM, SDE and PMI have no relevant disclosures. KW and MCL are the co-inventors of a mobile application to assist patients following low FODMAP diet. KW has received consultancy fees from Danone, and a research grant from Clasado.

Author contributions:

SRC and KW were grant holders; SRC, JOL, AJS, MCL, PMI and KW conceived and designed the study; SRC, PMI and JOL recruited participants; SRC collected, collated and analyzed the data; KW supervised data analysis; SRC and KW interpreted the data; SRC, AJS, NEM performed flow cytometry and analysis; SF, SBI, NM, NP, HR, NG, FL and SDE advised on and performed metagenomic sequencing and bioinformatics analysis; SRC wrote the manuscript; KW performed extensive editing of the manuscript; all authors reviewed and approved the final manuscript for submission.

Abstract

Background & Aims: There is limited evidence that a diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) reduces gut symptoms in quiescent inflammatory bowel disease (IBD). We performed a randomized, controlled trial to investigate the effects of a low-FODMAP diet on persistent gut symptoms, the intestinal microbiome, and circulating markers of inflammation in patients with quiescent IBD.

Methods: We performed a single-blind trial of 52 patients with quiescent Crohn's disease or ulcerative colitis and persistent gut symptoms at 2 large gastroenterology clinics in the United Kingdom. Patients were randomly assigned to groups that followed a diet low in FODMAPs (n=27) or a control diet (n=25), with dietary advice, for 4 weeks. Gut symptoms and health-related quality of life were measured using validated questionnaires. Stool and blood samples were collected at baseline and end of trial. We assessed fecal microbiome composition and function using shotgun metagenomic sequencing and phenotypes of T cells in blood using flow cytometry.

Results: A higher proportion of patients reported adequate relief of gut symptoms following the low-FODMAP diet (14/27, 52%) than the control diet (4/25, 16%, *P*=.007). Patients had a greater reduction in irritable bowel syndrome severity scores following the low-FODMAP diet (mean reduction of 67; standard error, 78) than the control diet (mean reduction of 34; standard error, 50), although this difference was not statistically significant (*P*=.075). Following the low-FODMAP diet, patients had higher health-related quality of life scores (81.9±1.2) than patients on the control diet (78.3±1.2, *P*=.042). A targeted analysis revealed that in stool samples collected at the end of the study period, patients on the low-FODMAP diet had significantly lower abundance of *Bifidobacterium adolescentis*, *B longum*, and *Faecalibacterium*

prausnitzii than patients on control diet. However, microbiome diversity and markers of inflammation did not differ significantly between groups.

Conclusions: In a trial of the low-FODMAP diet vs a control diet in patients with quiescent IBD, we found no significant difference after 4 weeks in change in irritable bowel syndrome severity scores, but significant improvements in specific symptom scores and numbers reporting adequate symptom relief. The low-FODMAP diet reduced fecal abundance of microbes believed to regulate the immune response, compared with the control diet, but had no significant effect on markers of inflammation. We conclude that a 4-week diet low in FODMAPs is safe and effective for managing persistent gut symptoms in patients with quiescent IBD. www.isrctn.com no: ISRCTN17061468

KEY WORDS: CD, UC, IBS, HR-QOL

Introduction

An estimated 35% of patients with inflammatory bowel disease (IBD) experience gut symptoms despite having quiescent disease with minimal objective evidence of gastrointestinal (GI) inflammation [1] The etiology of these gut symptoms in quiescent IBD is unclear but they are hypothesized to relate to coexistent irritable bowel syndrome (IBS), the legacy of previous GI inflammation on gut function, persistent unidentified low-grade inflammation, or the psychological impact of IBD [2]. These persistent gut symptoms have a significant impact upon health-related quality of life (HR-QOL) [3] and pose a treatment dilemma since escalating immune-modulating agents is likely to be ineffective. Limited evidence exists to support the pharmacological management of persistent gut symptoms in quiescent IBD.

Dietary fermentable carbohydrates increase small intestinal water through osmotic potential (e.g. fructose, mannitol) and colonic gas through microbial fermentation (e.g. fructans, galacto-oligosaccharides) [4]. Randomized, crossover re-challenge trials, which overcome the limitations of masking and confounding in dietary intervention studies, have shown that fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs) can induce gut symptoms in both IBS and quiescent IBD [5, 6].

Dietary restriction of FODMAPs (low FODMAP diet) is thought to ameliorate functional gut symptoms by reducing diet-induced luminal water and colonic gas and consequently, luminal distension, in those with visceral hypersensitivity [7, 8]. Randomized, placebo-controlled trials of low FODMAP diet in IBS, delivered through a feeding study or as dietary advice, reported improvement of gut symptoms in 70% and 57% of patients, respectively [9, 10]. In IBD, retrospective and prospective uncontrolled studies suggest potential benefit of low FODMAP diet as a therapy for persistent gut symptoms [11, 12] and more recently, a randomized controlled trial reported that gut symptoms improved in 81% of patients with IBD during low FODMAP diet compared with 46% in control [13]. However, the trial was unblinded, therefore cannot

account for the considerable placebo response that occurs in both IBS and IBD [14] particularly in response to diet interventions.

Low FODMAP diet reduces fermentable substrate in the colon, and in IBS this alters microbiome composition, resulting in reduced Bifidobacteria [9, 15] and *Faecalibacterium prausnitzii* [16] abundance. Bifidobacteria abundance in the mucosal microbiome is positively associated with the proportion of IL-10 expressing dendritic cells in Crohn's disease (CD) [17]. Furthermore, low abundance of *F. prausnitzii* is associated with active IBD, and is associated with greater post-operative relapse at 6 months in CD [18-20]. Therefore, the microbiological impact of low FODMAP diet could theoretically have an adverse effect on the mucosal immune response and disease course in IBD, but to date has only been investigated in one trial of nine patients with Crohn's disease [21].

Accordingly, clinical trials to establish the therapeutic benefit of low FODMAP diet in managing gut symptoms in IBD must be placebo-controlled and must assess the impact on the microbiome, GI inflammation and disease activity. To this end, we designed a randomized controlled trial to investigate the effects of low FODMAP dietary advice compared to placebo (sham) dietary advice on persistent gut symptoms, disease activity, GI microbiome and peripheral T-cell phenotypes in quiescent IBD.

Methods

Study design and participants

Patients were recruited from two large gastroenterology clinics in London, United Kingdom in a multi-center, randomized, parallel, single-blinded, placebo-controlled trial. Eligible patients were aged ≥18 years, with quiescent CD or ulcerative colitis (UC), experiencing ongoing gut symptoms and were naïve to low FODMAP diet. Quiescent IBD was defined by all of the following: physician global assessment; stable medications; no IBD flare in the previous 6 months; fecal calprotectin <250 µg/g; and serum CRP <10 mg/L. The threshold for fecal calprotectin was chosen according to evidence proposing optimal sensitivity and

specificity for detecting endoscopically quiescent disease [22]. Ongoing gut symptoms were required to meet the Rome III criteria for either diarrhea predominant (IBS-D), mixed subtype (IBS-M) or unsubtyped IBS (IBS-U), functional bloating (FB) or functional diarrhea (FD), experiencing abdominal pain, bloating and/or diarrhea on ≥2 days during the baseline screening week and reporting inadequate relief of GI symptoms [23].

Patients with dose changes of azathioprine, mercaptopurine, methotrexate or biologics in the preceding 12 weeks, oral 5-aminosalicylic acid in the preceding 4 weeks or antibiotics, probiotics or prebiotics in the preceding 8 weeks were excluded. Patients with pure perianal CD, a current stoma, previous extensive GI resection or a current stricture were excluded. Patients with established bile acid malabsorption (BAM) were excluded since gut symptoms relating directly to BAM may not be modifiable by low FODMAP diet. Patients with constipation-predominant symptoms were excluded, since low FODMAP diet could exacerbate this symptom. Patients with self-reported lactose intolerance were included if they continued to experience gut symptoms despite low lactose diet. Patients were excluded if they had significant comorbidities, or if they were pregnant or lactating.

Research ethics committee approval was received from the London Dulwich ethics committee (Reference 15/LO/1684) and the trial was registered on the ISRCTN registry (ISRCTN17061468) prior to participant recruitment. All authors had access to the study data and reviewed and approved the final manuscript.

Randomization and masking

A random allocation sequence was prepared online (www.sealedenvelope.com) by an independent researcher using block randomization, with a 1:1 ratio of low FODMAP to placebo sham diet. Randomization was stratified by diagnosis (CD or UC) and fecal calprotectin at screening (≤100 μg/g and 101-249 μg/g). Allocation sequences were sealed in opaque envelopes.

147 Participants were blinded to diet allocation and informed that both diets would change the types of
148 carbohydrates consumed, but that one was the diet under investigation, while the other was a sham diet.
149 The terms 'fermentable carbohydrates', 'low FODMAP diet' or the mechanisms of the diet were not
150 mentioned to participants.

151 ***Study visits***

152 Patients were identified via gastroenterology clinics and referrals to the dietetic department for the
153 management of gut symptoms in quiescent IBD. Fecal calprotectin and CRP were assessed during
154 screening and a 7-day food, stool and GI symptom diary was completed, from which the frequency and
155 severity of gut symptoms were assessed for eligibility. Eligible participants attended a baseline visit, during
156 which questionnaires were completed and stool and blood samples were collected to assess microbiome
157 and immunology. Patients were randomized to follow either low FODMAP or sham dietary advice for 4
158 weeks and completed a 7-day food, stool and GI symptom diary in the final week. Finally, all outcomes
159 were re-assessed at an end of trial visit which was conducted within 3-days of the end of the 4-week
160 period, during which diet allocation was continued.

161 ***Intervention and control***

162 Low FODMAP and sham dietary advice were provided to all participants by the same research dietician
163 (SC) with extensive training and experience in delivering low FODMAP diet. The diet involves the
164 restriction of dietary fructans, galacto-oligosaccharides (GOS), lactose, fructose in excess of glucose, and
165 polyols, including sorbitol and mannitol, and is described in detail elsewhere [(24)]. The selection of an
166 appropriate control group and difficulties in masking intervention and control are challenging in dietary
167 intervention studies, but for research on dietary advice (which most closely mimics clinical practice),
168 'sham' dietary advice is considered gold standard [(25)]. The sham diet in this trial aimed to provide patients
169 in the control group with an exclusion diet of similar intensity and burden to low FODMAP diet, while not

impacting upon nutrient, fiber or FODMAP intakes. The sham diet has been used successfully in the only randomized, placebo-controlled trial of low FODMAP dietary advice in IBS [9]. Dietary counselling for both low FODMAP diet and sham diet lasted approximately 20 minutes and both groups received written information.

Dietary compliance to both diets was encouraged at weekly telephone contact. Compliance with the diet was assessed at end of trial using the single question: 'During the 4-week trial I have followed the diet…': never/rarely (<25% of the time), sometimes (25-50% of the time), frequently (51-75% of the time) or always (76-100% of the time). For the purposes of per protocol analysis, compliance was defined as following diet 'always' (76-100% of the time) during the trial.

Outcomes

The primary outcome was the change in IBS Severity Scoring System (IBS-SSS) during the trial, compared between groups. Pre-defined secondary outcomes included other measures of gut symptoms (total IBS-SSS score, proportion of patients achieving a 50-point IBS-SSS reduction, global symptom question; GSQ, GI symptom rating scale; GSRS), disease-specific HR-QOL, stool frequency and consistency, clinical disease activity, inflammatory markers, dietary intake, microbiome composition and function, short chain fatty acid (SCFA) concentrations and peripheral T-cell phenotype. All pre-defined secondary outcomes were included in the study protocol prior to study commencement. Exploratory outcomes included responders defined as achieving at least a 50% reduction in total IBS-SSS score during the trial.

Clinical outcomes

Gut symptoms were evaluated at baseline and end of trial using the IBS-SSS [26] and the GSRS [27]. The GSQ was used to assess adequate relief of GI symptoms at end of trial. Disease-specific HR-QOL was assessed using the UK-specific IBD questionnaire (IBDQ) [28]. Stool frequency and consistency were measured using the Bristol Stool Form Scale (BSFS) [29] which has undergone extensive validation [30].

Disease activity

At baseline and end of trial, disease activity was assessed using the Harvey Bradshaw Index for CD [31] and the Partial Mayo Score for UC [32]. Patient-perceived IBD control was assessed in all patients using the IBD Control questionnaire [33]. Fecal calprotectin concentrations were determined using enzyme-linked immunosorbent assay and serum CRP concentrations were determined using a standard assay in the hospital laboratory.

Dietary intake

Dietary intake was measured at baseline and end of trial using 7-day food records. A nutrient composition database (Nutritics, Dublin, Ireland) was used for assessment of nutrient and fiber intakes, and into a bespoke database to assess FODMAP intake (Monash University, Melbourne, Australia).

Microbiome composition, function and SCFA

A quantitative metagenomic pipeline following the International Human Microbiome Standards (IHMS; http://www.microbiome-standards.org) was used to assess GI microbiome composition and function [34].

A fresh stool sample was collected at baseline and end of trial and stored immediately on ice. The sample was homogenized and stored at -80°C (IHMS SOP 04 V2). DNA extraction was performed following IHMS SOP 07 V2. DNA was quantitated using Qubit Fluorometric Quantitation (ThermoFisher Scientific, Waltham, US) and qualified on a Fragment Analyzer (Agilent Technologies, Santa Clara, US). The sequencing library was built using 3 µg of high molecular weight DNA (>10 kbp). DNA was sheared into fragments of approximately 150 bp using an ultrasonicator (Covaris, Woburn, US) and fragment library construction was performed using the 5500 Solid Fragment 48 Library Core Kit (ThermoFisher Scientific, Waltham, US). Fragment libraries were sequenced using the Ion Proton Sequencer (ThermoFisher Scientific, Waltham, US), generating a minimum of 20 million high-quality reads of 150 bp per library.

Gene abundance profiling was performed by mapping high-quality reads to the 9.9 million gene integrated reference catalog of the human microbiome [35] using Bowtie 2 with a 95% identity threshold [36]. The gene abundance profiling table was generated via a two-step procedure using METEOR. The gene abundance table was processed for rarefaction and normalization using the MetaOMineR (momr) R package [37]. To decrease technical bias due to different sequencing depth and artifacts of sample size on low abundance genes, read counts were rarefied to 14 million reads per sample by random sampling without replacement. The resulting rarefied gene abundance table was normalized according to the FPKM (fragments per kilobase of exon model per million reads mapped) strategy. Metagenomic species (MGS) are co-abundant gene groups with more than 500 genes corresponding to microbial species. Taxonomical annotation was performed on all genes by sequence similarity using NCBI blast N; a species-level assignment was given if >50% of the genes matched the same reference genome of the NCBI database (November 2016 version) at a threshold of 95% of identity and 90% of gene length coverage. The remaining MGS were assigned to a given taxonomic level from genus to superkingdom level, where more than 50% of their genes had the same assignment level. Microbial gene richness (gene count) was calculated by counting the number of genes detected at least once in a given sample. MGS richness (MGS count) was calculated directly from the MGS abundance matrix.

The functional analysis is led using a MGP pipeline FantoMET (unpublished). Genes of the catalog were annotated using KEGG82 database. KEGG and GMM modules (Gut Metabolic Module) were reconstructed in each metagenomic species using their pathway structures (and potential alternative pathways) (39). Abundance of each detected module in a metagenomic species corresponds to the abundance of the metagenomic species as described in the method section. Abundance of a given module in a sample is computed as the sum of the abundances of the module in each metagenomic species.

Fecal short-chain fatty acid (SCFA) concentrations were assessed using a standard gas-liquid chromatography (GLC) protocol, using the 9890A series GLC system (Agilent Technologies, Santa Clara,

US) and fecal pH was measured using a pH probe (InLab®, Mettler Toledo probe and FE20 FiveEasy™ Benchtop pH meter).

Peripheral T-cell phenotype

Blood samples were collected at baseline and end of trial in sodium-heparin vacutainer tubes (BD Bioscience) and processed within 3 hours. Whole blood was labelled with fluorescently conjugated monoclonal antibodies to detect CD3 T-cells, as well as naïve (CD45RA+) and effector/memory (CD45RA-) CD4 and CD8 T-cells, and Vδ2 unconventional T-cells. The gut-homing integrin α4β7 was detected by labelling with anti-β7 [40, 41]. The BD FACSCanto II flow cytometer was used to acquire data, the FACS DIVA software (BD Bioscience) used to collect the data, and Winlist software (Verity, Topsham, ME, US) used to analyze the data.

Statistical analysis

Sample size was calculated based on the primary outcome, with expected values taken from a previous trial in IBS comparing low FODMAP (mean IBS-SSS change -117 points, SD 86) with sham advice (-44 points, SD 72) [9]. With a power of 80% and two-sided significance of 5%, a sample size of 44 participants was required. Assuming 15% attrition, a sample size of 52 participants (26 per group) was required.

Pre-planned comparisons of the primary (change in IBS-SSS score during trial) and secondary outcomes between the low FODMAP and sham diet at end of trial were performed. Sub-group analysis for UC and CD were pre-planned in the protocol and were conducted for all outcomes. The proportion of participants achieving at least a 50% reduction in total IBS-SSS score during the trial was an exploratory outcome compared between the diet groups.

Data on gut symptoms, HR-QOL, disease activity, inflammatory markers and peripheral T-cell phenotype were analyzed intention-to-treat (ITT), followed by per protocol (PP), the latter consisting of patients who

completed the trial, did not violate protocol and were 'always' compliant with dietary intervention. Data on microbiome composition and SCFA concentrations are presented for the PP population.

Clinical variables, SCFA and T-cell phenotype data were compared between groups at end of trial using ANCOVA, with corresponding baseline values as a covariate, and are therefore presented as estimated marginal mean (standard error of the mean; SEM). Categorical variables, presented as number (%), were compared between groups using the Chi-squared or Fisher's Exact Test. Statistical analysis was performed using SPSS Version 24.0 (IBM, Chicago, US).

Differences in gut microbial alpha and beta diversity between low FODMAP and sham diet were calculated using Mann-Whitney tests while comparisons of taxonomical and functional composition were assessed using likelihood ratio tests. Microbiome composition was analyzed using two approaches. First, an untargeted analysis of the relative abundance of all characterized bacteria (a total of 616 species and strains) was performed. Then, a targeted analysis of the specific species and strains of interest with regards to the low FODMAP diet or IBD was performed. *P*-values were adjusted for multiple comparisons using the Benjamini Hochberg approach for both the untargeted and targeted analyses. Microbiome bioinformatics was performed using R version 1.0.136 (Vienna, Austria). Differences are stated as statistically significant where $P \leq .05$.

Results

Recruitment occurred between February 2016 and May 2017. Of 155 screened participants, 103 were ineligible (**Figure 1**). Fifty-two patients were randomized to low FODMAP (n=27) and sham diets (n=25). All 52 randomized patients were included in the ITT analysis. Six participants were withdrawn; two withdrew consent during the trial (one in each group), one became pregnant (sham diet), two commenced steroids due to an IBD flare (one in each group), and one commenced antibiotics for an unrelated infection

283 (low FODMAP diet). Of the 46 patients completing the trial, three were non-compliant with the diet,
284 leaving 43 participants (21 low FODMAP diet, 22 sham diet) in the PP analysis.

285 Baseline characteristics are displayed in **Table 1**. There were no differences in IBD characteristics between
286 diet groups. However, participants in low FODMAP group were younger (33, SD 11 years) than in the sham
287 diet (40, SD 13 years, *P*=.031). There was a greater proportion of participants of white ethnicity in low
288 FODMAP (25/27, 92%) than the sham group (19/25, 76%, *P*=.029).

289 *Adverse events*

290 There were six adverse events during the trial. Two participants had an IBD relapse (one in each group)
291 and one commenced antibiotics unrelated to IBD (low FODMAP). All three participants were withdrawn
292 from the trial due to meeting exclusion criteria. One participant reported a worsening of abdominal pain
293 lasting two days that resolved (sham diet). Flu-like symptoms and sinusitis were reported (one in each
294 group), both of which were unrelated to the diet. No serious adverse events were recorded.

295 *Gut symptoms and HR-QOL*

296 There was a greater reduction in total IBS-SSS score following low FODMAP (-67, SEM 12) compared to
297 sham diet (-34, SEM 13), although the difference was not statistically significant (*P*=.075) (Table 2). There
298 was a significantly lower score for bloating severity (IBS-SSS) following low FODMAP (23, SEM 3) than
299 sham diet (34, SEM 3, *P*=.021). The PP analysis showed similar results to the ITT analysis for all IBS-SSS
300 outcomes. The exploratory analysis revealed that significantly more participants achieved a 50% reduction
301 in IBS-SSS following low FODMAP (9/27, 33%) than sham diet (1/25, 4%, *P*=.012) (**Table 2**).

302 Pre-defined sub-group analyses of UC (n=26) and CD (n=26) were performed for all clinical outcomes
303 (**Table 2**). In UC, there was a significantly greater reduction in IBS-SSS score following low FODMAP
304 compared to sham diet (*P*=.031), as well as a significantly lower end of trial IBS-SSS score (*P*=.031). In CD,

there was no difference in change in IBS-SSS score following low FODMAP compared to sham diet (*P*=.515), or in end of trial IBS-SSS score (*P*=.515).

Significantly more patients reported adequate relief of gut symptoms following low FODMAP (14/27, 52%) than sham diet (4/25, 16%, *P*=.007). There were no differences in the proportion of patients reporting adequate relief between low FODMAP and sham diet in the sub-group analysis of UC (7/13, 54% vs. 2/13, 15%, *P*=.097) or CD (7/14, 50% vs. 2/12, 17%, *P*=.110).

The severity of flatulence, as measured using the GSRS, was significantly lower during low FODMAP (0.9, SEM 0.1) compared to sham diet (1.2, SEM 0.1, *P*=.035), however no other symptoms, including abdominal pain, were different between groups (**Supplementary information**). Significantly lower daily stool frequency was reported following low FODMAP (1.7, SEM 0.1) than sham diet (2.1, SEM 0.1, *P*=.012), but there was no difference in the proportion of stools of normal consistency (types 3-5) between low FODMAP (65% normal consistency, SEM 5%) and sham diet (69%, SEM 5%, *P*=.478) **(Table 2).**

Total IBDQ score was significantly greater (indicating better HR-QOL) following low FODMAP (81.9, SEM 1.2) than sham diet (78.3, SEM 1.2, *P*=.042). Specifically, the Bowel II domain score (effects of GI symptoms on HR-QOL) was significantly greater following low FODMAP (76.5, SEM 2.0) than sham diet (70.0, SEM 2.1, *P*=.031).

Disease activity

At baseline, the majority of participants had CRP <5 mg/L (50/52, 96%) and fecal calprotectin <100 µg/g (43/52, 83%).

In CD, there was no difference in HBI score between low FODMAP (3.2, SEM 0.4) and sham diet (3.4, SEM 0.5, *P*=.814) at end of trial. In UC, there was no difference in Partial Mayo score between low FODMAP (0.2, SEM 0.2) and sham diet (0.2, SEM 0.2, *P*=.951). The IBD-control score demonstrated greater patient-

perceived control of IBD following low FODMAP (88.3, SEM 4.3) compared to sham diet (74.3, SEM 4.5, *P*=.028), these differences were seen specifically in UC (94.2, SEM 6.6 vs. 71.3, SEM 6.6, *P*=.022) but not in CD (81.4, SEM 5.2 vs. 79.1, SEM 5.7, *P*=.768).

Importantly, there was no difference in end of trial fecal calprotectin between low FODMAP (60.0 µg/g, SEM 9.4) and sham diet (59.6 µg/g, SEM 9.8, *P*=.976) or in serum CRP concentration between low FODMAP (2.0 mg/L, SEM 0.3) and sham diet (1.6 mg/L, SEM 0.3, *P*=.246).

Further fecal calprotectin concentration data (including UC and CD sub-group analyses and baseline compared to end of trial comparisons) are presented in the **Supplementary information.**

Dietary intake and compliance

In low FODMAP and sham diet groups, 24/27 (88%) and 25/25 (100%) of participants reported following the diet 'always' (76-100% of the time) (*P*=.230). In support of high levels of self-reported compliance, intakes of fructans, GOS, lactose, excess fructose, sorbitol and mannitol were significantly lower in the low FODMAP compared to sham diet (**Supplementary information**).

Seven-day food diaries revealed significantly lower energy, protein, fat, sugars, calcium, phosphorous and iodine intake in low FODMAP compared to sham diet (**Supplementary information**). There were no significant differences in intakes of any other nutrients between diet groups.

Microbiome composition, function and SCFA

An average of 22,690,418 sequencing reads of 150 bp were obtained for each sample, with an average 14,310,652 reads mapping uniquely to the gene catalogue (67% of reads).

There was no difference in gene count, species count, phyla distribution or any index of α-diversity or β-diversity between diet groups at end of trial (**Figure 2a-d**).

Of 616 species present in more than 5% of subjects, the abundance of 29 species (4.7%) was significantly impacted ($P \leq .05$) by the diet (untargeted microbiome analysis) (**Figure 3**). None of these remained significant when adjusted for multiple comparisons. In the targeted microbiome analysis (**Table 3**), relative abundance of total Bifidobacteria was not significantly different between low FODMAP and sham diet ($P = .073$), however *Bifidobacterium longum* ($P = .005$, $Q = .017$) and *B. adolescentis* ($P = .003$, $Q = .017$) were significantly lower, and *B. dentium* abundance was higher ($P = .035$, $Q = .096$) following the low FODMAP diet. Abundance of total *F. prausnitzii* species was significantly lower following low FODMAP compared to sham diet ($P = .038$). However, no *F. prausnitzii* strains were significantly lower and interestingly, *F. prausnitzii* SL3/3-M21/2 was higher following low FODMAP compared to sham diet (**Table 3**).

Differences in microbial abundance in the UC and CD sub-group analyses are presented in supplementary information (**Supplementary information**).

The metabolic potential of the microbiome was assessed using functional metagenomics. The abundance of 34 KO (KEGG orthology) groups were significantly different ($P \leq .05$) between low FODMAP and sham diet groups (**Figure 4**). Among the modules significantly higher in abundance following low FODMAP compared to sham diet were cellobiose transport system and propionate production, and among modules lower in abundance were lactose and galactose degradation pathways and glutamate transport system and the putative zinc/manganese transport system. None of these remained significant following FDR correction.

There were lower fecal concentrations of total SCFA following low FODMAP (398 mg/100g feces, SEM 37) compared to sham diet (505 mg/100g feces, SEM 36, $P = .049$) in the PP population. In UC, total SCFA were significantly lower following low FODMAP (386 mg/100g feces, SEM 53) than sham diet (553 mg/100g feces, SEM 55, $P = .041$). However, in CD there was no difference between diet groups (409 mg/100g feces,

SEM 51) and sham diet (463 mg/100g feces, SEM 46, *P*=.453). Individual SCFA concentrations and fecal pH in the ITT and PP populations, and in UC and CD, are provided in the **Supplementary information**.

Peripheral T-cell phenotype

There were no differences in absolute numbers or proportions of circulating naïve or effector/memory CD4 and CD8 T-cell subsets, or in cells within these subsets expressing α4β7, between diet groups at the end of the trial (**online supplementary Table 5**). Although there was no difference in the total number of Vδ2 T cells between groups, there were significantly fewer α4β7 positive Vδ2 T cells following low FODMAP compared to sham diet (**online supplementary Table 5**).

Discussion

This is the first randomized, placebo-controlled trial demonstrating that low FODMAP dietary advice improves aspects of gut symptoms and HR-QOL in patients with quiescent IBD compared to sham dietary advice. Low FODMAP diet did not alter overall microbiome diversity or any species or strains on an untargeted analysis, though it altered some immune-regulatory components of the GI microbiome during a targeted analysis. Nonetheless, there was no impact on clinical disease activity or markers of inflammation.

The finding of no significant difference in change in IBS-SSS despite higher rates of adequate relief following low FODMAP diet contrasts with a recent trial in IBS that reported a significant reduction in IBS-SSS but no difference in adequate relief [9]. The effectiveness of low FODMAP diet in the current trial confirms the findings of a non-blinded RCT in IBD in which more patients responded to low FODMAP diet than the normal diet group [13], although the IBS-SSS response rate to low FODMAP diet in the current trial was significantly lower, which likely relates to the lack of blinding in the previous trial.

The subgroup of patients with UC, but not CD, reported a significantly greater reduction in IBS-SSS score after low FODMAP compared to sham diet. Differing efficacy of drug [42] and dietary [43] interventions has been demonstrated between CD and UC previously, and may be explained by differing disease pathophysiology and location. Furthermore, patients with CD are more likely to have intestinal inflammation not detected through fecal calprotectin [44], which could have abrogated GI symptom responses to the diet. This sub-group analysis although planned *a priori* should be interpreted with caution since the trial was not powered for this comparison.

As expected from the proposed mechanism of action of low FODMAP diet, and consistent with previous studies in both IBS and IBD [9, 13, 15, 45], the greatest impact was on bloating and flatulence. Interestingly, abdominal pain was not different between diet groups following the diet. Unlike IBS, there is only limited

evidence that abdominal pain in quiescent IBD relates to luminal distension [46]. Furthermore, at trial entry, 62% of participants fulfilled functional bloating or functional diarrhea criteria, but not IBS, and therefore had minimal abdominal pain.

In both the untargeted and targeted microbiome analyses, the abundance of fecal *Bifidobacterium longum*, *B. adolescentis* and total *F. prausnitzii* were lower following low FODMAP compared with sham diet, in agreement with the findings of some previous IBS trials [9, 16] but in contrast with a previous trial in which no changes in these bacteria were demonstrated in a small (n=9) sub-group of patients with Crohn's disease following low FODMAP diet [21]. Following adjustment for multiple comparisons, these findings remained significant in only the targeted microbiome analysis, as a result of fewer comparisons. These microbial alterations are likely a result of changes in colonic fermentable substrate; Bifidobacteria preferentially ferment fructans and GOS, while *F. prausnitzii* indirectly utilizes them through cross-feeding [47].

The reduction in Bifidobacteria and *F. prausnitzii* during low FODMAP diet are of potential concern as these bacteria have immune-regulatory effects, including consistent evidence that Bifidobacteria and *F. prausnitzii* increase peripheral blood mononuclear cell (PBMC) IL-10 production *in vitro* [18, 48]. Furthermore, *F. prausnitzii* is associated with lower post-operative Crohn's disease recurrence [18]. Despite this, there were no detrimental effects of low FODMAP diet on fecal calprotectin or CRP. The lower proportion of α4β7+ Vδ2+ T-cells following low FODMAP diet may relate to variability in and the possible effect of thiopurine exposure on Vδ2+ T-cell numbers between individuals [49], since there was no difference in absolute numbers of this T-cell subgroup between diet groups.

The lack of effect of low FODMAP diet on inflammation, despite microbiome alterations, may be explained in several ways. Firstly, much of the evidence of immune-regulatory effects of *F. prausnitzii* relate to strain A2-165 [18, 50], which was not different between diet groups. Secondly, other GI bacteria, such as *Roseburia*

intestinalis and Lactobacillus species, also exert immune-modulatory effects and were not altered by the diet [48, 51]. Finally, the impact of longer-term restriction on inflammation in IBD is unknown since trial duration was four weeks.

Abundance of hydrogen-consuming *Adlercreutzia equolifaciens* was higher following low FODMAP compared with sham diet, confirming findings in IBS [52]. An emerging hypothesis is that low FODMAP diet may reduce luminal gas through both reduced fermentation and increased abundance of hydrogen-consuming bacteria, however this requires confirmation.

The reduced SCFA concentrations in UC specifically may be explained by differences in baseline microbiome composition between UC and CD [53] and also the greater GI symptom responses to low FODMAP diet in UC. Furthermore, since the colon is the site of SCFA generation, the degree of colonic disease involvement may contribute to differences in SCFA generation between CD and UC. It is tempting to speculate that the UC microbiome possesses greater saccharolytic potential, which is thus more likely to respond to reduced fermentable substrate with a decline in GI symptoms and a concomitant decline in SCFA. However, this requires confirmation in studies powered to detect differential effects of the diet in UC and CD.

The analysis revealed differing abundance in numerous microbial genomic functional pathways between diet groups at end of trial. The abundance of acetyl-CoA to acetate pathway was lower following low FODMAP diet, in line with lower fecal acetate concentrations (supplementary information). Although fecal propionate concentrations were not affected by diet, the abundance of propionate production pathway was greater following low FODMAP diet.

A major strength of this trial is that low FODMAP dietary advice was compared to sham dietary advice, providing the first placebo-controlled evidence of effectiveness in IBD. Unlike feeding studies, which are ideal for proof-of-concept, the current trial methodology assesses the effectiveness of a dietary

intervention as used in clinical practice. This trial also represents the first use of metagenomic sequencing providing a comprehensive assessment of GI microbiome composition and functional potential following low FODMAP diet. Furthermore, this is the first assessment of the effects of low FODMAP diet on immune function in IBD.

The trial design did not permit blinding of the investigator to treatment allocation. Furthermore, the observed alterations in certain nutrient intakes following low FODMAP diet, as demonstrated in previous low FODMAP diet trials [54, 55], may be confounders in interpreting the effects of low FODMAP diet in this trial. Finally, although not all patients fulfilled the IBS criteria at baseline, the IBS-SSS was chosen for gut symptom assessment since it encompasses the predominant symptoms of IBS (abdominal pain/altered bowel habit), functional bloating (bloating/distension) and functional diarrhea (altered bowel habit).

Quiescent IBD was defined, in part, as having fecal calprotectin ≤250 μg/g, as this has been shown to have optimal sensitivity and specificity for the identification of quiescent IBD [22]. Theoretically, this may have resulted in recruitment of some participants with very mildly active disease. However, only 16/52 (31%) had a fecal calprotectin above 50 μg/g and 9/52 (17%) above 100 μg/g at enrolment, thus likely having minimal effects on trial outcomes.

In conclusion, the first randomized, placebo-controlled dietary advice trial of low FODMAP diet in quiescent IBD reports improvement in some GI symptoms and HR-QOL. Despite a decline in Bifidobacteria and *F. prausnitzii* abundance, the diet did not adversely impact disease activity. Therefore, we propose that a 4-week low FODMAP diet with expert advice and intensive follow-up is safe and effective in the management of persistent gut symptoms in quiescent IBD, but caution should be taken in longer term use.

469 **Tables**

470 **Table 1 Baseline demographic and IBD characteristics of the study groups**

Variable	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value
Age (years)	33 (11)	40 (13)	**.031**
Male, n (%)	10 (37)	13 (52)	.278
BMI (kg/m^2)	24 (3)	25 (4)	.526
Ethnicity, white, n (%)	25 (92)	19 (76)	**.029**
Rome III criteria, n (%)			.150
IBS-D	10 (37)	5 (20)	
IBS-M	2 (7)	2 (8)	
IBS-U	0 (0)	1 (4)	
Functional bloating	15 (56)	13 (52)	
Functional diarrhoea	0 (0)	4 (16)	
Baseline IBS-SSS score	222 (76)	227 (81)	.847
Crohn's disease, n (%)	14 (52)	12 (48)	.781
Time since diagnosis, years	7 (8)	11 (11)	.187
Montreal classification			
Crohn's disease location, n (% of CD)			.773
Ileal	4/14 (29)	2/12 (17)	
Colonic	4/14 (29)	4/12 (33)	
Ileocolonic	6/14 (42)	6/12 (50)	
Crohn's disease behaviour, n (% of CD)			.949

Variable	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value
Non-stricturing, non-penetrating	9/14 (64)	8/12 (66)	
Stricturing	3/14 (21)	2/12 (17)	
Penetrating	2/14 (14)	2/12 (17)	
Perianal disease, n (% of CD)	4/14 (29)	3/12 (25)	1.000
Ulcerative colitis extent, n (% of UC)			.403
Proctitis	6/13 (46)	3/13 (23)	
Left-sided	4/13 (31)	7/13 (54)	
Extensive	3/13 (23)	3/13 (23)	
Medication, n (%)			
5-ASA	12 (44)	11 (44)	.974
Thiopurine	9 (33)	12 (48)	.282
Infliximab	10 (37)	4 (16)	.087
Adalimumab	2 (7)	4 (16)	.411
Vedolizumab	0 (0)	1 (4)	.481
Methotrexate	2 (7)	1 (4)	1.000
Clinical symptoms			
Total IBS-SSS score, mean (SD)	222 (76)	227 (81)	.847
Stool frequency, mean (SD)	1.8 (1.3)	2.1 (1.0)	.282
Stool consistency, proportion normal stools (type 3, 4, 5), mean (SD)	66 (29)	64 (32)	.869

Variable	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value

Continuous variables are presented as mean (SD) and were compared between groups using unpaired t-test, and categorical variables are presented as n (%) and were compared between groups using Chi-squared test

471

472 **Table 2**

IBS Severity Scoring System scores, global symptom question and stool frequency and consistency at end of trial

	All participants			Ulcerative colitis			Crohn's disease		
	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value	Low FODMAP diet (n=13)	Sham diet (n=13)	*P*-value	Low FODMAP diet (n=14)	Sham diet (n=12)	*P*-value
Change in IBS-SSS score, mean (SEM)	-67 (12)	-34 (13)	.075	-77 (15)	-29 (15)	**.031**	-55 (99)	-42 (43)	.515
Total IBS-SSS score, mean (SEM)	158 (12)	190 (13)	.075	135 (15)	183 (15)	**.031**	170 (96)	208 (95)	.515
Pain severity	22 (3)	30 (3)	.098	20 (4)	29 (4)	.123	24 (22)	32 (20)	.475
Days of pain (days)	36 (5)	38 (5)	.781	31 (6)	35 (6)	.645	36 (37)	48 (37)	.871
Bloating severity	23 (3)	34 (3)	**.021**	21 (4)	31 (4)	.113	22 (20)	39 (17)	.071
Satisfaction with bowels	39 (3)	47 (4)	.103	31 (5)	45 (5)	.068	52 (18)	43 (26)	.487
Impact on life	38 (3)	41 (3)	.521	34 (4)	41 (4)	.199	36 (25)	46 (25)	.799
IBS-SSS 50% reduction, n (%)	9 (33)	1 (4)	**.012**	4 (31)	0 (0)	.096	5 (36)	1 (8)	.170
Adequate relief, n (%)	14 (52)	4 (16)	**.007**	7 (54)	2 (15)	.097	7 (50)	2 (17)	.110
Stool frequency (per d), mean (SEM)	1.7 (0.1)	2.1 (0.1)	**.012**	1.8 (0.1)	2.0 (0.1)	.501	1.7 (0.1)	2.1 (0.1)	**.019**
Stool consistency									
Daily BSFS score, mean (SEM)	4.3 (0.2)	4.4 (0.2)	.606	4.0 (0.2)	4.4 (0.2)	.191	4.6 (0.2)	4.4 (0.2)	.673

Stool consistency, proportion normal stools (Type 3, 4, 5), mean proportion (SEM)	65 (5)	69 (5)	.478	66 (6)	73 (6)	.487	63 (6)	65 (7)	.815

Continuous variables are presented as estimated marginal mean (SEM) and were compared between groups using an ANCOVA with the corresponding baseline values as a covariate, and categorical variables are presented as n (%) and were compared between groups using Chi-squared test

IBS-SSS, Irritable bowel syndrome severity scoring system; BSFS, Bristol Stool Form Scale

Table 3 Targeted microbiome analysis: relative abundance of Bifidobacteria species and *Faecalibacterium prausnitzii* strains between diet groups at end of trial

	Low FODMAP diet (n=21)	Sham diet (n=22)	*P*-value	*Q*-value
Bifidobacteria (total)	8.63^{-7} (4.41^{-7})	3.19^{-6} (3.59^{-6})	.073	-*
Bifidobacterium adolescentis	1.99^{-7} (2.78^{-7})	2.55^{-6} (5.48^{-6})	**.003**	**.017**
Bifidobacterium longum	1.24^{-7} (1.81^{-7})	6.95^{-7} (1.03^{-6})	**.005**	**.017**
Bifidobacterium animalis	1.87^{-9} (8.59^{-9})	1.00^{-8} (4.58^{-8})	.746	.768
Bifidobacterium bifidum	6.77^{-8} (1.35^{-7})	1.79^{-7} (3.38^{-7})	.066	.146
Bifidobacterium breve	2.39^{-8} (1.09^{-7})	2.21^{-9} (1.09^{-7})	.768	.768
Bifidobacterium dentium	1.68^{-8} (5.23^{-8})	4.72^{-9} (1.75^{-8})	**.035**	.096
Bifidobacterium pseudocatenulatum	3.55^{-8} (1.17^{-7})	1.48^{-7} (4.42^{-7})	.473	.651
Faecalibacterium prausnitzii (total)	1.12^{-5} (1.42^{-5})	1.65^{-5} (1.35^{-5})	**.038**	**-***
Faecalibacterium prausnitzii A2-165	2.33^{-6} (1.93^{-6})	2.81^{-6} (2.81^{-6})	.186	.341
Faecalibacterium prausnitzii SL3/3-M21/2	1.52^{-6} (2.08^{-6})	1.35^{-6} (1.68^{-6})	**.003**	**.017**
Faecalibacterium prausnitzii L2-6	3.61^{-6} (4.26^{-6})	1.30^{-6} (1.32^{-6})	.750	.768
Faecalibacterium prausnitzii cf. KLE1255	2.68^{-6} (3.48^{-6})	3.41^{-6} (3.89^{-6})	.310	.488

All data are presented as mean (SD) relative abundance and were compared between groups adjusted for baseline abundance and end of trial stool consistency

*Total Bifidobacteria and *Faecalibacterium prausnitzii* abundance were not adjusted for multiple comparisons since these were analyzed separately at the genus level

481

Figures

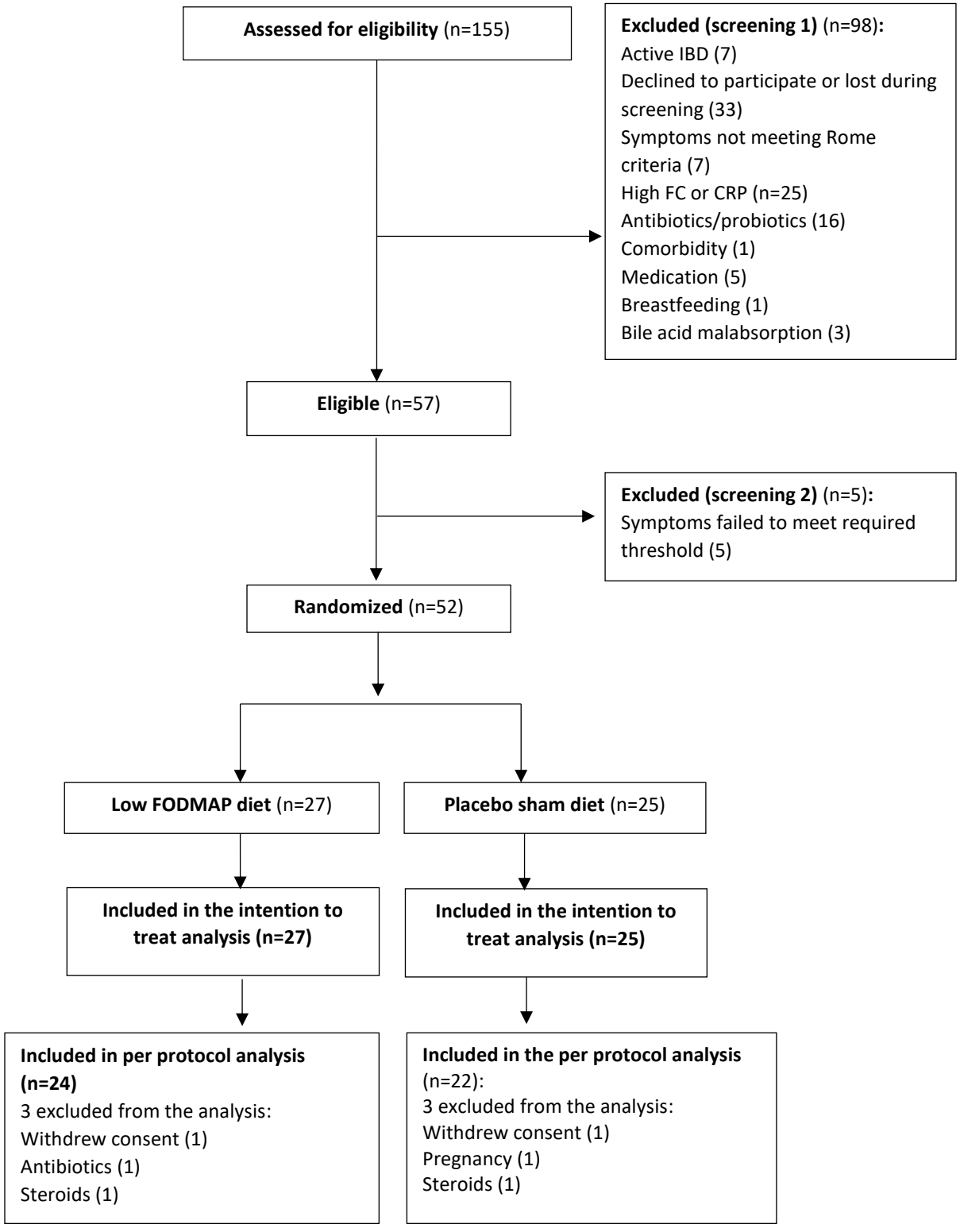


Figure 1 CONSORT diagram of participant flow through the trial

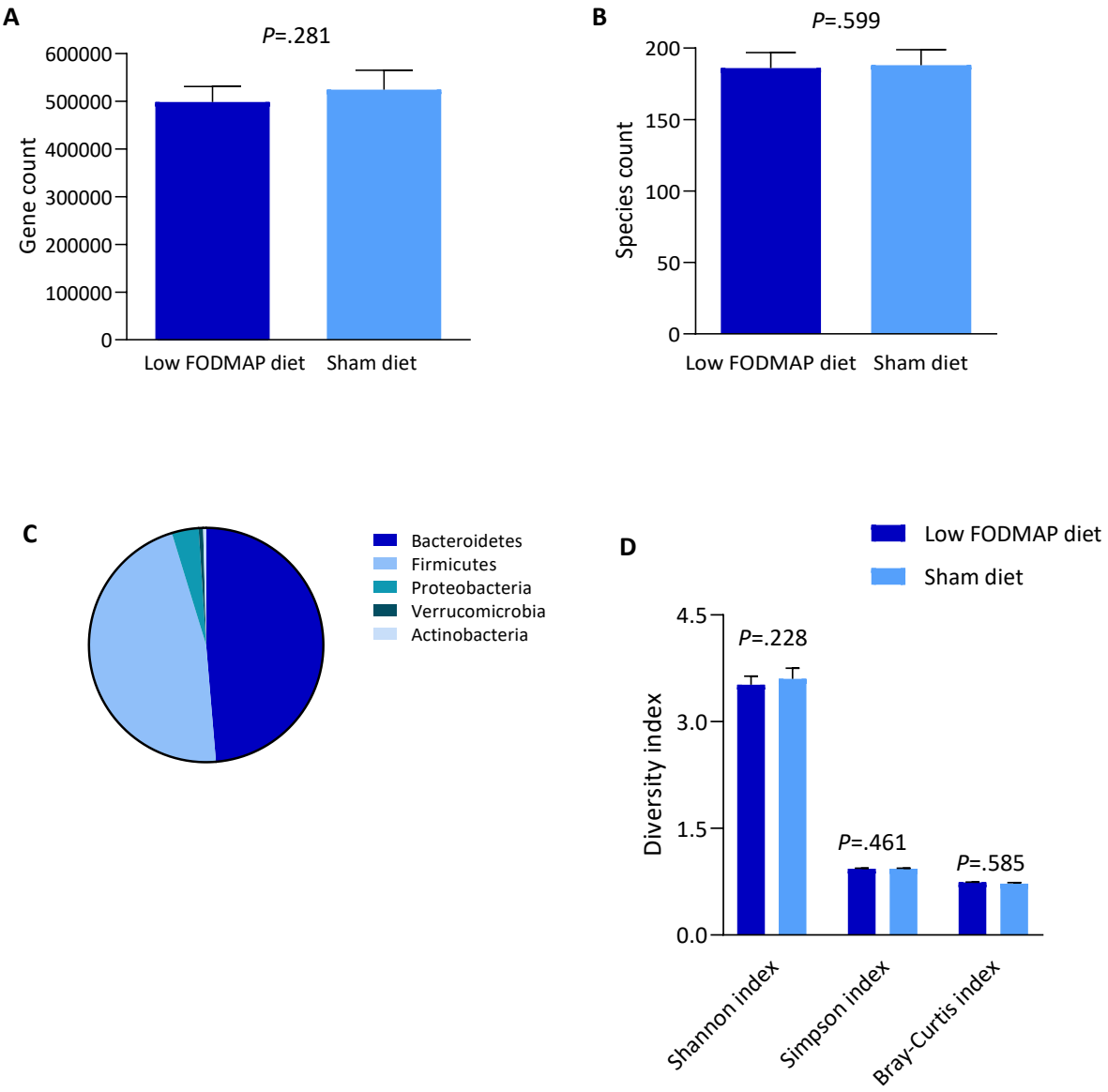


Figure 2 Alpha and beta diversity and phyla distribution at end of trial. (A) microbial gene richness, (B) microbial species richness, (C) phyla distribution, (D) Shannon index, Simpson index and Bray-Curtis index

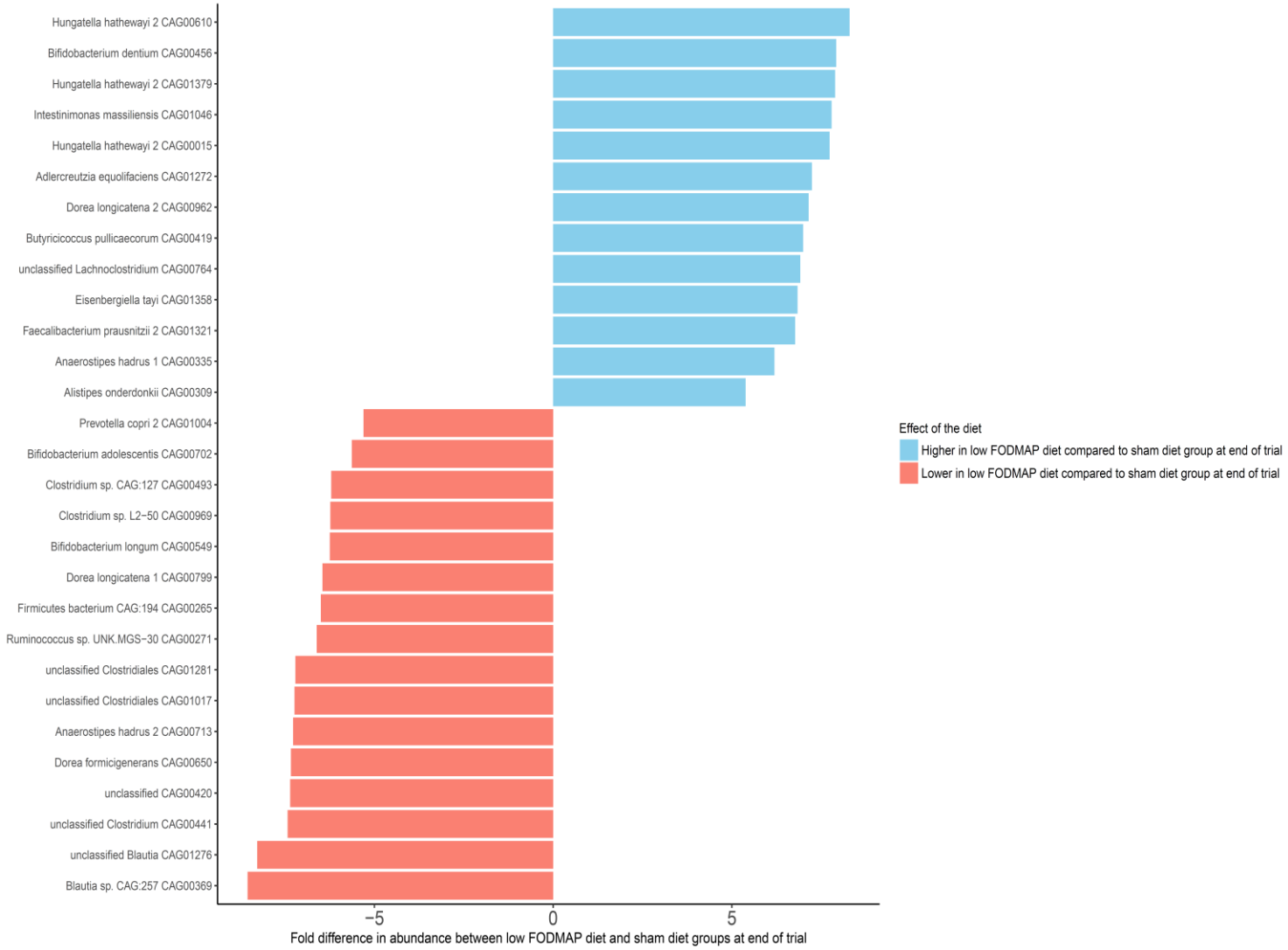
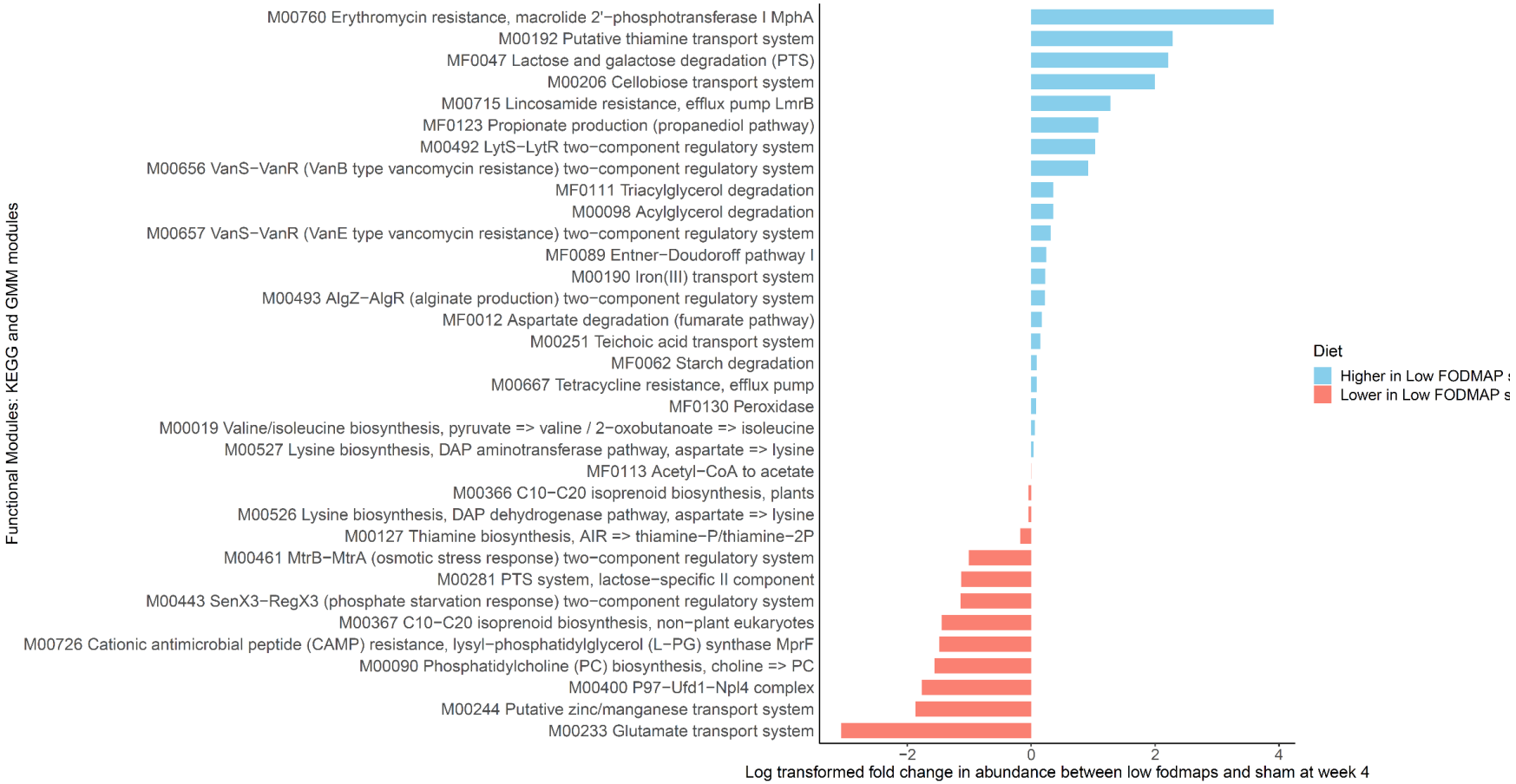


Figure 3 Untargeted microbiome analysis: fold difference in abundance of 29 species that were significantly different (*P*<.05) between diet groups at end of trial. None of these remained significant after FDR correction



535

536 **Figure 4 Fold difference in abundance of 34 functional modules with significantly different (*P*<.05) abundance between diet groups at end of**

537 **trial. None of these remained significant after FDR correction**

538

References

1. Halpin SJ, Ford AC. Prevalence of Symptoms Meeting Criteria for Irritable Bowel Syndrome in Inflammatory Bowel Disease: Systematic Review and Meta-Analysis. The American journal of gastroenterology. 2012;107(10):1474-82.
2. Quigley EMM. Overlapping irritable bowel syndrome and inflammatory bowel disease: less to this than meets the eye? Therap Adv Gastroenterol. 2016;9(2):199-212.
3. Gracie DJ, Williams CJ, Sood R, Mumtaz S, Bholah MH, Hamlin PJ, et al. Negative Effects on Psychological Health and Quality of Life of Genuine Irritable Bowel Syndrome-type Symptoms in Patients With Inflammatory Bowel Disease. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2017;15(3):376-84.e5.
4. Staudacher HM, Whelan K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. Gut. 2017;66(8):1517-27.
5. Shepherd SJ, Parker FC, Muir JG, Gibson PR. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: randomized placebo-controlled evidence. Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association. 2008;6(7):765-71.
6. Cox SR, Prince AC, Myers CE, Irving PM, Lindsay JO, Lomer MC, et al. Fermentable carbohydrates (FODMAPs) exacerbate functional gastrointestinal symptoms in patients with inflammatory bowel disease: a randomised, double-blind, placebo-controlled, cross-over, re-challenge trial. Journal of Crohn's & colitis. 2017;11:1420-29.
7. Major G, Pritchard S, Murray K, Alappadan JP, Hoad CL, Marciani L, et al. Colon Hypersensitivity to Distension, Rather Than Excessive Gas Production, Produces Carbohydrate-Related Symptoms in Individuals With Irritable Bowel Syndrome. Gastroenterology. 2017;152(1):124-33.e2.
8. Barrett J, Gearry R, Muir J, Irving P, Rose R, Rosella O, et al. Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon. Aliment Pharmacol Ther. 2010;31(8):874-82.
9. Staudacher HM, Lomer MCE, Farquharson FM, Louis P, Fava F, Franciosi E, et al. Diet Low in FODMAPs Reduces Symptoms in Patients with Irritable Bowel Syndrome and Probiotic Restores Bifidobacterium Species: a Randomized Controlled Trial. Gastroenterology. 2017;67:895-903.
10. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. Gastroenterology. 2014;146(1):67-75. e5.
11. Gearry RB, Irving PM, Barrett JS, Nathan DM, Shepherd SJ, Gibson PR. Reduction of dietary poorly absorbed short-chain carbohydrates (FODMAPs) improves abdominal symptoms in patients with inflammatory bowel disease—a pilot study. J Crohn's Colitis. 2009;3(1):8-14.
12. Prince AC, Myers CE, Joyce T, Irving P, Lomer M, Whelan K. Fermentable Carbohydrate Restriction (Low FODMAP Diet) in Clinical Practice Improves Functional Gastrointestinal Symptoms in Patients with Inflammatory Bowel Disease. Inflamm Bowel Dis. 2016.
13. Pedersen N, Ankersen DV, Felding M, Wachmann H, Vegh Z, Molzen L, et al. Low-FODMAP diet reduces irritable bowel symptoms in patients with inflammatory bowel disease. World journal of gastroenterology. 2017;23(18):3356-66.
14. Elsenbruch S, Enck P. Placebo effects and their determinants in gastrointestinal disorders. Nature reviews Gastroenterology & hepatology. 2015;12(8):472-85.
15. Staudacher HM, Lomer MC, Anderson JL, Barrett JS, Muir JG, Irving PM, et al. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. The Journal of nutrition. 2012;142(8):1510-8.
16. Halmos EP, Christophersen CT, Bird AR, Shepherd SJ, Gibson PR, Muir JG. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. Gut. 2014;64:93-100.
17. Ng S, Benjamin J, McCarthy N, Hedin C, Koutsoumpas A, Plamondon S, et al. Relationship between human intestinal dendritic cells, gut microbiota, and disease activity in Crohn's disease. Inflammatory Bowel Diseases. 2011;17(10):2027-37.

18. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux J-J, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. PNAS. 2008;105(43):16731-6.
19. Varela E, Manichanh C, Gallart M, Torrejón A, Borruel N, Casellas F, et al. Colonisation by Faecalibacterium prausnitzii and maintenance of clinical remission in patients with ulcerative colitis. Aliment Pharmacol Ther. 2013;38(2):151-61.
20. Halfvarson J, Brislawn CJ, Lamendella R, Vázquez-Baeza Y, Walters WA, Bramer LM, et al. Dynamics of the human gut microbiome in inflammatory bowel disease. Nat Microbiol. 2017;2:17004.
21. Halmos EP, Christophersen CT, Bird AR, Shepherd SJ, Muir JG, Gibson PR. Consistent Prebiotic Effect on Gut Microbiota With Altered FODMAP Intake in Patients with Crohn's Disease: A Randomised, Controlled Cross-Over Trial of Well-Defined Diets. Clin Transl Gastroenterol. 2016;7:e164.
22. Lin J-F, Chen J-M, Zuo J-H, Yu A, Xiao Z-J, Deng F-H, et al. Meta-analysis: Fecal Calprotectin for Assessment of Inflammatory Bowel Disease Activity. Inflamm Bowel Dis. 2014;20(8):1407-15
23. Mangel A, Hahn B, Heath A, Northcutt A, Kong S, Dukes G, et al. Adequate relief as an endpoint in clinical trials in irritable bowel syndrome. J Int Med Res. 1998;26(2):76-81.
24. Whelan K, Martin LD, Staudacher HM, Lomer MCE. The low FODMAP diet in the management of irritable bowel syndrome: an evidence-based review of FODMAP restriction, reintroduction and personalisation in clinical practice. Journal of human nutrition and dietetics : the official journal of the British Dietetic Association. 2018;31:239-55.
25. Staudacher HM, Irving PM, Lomer MCE, Whelan K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. The Proceedings of the Nutrition Society. 2017;76(3):203-12.
26. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997;11(2):395-402.
27. Svedlund J, Sjödin I, Dotevall G. GSRS—a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. Digestive diseases and sciences. 1988;33(2):129-34.
28. Cheung W-y, Garratt AM, Russell IT, Williams JG. The UK IBDQ—A British version of the inflammatory bowel disease questionnaire: development and validation. J Clin Epidemiol. 2000;53(3):297-306.
29. Lewis SJ, Heaton KW. Stool Form Scale as a Useful Guide to Intestinal Transit Time. Scandinavian journal of gastroenterology. 1997;32(9):920-4.
30. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther. 2016;44(7):693-703.
31. Harvey R, Bradshaw J. A simple index of Crohn's-disease activity. The Lancet. 1980;315(8167):514.
32. Lewis JD, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. Inflamm Bowel Dis. 2008;14(12):1660-6.
33. Bodger K, Ormerod C, Shackcloth D, Harrison M, Collaborative obotIC. Development and validation of a rapid, generic measure of disease control from the patient's perspective: the IBD-Control questionnaire. Gut. 2013;63:1092-102.
34. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, et al. Towards standards for human fecal sample processing in metagenomic studies. Nature biotechnology. 2017;35(11):1069-76.
35. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, et al. An integrated catalog of reference genes in the human gut microbiome. Nature biotechnology. 2014;32(8):834-41.
36. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. Nature methods. 2012;9(4):357-9.

641 37. Cotillard A, Kennedy SP, Kong LC, Prifti E, Pons N, Le Chatelier E, et al. Dietary intervention
642 impact on gut microbial gene richness. Nature. 2013;500(7464):585-8.
643 38. Nielsen HB, Almeida M, Juncker AS, Rasmussen S, Li J, Sunagawa S, et al. Identification and
644 assembly of genomes and genetic elements in complex metagenomic samples without using reference
645 genomes. Nature biotechnology. 2014;32:822.
646 39. Vieira-Silva S, Falony G, Darzi Y, Lima-Mendez G, Garcia Yunta R, Okuda S, et al. Species-
647 function relationships shape ecological properties of the human gut microbiome. Nat Microbiol.
648 2016;1(8):16088.
649 40. Hedin CR, McCarthy NE, Louis P, Farquharson FM, McCartney S, Taylor K, et al. Altered
650 intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their
651 unaffected siblings. Gut. 2014;63(10):1578-86.
652 41. Fischer A, Zundler S, Atreya R, Rath T, Voskens C, Hirschmann S, et al. Differential effects of
653 α4β7 and GPR15 on homing of effector and regulatory T cells from patients with UC to the inflamed
654 gut in vivo. Gut. 2015;65:1642-64.
655 42. Cholapranee A, Hazlewood GS, Kaplan GG, Peyrin-Biroulet L, Ananthakrishnan AN. Systematic
656 review with meta-analysis: comparative efficacy of biologics for induction and maintenance of
657 mucosal healing in Crohn's disease and ulcerative colitis controlled trials. Aliment Pharmacol Ther.
658 2017;45(10):1291-302.
659 43. Derwa Y, Gracie DJ, Hamlin PJ, Ford AC. Systematic review with meta-analysis: the efficacy of
660 probiotics in inflammatory bowel disease. Aliment Pharmacol Ther. 2017;46(4):389-400.
661 44. Costa F, Mumolo M, Ceccarelli L, Bellini M, Romano M, Sterpi C, et al. Calprotectin is a stronger
662 predictive marker of relapse in ulcerative colitis than in Crohn's disease. Gut. 2005;54(3):364-8.
663 45. Halmos EP, Power VA, Shepherd SJ, Gibson PR, Muir JG. A diet low in FODMAPs reduces
664 symptoms of irritable bowel syndrome. Gastroenterology. 2014;146(1):67-75.e5.
665 46. van Hoboken EA, Thijssen AY, Verhaaren R, van der Veek PPJ, Prins FA, Verspaget HW, et al.
666 Symptoms in patients with ulcerative colitis in remission are associated with visceral hypersensitivity
667 and mast cell activity. Scandinavian journal of gastroenterology. 2011;46(7-8):981-7.
668 47. Moens F, Weckx S, De Vuyst L. Bifidobacterial inulin-type fructan degradation capacity
669 determines cross-feeding interactions between bifidobacteria and Faecalibacterium prausnitzii.
670 International journal of food microbiology. 2016;231:76-85.
671 48. Dong H, Rowland I, Yaqoob P. Comparative effects of six probiotic strains on immune function
672 in vitro. The British journal of nutrition. 2012;108(3):459-70.
673 49. McCarthy NE, Hedin CR, Sanders TJ, Amon P, Hoti I, Ayada I, et al. Azathioprine therapy
674 selectively ablates human Vδ2(+) T cells in Crohn's disease. The Journal of clinical investigation.
675 2015;125(8):3215-25.
676 50. Quévrain E, Maubert MA, Michon C, Chain F, Marquant R, Tailhades J, et al. Identification of
677 an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in
678 Crohn's disease. Gut. 2015;65(3):415-25.
679 51. Shen Z, Zhu C, Quan Y, Yang J, Yuan W, Yang Z, et al. Roseburia intestinalis alleviates
680 experimental colitis pathology by inducing anti-inflammatory responses. J Gastroenterol Hepatol.
681 2018;Epub ahead of print.
682 52. McIntosh K, Reed DE, Schneider T, Dang F, Keshteli AH, De Palma G, et al. FODMAPs alter
683 symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut.
684 2016;66(7):1241-51.
685 53. Pascal V, Pozuelo M, Borruel N, Casellas F, Campos D, Santiago A, et al. A microbial signature
686 for Crohn's disease. Gut. 2017;66(5):813-22.
687 54. Staudacher HM, Ralph FSE, Irving PM, Whelan K, Lomer MCE. Nutrient Intake, Diet Quality,
688 and Diet Diversity in Irritable Bowel Syndrome and the Impact of the Low FODMAP Diet. J Acad Nutr
689 Diet. 2019.

690 55. Eswaran S, Dolan RD, Ball SC, Jackson K, Chey W. The Impact of a 4-Week Low-FODMAP and
691 mNICE Diet on Nutrient Intake in a Sample of US Adults with Irritable Bowel Syndrome with Diarrhea.
692 J Acad Nutr Diet. 2019.

693

Supplementary information

Supplementary methods

Microbiome composition and function

The gene abundance profiling table was generated via a two-step procedure using METEOR. First, reads uniquely mapping to a gene in the catalogue were attributed to their corresponding genes. Second, reads mapped to multiple shared genes in the catalogue were attributed according to the ratio of the genes unique mapping counts.

The gene abundance profiling table was generated via a two-step procedure using METEOR. First, reads uniquely mapping to a gene in the catalogue were attributed to their corresponding genes. Second, reads mapped to multiple shared genes in the catalogue were attributed according to the ratio of the genes unique mapping counts.

The 9.9 million-gene catalogue was constructed by clustering 1436 MGS from 1267 human gut microbiome samples, as previously described (1). MGS abundances were estimated as the mean abundance of the 50 genes defining a robust centroid of the cluster.

Supplementary results

Gut symptoms

The incidence of moderate or severe gastrointestinal symptoms and 7-day severity of symptoms (as assessed using the Gastrointestinal Symptom Rating Scale, GSRS) is presented in online supplementary Table 1. There were no differences between the diet groups in the incidence or severity of any symptoms, except for lower flatulence severity following low FODMAP compared to sham diet

Online Supplementary Table 1 Incidence and severity of gastrointestinal symptoms, as measured by the Gastrointestinal Symptom Rating Scale, at end of trial

	Incidence of moderate or severe symptoms[a]			Severity of GI symptoms[b]		
Symptom	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value
Pain	1.5 (0.3)	1.1 (0.3)	.220	0.9 (0.5)	0.7 (4.5)	.243
Heartburn	0.3 (0.1)	0.2 (0.1)	.514	0.2 (0.5)	0.1 (0.3)	.344
Acid regurgitation	0.3 (0.1)	0.2 (0.1)	.359	0.2 (0.5)	0.2 (0.5)	.504
Nausea	0.5 (0.1)	0.3 (0.1)	.283	0.3 (0.5)	0.3 (0.5)	.335
Gurgling	0.7 (0.2)	0.8 (0.2)	.858	0.6 (0.5)	0.6 (0.5)	.995
Bloating	1.4 (0.3)	1.7 (0.3)	.595	0.9 (0.5)	0.9 (0.5)	.628
Belching	0.2 (0.1)	0.5 (0.1)	.141	0.4 (0.5)	0.5 (0.5)	.312
Flatulence	1.4 (0.3)	2.1 (0.4)	.152	0.9 (0.5)	1.1 (0.6)	**.035**
Constipation	0.5 (0.2)	0.6 (0.2)	.768	0.3 (0.5)	0.3 (0.5)	.513
Diarrhoea	0.4 (0.1)	0.5 (0.1)	.507	0.2 (0.5)	0.3 (0.5)	.214
Loose stools	0.9 (0.2)	0.9 (0.2)	.914	0.5 (0.5)	0.5 (0.5)	.981
Hard stools	0.1 (0.1)	0.3 (0.1)	.293	0.2 (0.4)	0.2 (0.5)	.656
Urgency	0.9 (0.2)	0.8 (0.2)	.756	0.6 (0.5)	0.5 (0.5)	.635
Incomplete evacuation	0.7 (0.2)	0.5 (0.2)	.592	0.5 (0.5)	0.4 (0.5)	.166
Tiredness	2.3 (0.3)	2.0 (0.4)	.692	1.1 (0.5)	1.0 (0.5)	.694

Overall symptoms	1.2 (0.5)	1.7 (0.7)	.439	1.0 (0.5)	1.1 (0.5)	.493

Data are presented as estimated marginal mean (SEM) and groups were compared using ANCOVA with baseline values as a covariate

[a] Number of days on which each symptom was reported at moderate or severe during the final week of the diet

[b] Average severity across 7 days; 0=absent, 1=mild, 2=moderate, 3=severe

Dietary intake

Daily intakes of energy, protein, fat, sugars, calcium, phosphorous and iodine were significantly lower following the low FODMAP compared to sham diet at end of trial (online supplementary Table 2).

Online Supplementary Table 2 Daily intake of nutrients and FODMAPs in the diet groups at end of trial (7-day average intakes)

	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value
Energy (kcal/d)	1697 (47)	1918 (49)	**.002**
Protein (g/d)	74 (2)	83 (2)	**.008**
Fat (g/d)	68 (4)	80 (4)	**.035**
Saturated fat (g/d)	24 (1)	27 (2)	.102
Carbohydrate (g/d)	180 (6)	197 (6)	.058
Starch (g/d)	116 (4)	117 (5)	.841
Sugars (g/d)	63 (4)	76 (4)	**.022**
Fiber, AOAC (g/d)	17.8 (0.8)	19.2 (0.9)	.249
Calcium (mg/d)	692 (39)	911 (41)	**<.001**
Iron (mg/d)	10.9 (0.6)	12.0 (0.6)	.170
Zinc (mg/d)	9 (1)	10 (1)	.470
Sodium (mg/d)	1532 (85)	2195 (89)	**<.001**
Potassium (mg/d)	2938 (148)	3034 (154)	.658
Phosphorous (mg/d)	1140 (36)	1312 (37)	**.002**
Magnesium (mg/d)	290 (13)	297 (13)	.709
Iodine (µg/d)	124 (15)	176 (16)	**.022**
Selenium (µg/d)	59 (4)	57 (4)	.823
Vitamin A (µg/d)	1358 (207)	1328 (215)	.921
Vitamin C (mg/d)	90 (7)	75 (8)	.166
Vitamin D (µg/d)	6.4 (0.4)	6.3 (0.4)	.818
Vitamin B_9 (folate) (µg/d)	229 (12)	257 (12)	.110

	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value
Vitamin B_{12} (cobalamin) (μg/d)	6.0 (0.9)	5.6 (0.9)	.782
FODMAPs			
Fructans (g/d)	1.3 (0.2)	2.9 (0.2)	**<.001**
GOS (g/d)	0.4 (0.1)	0.8 (0.1)	**<.001**
Lactose (g/d)	5.6 (1.0)	10.9 (1.1)	**.001**
Excess fructose (g/d)	0.5 (0.2)	1.4 (0.2)	**.001**
Sorbitol (g/d)	0.1 (0.1)	0.6 (0.1)	**.001**
Mannitol (g/d)	0.1 (0.0)	0.3 (0.0)	**.002**

Data are presented as estimated marginal mean (SEM) and groups were compared using ANCOVA with baseline values as a covariate. AOAC, Association of Official Analytical Chemists

There were no differences in the proportion of patients meeting national macronutrient, micronutrient and fiber recommendations between the low FODMAP and sham diet groups at end of trial, or between baseline and end of trial in either diet group (data not shown).

Microbiome composition and SCFA

Online supplementary table 3 displays the relative abundance of the bacterial species or strains that were significantly different between the diet groups at end of trial in the untargeted UC and CD sub-group microbiome analyses.

Online Supplementary Table 3 Untargeted microbiome analysis: relative abundance of species and strains that were significantly different between the diet groups (*P*≤.05) at end of trial in patients with ulcerative colitis and Crohn's disease. None of these species were significantly different between diet groups after FDR correction

	Ulcerative colitis				Crohn's disease			
Genus or species	**Low FODMAP diet (n=13)**	**Sham diet (n=11)**	***P*-value**	***Q*-value**	**Low FODMAP diet (n=8)**	**Sham diet (n=11)**	***P*-value**	***Q*-value**
Bifidobacterium. adolescentis	1.52^{-7} (2.65^{-7})	1.72^{-7} (2.79^{-6})	**.004**	.592	2.73^{-7} (3.02^{-7})	3.31^{-6} (7.19^{-6})	.216	.690
B. longum	1.60^{-7} (2.18^{-7})	7.21^{-7} (1.13^{-6})	**<.001**	.115	6.53^{-8} (7.46^{-8})	6.73^{-7} (9.83^{-7})	.201	.682
F. prausnitzii								
SL3/3-M21/2	1.30^{-6} (1.93^{-6})	1.55^{-6} (1.47^{-6})	**.017**	.592	1.87^{-6} (2.39^{-6})	1.17^{-6} (1.90^{-6})	**.031**	.654
A2-165	2.38^{-6} (2.02^{-6})	2.97^{-6} (2.35^{-6})	.563	.806	2.26^{-6} (1.91^{-6})	2.66^{-6} (3.29^{-6})	.094	.654
L2-6	3.76^{-6} (4.67^{-6})	1.68^{-6} (1.19^{-6})	.356	.693	3.37^{-6} (3.79^{-6})	9.56^{-7} (1.39^{-6})	.443	.752
KLE1255	3.63^{-6} (4.14^{-6})	4.43^{-6} (3.81^{-6})	.562	.806	1.13^{-6} (8.88^{-7})	2.48^{-6} (3.89^{-6})	**.025**	.654
Ruminococcus sp. UNK.MGS-30	0.00 (0.00)	5.14^{-7} (9.13^{-7})	**.024**	.592	0.00 (0.00)	0.00 (0.00)	.393	.729
Rumincoccus bicirculans	8.78^{-7} (2.18^{-6})	2.97^{-6} (5.15^{-6})	**.005**	.592	1.40^{-6} (2.58^{-6})	1.05^{-6} (1.97^{-6})	.984	.993
Ruminococcaceae unclassified CAG00957	2.19^{-8} (7.21^{-8})	1.44^{-8} (3.49^{-8})	**.010**	.592	1.63^{-9} (4.61^{-9})	1.31^{-7} (4.10^{-7})	.475	.768
Clostridium sp. AT4	4.91^{-7} (1.44^{-6})	5.35^{-8} (9.36^{-8})	**.015**	.592	1.02^{-7} (2.10^{-7})	1.31^{-7} (3.51^{-7})	.596	.849
Clostridium unclassified CAG00441	3.44^{-8} (3.72^{-8})	7.92^{-8} (1.31^{-7})	.107	.592	2.63^{-8} (1.89^{-8})	5.95^{-8} (1.30^{-7})	**.009**	.563
Clostridium bolteae	1.01^{-6} (2.99^{-6})	3.87^{-8} (4.40^{-8})	**.049**	.592	5.41^{-8} (2.71^{-7})	2.04^{-7} (2.71^{-7})	.800	.966
Clostridium citroniae	8.52^{-8} (1.03^{-7})	3.21^{-8} (3.29^{-8})	.799	.927	1.01^{-7} (1.03^{-7})	4.90^{-8} (6.40^{-8})	**.001**	.311
Clostridium sp. KLE 1755	9.04^{-8} (1.55^{-7})	2.80^{-8} (5.72^{-8})	.201	.597	2.40^{-7} (2.70^{-7})	1.62^{-7} (4.46^{-7})	**.035**	.654
Clostridiales unclassified CAG01017	0.00 (0.00)	7.73^{-8} (1.25^{-7})	.075	.592	1.17^{-8} (2.20^{-8})	4.98^{-8} (1.28^{-7})	**.049**	.654
Clostridiales unclassified CAG01281	2.42^{-8} (8.05^{-8})	1.57^{-8} (3.90^{-8})	**.006**	.592	4.44^{-10} (1.26^{-9})	1.33^{-7} (4.39^{-7})	.087	.654
Roseburia intestinalis CAG00291	5.09^{-6} (8.80^{-6})	4.71^{-6} (8.35^{-6})	**.028**	.592	2.98^{-6} (6.09^{-6})	6.39^{-7} (1.37^{-6})	.300	.726
Roseburia intestinalis CAG01369	4.94^{-6} (8.59^{-6})	4.42^{-6} (7.70^{-6})	**.032**	.592	2.90^{-6} (5.94^{-6})	5.92^{-7} (1.27^{-6})	.307	.726
Roseburia unclassified CAG00869	7.95^{-8} (1.50^{-7})	5.65^{-8} (6.71^{-8})	.649	.871	4.14^{-8} (8.93^{-8})	1.45^{-7} (2.47^{-7})	**.043**	.654

	Ulcerative colitis				Crohn's disease			
Genus or species	**Low FODMAP diet (n=13)**	**Sham diet (n=11)**	***P*-value**	***Q*-value**	**Low FODMAP diet (n=8)**	**Sham diet (n=11)**	***P*-value**	***Q*-value**
Flavonifractor sp. 2789STDY5834895	1.40^{-7} (1.55^{-7})	1.52^{-7} (1.71^{-7})	**.018**	.592	2.44^{-7} (5.96^{-7})	4.12^{-7} (5.54^{-7})	.148	.654
Prevotella unclassified CAG00517	5.62^{-8} (2.03^{-7})	3.24^{-8} (1.03^{-7})	**.018**	.592	0.00 (0.00)	1.37^{-6} (4.53^{-6})	.335	.726
Prevotella sp. CAG:520	8.29^{-7} (2.99^{-6})	4.38^{-7} (1.39^{-6})	**.018**	.592	0.00 (0.00)	6.59^{-7} (2.19^{-6})	.148	.654
Eubacterium ventriosum	3.01^{-7} (5.45^{-7})	4.69^{-8} (7.85^{-8})	**.021**	.592	3.74^{-8} (1.01^{-7})	3.86^{-7} (5.64^{-7})	**.043**	.654
Eubacterium hallii	2.02^{-7} (2.57^{-7})	1.66^{-7} (1.62^{-7})	.369	.694	5.35^{-8} (6.15^{-8})	1.73^{-7} (1.57^{-7})	**.036**	.654
Catenibacterium mitsuokai	6.12^{-9} (2.21^{-8})	3.45^{-7} (1.09^{-6})	**.024**	.592	1.25^{-7} (3.53^{-7})	0.00 (0.00)	.311	.726
Barnesiella intestinihominis	3.49^{-6} (5.64^{-6})	1.99^{-6} (2.93^{-6})	**.024**	.592	2.73^{-6} (3.36^{-6})	3.97^{-6} (5.50^{-6})	.638	.862
Firmicutes unclassified CAG00808	9.75^{-8} (2.04^{-7})	1.62^{-8} (4.34^{-8})	.886	.958	2.63^{-8} (3.74^{-8})	4.77^{-8} (1.01^{-7})	**.012**	.654
Firmicutes bacterium CAG:194	0.00 (0.00)	2.02^{-7} (4.02^{-7})	**.036**	.592	0.00 (0.00)	4.25^{-7} (1.41^{-6})	.402	.729
Bacteroides xylanisolvens	2.57^{-6} (6.30^{-6})	1.66^{-6} (2.11^{-6})	.481	.771	1.43^{-5} (2.43^{-5})	2.58^{-6} (4.99^{-6})	**.009**	.563
Bacteroides cellulosilyticus	1.46^{-7} (3.71^{-7})	1.59^{-8} (3.06^{-8})	**.038**	.592	6.14^{-8} (1.74^{-7})	5.69^{-7} (1.10^{-6})	.247	.706
Parabacteroides distasonis	7.40^{-6} (1.61^{-5})	1.15^{-6} (9.61^{-7})	.798	.927	3.99^{-6} (3.84^{-6})	3.25^{-6} (3.22^{-6})	**.007**	.563
Candidatus gastranaerophilales bacterium HUM_2	1.16^{-6} (2.86^{-6})	2.07^{-7} (6.55^{-7})	**.032**	.592	5.99^{-7} (1.69^{-6})	6.49^{-7} (2.11^{-6})	.219	.693
Coprobacter secundus	2.03^{-8} (4.44^{-8})	3.65^{-8} (7.37^{-8})	**.046**	.592	1.80^{-7} (3.06^{-7})	2.63^{-8} (8.74^{-8})	.195	.682
Coprobacter fastidiosus	5.85^{-8} (1.37^{-7})	9.51^{-8} (1.95^{-7})	.951	.975	3.04^{-9} (6.17^{-9})	2.57^{-7} (4.49^{-7})	**.027**	.654
Dorea longicatena 1	3.61^{-7} (5.35^{-7})	6.77^{-7} (9.24^{-7})	.634	.860	1.19^{-7} (7.84^{-8})	5.72^{-7} (5.70^{-7})	**.001**	.311
Dorea longicatena 2 CAG00962	2.61^{-7} (6.72^{-7})	8.13^{-8} (1.16^{-7})	**.009**	.592	3.93^{-8} (5.78^{-8})	1.27^{-7} (3.23^{-7})	.353	.727
Dorea formicigenerans	3.03^{-7} (2.85^{-7})	3.49^{-7} (2.13^{-7})	.512	.785	1.00^{-7} (6.40^{-8})	2.02^{-7} (1.86^{-7})	**.005**	.453
Dorea sp. CAG:105	1.21^{-8} (1.92^{-8})	2.66^{-8} (3.73^{-8})	.924	.973	1.12^{-8} (1.60^{-8})	2.13^{-8} (2.16^{-8})	**.021**	.654

	Ulcerative colitis				Crohn's disease			
Genus or species	**Low FODMAP diet (n=13)**	**Sham diet (n=11)**	***P*-value**	***Q*-value**	**Low FODMAP diet (n=8)**	**Sham diet (n=11)**	***P*-value**	***Q*-value**
Hungatella hathewayi 2 CAG00015	2.50^{-8} (2.60^{-8})	3.83^{-9} (9.37^{-9})	**.052**	.592	2.56^{-8} (3.91^{-8})	9.46^{-9} (1.22^{-8})	**.021**	.654
Blautia unclassified CAG00235	1.74^{-7} (4.60^{-7})	9.77^{-9} (2.87^{-8})	.108	.592	8.91^{-10} (2.52^{-9})	5.31^{-8} (9.61^{-8})	**.024**	.654
Anaerostipes hadrus	1.80^{-6} (5.47^{-8})	3.92^{-7} (3.28^{-7})	.209	.597	1.48^{-7} (1.19^{-7})	6.37^{-7} (6.58^{-7})	**.005**	.453
Haemophilus parainfluenzae CAG00950	9.40^{-8} (1.32^{-7})	4.06^{-8} (7.41^{-8})	.715	.901	1.24^{-7} (2.52^{-7})	2.49^{-8} (5.14^{-8})	**.002**	.311
Haemophilus parainfluenzae CAG01056	6.50^{-7} (1.08^{-6})	3.58^{-7} (6.93^{-7})	.542	.798	9.61^{-7} (2.14^{-6})	1.94^{-7} (3.77^{-7})	**.033**	.654
Streptococcus thermophilus	4.93^{-8} (6.58^{-8})	1.59^{-8} (2.31^{-8})	.245	.628	2.81^{-9} (7.95^{-9})	6.21^{-8} (1.48^{-7})	**.019**	.654
Massiliomicrobiota CAG00816	5.65^{-8} (1.75^{-7})	3.22^{-9} (7.35^{-9})	.318	.660	0.00 (0.00)	8.64^{-9} (1.45^{-8})	**.025**	.654
Fusicatenibacter saccharivorans	1.26^{-6} (1.29^{-6})	1.00^{-6} (1.07^{-6})	.704	.901	4.67^{-7} (2.90^{-7})	1.76^{-6} (1.73^{-6})	**.027**	.654
Eisenbergiella tayi	1.24^{-7} (3.02^{-7})	7.64^{-9} (1.36^{-8})	.075	.592	2.28^{-7} (4.92^{-7})	1.69^{-8} (4.08^{-8})	**.019**	.654
Adlercreutzia equolifaciens	1.75^{-7} (2.18^{-7})	6.69^{-8} (7.42^{-8})	.471	.762	2.76^{-8} (2.74^{-8})	5.54^{-8} (6.39^{-8})	**.003**	.447
Alistipes onderdonkii	9.11^{-7} (1.25^{-6})	4.06^{-7} (1.06^{-6})	**.015**	.592	1.29^{-5} (2.68^{-5})	2.18^{-6} (4.41^{-6})	.336	.726
Intestinimonas massiliensis	1.08^{-7} (2.57^{-7})	1.71^{-9} (5.42^{-9})	**.023**	.592	2.17^{-8} (3.66^{-8})	1.11^{-7} (2.41^{-7})	.128	.654
Lachnoclostridium unclassified CAG00764	3.36^{-7} (6.64^{-7})	5.11^{-8} (9.28^{-8})	**.022**	.592	1.37^{-7} (2.56^{-7})	2.17^{-7} (3.47^{-7})	.307	.726
Unclassified CAG00420	2.69^{-8} (5.38^{-8})	7.54^{-8} (1.63^{-7})	**.024**	.592	1.43^{-8} (2.85^{-8})	5.85^{-8} (1.17^{-7})	.128	.654

748 Data are presented as mean (SD) relative abundance and were compared between groups adjusted for baseline abundance and end of trial stool consistency

749

There were no differences in α-diversity or β-diversity between the diet groups in UC or CD (data not shown).

There were no differences in concentrations of individual fecal short-chain fatty acids (SCFA) between diet groups at end of trial in the ITT population (online supplementary Table 4). However, in the PP population, there were significantly lower concentrations of total SCFA following low FODMAP diet compared to sham diet (online supplementary table 4). Specifically, fecal acetate was significantly lower following low FODMAP diet compared to sham diet.

In patients with UC on the low FODMAP diet, compared to sham diet, there were lower concentrations of acetate (209 mg/100g, SD 109 vs. 328 mg/100g, SD 154, *P*=.037), butyrate (66 mg/100g, SD 40 vs. 111 mg/100g, SD 75, *P*=.050) and valerate (6 mg/100g, SD 4 vs. 13 mg/100g, SD 10, *P*=.044) in the PP population. In patients with CD, there was a significantly lower end of trial isobutyrate concentration following the low FODMAP diet (7 SD 3 mg/100g) compared to the sham diet (11 mg/100g, SD 3, *P*=.024). There were no differences in the concentrations of any other individual SCFA in patients with CD in the PP population (data not shown).

765 **Online Supplementary Table 4 Total and individual SCFA concentrations in the ITT and PP analysis**

	ITT analysis			PP analysis		
	Low FODMAP diet (n=27)	Sham diet (n=25)	*P*-value	Low FODMAP diet (n=21)	Sham diet (n=22)	*P*-value
Total SCFA	398 (192)	556 (245)	.080	366 (174)	536 (251)	**.049**
Acetate	232 (117)	323 (138)	.073	213 (109)	313 (140)	**.044**
Butyrate	67 (42)	92 (58)	.102	62 (40)	86 (60)	.094
Propionate	76 (41)	108 (71)	.190	69 (36)	104 (71)	.138
Valerate	7 (5)	11 (8)	.169	7 (4)	10 (8)	.164
Isobutyrate	7 (3)	9 (6)	.142	6 (3)	9 (6)	.084
Isovalerate	10 (5)	13 (9)	.468	9 (4)	13 (9)	.304
pH	6.7 (0.6)	6.4 (0.6)	.329	6.7 (0.6)	6.5 (0.6)	.409

766 Data are presented as estimated marginal mean (SEM) and were compared between groups using an ANCOVA
767 with baseline values as a covariate

768 **Peripheral T-cell phenotype**

769 There were no differences in proportion of T-cells expressing α4β7 between diet groups in patients
770 with UC. In CD there were significantly fewer naïve CD4+ T-cells (58.2%, SEM 4.5% vs. 79.8%, SEM
771 5.7%; *P*=.008), naïve CD8+ T-cells (62.6%, SEM 4.0% vs. 76.4%, SEM 4.9%; *P*=.042) and
772 effector/memory CD8+ T-cells (59.5%, SEM 3.0% vs. 70.3%, SD 3.7%; *P*=.036) expressing α4β7+ on low
773 FODMAP compared to sham diet.

774

Online supplementary table 5 T-cell subset analysis: proportion of each population expressing α4β7+ and absolute number of α4β7+ cells at end of trial

	Low FODMAP diet (n=27)	Sham diet (n=23)	*P*-value
Naïve CD4+			
Proportion (%)	67.1 (2.9)	74.0 (3.2)	.116
Absolute	333,815 (4024)	279,761 (4466)	.377
Effector/memory CD4+			
Proportion (%)	38.7 (1.2)	41.1 (1.3)	.164
Absolute	166,034 (1634)	164,934 (1821)	.965
Naïve CD8+			
Proportion (%)	68.9 (2.5)	74.6 (2.7)	.135
Absolute	225,275 (2486)	172,076 (2759)	.163
Effector/memory CD8+			
Proportion (%)	63.6 (2.3)	69.9 (2.3)	.054
Absolute	81,845 (8812)	80,040 (9803)	.894
Vδ2+			
Proportion (%)	71.6 (2.0)	79.1 (2.2)	**.017**
Absolute	30,535 (3897)	31,140 (4419)	.377

Data are presented as estimated marginal mean (SEM) and were compared between groups using an ANCOVA with baseline values as a covariate

Fecal calprotectin between baseline and end of trial

There was no difference in fecal calprotectin concentrations between low FODMAP and sham diet groups at end of trial in either the CD (61.2 µg/g SEM 6.3 vs. 68.4 µg/g SEM 6.8, *P*=.448) or the UC (55.9 µg/g SEM 18.2 vs. 54.2 µg/g SEM 18.2, *P*=.950) sub-groups.

There were no differences in fecal calprotectin at baseline compared to end of trial in low FODMAP or sham diet groups, and the same was true for the UC and CD sub-groups (online supplementary Table 6).

Online Supplementary Table 6 Baseline compared to end of trial fecal calprotectin concentrations in the low FODMAP and sham diet groups in all patients and the UC and CD sub-groups

	All patients (low FODMAP n=27, sham n=25)			UC (low FODMAP n=13, sham n=13)			CD (low FODMAP n=14, sham n=12)		
	Baseline	End of trial	*P*-value	Baseline	End of trial	*P*-value	Baseline	End of trial	*P*-value
Low FODMAP (µg/g)	54.8 (84.8)	53.3 (84.8)	.857	21.9 (69.7)	10.9 (30.7)	.087	22.8 (66.1)	35.2 (26.8)	.674
Sham (µg/g)	70.9 (117.3)	66.9 (106.4)	.727	25.2 (67.3)	28.6 (67.7)	.721	22.8 (52.5)	15.9 (87.8)	.929

Data are presented as median (interquartile range) and were compared between baseline and end of trial using a Wilcoxon signed rank test

Supplementary references

1. Nielsen HB, Almeida M, Juncker AS, Rasmussen S, Li J, Sunagawa S, et al. Identification and assembly of genomes and genetic elements in complex metagenomic samples without using reference genomes. Nature biotechnology. 2014;32:822.