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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

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OBJECTIVES: Altering FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) intake has substantial effects on gut microbiota. This study aimed to investigate effects of altering FODMAP intake on markers of colonic health in patients with Crohn's disease.

METHODS: After evaluation of their habitual diet, 9 patients with clinically quiescent Crohn's disease were randomised to 21 days of provided low or typical ("Australian") FODMAP diets with $\geq$ 21-day washout in between. Five-day fecal samples were collected at the end of each diet and analyzed for calprotectin, pH, short-chain fatty acids (SCFA) and bacterial abundance. Gastrointestinal symptoms were recorded daily.

RESULTS: Eight participants collected feces and were adherent to the diets. FODMAP intake differed across the three dietary periods with low $<$ habitual $<$ Australian diet. SCFA, pH and total bacterial abundance remained unaltered, but relative abundance was higher for butyrate-producing *Clostridium* cluster XIVa ($P = 0.008$) and mucus-associated *Akkermansia muciniphila* ($P = 0.016$), and lower for *Ruminococcus torques* ($P = 0.034$) during the Australian compared with low FODMAP diet. Results during habitual diet were similar to the low FODMAP intervention, but significantly different to the Australian diet. The diets had no effects on calprotectin, but symptoms doubled in severity with the Australian diet ($n = 9$; $P < 0.001$).

CONCLUSIONS: In clinically quiescent Crohn's disease, altering dietary FODMAP intake is associated with marked changes in fecal microbiota, most consistent with a prebiotic effect of increasing FODMAPs as shown in an irritable bowel/healthy cohort. This strategy might be favorable for gut health in Crohn's disease, but at the cost of inducing symptoms.

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INTRODUCTION

A diet low in FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols) is increasingly being used to manage symptoms in patients with irritable bowel syndrome (IBS) and other functional gastrointestinal conditions.[1] Evidence of efficacy has been mounting worldwide, with several observational trials as well as randomised controlled trials showing the low FODMAP diet reduces gastrointestinal symptoms to a satisfactory level.[2–5] Most recently, a carefully controlled randomised cross-over feeding trial in which two diets of varying FODMAP content (and matched for other nutrients) were provided to 30 IBS and 8 healthy subjects demonstrated that the low FODMAP diet halved gastrointestinal symptoms compared with a typical diet, and that the effects were specific to IBS subjects.[6]

Functional gastrointestinal symptoms are present three times more commonly in patients with inflammatory bowel disease (IBD) compared with the general population.[7] Whether these symptoms represent true functional symptoms or a manifestation of ongoing inflammatory disease (or other cause) remains controversial. However, reducing FODMAP intake in 72 IBD patients (including 52 with Crohn's disease) who had IBS-like symptoms and quiescent disease was associated with considerable reduction of symptoms in greater than one-half in a retrospective study.[8] Only evidence from a randomised placebo-controlled trial will truly ascertain whether this benefit represents placebo effect or not.

One putatively negative effect of reducing FODMAP intake is the change it induces in the gut microbiota in patients with IBS and healthy controls. One study showed a reduction of the relative abundance of *Bifidobacteria* in the feces.[9] In a more detailed analysis, a low compared with moderate (typical) FODMAP intake was associated with one-third reduction in the total abundance of fecal bacteria, specific relative

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reduction of the strongly butyrate-producing bacteria—*Clostridium cluster XIVa*—by sevenfold and the mucus-associated bacteria, *Akkermansia muciniphila* by fivefold, and a 1.5-fold increase in the mucus-degrading bacteria *Ruminococcus torques*, but no change in *Faecalibacterium prausnitzii* in the 27 IBS and six healthy subjects.[10] Some of these findings raise concern as many of the changes mimic those reported to occur in patients with Crohn's disease.[11,12] Although it is not known whether such dysbiosis is cause or effect of the disease, if such changes in the microbiota also occur in patients with Crohn's disease when treated with the low FODMAP diet, it might have deleterious effects.

The present study aimed, therefore, to investigate the effects of two controlled diets varying only in FODMAP content on fecal microbiota and other biomarkers of colonic health in a randomised controlled, cross-over trial of patients with quiescent Crohn's disease. Secondary end points were to compare fecal measures with those previously determined in patients with IBS and healthy controls, to compare the fecal indices with those associated with the patients' habitual diet and to assess the effect of varying FODMAP intake on gastrointestinal symptoms. We hypothesized that a low FODMAP diet will reduce abundance of total and specific bacteria with putative good health effects and reduce gastrointestinal symptoms compared with a diet of higher FODMAP content in Crohn's disease subjects and that data will be similar to that seen in IBS and healthy subjects.

METHODS

Participants. Patients with quiescent Crohn's disease according to Harvey–Bradshaw Index of ≤ 5[13] who had been clinically stable and were on stable therapy for at least 8 weeks were recruited between March 2009 and May 2011 via advertisements in gastroenterology clinics, community newspapers, and through word of mouth. Subjects had not previously consulted a dietitian for management of symptoms and were naive to the low FODMAP diet. All subjects had celiac disease excluded by duodenal biopsy and/or negative celiac serological testing while consuming a gluten-rich diet and/or negative HLA-DQ2/DQ8. Other exclusion criteria comprised previous abdominal surgery and co-morbid conditions such as diabetes. Besides their usual medications to maintain their stable IBD, patients were not permitted to take pharmacological agents that might alter their symptoms (such as laxatives or antidiarrheal agents). Antibiotic or probiotic intake in the previous 2 months was also an exclusion criterion.

Study protocol. The study protocol has been previously described in detail, in which 27 IBS and 6 healthy subjects underwent the same study protocol.[6] Briefly, a gastroenterologist assessed all patients prior to enrollment to verify the diagnosis and disease activity, to ensure that inclusion and exclusion criteria were met, and to document previous investigations. For 1 week, participants recorded their habitual dietary intake and symptoms. Gastrointestinal symptoms were measured daily using a 100 mm visual analog scale, where 0 indicated no symptoms and 100 mm represented worst symptoms ever experienced. The visual analog scale score was used to measure overall gastrointestinal symptoms, abdominal pain, bloating, and passage of wind as previously applied.[14,15]

Participants were then randomised according to a computer-generated order to receive 21 days of a diet low in FODMAPs or 21 days of a diet containing FODMAP content of a typical Australian diet.[16] Participants were blinded to the diets and almost all food was provided. After this 21-day diet, each participant entered a washout period of at least 21 days in which they resumed their usual diet and then crossed-over to the alternate diet. The second interventional diet was not commenced until the symptoms had returned to the same level as during their habitual diet, as determined by direct questioning by a study investigator. From days 3–7 of the participants' habitual diet and days 17–21 of both interventional diet periods, participants collected all feces passed. Just prior to the fecal collection (morning of day 3 of habitual and day 17 of interventional diets), participants swallowed a capsule containing 24 radiopaque markers (Sitzmarks, Konsyl Pharmaceuticals, MD). The time and date of capsule ingestion was noted. Participants were instructed to collect each stool in a supplied plastic container and to avoid urine contamination. The containers were sealed and immediately stored in a supplied portable – 4 °C freezer. Each container was marked with the date and time of stool passage. The freezers were transported and delivered to the laboratory within the week following the 5-day collection. Stools were X-rayed and radiopaque markers counted to determine whole-gut transit time based on time of stool passage.

All participants gave written informed consent prior to commencement of the study. The study protocol was approved by the Eastern Health and Monash University Human Research and Ethics Committees.

Interventional diets. Almost all food, comprising three main meals and three snacks daily, was provided. Detailed meal plans specifying meals and quantities were supplied (given in Supplementary Table S1 online). Participants were instructed to eat to their appetite, and additional food lists were provided so that they could purchase fresh perishable items and additional food if participants wanted more. The supplemented foods contained at least one FODMAP for those following the typical Australian diet or were low FODMAP of ≤ 0.5 g per sitting on the low FODMAP diet. If participants ate out or wanted to include foods that were not specified on the supplied lists, they contacted the study investigator for guidance. The study investigator (EPH) and university research chef, assisted by two hospitality students, prepared all food in commercial kitchens. Meals were cooked and provided as frozen complete meals with instructions to thaw and warm either via microwave or oven. They were free of charge and delivered to participants' homes weekly. All food consumed was recorded in food diaries and adherence to the diet was based on these records. If a participant consumed a high FODMAP meal during the low FODMAP diet or had a day of no high FODMAP-containing foods during the typical Australian diet, that participant was considered non-compliant for that day. A dietitian study investigator (EPH) determined non-compliance based on consumed foods that were not supplied and appeared to be either high or low FODMAP.

The composition of the interventional diets was matched for energy, macronutrients, sugars, starch and fiber. Because the low FODMAP diet was also estimated to be lower in total fiber and resistant starch, small quantities of psyllium and resistant starch were added to the low FODMAP diet (daily average of 3 g psyllium and 5 g Hi-Maize 220 (National Starch & Chemical Company, Bridgewater, NJ), respectively) to ensure only FODMAP content was altered between the two diets and both diets contained gluten. The meal plans were aimed to provide an average 8 MJ daily and to meet the recommended serves of all food groups according to the Australian dietary guidelines.[17] Both diets were also aimed to be low lactose (<5 g per sitting).[18] The low FODMAP diet aimed to keep oligosaccharide, fructose in excess of glucose and polyol content of <0.5 g each per sitting based on previously published data[15] and the typical Australian diet aimed to mimic the FODMAP content previously estimated by a validated food frequency questionnaire to be typically a daily combined oligosaccharide and polyol content of 7.0 g.[16] FODMAP content for all provided food underwent FODMAP analysis via high-performance liquid chromatography and enzymatic assays.

Fecal assessment. A single independent observer noted the fecal frequency and weighed each stool. Stools were also analyzed for fecal water content (FWC), whereby the 5-day fecal samples were defrosted, pooled, and thoroughly mixed, then transferred into a small specimen container. Each sample was weighed and freeze-dried using Operon (Thermo Fisher Scientific Australia, Scoresby, VIC, Australia), then reweighed which enabled the calculation of wet weight and dry weight. FWC was expressed as percentage.

The 5-day fecal samples were defrosted, pooled, and mixed, then transferred into small specimen containers. Samples were packed on dry ice and delivered to the Commonwealth Scientific and Industrial Research Organisation (CSIRO) Animal, Food and Health Sciences (Adelaide, SA, Australia), where they were thawed at 4 °C, then transferred to an anaerobic chamber and aliquoted for further analysis. All samples were stored at − 20 °C until analyzed for SCFA and bacteria abundances. CSIRO investigators were blinded to the treatments.

Fecal contents were analyzed in duplicate for SCFA by gas chromatography and pH, as described previously.[19] Concentrations of the total SCFA (sum of SCFA) and individual SCFA including branched-chain fatty acids were reported as μmol/g of fecal matter.

DNA was extracted from 0.25 g fecal matter using a repeat-bead-beating plus column method.[20] Total bacteria, butyrate-producing bacteria that comprised *Clostridium* cluster IV (*Clostridium leptum* group) including *Faecalibacterium prausnitzii* and *Clostridium* cluster XIVa (*Clostridium coccoides* group) and *Roseburia* spp., traditionally prebiotic bacteria (*Lactobacilli* and *Bifidobacteria* spp.), and mucus-degrading bacteria (*Akkermansia muciniphila*, *Ruminococcus gnavus* and *Ruminococcus torques*) were analyzed. These bacteria were chosen for analysis to specifically examine the role of dietary FODMAPs on bacteria thought to be markers of inflammation and bacteria thought to have health benefits. Total bacterial abundance, and absolute and relative abundance of specific bacteria were measured to analyze the absolute and proportional changes in bacteria putatively beneficial or detrimental to health, thereby indicating the relative degree of prebiosis. Detailed methodology of the primers and optimized quantitative real-time PCR conditions has been previously outlined in detail.[10]

Pooled samples also underwent analysis for the content of calprotectin. This was assessed in duplicate by ELISA using a commercial kit (Buhlmann EK-Cal, Schönenbuch, Switzerland) as per manufacturer's instructions. The results were expressed as an average of duplicate samples as μg/g feces.

End points. The primary end point was the difference in fecal microbiota on the low FODMAP compared with typical Australian diets as measured by total and specific bacterial abundance in the Crohn's disease participants. Secondary end points included differences in fecal pH, total and specific fecal SCFA concentration, gastrointestinal symptoms, fecal frequency, fecal weight, FWC, whole-gut transit time, comparison of data during interventional diets with subjects' habitual diet, and comparison of data from Crohn's disease subjects to IBS/healthy subjects who followed the same study protocol.

Statistical analysis. Power calculations were based upon a cohort of patients with IBS, of which the data have been previously published.[6] Crohn's disease participants were recruited up until the adequate sample size of IBS participants was reached. At that time, nine Crohn's disease participants had completed the study. Data were analyzed on an intention-to-treat basis.

All descriptive data, including participant demographics, were parametric and presented as mean and 95% confidence interval (CI) unless otherwise specified. Data of fecal characteristics were parametric, except for bacterial abundance, which were normalized by $\log_{10}$ conversion before further analysis. All raw fecal characteristic data were presented as mean (95% CI), unless otherwise specified. Differences of fecal characteristics between the interventional diets were shown as a ratio of low FODMAP compared with typical Australian diet and did not fit a normal distribution. Hence, changes in bacterial abundance and SCFA were compared between diets by Wilcoxon matched-pairs signed rank test. Bacterial abundance of Crohn's disease and IBS/healthy cohorts were compared using Mann–Whitney test. A P-value ≤ 0.05 was considered statistically significant. All statistical tests, unless specified, were analyzed with GraphPad Prism version 6 (La Jolla, CA) and SPSS version 20 (IBM Corporation, Armonk, NY) programs. Comparisons in microbiological data were qualitatively made with the results previously published for the cohort of healthy subjects and patients with IBS.

RESULTS

Participants. The nine participants completed all dietary arms, but one did not collect feces. The subjects included six women (67%) and had a median (interquartile range) age of 35 (29–41) years and body mass index 24 (23–27) kg/m^2.

Four subjects had colonic disease and the remaining five had ileo-colonic disease, one of whom also had upper gastrointestinal Crohn's disease. Median (interquartile range) time since diagnosis was 7 (3.5–13) years. As shown in Figure 1, fecal calprotectin was also similar across the three phases of the study, but, in three subjects, it was consistently >150 μg/g, indicating active disease despite the Harvey–Bradshaw Index being <5. Four subjects were not on any medications to manage their Crohn's disease, one subjects was taking balsalazide, one was taking azathioprine monotherapy, one mesalazine and azathioprine, and two were on anti-tumour necrosis factor monotherapy therapy (infliximab and adalimumab). No changes in medication occurred during the study.

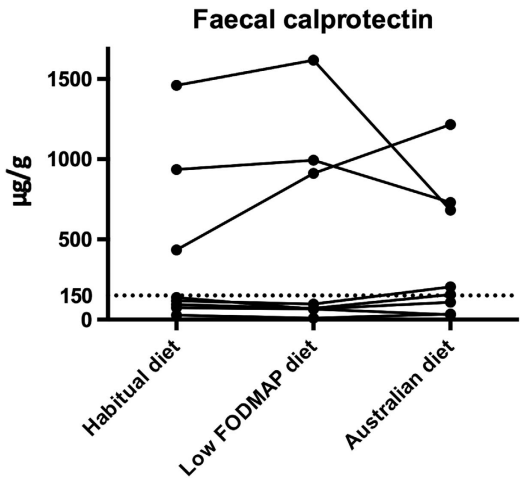


Figure 1 Mean fecal calprotectin (μg/g) analyzed in duplicate from pooled samples of 8 subjects with Crohn's disease following their habitual diet and 17–21 days of low FODMAP and typical Australian diets. A limit of 150 μg/g is considered quiescent disease as indicated by a dotted line. No differences were observed between diets.

Dietary adherence during the interventional diets was good with all participants adhering to the typical Australian diet, and all except one participant (the participant who did not collect fecal samples) adhering to the low FODMAP diet for at least 17 out of the 21-day interventional period. The only measured nutrient that differed between the two interventional diets was the average daily intake of FODMAPs (Table 1).

Symptoms. The severity of overall gastrointestinal symptoms was significantly less on the last 14 days of the low FODMAP diet at mean (95% CI) 13.5 (5.9–21.1) mm compared with the last 14 days of the typical Australian diet 24.8 (12.6–37) mm ($P<0.001$; repeated measures ANOVA (see Figure 2a)). This represented a fall of 11.4 (0.85–21.9) mm. As illustrated in Figure 2b–d, similar results were seen in abdominal pain (fall of 12.0 (7.5–16.4) mm), bloating, (fall of 13.8 (6.0–21.6) mm), and passage of wind (fall of 15.5 (11.9–19.0) mm). The paired differences in symptoms were similar in those who received the low FODMAP diet first or second (data not shown).

Fecal indices during the interventional diets. The results of complete fecal samples that were collected over the last 5 days of each dietary period are shown in Table 2. The one participant who did not collect fecal samples could not be included in fecal data. There were no significant differences in fecal frequency, weight, FWC, or whole-gut transit time across the dietary periods. There were no differences in fecal pH, total or specific fecal SCFA across diets (Table 3). Molar proportions of the major SCFA were also unchanged (data not shown).

Total bacterial abundance was similar with the two interventional diets (Table 4). During the typical Australian diet, absolute and relative abundance of the butyrate-producing *C.* cluster XIVa and the mucus-associated *A. muciniphila* were greater compared with those associated with the low

Table 1 The mean daily nutrition information of provided low and typical Australian FODMAP interventional diets and habitual diets of subjects with Crohn's disease

Per day	Typical Australian diet	Low FODMAP diet	*P*-value	Habitual diet
Energy (MJ)	8.17 (7.37–8.97)	8.17 (7.09–9.24)	0.979	8.90 (7.47–10.3)
Protein (g)	96.1 (84.7–107)	98.1 (83.7–113)	0.361	89.2 (70.0–108)
Fat (g)	71.6 (49.4–93.8)	74.4 (51.9–97.0)	0.337	79.2 (62.1–96.4)
Carbohydrate (g)	219 (180–259)	215 (181–249)	0.579	254 (216–292)
Sugars (g)	120 (103–137)	122 (106–139)	0.468	113 (92.4–134)
Starch (g)	94.0 (52.8–135)*	95.4 (59.7–131)†	0.783	**153 (110–197)***†
Fiber (g)	25.9 (21.3–30.6)	23.4 (18.7–28.2)	0.085	22.1 (15.2–28.9)
Oligosaccharides (g)	**5.49 (2.34–8.65)**	**1.57 (0.47–2.66)***	**0.009**	**3.45 (2.31–4.60)***
Polyols (g)	**4.21 (2.57–5.85)***	**0.20 (–0.04–0.44)†**	**0.002**	**1.30 (0.52–2.07)*†**
Lactose (g)	**1.35 (0.20–2.49)***	**0.05 (–0.01–0.10)†**	**0.033[a]**	**9.11 (5.20–13.0)*†**
Fructose in excess of glucose (g)[b]	**12.7 (8.06–17.3)**	**1.24 (0.41–2.07)**	**0.001**	—
Total FODMAPs (g)[b]	**23.7 (16.9–30.6)**	**3.05 (1.86–4.25)**	**<0.001**	—
Gluten	Present	Present		Present

FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols.
All nutrients were estimated by the FoodWorks program, except for the FODMAP content of the typical Australian diet and low FODMAP diets, which were analyzed by high-performance liquid chromatography and enzymatic assays. Interventional diets were analyzed by paired *t*-tests.
Statistically significant differences are shown in bold and are based on $P \leq 0.05$.
Differences between *habitual diet and †interventional diets.
[a]Although there is a significant difference in lactose between the interventional diets, 5 g lactose per sitting is considered to be well-absorbed and tolerated in majority of people.[18]
[b]Fructose in excess of glucose, and therefore total FODMAPs, cannot be estimated by the FoodWorks program for the habitual diet.

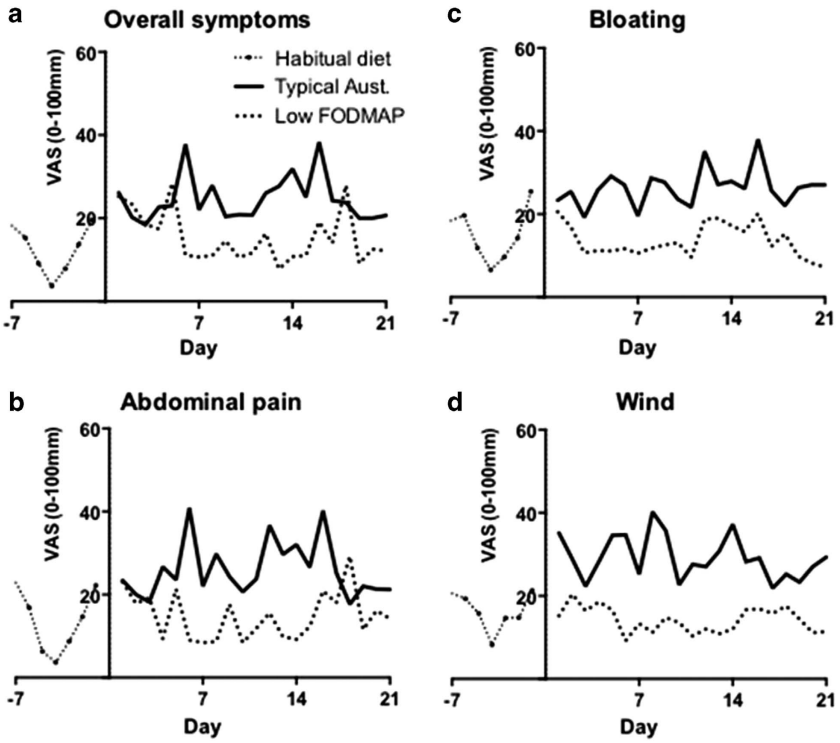


Figure 2 Mean daily symptoms in subjects with quiescent Crohn's disease rated using 100 mm visual analog scale (VAS) for (**a**) overall gastrointestinal symptoms, (**b**) abdominal pain, (**c**) bloating, and (**d**) passage of wind during habitual diets and interventional low FODMAP and typical Australian diets. Symptoms were significantly lower on both habitual and low FODMAP diets compared with the typical Australian diet (see text).

Table 2 Daily fecal frequency, weight, fecal water content, and whole-gut transit time in eight subjects with quiescent Crohn's disease after following a habitual diet and a low FODMAP and typical Australian diet for 17–21 days

Subject group	Diet	Fecal frequency (number of stool per day)				Fecal weight (g per day)		Fecal water content (percentage of total weight)		Whole-gut transit time (h)	
		0	1–2	3–4							
Crohn's disease ($n=8$)	Habitual	0	6	2	$P=0.626$	159 (86–232)	$P=0.271$	78.5 (73.7–83.3)	$P=0.967$	33.4 (23.0–43.8)	0.794
	Australian	1	6	1		151 (89–214)		76.9 (70.4–83.4)		43.5 (23.0–64.0)	
	Low FODMAP	0	7	1		149 (88–211)		77.7 (72.3–83.1)		39.2 (23.5–54.8)	

FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols.
Data are presented as mean (95% CI); χ^2-test for fecal frequency and Friedman test for fecal weight, fecal water content, and whole-gut transit time.

FODMAP diet (Table 4 and Figure 3). Relative abundance of *R. torques* was decreased on the typical Australian diet in comparison with the low FODMAP diet (Table 4 and Figure 3b). The effects on these bacteria were similar in those who received the low FODMAP diet first or second (data not shown). There were no significant differences in absolute and relative abundance of *Lactobacilli* and *Bifidobacteria* spp. between the two controlled diets.

Habitual diets. Participants' habitual diets were similar to the two interventional diets, except for starch intake, which was higher than in the interventional diets. None of the subjects were restricting dietary FODMAPs during their habitual diet, but oligosaccharides and polyols were not as high as the typical Australian diet (Table 1). Lactose was unrestricted during the habitual diet. Compared with symptoms while consuming the participants' habitual diet (12.5 (8.7–16.3) mm), the low FODMAP diet did not alter the severity of symptoms ($P=0.285$), but, the typical Australian diet increased overall gastrointestinal symptoms ($P<0.001$; Figure 2a) and similar patterns were seen with the other measured gastrointestinal symptoms (Figure 2b–d).

There were no differences in fecal pH, total or specific fecal SCFA on the habitual diet compared with the provided diets (Table 3). Absolute and relative abundance of bacteria in association with the habitual diet are shown in Table 4. Total bacterial abundance was similar on the habitual diet compared

Table 3 Fecal pH, total and specific SCFA (μmol/l) on pooled 5-day fecal samples after following a habitual diet and low FODMAP and typical Australian diets for 17–21 days in 8 subjects with quiescent Crohn's disease in a cross-over trial

Measure	Typical Australian diet	Low FODMAP diet	*P*-value	Habitual diet
pH	6.80 (6.36–7.24)	6.92 (6.42–7.43)	0.313	6.92 (6.62–7.22)
Total SCFA	68.6 (45.8–91.5)	78.1 (57.5–98.6)	0.195	76.6 (62.4–90.8)
Butyrate	11.3 (5.4–17.3)	13.0 (6.8–19.3)	0.195	12.0 (6.1–17.9)
Propionate	12.6 (6.1–16.4)	15.1 (10.3–19.8)	0.148	16.1 (11.4–20.7)
Acetate	38.0 (25.1–50.8)	42.9 (29.0–56.8)	0.195	42.2 (34.1–50.4)
Isobutyrate[a]	2.27 (0.98–3.56)	2.10 (1.12–3.08)	1.00	1.95 (1.11–2.79)
Isovalerate	3.13 (1.09–5.17)	3.06 (1.49–4.63)	0.844	2.82 (1.35–4.29)
Valerate[a]	2.05 (0.28–3.83)	2.71 (1.67–3.74)	0.438	1.90 (0.52–3.29)
Caproate[a]	0.90 (–0.42–2.21)	0.86 (0.48–1.23)	1.00	1.35 (–1.98–4.64)

FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols; SCFA, short-chain fatty acids.
Interventional diets were analyzed by Wilcoxon matched-pairs signed rank test. There were no statistically significant differences seen based on $P \leq 0.05$.
[a]Due to difficulties in analysis for isobutyrate, $n=7$; valerate, $n=5$; and caproate, $n=3$.

Table 4 Absolute and relative bacterial abundance on pooled fecal samples after following a habitual diet and low FODMAP and typical Australian diets for 17–21 days in cross-over trial ($n=8$)

Measure	Bacteria	Australian diet	Low FODMAP diet	*P*-value	Habitual diet
Absolute abundance (Log_{10} copies of 16S rRNA gene/g)	Total bacteria	9.48 (9.17–9.79)	9.53 (9.20–9.86)	0.641	9.60 (9.19–10.0)
	Clostridium cluster IV	7.58 (6.65–8.50)	7.59 (6.49–8.68)	0.773	7.56 (6.34–8.78)
	F. prausnitzii	6.79 (5.77–7.80)	6.81 (5.64–7.98)	1.00	6.96 (5.73–8.20)
	Clostridium cluster XIVa	**8.73**[a] (**8.42–9.03**)	**7.94** (**7.55–8.34**)	**0.008**	**8.01** (**7.57–8.46**)
	Roseburia	7.02 (6.03–8.00)	6.95 (5.80–8.09)	0.313	7.16 (6.32–8.00)
	Lactobacilli	6.11 (5.70–6.52)	6.48 (6.01–6.96)	0.078	6.29 (5.84–6.73)
	Bifidobacteria	7.33 (6.87–7.80)	7.12 (6.54–7.70)	0.078	7.33 (6.60–8.07)
	A. muciniphila[b]	**5.08**[a] (**3.19–6.97**)	**3.75** (**1.97–5.54**)	**0.016**	**4.11** (**2.44–5.78**)
	R. gnavus	7.11 (6.79–7.44)	7.20 (6.90–7.50)	0.547	7.26 (6.81–7.71)
	R. torques	5.65 (4.65–6.65)	6.13 (5.28–6.99)	0.195	6.14 (5.41–6.86)
Relative abundance (percentage of total bacteria)	*Clostridium* cluster IV	2.70 (0.63–4.78)	2.69 (0.93–4.46)	0.383	3.02 (0.77–5.26)
	F. prausnitzii	0.47 (0.21–0.72)	0.66 (0.14–1.19)	0.250	0.81 (0.09–1.54)
	Clostridium cluster XIVa	**19.2**[a] (**11.2–27.3**)	**2.81** (**1.88–3.74**)	**0.008**	**2.90** (**1.80–4.01**)
	Roseburia	0.94 (0.21–1.68)	0.81 (0.18–1.44)	1.00	0.68 (0.23–1.13)
	Lactobacilli	0.06 (0.01–0.10)	**0.17**[a] (**–0.01 to 0.35**)	0.195	**0.06** (**0.03–0.08**)
	Bifidobacteria	1.27 (0.11 to 2.43)	1.11 (–0.55 to 2.76)	0.383	2.09 (–0.54 to 4.71)
	A. muciniphila[b]	**0.15**[a] (**–0.05 to 0.35**)	**0.01** (**–0.02 to 0.04**)	**0.016**	**0.01** (**–0.01 to 0.03**)
	R. gnavus	1.17 (–0.25 to 2.59)	1.39 (–0.38 to 3.17)	0.461	2.26 (–1.51 to 6.03)
	R. torques	**0.10** (**–0.04 to 0.24**)	**0.16** (**–0.03 to 0.34**)	**0.039**	0.15 (–0.05 to 0.35)

FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols.
Interventional diets were analyzed by Wilcoxon matched-pairs signed rank test.
Statistically significant differences are shown in bold and are based on $P \leq 0.05$.
*Differences between habitual diet and interventional diets.
[a]$P<0.05$ compared with habitual diet; Wilcoxon matched-pairs signed rank test.
[b]Due to difficulties in microbial analysis for *A. muciniphila*, one subject could not be included so $n=7$.

with the other two dietary periods. However, the habitual diet was similar to the low FODMAP diet and had a reduced absolute and relative abundance of the butyrate-producing *C.* cluster XIVa and the mucus-associated *A. muciniphila* compared with the typical Australian diet (Table 4). The habitual diet also had a lower relative abundance of *Lactobacilli* compared with that with the low FODMAP diet ($P=0.039$; Wilcoxon matched-pairs signed rank test).

Bacterial abundance compared to IBS and healthy subjects. The Crohn's disease subjects had similar bacterial profiles during all three dietary periods than those seen from a combined IBS and healthy population who had followed a study of identical protocol (habitual diet shown in Figure 4), although the low FODMAP diet did not reduce total bacterial load as seen in the IBS/healthy cohort.[10] It is evident that two Crohn's disease subjects had marked reduction in abundance of *C.* cluster IV and *F. prausnitzii* on their habitual diets (Figure 4). One had a fecal calprotectin of 1,460 μg/g. The other bacterial groups studied were present in similar absolute and relative abundance.

DISCUSSION

Altering the carbohydrate content of the diet potentially alters the gut microbiota, which is of great interest in a Crohn's disease population that is already at risk of dysbiosis. Literature would suggest that the bacterium of most relevance in a

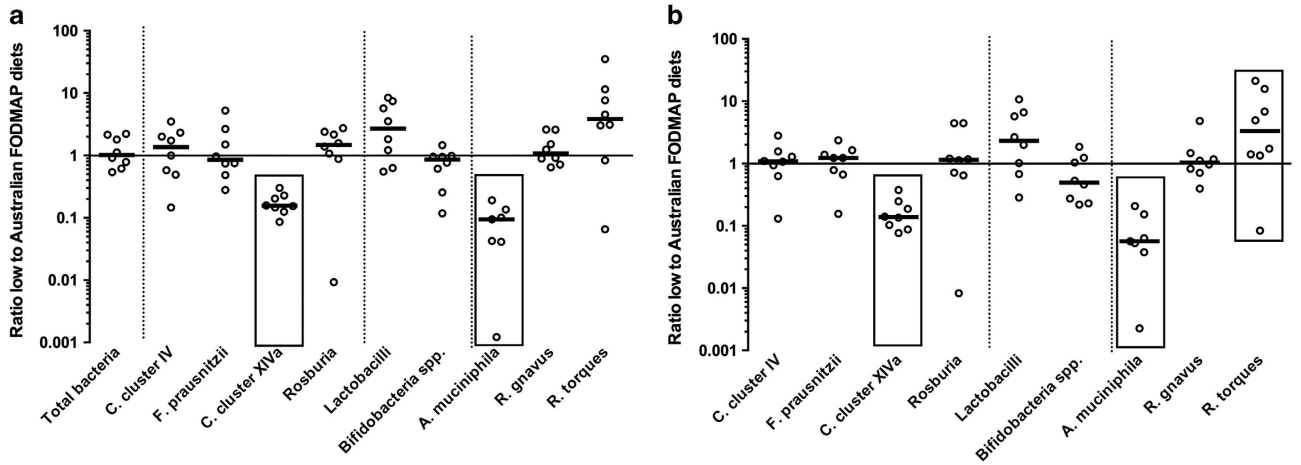


Figure 3 Absolute bacterial abundance of subjects with Crohn's disease during the provided low FODMAP and typical Australian diets, presented as ratio of low-to-typical Australian diet for (**a**) absolute bacterial abundance and (**b**) relative bacterial abundance. Data comparing low and typical Australian diets were analyzed by Wilcoxon matched-pairs signed rank test and statistically significant differences between the diets are indicated with a box based upon $P \leq 0.05$. Due to difficulties in analysis *A. muciniphilia* $n = 7$ for provided diets.

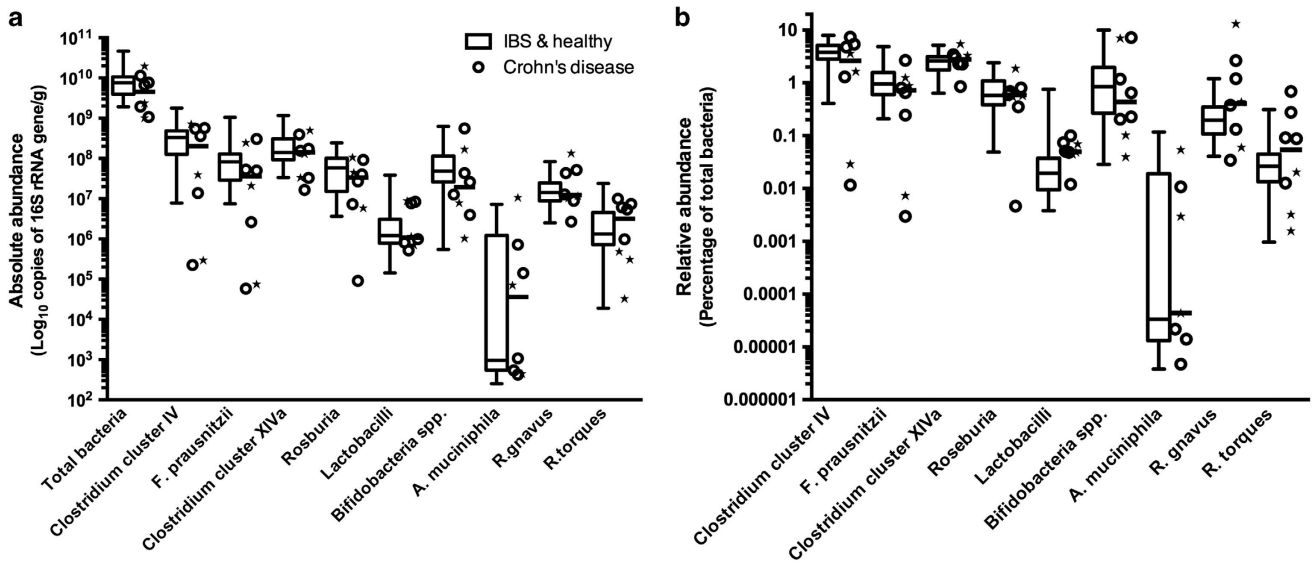


Figure 4 Bacterial abundance of subjects with Crohn's disease compared with irritable bowel syndrome and healthy subjects during their habitual diet. Data are presented as (**a**) absolute bacterial abundance and (**b**) relative bacterial abundance. Due to difficulties in analysis *A. muciniphilia* $n = 7$ for provided diets. Subjects with fecal calprotectin > 250 μg/g are indicated with a star. No differences in bacterial abundance were observed between the subject groups.

Crohn's disease population is *F. prausnitzii*. Reduced mucosal *F. prausnitzii* has predicted onset of active disease[21] and is altered in new-onset Crohn's disease,[22] and fecal *F. prausnitzii* is reduced in unaffected siblings of patients with Crohn's disease, thought to be at high risk of Crohn's disease development.[23] Similarly, *in vitro* and animal models suggest that *A. muciniphila* may also predict Crohn's disease activity.[12,24,25] Indeed, two subjects with Crohn's disease in the present study had, in comparison with our own comparator IBS/healthy cohort, a markedly lower absolute and relative abundance of *C.* cluster IV and *F. prausnitzii* (Figure 4), together with a reduced absolute and relative abundance of *A. muciniphila.* One of them also had an increased absolute and relative abundance of *R. gnavus*. This pattern has been previously reported to be associated with active disease.[11] Evidence of fecal dysbiosis may be present in these two Crohn's disease subjects.

The patterns of change in the measured bacteria were almost identical to those observed in the IBS/healthy cohort who underwent the same interventions and had identical methodological dissection of the fecal microbiota[10]

(Figure 4). It is reassuring that the same dietary manipulations of FODMAPs produce consistent effects irrespective of the underlying disease state. Few patients with active disease were found in the current cohort as they were selected on the basis of clinical remission. Nevertheless, the three who had active intestinal disease on the basis of an elevated fecal calprotectin also had the same patterns of change in microbiota in response to dietary change (Figure 3).

The typical Australian diet had a mean oligosaccharide content of 2.04 g/day and a polyol content of 2.91 g/day (although only perhaps 30% of that might have been expected to reach the colonic bacteria[26]) above the habitual diet. It is likely that this relatively small increase in dietary components that have prebiotic effects were responsible of the changes in microbiota in the typical Australian dietary arm, especially as an increase in oligosaccharides of as little as 2.5 g daily can induce a prebiotic effect.[27] The prebiotic effect may have been exaggerated because the comparator arm had such a low FODMAP intake. This confounder, that is, the background habitual dietary FODMAP intake, has not been previously considered in clinical trials of prebiotics. This is so important as the concept of prebiotics is that there is a ceiling effect; doses above this will lead to non-specific, fermentative effects only. This is consistent with the randomised double-blinded study of fructo-oligosaccharides supplementation in a large cohort of patients with Crohn's disease in which no prebiotic effect was documented, disease activity did not change, but symptoms likely related to fermentation events were worsened.[28] The usual FODMAP intake of the nine subjects with Crohn's disease was lower than expected, on the basis of a previous study of the FODMAP intake of a healthy population.[16] None of the recruited patients had intentionally altered their habitual diet and were selected on the basis of being 'FODMAP naive'. However, there are survey data showing 57% of patients with ulcerative colitis restrict food during remission, mostly to alleviate symptoms[29] and a case-control study showed that patients with Crohn's disease who were apparently naive to the low FODMAP diet eat lower amounts of fructans.[30]

The differences in microbiota between the low FODMAP and the habitual diets were characterized by three observations. First, the higher relative abundance of *Lactobacilli* on the low FODMAP diet, a finding that was not expected, was, in view of its small effect size, probably a chance observation due to multiple comparisons with small number of subjects. Secondly, the habitual diet was associated with total bacterial abundance that was similar to that seen with the low FODMAP diet. This was very different to the effects observed in the IBS/healthy cohort in which the low FODMAP diet reduced total bacterial abundance. Whether this is a disease effect or due to differences in the habitual diet cannot be ascertained. Thirdly, there was no relative expansion of any of the targeted specific bacteria, even though there was greater than twofold more FODMAPs consumed. It is unlikely that inclusion of small amounts of psyllium and resistant starch in the low FODMAP diet could have exerted this effect on the microbiota as they were also included in the diets of the IBS/healthy cohort. These issues need further study.

The primary endpoint of comparison of the controlled low FODMAP and typical Australian diets showed a considerable difference in *C.* cluster XIVa and *A. muciniphila,* bacterial populations with favorable health effects[31,32] that have great significance in Crohn's disease as a reduced production of butyrate and their precursors is likely to support an inflammatory environment.[11] In patients with ulcerative colitis, butyrate enemas may be effective in reducing disease activity[33] although similar studies in patients with Crohn's disease have not been reported. The general consensus on *A. muciniphila* is that it supports healthy bacteria adjacent to the epithelium,[12,24,25,31] which again, may be conducive to reducing risk of inflammation. The lower relative abundance of *A. muciniphila* on the low FODMAP diet was also accompanied by a higher relative abundance of *R. torques* as previously observed by Png *et al.*[12] *R. torques* is commonly seen in higher abundance in patients with IBD,[11,12] suggests that a reduced FODMAP intake may encourage an environment that is unfavorable to health. Altering dietary FODMAPs did not change the abundance of *Lactobacilli* and *Bifidobacteria* spp., which are traditionally thought to be markers of prebiotic activity. Again, this seems consistent with a previous observation in patients with Crohn's disease who received fructo-oligosaccharides supplementation, in which no specific increase in fecal *Bifidobacteria* spp. ahead of total bacterial abundance was observed.[28] The lack of change in fecal SCFA in this study does not support the notion that a low FODMAP diet reduces butyrate production.[9] Likely reasons for this is that fecal SCFA does not accurately reflect SCFA production as $>95\%$ of SCFA are rapidly absorbed and metabolized and SCFA production occurs mostly in the proximal colon.[34] These data suggest that if, patients with Crohn's disease had a FODMAP intake similar to that of the designed typical Australian diet, their microbiome would approach a putatively better structure. The observed differences in fecal indices between the different diets were almost identical to those observed in IBS/healthy patients undergoing the same trial procotol,[10] except for the differences in overall bacterial abundance observed in IBS/healthy control group. This may be because the majority of patients in the IBS/healthy cohort had IBS. Dysbiosis has been described in some studies in patients with IBS.[35,36] Unfortunately, this study was not powered to observe differences between Crohn's disease and healthy subjects.

The subjects were relatively asymptomatic on their habitual diet and remained so on the low FODMAP diet, but gastrointestinal symptoms significantly increased with the higher FODMAP intake on the typical Australian diet without changing disease activity. The efficacy of the low FODMAP diet in managing functional gastrointestinal symptoms in patients with IBS is well-established,[1] but published evidence for the efficacy of this treatment in patients with quiescent IBD and IBS-like symptoms is limited to an observational study.[8] The increase in gastrointestinal symptoms with increasing dietary FODMAP intake without change to disease activity, as shown by stable fecal calprotectin, provides evidence that FODMAPs do induce functional symptoms in patients with Crohn's disease. The increase in symptoms of >10 mm on the typical Australian diet was considered clinically significant based on previous literature.[6] This might be considered as

evidence that there is merit in reducing dietary FODMAPs in patients with Crohn's disease and co-existing functional gastrointestinal symptoms. However, such efficacy can only be more definitively proven by studying patients with quiescent Crohn's disease who are complaining of functional symptoms.

A major limitation of the current study is the small number of patients examined. Most importantly, this can lead to type I errors occurring in multiple comparisons. Indeed, correction for multiple comparisons using, for example, the false-discovery rate methodology yields a *P*-value threshold (0.006) that is not even reached by comparison of the relative abundance of *C. cluster* XIVa between the two controlled diets, despite the relative abundance being markedly lower in association with the low FODMAP diet in every patient with wide differences in 95% CIs and a *P*-value of 0.008. The confidence that the differences observed are not errors lies in the almost identical pattern of differences that were seen in a larger sample of non-inflamed subjects (healthy and IBS), wherein correction for multiple comparisons did not negate the statistical significance of the findings.

In conclusion, the findings show that altering the FODMAP content with a standardised diet consistently and predictably altered the faceal microbiota in similar ways that those diets did in a cohort of healthy subjects and patients with IBS and Crohn's disease, despite the presence of dysbiosis. When compared with the fecal findings in association with the patients' habitual diets, the changes better reflected a prebiotic effect of increasing FODMAPs in this Crohn's disease cohort who had a relatively low habitual FODMAP intake. Increasing FODMAP intake did induce gastro-intestinal symptoms of greater severity without change in inflammatory activity. Although these results might support the use of a low FODMAP diet in an attempt to reduce presumed functional gastrointestinal symptoms in patients with Crohn's disease, caution should be exercised in long-term strict restriction of FODMAP intake as the implications of the loss of the prebiotic effects of FODMAPs on disease activity and other aspects of colonic health remain unknown. On the contrary, the findings support a modest increase in FODMAP intake in putatively improving the colonic microenvironment.

CONFLICT OF INTEREST

Guarantor of the article: Peter R. Gibson, MD, FRACP.
Specific author contributions: Study concept and design: Emma P. Halmos, Susan J. Shepherd, Jane G. Muir, and Peter R. Gibson; acquisition of data: Emma P. Halmos, Claus T. Christophersen, Anthony R. Bird, and Jane G. Muir. All the authors were involved in the analysis and interpretation of the data, drafting of the article or revising it critically for important intellectual content, and final approval of the version to be submitted. All authors had access to study data and reviewed and approved the final manuscript.
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Potential competing interests: Susan J. Shepherd has published a book on food intolerances and several cookbooks related to the topic of the manuscript. Peter R. Gibson has published a book on food intolerances. The Department of Gastroenterology has developed an App on the Monash University Low FODMAP Diet, the proceeds of which partly go to the Department, but not to the individuals. The remaining authors declare no conflict of interest.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ A low FODMAP diet (LFD) reduces symptoms in patients with irritable bowel syndrome (IBS).
- ✓ A LFD is frequently applied to patients with Crohn's disease (CD) and functional symptoms.
- ✓ A LFD reduces abundance of total fecal bacteria in IBS and healthy subjects.
- ✓ A LFD reduces specific bacteria with putative health benefits in IBS and healthy subjects.

WHAT IS NEW HERE

- ✓ In patients with CD, a LFD does not reduce total fecal bacterial abundance.
- ✓ Altering FODMAPs in patients with CD has marked changes in specific fecal bacteria.
- ✓ Changes in specific fecal bacteria of CD subjects is consistent with IBS/healthy subjects.
- ✓ A LFD may be beneficial in reducing functional symptoms in patients with CD.

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