ORIGINAL ARTICLE

Diets that differ in their FODMAP content alter the colonic luminal microenvironment

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ABSTRACT

Objective A low FODMAP (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols) diet reduces symptoms of IBS, but reduction of potential prebiotic and fermentative effects might adversely affect the colonic microenvironment. The effects of a low FODMAP diet with a typical Australian diet on biomarkers of colonic health were compared in a single-blinded, randomised, cross-over trial.

Design Twenty-seven IBS and six healthy subjects were randomly allocated one of two 21-day provided diets, differing only in FODMAP content (mean (95% CI) low 3.05 (1.86 to 4.25) g/day vs Australian 23.7 (16.9 to 30.6) g/day), and then crossed over to the other diet with ≥21-day washout period. Faeces passed over a 5-day run-in on their habitual diet and from day 17 to day 21 of the interventional diets were pooled, and pH, short-chain fatty acid concentrations and bacterial abundance and diversity were assessed.

Results Faecal indices were similar in IBS and healthy subjects during habitual diets. The low FODMAP diet was associated with higher faecal pH (7.37 (7.23 to 7.51) vs 7.16 (7.02 to 7.30); p=0.001), similar short-chain fatty acid concentrations, greater microbial diversity and reduced total bacterial abundance (9.63 (9.53 to 9.73) vs 9.83 (9.72 to 9.93) $\log_{10}$ copies/g; p<0.001) compared with the Australian diet. To indicate direction of change, in comparison with the habitual diet the low FODMAP diet reduced total bacterial abundance and the typical Australian diet increased relative abundance for butyrate-producing *Clostridium* cluster XIVa (median ratio 6.62; p<0.001) and mucus-associated *Akkermansia muciniphila* (19.3; p<0.001), and reduced *Ruminococcus torques*.

Conclusions Diets differing in FODMAP content have marked effects on gut microbiota composition. The implications of long-term reduction of intake of FODMAPs require elucidation.

Trial registration number ACTRN12612001185853.

Significance of this study

What is already known on this subject?

- A diet low in Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols (FODMAPs) reduces GI symptoms in approximately 75% of patients with IBS.
- FODMAPs are fermentable substrates and some have prebiotic effects with putative benefits on colonic health.
- A randomised parallel group study showed a reduction of the proportion and concentration of faecal *Bifidobacteria* spp on a dietitian-taught low FODMAP diet compared with a habitual diet.

What are the new findings?

- Diets that differ in their FODMAP content are associated with considerable changes to the structure of the faecal microbiota.
- A provided low FODMAP diet reduces total bacterial abundance but has no effect on relative abundance of bacteria putatively associated with colonic health.
- The higher FODMAP intake of the provided typical Australian diet compared with that of the low FODMAP or habitual diets was associated with specific stimulation of the growth of bacterial groups with putative health benefits.
- No alterations in faecal short-chain fatty acid concentrations were associated with differences in FODMAP ingestion.

How might it impact on clinical practice in the foreseeable future?

- A low FODMAP diet should not be recommended for asymptomatic populations.
- Caution should be taken when recommending the low FODMAP diet long-term.
- Liberalising FODMAP restriction to the level of adequate symptom control should be exercised to use the potential health benefits of higher FODMAP intake on the gut microbiota.

INTRODUCTION

IBS often requires multimodal management approaches that include psychological, dietary and pharmacological domains. Dietary therapies are gaining popularity as evidence of efficacy for specific diets have emerged. One strategy is to restrict intake of poorly absorbed short-chain carbohydrates, termed FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols). The evidence-base for efficacy of the low FODMAP diet is building[1–5] with a recent blinded placebo-controlled cross-over study confirming previous studies that about 75% of patients gain clinically significant benefit.[6] The low FODMAP diet is increasingly being applied by health professionals in patients with IBS as first-line therapy.[5 6] Given the high prevalence and chronic nature of IBS, it is possible that many people will restrict intake of FODMAPs over months to years.[2]

FODMAPs are being increasingly used in food industry as prebiotics, either formulated into various types of products or manufactured as supplements, to promote colonic health. Evidence is strong for the prebiotic actions of oligosaccharides.[7–9] Reduced FODMAP delivery to colonic microbiota might, therefore, have deleterious effects on the growth of bacteria with potentially favourable health effects. Indeed, a randomised parallel group study where the effects on faecal microbiota of a dietitian-taught low FODMAP diet compared with those of a habitual diet indicated a reduction of the proportion and concentration of *Bifidobacteria* spp,[4] providing the first evidence for potentially unfavourable effects of the diet.

FODMAPs are also substrates for fermentation by bacteria not considered to be prebiotic.[10] Bacterial fermentation of carbohydrates yields short-chain fatty acids (SCFAs), including butyrate, the major energy substrate for the colonic epithelium.[11 12] Butyrate is also a key regulator of colonocyte proliferation and apoptosis, and has immunomodulatory effects.[10] In these ways, fermentable carbohydrates delivered to the colon have potential anticarcinogenic and anti-inflammatory actions.[13] Restriction of FODMAPs delivered to the colon to reduce gas production, subsequent luminal distension and GI symptoms might consequently have adverse effects on colonic health.

The present study aimed to address the hypothesis that a low FODMAP diet recommended for reduction of IBS symptoms will have adverse effects on colonic luminal microenvironment. This was investigated by comparing the effects of a low FODMAP diet with those of a FODMAP content of a typical Australian diet on faecal microbiota and biomarkers related to colonic health. Additional comparison with faecal indices while subjects consumed their own diet was also performed. The bacteria targeted were chosen on the basis of being avid butyrate-producers with anti-inflammatory association for some, traditional 'prebiotic' bacteria and representatives of mucus-associated bacteria that have putative health-promoting or detrimental effects.[9 14] Subjects studied were participating in a randomised controlled efficacy trial of the two diets where almost all food was provided and included patients with IBS and healthy subjects.[6]

METHODS

Participants

The study participants have been previously described in detail.[6] Briefly, patients with IBS as defined by Rome III criteria[6] and healthy controls were recruited via advertisements and word of mouth. Exclusion criteria comprised coeliac disease, previous abdominal surgery, comorbid conditions such as diabetes, and inability to understand English. No participant had previously visited a dietitian for management of GI symptoms, and had not used antibiotics or probiotics for 2 weeks prior to study commencement. Fibre supplements, laxatives and antidiarrhoeal medications were not taken during the trial.

Study protocol

The study protocol has also been recently described in detail.[6] Briefly, for 1 week, participants recorded their habitual dietary intake and a 5-day faecal sample was collected (as outlined below). 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. Participants were blinded to the diets and almost all food was provided.[6] 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. From day 3 to day 7 of the habitual diet and day 17 to day 21 of interventional diets, participants collected all faeces passed. Just prior to the faecal collection (morning of day 3 of habitual and day 17 of interventional diets), participants swallowed a capsule containing 24 radiopaque markers (Sitzmarks, Konsyl Pharmaceuticals, Maryland, USA). 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 portable freezer (Waeco Pacific, Queensland, Australia). Each container was marked with the date and time of stool passage. The freezers were delivered to the laboratory within the week following collection. Stools were X-rayed and radiopaque markers counted to determine whole gut transit time (WGTT) based on time of stool passage.

All participants gave written informed consent prior to study commencement. The study protocol was approved by the Eastern Health and Monash University Human Research and Ethics Committees. The protocol was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12612001185853).

Interventional diets

Participants were supplied with three main meals and three snacks daily as previously described.[6] The provided food was free of charge and delivered to participants' homes weekly. All food consumed was recorded in diaries; dietary adherence was based on these records.

The nutritional contents of the interventional diets were analysed using the Foodworks program (Xyris Software Pty Ltd; Brisbane, Queensland, Australia) except for FODMAPs, which were analysed using high performance liquid chromatography and enzymatic assays.[15 16] The interventional diets differed only in FODMAP-content6 as shown in table 1. Matching of the diets for total fibre and resistant starch was achieved adding psyllium and Hi-Maize 220 (National Starch and Chemical

Table 1 The mean daily nutrition information of provided low and typical Australian FODMAP diets

Per day	Typical Australian diet	Low FODMAP diet	p Value
Energy (MJ)	8.17 (7.37–8.97)	8.17 (7.09–9.24)	NS
Protein (g)	96.1 (84.7–107)	98.1 (83.7–113)	NS
Fat (g)	71.6 (49.4–93.8)	74.4 (51.9–97.0)	NS
Total carbohydrate (g)	219 (180–259)	215 (181–249)	NS
Sugars (g)	120 (103–137)	122 (106–139)	NS
Starch (g)	94.0 (52.8–135)	95.4 (59.7–131)	NS
Total dietary fibre* (g)	29.7 (23.9–35.7)	30.4 (24.2–36.5)	NS
Fibre† (g)	25.9 (21.3–30.6)	23.4 (18.7–28.2)	NS
Resistant starch‡ (g)	3.74 (1.85–5.63)	6.93 (3.56–10.3)	NS
Total FODMAPs (g)	**23.7 (16.9–30.6)**	**3.05 (1.86–4.25)**	**<0.001**
Oligosaccharides (g)	**5.49 (2.34–8.65)**	**1.57 (0.47–2.66)**	**0.009**
Polyols (g)	**4.21 (2.57–5.85)**	**0.20 (−0.04–0.44)**	**0.002**
Lactose (g)	**1.35 (0.20–2.49)**	**0.05 (−0.01–0.10)**	**0.033§**
Fructose in excess of glucose (g)	**12.7 (8.06–17.3)**	**1.24 (0.41–2.07)**	**0.001**

Diets were matched for all nutrients except daily FODMAPs, indicated in bold (paired t test).
*Total dietary fibre comprises fibre and resistant starch.
†The low FODMAP diet was supplemented with a daily average of 3 g psyllium.
‡The low FODMAP diet was supplemented with a daily average of 5 g Hi-Maize 220 (National Starch and Chemical Company; Bridgewater, New Jersey, USA).
§While there is a significant difference in lactose, 5 g lactose per sitting is considered well absorbed and tolerated in majority of people[18].
FODMAP, Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols; NS, not significant.

Company, New Jersey, USA), respectively.[6] For the typical Australian diet, 4.4 g oligosaccharides, 2.6 g polyols and 23.7 g total FODMAPs were consumed daily as guided by the Monash Complete Nutritional Assessment Questionnaire.[17] The low FODMAP diet aimed to keep oligosaccharide, fructose in excess of glucose and polyol content <0.5 g per sitting.[3] Average daily intake was estimated 1.6 g oligosaccharides, 0.2 g polyols and 3.1 g total FODMAPs. Both diets were low in lactose (<5 g per sitting).[18]

Faecal assessment

On delivery to the laboratory, the 5-day faecal 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 Animal, Food and Health Sciences (Adelaide, South 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 analysed for SCFA and bacteria abundances. Commonwealth Scientific and Industrial Research Organisation investigators were blinded to the treatments, but not the patient cohorts.

Faecal contents were analysed 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 (BCFA) were reported as μmol/g of faecal matter.

DNA was extracted from 0.25 g faecal matter using a repeat-bead-beating plus column method.[20] Total bacteria as well as butyrate-producing bacteria *Clostridium* cluster IV (*Clostridium leptum* group) including *Faecalibacterium prausnitzii* and *Clostridium* cluster XIVa (*Clostridium coccoides* group) and *Roseburia* spp, prebiotic bacteria (*Lactobacilli* and *Bifidobacteria* spp), and mucus-degrading bacteria (*Akkermansia muciniphila*, *Ruminococcus gnavus* and *Ruminococcus torques*) were analysed. These bacteria were chosen for analysis to specifically examine the role of dietary FODMAPs on bacteria thought to be markers of inflammation and bacteria traditionally thought to be good markers of prebiotic effects.

Detailed methodology of the primers and optimised quantitative real-time PCR conditions used are summarised in online supplement 1 in addition to microbial diversity and lactate and succinate.

Comparison of microbiota with symptom response

In order to assess whether patterns of microbiota predicted differences in symptoms experienced during the two interventional diets, IBS subjects were arbitrarily categorised as non-responders or good responders as defined by mean overall symptom scores in the last 14 days of the low FODMAP diet <10 mm or >20 mm below those in the typical Australian diet, respectively.[6]

Whole gut transit time

WGTT was calculated as previously described.[21] For the purpose of WGTT analysis, patients with IBS were further subclassed as diarrhoea-predominant (IBS-D), constipation-predominant (IBS-C), patients with IBS with existing diarrhoea and constipation (IBS-M) and patients with IBS with neither diarrhoea nor constipation (IBS-U).

Statistical analysis

Sample size estimations were calculated based on GI symptoms which have been previously published.[6] The data from patients with IBS and healthy subjects were examined separately and pooled (given the similarity between the results in the two groups). Subject age and body mass index were presented as median (IQR). A p value ≤0.05 was considered statistically significant for data describing participant demographics, WGTT, faecal pH and lactate and succinate. Data of faecal characteristics were parametric, except for bacterial abundance, which were normalised by $\log_{10}$ conversion before further analysis. All raw faecal characteristic data were presented as mean (95% CI). Differences of faecal 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 individual diets by Wilcoxon matched-pairs signed rank test with Bonferroni corrections. Habitual diet data comparing participant groups were analysed by unpaired t test with Bonferroni correction; p≤0.006 was considered statistically significant for SCFA observations, p≤0.005 for absolute bacterial abundance and p≤0.006 for relative bacterial abundance observations. Multivariate analysis of denaturing gradient gel electrophoresis (DGGE)-banding patterns was performed using the Primer 6+Permanova package (PRIMER-E Limited, Plymouth, UK), with the assumption that each DGGE band represents one phylotype. Data were analysed using a Bray-Curtis similarity matrix on fourth root-transformed data. Differences between diets on the basis of DGGE-banding patterns were calculated with a one-way and pairwise permanova analysis. All statistical tests, unless specified, were analysed with GraphPad Prism V.6 and SPSS V.20 programs.

RESULTS

Participants

Thirty-eight participants (30 IBS and 8 healthy controls) completed the study. Of the six IBS subjects who ceased the typical

Table 2 Comparison of subject demographics and habitual diet characteristics between IBS and healthy cohorts

	IBS (n=27)	Healthy controls (n=6)	p Value
Demographics			
Female*	21 (78%)	5 (83%)	NS
Age (years)†	43 (29–54)	31 (23–61)	NS
Body mass index (kg/m^2)†	24 (23–27)	24 (23–29)	NS
Habitual dietary intake			
Energy (MJ)	9.1 (8.3 to 10)	8.3 (7.1 to 9.5)	NS
Protein (g)	94.7 (84.3 to 105)	91.4 (72.3 to 110)	NS
Fat (g)	87.6 (76.9 to 98.3)	81.7 (63.2 to 100)	NS
Carbohydrate (g)	236 (207 to 266)	204 (144 to 264)	NS
Sugars (g)	110 (88.8 to 130)	93.4 (58.4 to 128)	NS
Starch (g)	140 (124 to 157)	118 (76.8 to 160)	NS
Fibre (g)	24.1 (21.2 to 27.0)	22.5 (17.6 to 27.3)	NS
Total FODMAPs (g)‡	16.6 (14.2 to 18.9)	18.2 (11.9 to 24.9)	NS
Oligosaccharides (g)	3.9 (3.3 to 4.4)	3.9 (3.2 to 4.7)	NS
Polyols (g)	1.6 (1.2 to 2.1)	2.5 (1.1 to 4.0)	NS
Lactose (g)	11.1 (8.7 to 13.5)	11.8 (6.1 to 17.4)	NS
Fructose (g)	18.7 (14.1 to 23.3)	16.5 (7.1 to 25.9)	NS
Glucose (g)	24.0 (17.7 to 30.3)	22.6 (12.9 to 32.4)	NS

Data are presented as mean (95% CI) and compared by unpaired t test except where specified.
*n (percentage of total); Fisher's exact analysis used.
†Median (IQR).
‡Total FODMAP content does not include fructose in excess of glucose which cannot be estimated from Foodworks program.
FODMAP, Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols; NS, not significant.

Australian diet early due to unbearable symptoms, four completed faecal collection prior to exiting the study (range 7–12 days). One IBS and two healthy subjects did not complete faecal collection. Thus, data from 27 IBS and 6 healthy subjects were included in analysis. As shown in table 2, the cohorts were well matched for sex, age and body mass index. Their habitual diets were also matched in nutrients including FODMAP content (table 2). Dietary adherence during the interventional diets was good with all participants adhering to the typical Australian diet, and 81% of IBS and all healthy participants adhering to the low FODMAP diet for at least 81% of the interventions.

Faecal analysis during habitual diet

Faecal pH, lactate, succinate, SCFA and absolute and relative bacterial abundance in IBS and healthy subjects during their habitual diet are shown in table 3. Besides lower isobutyrate and isovalerate concentrations in patients with IBS when compared with healthy subjects, there were no differences in other measured indices. On the habitual diets, WGTT was significantly slower in healthy (67.1 (47.0 to 87.2) h) participants when compared with IBS-D (31.9 (22.4 to 41.4) h; p=0.001) and IBS-M subjects (40.6 (21.5 to 59.6) h; p=0.034) (see online supplement 2).

Comparison of biochemical indices on habitual and interventional diets

As there were few differences in faecal measures during the habitual diet, IBS and healthy cohorts were combined. Faecal pH was 0.2 units higher on the low FODMAP in comparison with the habitual and typical Australian diets (p=0.008). No differences were seen in total or specific faecal SCFA or succinate between diets (table 4 and figure 1A). Only one participant had complete data for faecal lactate, so could not be analysed. Molar proportions of the major SCFA were also unchanged (data not shown).

Absolute and relative abundance of bacteria are shown in table 5. When analysed as absolute abundance, the low FODMAP diet had a lower total bacterial load compared with habitual and typical Australian diets (table 5 and figure 1B). Furthermore, absolute abundance of the butyrate-producing bacteria, the prebiotic bacteria, *Bifidobacteria* spp and the mucus-associated bacterium, *A. muciniphila*, was greater on the typical Australian diet compared with the other two diets (table 5 and figure 1B). In terms of relative abundance, the typical Australian diet increased *Clostridium* cluster XIVa and *A. muciniphila* in comparison with habitual and low FODMAP diets, but decreased *R. torques* in comparison with the low FODMAP diet (table 5 and figure 1C). Microbial diversity in the *Clostridium* cluster XIV was greater in subjects on the low FODMAP compared with the typical Australian diet and the habitual diet (table 5). Similar patterns were seen when data were separated into IBS and healthy cohorts.

Neither dietary changes nor the habitual diet pattern in absolute and relative bacterial abundances predicted symptomatic difference between the interventional diets in non-responders and good responders (see online supplement 3).

Altering dietary FODMAPs did not affect WGTT in any subject cohort, including specific IBS subtypes. The healthy subjects had a slower WGTT when compared with IBS-D and IBS-M subjects on all diets as well as IBS-C subjects during the interventional diets (see online supplement 2). No correlation between WGTT and the composition of the gut microbiota were observed (data not shown).

Table 3 Faecal pH, lactate, succinate, SCFA, absolute and relative bacterial abundance and bacterial diversity on pooled 5-day samples in IBS and healthy subjects during their habitual diet

	IBS (n=27)	Healthy control (n=6)	p Value
pH	7.19 (7.07–7.31)	7.17 (6.63–7.72)	0.916
Lactate (g/100 g)*	0.02 (0.02–0.03)	0.02 (0–0.04)	0.375
Succinate (g/100 g)*	0.03 (0.02–0.03)	0.04 (0.02–0.06)	0.063
SCFA concentration (μmol/g)			
Total SCFA	81.9 (72.3–91.5)	93.4 (58.6–128)	0.334
Butyrate	15.6 (13.0–18.1)	18.9 (9.61–28.3)	0.283
Propionate	15.8 (13.6–18.0)	17.6 (9.53–25.6)	0.526
Acetate	42.5 (37.4–47.5)	44.7 (27.5–61.9)	0.715
Isobutyrate	**1.92 (1.66–2.17)**	**3.10 (2.52–3.68)**	**<0.001**
Isovalerate	**2.99 (2.56–3.43)**	**5.06 (3.92–6.19)**	**<0.001**
Valerate	2.28 (1.73–2.83)	3.70 (1.62–5.78)	0.044
Caproate†	0.95 (0.58–1.32)	0.46 (−0.12–1.04)	0.175
Absolute abundance (Log_{10} copies of 16S rRNA gene/g)			
Total bacteria	9.85 (9.72–9.98)	9.82 (9.61–10.0)	0.861
Clostridium cluster IV	8.40 (8.20–8.59)	8.37 (7.97–8.76)	0.892
Faecalibacterium prausnitzii	7.86 (7.68–8.05)	7.76 (7.12–8.39)	0.643
Clostridium cluster XIVa	8.26 (8.11–8.41)	8.04 (7.66–8.41)	0.193
	7.64 (7.46–7.83)	7.50 (6.90–8.10)	0.518
Lactobacilli	6.27 (6.03–6.51)	5.94 (5.49–6.39)	0.219
Bifidobacteria	7.61 (7.37–7.86)	8.07 (7.55–8.58)	0.104
Akkermansia muciniphila	4.07 (3.42–4.72)	4.50 (2.53–6.47)	0.571
Ruminococcus gnavus	7.20 (7.06–7.34)	6.96 (6.70–7.22)	0.129
Ruminococcus torques	6.09 (5.83–6.34)	6.74 (6.37–7.11)	0.025
Relative abundance (percentage of total bacteria)			
Clostridium cluster IV	4.05 (3.34–4.77)	3.73 (2.39–5.07)	0.687
F. prausnitzii	1.31 (0.89–1.74)	1.18 (0.19–2.16)	0.778
Clostridium cluster XIVa	2.81 (2.40–3.22)	1.82 (0.97–2.67)	0.037
Roseburia	0.82 (0.57–1.06)	0.67 (0.13–1.22)	0.609
Lactobacilli	0.07 (0.01–0.13)	0.02 (0–0.03)	0.412
Bifidobacteria	1.33 (0.53–2.13)	2.18 (0.48–3.87)	0.348
A. muciniphila	0.01 (0–0.02)	0.01 (0–0.03)	0.756
R. gnavus	0.30 (0.20–0.40)	0.16 (0.04–0.28)	0.204
R. torques	0.03 (0.01–0.06)	0.12 (0.01–0.23)	0.008
Diversity (Shannon index)			
Clostridium cluster XIV	1.83 (1.67–1.98)	1.64 (1.35–1.93)	0.310

Differences in cohorts were analysed by unpaired t test. Statistically significant differences are shown in bold based upon p≤0.05 for pH, lactate and succinate, p≤0.006 for SCFA concentrations, p≤0.005 for absolute and p≤0.006 for relative bacterial abundance after Bonferroni correction.
*Due to difficulties in analysis, for lactate, IBS n=8 and healthy controls n=2.
†Due to difficulties in analysis, for caproate, IBS n=18 and healthy controls n=5.
SCFA, short-chain fatty acid.

DISCUSSION

The low FODMAP diet has good evidence of efficacy for symptom management in patients with IBS.[6] However, as FODMAPs, especially oligosaccharides, have shown positive effects on the colonic microenvironment and microbiota in healthy populations,[7 8] a low FODMAP diet might impact negatively on colonic health. The present study investigated its effects in 33 subjects compared with those of a carefully matched diet representing the typical FODMAP intake in Australia on markers linked to colonic health through the assessment of WGTT, soluble luminal microenvironment and faecal microbiota. Marked differences in absolute and relative bacterial abundance and diversity, but not SCFA or transit were observed.

Table 4 Faecal pH, succinate, total and specific SCFA (μmol/g) on pooled 5-day faecal samples after following a habitual diet for 5 days and low FODMAP and typical Australian diets for 17–21 days in cross-over trial (n=33)

Measure	Australian diet	Low FODMAP diet	p Value	Habitual diet
pH	**7.16 (7.02–7.30)**	**7.37* (7.23–7.51)**	**0.001**	**7.18 (7.07–7.31)**
Succinate†	0.03 (0.02–0.04)	0.03 (0.02–0.03)	0.178	0.03 (0.03–0.04)
Total SCFA	74.7 (65.9–83.4)	77.6 (68.8–86.5)	0.208	84.0 (74.8–93.2)
Butyrate	14.0 (11.8–16.2)	13.5 (11.3–15.7)	0.672	16.2 (13.7–18.6)
Propionate	14.4 (12.4–16.4)	15.2 (13.4–16.9)	0.145	16.2 (14.0–18.2)
Acetate	38.6 (34.1–43.1)	40.9 (36.1–45.6)	0.126	42.9 (38.2–47.6)
Isobutyrate	2.01 (1.66–2.37)	2.07 (1.69–2.45)	0.836	2.13 (1.86–2.41)
Isovalerate	3.15 (2.52–3.78)	3.22 (2.54–3.91)	0.857	3.37 (2.89–3.85)
Valerate	2.25 (1.82–2.67)	2.29 (1.90–2.69)	0.974	2.54 (1.97–3.10)
Caproate‡	1.03 (0.64–1.42)	1.13 (0.74–1.52)	0.454	1.20 (0.85–1.55)

Interventional diets were analysed by Wilcoxon matched-pairs signed rank test.
Statistically significant differences are shown in bold and based upon p≤0.05 for faecal pH and p<0.006 for SCFA concentrations after Bonferroni correction.
Differences between habitual diet and interventional diets are indicated with an asterisk.
*p=0.004 compared with habitual diet; Wilcoxon matched-pairs signed rank test.
†Due to difficulties in analysis for succinate, n=18.
‡Due to difficulties in analysis for caproate, n=15.
FODMAP, Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols; SCFA, short-chain fatty acid.

Comparisons with data during the participants' habitual diet were also made.

Colonic luminal concentrations of SCFA are of major importance to gut health given their role in secretion, absorption, motility and epithelial health. Because they are products of bacterial fermentation, a change in the delivery of fermentable substrates to the colon is anticipated to alter the concentrations and output of faecal SCFA. The low FODMAP diet reduced total bacterial abundance in the faeces by an average 47% in comparison with the typical Australian diet, which in turn could possibly lower SCFA production. However, faecal SCFA concentration was unaffected by the FODMAP content of the diet, although faecal pH was higher on the low FODMAP diet. This apparently paradoxical situation requires explanation. While SCFAs are the major anions in the large bowel, other bacterial metabolites not measured or alterations in protein catabolism may have contributed to the lower faecal pH associated with FODMAP intake. The higher average resistant starch content of the low FODMAP compared with that of the typical Australian diet (table 1) might have compensated for the lower FODMAP content as suggested by an animal trial comparing resistant starch with fructo-oligosaccharides (FOS).[22] However, the difference in resistant starch consumed in the present study was small (3.2 g/d) and all previous studies have shown that at least 16 g/d is required to alter faecal SCFA concentration, but only when combined with wheat bran.[22–24] Changes in colonic transit will influence faecal SCFA excretion, but WGTT was similar in each dietary period. This finding is consistent with previous data indicating that non-fermented or poorly fermented dietary fibres are more effective faecal bulking agents and so have a greater effect on hastening transit than readily fermented carbohydrates.[24–26] Although the methodology used measures whole gut transit, it is likely that this reflects large bowel transit as this is the longest duration. Indeed, faecal weight was not altered by FODMAP ingestion,[6] which might have been expected by such a reduction in total bacterial abundance. Supplementation of fibre and resistant starch to the low FODMAP diet may have concealed the expected change. The most likely explanation for the apparent paradox is, because FODMAPs are fermented in the proximal colon, that faecal concentrations of SCFA are poor markers of colonic fermentation. It is also known that more than 95% of SCFAs are rapidly absorbed and metabolised.[12]

It is likely that changes in the structure of the microbiome will translate into functional changes, although the nature of such a relationship remains undefined. The interventional diets were associated with several differences in faecal microbiota. Absolute abundances overall and of butyrate and prebiotic bacteria were less in association with the low FODMAP diet. This is not surprising as these bacteria metabolise carbohydrates, and a reduction of such substrates should lead to reduced proliferation non-specifically. Of the mucus-degrading bacterial species, *R. gnavus* and *A. muciniphila* were reduced in total abundance. As total bacterial abundance was altered on the interventional diets, the effects of the diets on relative abundance of specific bacteria were of more importance. Three bacterial groups of putative functional importance were targeted. First, *Clostridium* cluster XIVa showed a sevenfold difference between the two diets and this wide difference was observed in all participants (IBS or healthy). This observation is consistent with those in animal and human studies showing increased *Clostridium* cluster XIVa in faeces and digesta after consumption of oligosaccharides, or foods containing FODMAPs (wheat bran).[9 27 28]

Second, traditionally 'prebiotic' bacteria, namely *Bifidobacterium* spp, was similar between the two diets. The only previous study to investigate prebiotic bacteria in association with a low FODMAP diet identified a difference in absolute and relative abundance of faecal *Bifidobacterium* spp in patients with IBS compared with those in a parallel UK population consuming their habitual diet.[4] One possible explanation for this discrepancy might be that the habitual diet in the UK population contained a higher amount of galacto-oligosaccharides (GOS) (mean (95% CI) 2.0 (1.4 to 2.5) g/d) and total oligosaccharides than provided by the typical Australian (GOS 1.01 (0.09 to 1.94) g/d) and habitual diets (GOS 0.76 (0.55 to 0.96) g/d) of this Australian cohort. As oligosaccharides (including GOS) are thought to influence faecal concentrations of *Bifidobacterium* spp,[8 9] the greater decline from the habitual UK to the low FODMAP diet may have been responsible for the altered relative abundance in the UK subjects.

The third bacterial group studied was mucus-degrading bacteria, specifically *A. muciniphila* and two *Ruminococcus* spp. Simplistically, such bacteria are able to adhere to mucus and feed off glycans and mucin proteins as part of the mucus secreted by the gut epithelium.[29] Extensive degradation of the mucous layer might be detrimental by impairing gut barrier function. On the other hand, such foraging bacteria may provide substrates for other bacteria important for development of a healthy mucus-associated microbiota.[30] The diets were associated with marked differences in the relative abundance of *A. muciniphila*, (lower in the low FODMAP arm) and *R. torques* (higher in the low FODMAP arm). The pattern observed was similar as the difference seen in patients with IBD compared with controls.[31 32] Similarly in mice[33] and rats inoculated with human microbiota,[30] ingestion of oligosaccharides increase the excretion of *A. muciniphila*. However, the mechanism is uncertain as oligosaccharides do not directly promote the growth of *A. muciniphila* in vitro.[32] Faecal abundance appears to reflect distal colonic mucosal abundance,[32] but whether it reflects mucus-degrading microbiota in the proximal colon is uncertain

especially when caecal abundance of *A. muciniphila* was reduced in association with FOS-induced increase in faecal excretion of *A. muciniphila* in rodents.[30] Most evidence would suggest that mucus-associated *A. muciniphila* have favourable effects, perhaps via the provision of substrates such as acetate and propionate for the support of a healthy consortium of bacteria adjacent to the epithelium.[14] The study of the effect of the low FODMAP diet on mucus-degrading microbiota in the uninflamed proximal and distal colon is required to resolve this dilemma.

The diets differing in FODMAP content were also associated with differences in the diversity of a cluster of bacteria including many butyrate-producers, with greater diversity on the low FODMAP diet. Reduced diversity is a common finding in diseased colons, particular in association with IBD, where diversity is inversely proportional to the degree of intestinal inflammation.[34] However, the focus was specifically on the *Clostridium* cluster XIVa, which includes a large number of butyrate-producing bacteria. The abundance of this group (*Clostridium* cluster XIVa) declined significantly on the low FODMAP diet in IBS and healthy subjects. These two findings together may represent an alteration in dynamics of this cluster of bacteria from a cluster with fewer and more dominant species to one with more but less dominant species on the low FODMAP diet. However, functional and health significances cannot be attributed to this difference in diversity at the present time.

The key question regarding the differences in microbiota between diets that vary in their FODMAP intake is what is increased and what is decreased. The characterisation of faecal microbiota in the same participants while taking their habitual diet provided that opportunity. As anticipated, bacterial abundance was reduced in association with the low FODMAP diet. However, the marked changes in relative abundance of *Clostridium* cluster XIVa and *A. muciniphila* reflected an increase

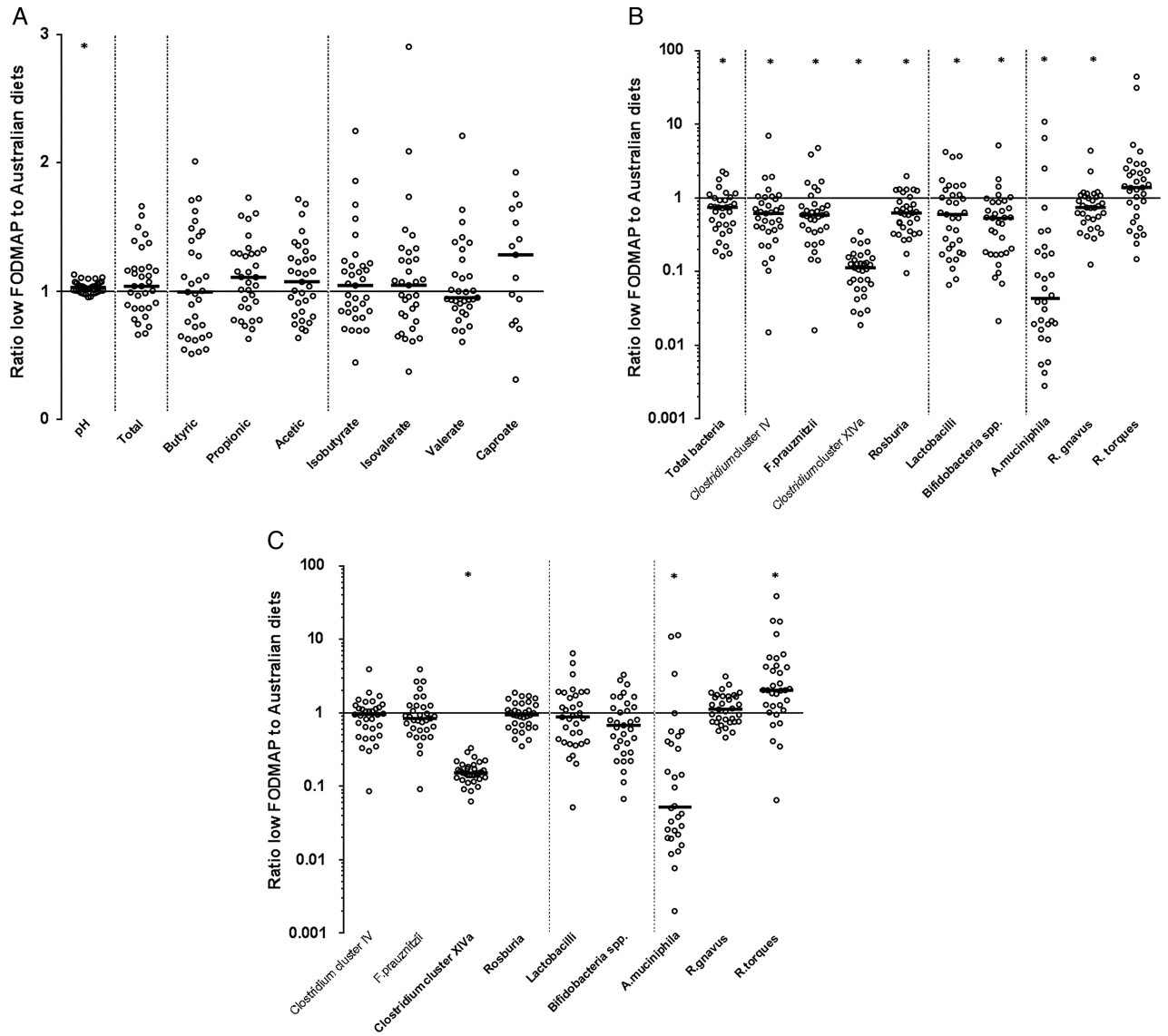

Figure 1 Comparison of faecal indices with the two interventional diets in subjects with IBS and healthy subjects. (A) Changes in pH, total and major short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs); (B) total and specific absolute bacterial abundance; and (C) relative bacterial abundance. All data are presented as a ratio of low Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols (FODMAP) to typical Australian diet and analysed by Wilcoxon matched-pairs signed rank test. Statistically significant differences between the diets are indicated with an asterisk based upon $p \leq 0.05$ for faecal pH, $p \leq 0.006$ for SCFA concentrations $p \leq 0.005$ for absolute and $p \leq 0.006$ for relative bacterial abundance after Bonferroni correction.

Table 5 Absolute and relative bacterial abundance and bacterial diversity on pooled faecal samples after following a habitual diet for 5 days and low FODMAP and typical Australian diets for 17–21 days in cross-over trial (n=33)

Measure	Bacteria	Australian diet	Low FODMAP diet	p Value	Habitual diet
Absolute abundance (Log_{10} copies of 16S rRNA gene/g)	Total bacteria	**9.83 (9.72–9.93)**	**9.63* (9.53–9.73)**	**<0.001**	**9.85 (9.73–9.96)**
	Clostridium cluster IV	**8.33 (8.15–8.52)**	**8.05* (7.88–8.23)**	**<0.001**	**8.39 (8.23–8.56)**
	Faecalibacterium prausnitzii	**7.72 (7.49–7.95)**	**7.45* (7.25–7.65)**	**<0.001**	**7.84 (7.67–8.01)**
	Clostridium cluster XIVa	**9.05* (8.93–9.16)**	**8.03 (7.91–8.15)**	**<0.001**	**8.22 (8.09–8.36)**
	Roseburia	**7.72 (7.59–7.85)**	**7.49 (7.34–7.63)**	**<0.001**	7.62 (7.45–7.79)
	Lactobacilli	**6.35 (6.20–6.50)**	**6.08 (5.91–6.24)**	**0.003**	6.21 (6.00–6.42)
	Bifidobacteria	**7.71 (7.53–7.88)**	**7.30* (7.11–7.50)**	**<0.001**	**7.70 (7.48–7.91)**
	Akkermansia muciniphila†	**5.46* (4.88–6.04)**	**4.29 (3.58–4.99)**	**<0.001**	**4.29 (3.67–4.92)**
	Ruminococcus gnavus	**7.26 (7.14–7.37)**	**7.10 (6.96–7.25)**	**0.002**	7.16 (7.04–7.28)
	Ruminococcus torques	6.08 (5.85–6.31)	6.23 (6.07–6.39)	0.140	6.20 (5.97–6.44)
Relative abundance (percentage of total bacteria)	*Clostridium* cluster IV	4.00 (3.21–4.71)	3.32 (2.70–3.94)	0.108	3.99 (3.39–4.60)
	F. prausnitzii	1.11 (0.82–1.40)	0.95 (0.69–1.22)	0.108	1.29 (0.92–1.66)
	Clostridium cluster XIVa	**18.1* (15.4–20.8)**	**2.72 (2.33–3.12)**	**<0.001**	**2.63 (2.26–3.01)**
	Roseburia	0.85 (0.585–1.11)	0.82 (0.68–0.96)	0.153	0.79 (0.58–1.00)
	Lactobacilli	0.05 (0.03–0.06)	0.04 (0.03–0.05)	0.634	0.06 (0.01–0.11)
	Bifidobacteria	1.33 (0.74–1.92)	0.87 (0.47–1.27)	0.028	1.48 (0.79–2.18)
	A. muciniphila†	**0.10* (0.03–0.16)**	**0.02 (0.01–0.03)**	**<0.001**	**0.01 (0–0.02)**
	R. gnavus	0.37 (0.23–0.50)	0.41 (0.27–0.53)	0.480	0.27 (0.19–0.36)
	R. torques	**0.04 (0.02–0.06)**	**0.06 (0.04–0.09)**	**0.001**	0.05 (0.02–0.08)
Diversity (Shannon index)	*Clostridium* cluster XIV	**1.47 (1.39–1.55)**	**1.79‡ (1.70–1.89)**	**<0.001**	**1.60 (1.46–1.73)**

Interventional diets were analysed by Wilcoxon matched-pairs signed rank test. Statistically significant differences are shown in bold and based upon $p \leq 0.05$ for bacterial diversity, $p \leq 0.005$ for absolute and $p \leq 0.006$ for relative bacterial abundance after Bonferroni correction. Differences between habitual diet and interventional diets are indicated with an asterisk.
*$p<0.001$ compared with habitual diet; Wilcoxon matched-pairs signed rank test.†Due to difficulties in microbial analysis for *A. muciniphila*, three IBS subjects could not be included; n=30.
‡$p<0.05$ compared with habitual diet; pairwise permanova test.
FODMAP, Fermentable Oligosaccharides, Disaccharides, Monosaccharides And Polyols.

in association with the Australian diet rather than a decrease in the low FODMAP diet. This could be arguably described as a prebiotic effect of the former diet. It is also reasonable to postulate that such a difference resided in the modest differences in oligosaccharide intake in the typical Australian diet compared with the habitual diet (approximately 1.6 g), although these two indices were measured using different methods. In concert with more oligosaccharides in the typical Australian than habitual diet, there was a small increase in GI symptoms.[6] Hence, the low FODMAP diet reduced absolute abundance of faecal bacteria, but did not have an 'antiprebiotic' effect.

The present study was not powered to compare faecal microbiota and biochemical indices in healthy subjects with those who have IBS. However, similar trends in microbiota and SCFA concentrations were noted. The only exceptions were the higher faecal concentrations of the BCFA. BCFAs are products of protein degradation, fermented increasingly through progression to the distal colon,[35] and associated with increased genotoxicity and possibly less cytotoxicity.[35] Reasons for differences in the faecal BCFA concentrations, such as differences in microbiota associated with protein fermentation, were not specifically investigated. However, dietary components with potential to increase faecal BCFAs, such as protein and calcium, which are thought to alter genotoxicity and cytotoxicity, respectively,[36 37] were similar between subject groups. Altering the dietary FODMAP content showed a similar lack of response in SCFAs across the two cohorts.

Post hoc analyses of faecal microbiota were performed to gain some insight as to whether changes in the microbiota might predict clinical response in patients with IBS (see online supplement 3). No differences were observed between non-responders and good responders indicating that the symptoms and microbiota were probably not directly associated in response to diet. Similarly, faecal content of SCFA and BCFA were not associated with response (data not shown).

In conclusion, this study is the first randomised controlled trial to compare faecal soluble milieu and microbiota in subjects with IBS while following two controlled diets differing in their FODMAP content in a cross-over design. There was a higher faecal pH, but the concentrations of faecal SCFAs were not different. In contrast, marked changes in the microbiota were found. The low FODMAP diet was associated with lower absolute abundance of total bacteria, butyrate-producing bacteria, prebiotic bacteria and *A. muciniphila* and *R. gnavus*. Marked lower relative abundances of *Clostridium* cluster XIVa and *A. muciniphila*, and a significantly higher abundance of *R. torques* were also observed. Finally, bacterial taxonomic diversity of a large cluster of primarily butyrate-producers was greater on the low FODMAP diet. Comparison with faecal microbiota on habitual diet indicated that the low FODMAP intake was associated with reduced absolute abundance of bacteria, but the higher FODMAP intake associated with the typical Australian diet showed evidence of specific stimulation of the growth of bacterial groups with putative health benefits. The functional significance and health implications of such changes might lead to caution about reducing FODMAP intake in the longer term. Liberalising FODMAP restriction to the level of adequate symptom control should be exercised. The low FODMAP diet should not be recommended for asymptomatic populations.

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Competing interests SJS has published a book on food intolerances and several cookbooks related to the topic of the manuscript. PRG has published a book on food intolerances. There were no conflicts of interest to declare for EPH, CTC, ARB, JGM.

Patient consent Obtained.

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