Long-term personalised low FODMAP diet improves symptoms and maintains luminal Bifidobacteria abundance in irritable bowel syndrome

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Short title: Long-term low FODMAP diet

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Conflict of interest statement
HMS reports non-financial support from CD Investments VSL Pharmaceuticals. MR is the owner of a breakfast cereal range. MCL is the coinventor of a mobile app with FoodMaestro to support patients following the low FODMAP diet. ED has received research funding from Almond Board of California. BW has received research funding from Clasado Biosciences. LDM has published a book regarding the low FODMAP diet. PL has received grants from Scottish Government Rural and Environment

Sciences and Analytical Services (RESAS). KW is the coinventor of a mobile app with FoodMaestro to support patients following the low FODMAP diet, has received research funding from Almond Board of California, Clasado Biosciences, Danone, International Nut and Dried Fruit Council, and has received consultancy fees from Danone. The remaining authors have nothing to disclose.

Author contributions

HMS and KW were grant holders; HS, MCL, PMI and KW conceived and designed the study; HMS, MR, LDM, MCL, PMI recruited participants; HMS, MR, FSER, LDM collected and analysed patient data, TK, ED, BW, PL performed laboratory analysis, HMS and TK analysed the data; KW supervised data collection and analysis; All authors interpreted the data; HMS and KW wrote the manuscript; All authors reviewed and approved the final manuscript for submission.

Data sharing

Data are not available for sharing as consent to provide raw data was not provided by patients nor the research ethics committee.

Abstract

Background: Short-term trials demonstrate the low FODMAP diet improves symptoms of irritable bowel syndrome (IBS) but impacts nutrient intake and the gastrointestinal microbiota. The aim of this study was to investigate clinical symptoms, nutrient intake and microbiota of patients with IBS 12 months after starting a low FODMAP diet.

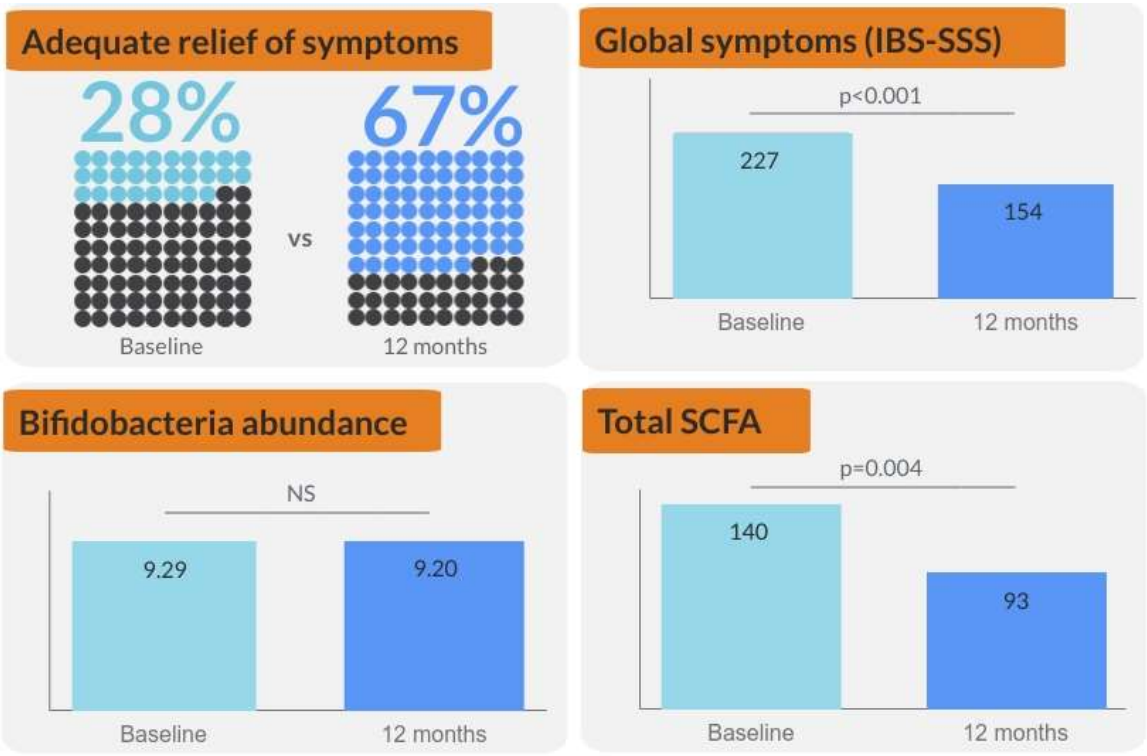
Methods: Participants enrolled in a previous short-term clinical trial and who had been through structured FODMAP restriction, reintroduction and personalisation were invited to participate in a follow-up study at one time point at 12months. Gastrointestinal symptoms, stool output, dietary intake and quality of life were recorded. Stool samples were collected and analysed for microbiota (qPCR) and short-chain fatty acids (SCFA). Data were compared with baseline (prior to any intervention in the original clinical trial) using non-parametric statistics.

Key results: Eighteen participants were included in the study. Adequate relief of symptoms occurred in 5/18 (28%) at baseline and increased to 12/18 (67%) following long-term personalised low FODMAP diet ($p=0.039$). There was a reduction in IBS-SSS total score between baseline (median 227, IQR 99) and long term (154, 89; $p<0.001$). Bifidobacteria abundance was not different between baseline (median 9.29, IQR 1.45) and long term (9.20, 1.41; $p=0.766$, $q=0.906$), however there were lower concentrations of total SCFA, acetate, propionate and butyrate.

Conclusions: In this long-term analysis, two thirds of patients reported adequate relief of symptoms after 12 months of personalised low FODMAP diet, that did not result in differences from baseline in Bifidobacteria. FODMAP reintroduction and personalisation may normalise some of the effects of short-term FODMAP restriction.

Keywords: FODMAP, diet, irritable bowel syndrome, microbiome, fructans, bifidobacteria

Graphical abstract



Introduction

Irritable bowel syndrome (IBS) is a prevalent functional gastrointestinal (GI) disorder with a global prevalence of 4.1% (1) and with a considerable patient burden. A number of dietary triggers have been explored, including a group of short-chain fermentable carbohydrates (2), termed fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs). Some FODMAPs increase small intestinal water and others increase colonic gas generation, which in a patient with IBS can induce symptoms (3).

Randomised controlled trials (RCT) demonstrate the efficacy of a short-term low FODMAP diet for IBS. Short periods (3-4 weeks) of low FODMAP diet lead to global clinical response (4-6), greater clinical response for individual gastrointestinal symptoms (e.g. bloating, pain) (4, 5, 7, 8) and improved quality of life outcomes (7, 9) compared with controls. However, there are possible disadvantages associated with a short-term low FODMAP diet including shifts in the GI microbiota (4, 7, 10), altered nutrient intake (11, 12) and lower diet quality (12) compared with controls who consume habitual or control diets.

The majority of research on the low FODMAP diet is focused on short-term clinical, nutritional and microbiota outcomes during the initial restriction phase only, where foods high in all FODMAPs are restricted from the diet. However, in clinical practice, the restriction phase is followed by a reintroduction phase where dietitian-supervised stepwise reintroduction of high FODMAP foods is undertaken to identify which FODMAPs and in what quantities trigger IBS symptoms. This is then followed in the longer term by the personalisation phase, such that the patient constructs and follows a 'personalised-FODMAP' diet that includes FODMAP-containing foods to tolerance whilst maintaining symptom control (13). Given the chronicity of symptoms in IBS, it is critical to determine whether patients with IBS experience clinical benefit from the low FODMAP diet in the long term, for which

there are numerous studies, and whether there are long-term impacts on nutrient intake, for which there are few studies, and also the impact on the microbiota, for which there are currently no studies.

Several cross-sectional and prospective studies have evaluated IBS symptoms during the low FODMAP diet in the long term, after FODMAP reintroduction and personalisation. Of the three cross-sectional studies, positive symptom responses were reported at follow-up 9-24 months following starting the diet (14-16). In a prospective questionnaire study most patients continued a personalised-FODMAP diet at 12 months and 57% reported satisfactory relief of overall symptoms (17). Three RCTs have reported IBS symptoms during the low FODMAP diet in the long term, all reporting sustained symptom improvement, albeit only at 4-6 months (18-20).

With regards to dietary adequacy, nutrient intake is somewhat restored when individuals move from the restriction phase of the low FODMAP diet to FODMAP personalisation in the long term. Despite reductions in intakes of energy (19, 20) and fibre (20) after 4-week FODMAP restriction, these were restored to baseline following longer term reintroduction and personalisation. One long-term follow-up study reported no difference in the proportion of patients meeting national recommendations for fibre and 13 micronutrients in those following a personalised-FODMAP diet after reintroduction and personalisation, compared with those who had returned to habitual diet (17).

These evaluations of the long-term effect of the low FODMAP diet on clinical and dietary endpoints are limited for a variety of reasons. Firstly, cross-sectional data are subject to recall bias whereby change in symptoms is reported after a period of 12-months or more. Secondly, objective measures of adherence to a low FODMAP diet (i.e. FODMAP intake) and nutrient intake have been measured only in selected studies (17, 19, 20). Finally, it is known that even very small doses (3.5 g/d) of prebiotic carbohydrates can modulate the microbiota in IBS (21), however, there are no reliable data reporting microbiota composition during long-term FODMAP personalisation. One RCT has attempted this,

although a technical failure impacted most of the baseline samples leaving the comparison with follow-up timepoints largely unreliable (19).

Therefore, the aim of this study was to investigate clinical symptoms, nutrient intake and microbiota of a carefully characterised group of patients with IBS at baseline (prior to FODMAP restriction) and twelve months later, after following all three phases of the low FOODMAP diet (restriction, reintroduction, personalisation).

Methods

Participants in this study had previously taken part in a RCT consisting of 104 patients with IBS randomised to a factorial design study of low FODMAP diet/control sham diet and probiotic/placebo for 4-weeks, described in full elsewhere (7)(ISRCTN02275221). The patients in the initial study were aged 18-65 years with Rome III IBS and recruited from Guy's and St Thomas' NHS Foundation Trust and St George's Healthcare NHS Trust (London, UK). They had no other major medical conditions (e.g. diabetes, major active psychiatric condition), other GI disease (e.g. inflammatory bowel disease, coeliac disease) or history of GI resection and were naïve to the low FODMAP diet. Those reporting consumption of antibiotics, prebiotics or probiotics four weeks prior to the RCT were excluded. Individuals were allocated to follow sham or low FODMAP dietary advice for four weeks. Those allocated to the low FODMAP diet group were provided indepth dietary counselling for the low FODMAP diet at baseline and then FODMAP reintroduction advice at four weeks together with explanation of the rationale for the diet (in order to maintain blinding in the original 4-week RCT). Those allocated to the sham diet group were provided with indepth dietary counselling for the low FODMAP diet together with explanation of the rationale for the diet at four weeks and subsequently provided FODMAP reintroduction advice 4-8 weeks later.

Recruitment to the current study involved contacting all patients (where possible) approximately 10 months after completing the RCT to ask about their interest in participating in this long-term follow-up study. Exclusion criteria for the long-term study was a new diagnosis of GI disease, GI resection since the RCT, and antibiotics, prebiotics, probiotics or bowel preparation in the previous 4 weeks. Eligible individuals attended the study centre 12-months after receiving low FODMAP advice (i.e. those initially randomised to the low FODMAP diet arm attended 12 months after starting the RCT; those randomised to the sham diet arm attended 12 months after completing the RCT). Therefore, for the current analysis, all patients had received indepth low FODMAP dietary advice from a registered dietitian, including all three phases (restriction, reintroduction, personalisation) starting 12 months earlier.

Informed consent was obtained prior to any study-related procedures, and this long-term follow-up study was approved by an NHS research ethics committee (NRES Committee London–Fulham, 12/LO/1402). Eligible and interested participants were sent diaries to record symptoms, stool output and food intake for seven days. At the long-term research visit, the seven-day symptom, stool and food diaries were collected, further questionnaires completed, bodyweight and medications recorded, and a fresh stool sample provided.

The symptom diary rated the presence and severity of 15 individual symptoms and overall symptoms on each of the seven days using the GI symptom rating scale (22). Symptom response was also measured using a global symptom question (‘did you have adequate relief of your symptoms over the last seven days’) and was completed on day seven only. The stool diary recorded the frequency and rated the stool consistency of all stools using the Bristol Stool Form Scale (23) and was completed daily for seven days.

Dietary intake was recorded in an unweighed 7-day food diary, using food labels, standard measures and food photographs to estimate portion size, and was completed daily for seven days. Nutrient intake was calculated using published tables of food composition in the United Kingdom (24) and FODMAP intake was calculated using bespoke online software populated with comprehensive FODMAP composition data (25).

At the long-term research visit, symptoms were also measured using the IBS severity scoring system (IBS-SSS) (26). Generic and disease-specific health-related quality of life were measured using the SF-36 (27) and the IBS-QoL (28) questionnaires, respectively.

A whole, fresh stool sample was collected in a sterile bag and homogenised in a stomacher for four minutes. An aliquot was stored in a lysis matrix tube at -80 °C until DNA extraction and subsequent microbiota analysis, an aliquot was stored immediately at -80 °C for analysis of short-chain fatty acid (SCFA) and another aliquot was used to immediately measure stool pH. Stool sample collection and processing at the long term visit were performed identically to those at baseline (e.g. collection method, delivery to the unit, timing and method of processing etc).

Microbiota analysis was performed using quantitative polymerase chain reaction (qPCR), with full methods reported in online supplementary information. Bacterial DNA was extracted using the FastDNA™ SPIN kit for soil (MP Biomedicals Europe, Illkirch-Graffenstaden, France) and DNA concentration measured using a NanoDrop ND1000 instrument prior to qPCR using primers for 11 bacterial taxa using a 7900HT fast qPCR system (Applied Biosystems, Foster City, CA). SCFA were analysed using gas liquid chromatography. SCFA were extracted from defrosted stool in buffer containing an internal standard, as previously described (29). Extracted SCFA (0.2 mL) were separated on a Hewlett Packard 6890 series GLC system (Agilent Technologies, Santa Clara, CA) equipped with a BP21 25-m capillary column with internal diameter of 0.22 mm and film thickness of 0.25 mm. Stool

pH was measured on fresh stool, which was diluted 1:4 (vol: vol) in pH buffer ($1x10^{-5}$mol/L Na_2HPO_4, KH_2PO_4, 0.1 g $HgCl_2$), homogenised, and incubated at room temperature for 1 h. The pH was measured using a pH meter and a pH electrode specifically designed for slurries (VWR, Pennsylvania, US). Full laboratory methods are provided in online supplementary information.

Statistical analysis

Data from the long-term research visit were compared with the same data from baseline, prior to any intervention in the original RCT. Conservative last observation carried forward approach was used to impute minor elements of missing data for symptoms and quality of life. Overall clinical, dietary and quality of life data were not normally distributed and therefore summary data are presented as median and interquartile range and the Wilcoxon ranked-signed tests used to compare data between baseline and following 12-months of low FODMAP diet. McNemar's test was used to compare the proportion of patients reporting adequate relief between baseline and following 12-months. Data were considered statistically significant where $p<0.05$. Microbiota data at long term were compared with baseline using Wilcoxon ranked-signed tests and corrections were made for multiple comparisons and false detection rate was considered significant when $q<0.05$.

Results

In total, 104 patients were recruited to the initial RCT at baseline and 95 completed that study. All patients were defined as compliant to the dietary interventions in the initial 4-week RCT, patients in the LFD group reduced FODMAP intake and those in the sham diet arm did not (7).

Of the 95 completing the original RCT, 44 responded to our contact, of whom 21 declined to participate (5 too busy, 16 no reason given). Of the 23 screened for long-term follow-up, 5 were excluded (3 never attempted low FODMAP diet, 1 still taking probiotics, 1 moved overseas). The remaining 18 consented to participate and completed the long-term follow study. Of these 18, in the

initial RCT, eight had been randomised to low FODMAP dietary advice and 10 had been randomised to sham diet (control group) and therefore received low FODMAP dietary advice four weeks after baseline, whilst nine had been randomised to the probiotic group and nine to the placebo group. Demographic data are presented in **Table 1**. The majority had diarrhoea-predominant IBS at baseline (14/18, 78%). Medication intake had remained stable for the majority of patients in the past 12 months (16/18, 89%). Of the two whose medication had changed, this was for non-IBS medication (one ceased a proton pump inhibitor and commenced over-the-counter reflux medication, one commenced a statin and a beta-blocker).

By the long-term research visit, following FODMAP restriction, reintroduction and personalisation, total FODMAP intake was not significantly different to baseline. Intake of individual FODMAPs was also not significantly different, except for sorbitol which was lower at 12 months (**Table 2**). Energy, carbohydrate, protein and fat intakes were lower in the long term compared with baseline. However, fibre intake was not significantly different between baseline (median 17.0 g/d, IQR 5.6) and long term (16.6 g/d, 5.7, p=0.349), but iron intake was reduced between baseline (median 11.4 mg/d, IQR 5.9) and long term (median 9.4 mg/d, IQR 5.7, p=0.005) (**Table 2**). Neither body weight (baseline median 70.9 kg, IQR 27.7 vs long term 72.7 kg, 22.4; p=0.711) nor BMI (baseline median 24.0 kg/m^2, IQR 10.1 vs long term 24.3 kg/m^2, 6.7; p=0.777) changed between baseline and long-term visit.

By the long-term research visit (following FODMAP restriction, reintroduction and personalisation), 5/18 (28%) participants had such symptom improvement that they no longer met Rome III criteria for IBS. Overall, compared with baseline (5/18, 28%), a higher proportion of patients reported adequate relief of symptoms in the long term (12/18, 67%, p=0.039). There was a significant reduction in IBS-SSS total score between baseline (median 227, IQR 99) and long term (median 154, IQR 89; p<0.001), together with significant reductions in all subscores (**Table 3**). In terms of individual symptoms,

measured using the GSRS, there were significantly lower severity scores for abdominal pain, borborygmi, bloating and flatulence (**Table 3**).

Compared with baseline, in the long term there was firmer stool consistency (median BSFS score 4.9, IQR 1.0 vs 4.1, 1.5; p<0.001) and lower stool frequency (median 13.5 per week, IQR 12.0 vs 9.0 per week, 5.8; p=0.038). Compared with baseline, there was improved quality of life in the long term as measured by the SF-36 pain subscale (p=0.011), overall IBS-QOL score and for several IBS-QOL sub-scores including interference with activity and health worry (**Table 4**).

In terms of stool microbiota absolute abundance, there was no difference in total bacterial abundance between baseline (median 11.13 $\log_{10}$ rRNA genes/g, IQR 0.04) and in the long term (11.33 rRNA genes/g, 0.42; p=0.054, q=0.108). In the long term, there were higher abundances of the genera Lactobacillus and Clostridium Cluster XIVa and the species Roseburia & *Eubacterium rectale*, *Ruminococcus bromii* and *Akkermansia muciniphila* compared with baseline, although these lost significance after correction for multiple comparisons (**Table 5**). Bifidobacteria abundance did not change between baseline (median 9.29 $\log_{10}$ rRNA genes/g, IQR 1.45) and in the long term (9.20 $\log_{10}$ rRNA genes/g, 1.41; p=0.766, q=0.906).

In terms of relative abundance, compared to baseline, there was lower relative abundance of Bacteroides spp. (median 30.33%, IQR 30.71 vs 13.34%, 16.22; p=0.004, q=0.022) and *Faecalibacterium prausnitzii* (median 7.11%, IQR 4.32 vs 2.73%, 1.78; p<0.001, q=0.001) and a higher abundance of *Ruminococcus bromii* (median 0.77%, IQR 1.62 vs 1.75%, 1.96; p=0.007, q=0.024) in the long term (**Table 5, Figure 1**). Bifidobacteria relative abundance did not change between baseline (median 1.34%, IQR 5.77) and in the long term (1.24%, 3.30; p=0.766, q=0.997).

SCFA and pH data were only available for 17 participants, due to insufficient sample from one participant. There was a reduction in total SCFA, acetate, propionate and butyrate concentrations between baseline and in the long term ($p<0.05$, **Table 6, Figure 2**), but there was no change in faecal pH.

Discussion

This is the first prospective study to report long-term data for symptoms, nutrient intake and microbiota for people with IBS following a low FODMAP diet, following all three phases (restriction, reintroduction, personalisation). Overall, reintroduction and personalisation of FODMAP intake into the diet led to total FODMAP intakes that were not different from habitual baseline intakes (except for reduced sorbitol intake) and was associated with long-term improvement in overall and specific GI symptoms, quality of life, and maintenance of absolute and relative abundance of Bifidobacterium in stool. However, in the long term, there were reductions in intake of some nutrients, and alterations in relative abundance of some bacteria and absolute abundance of some SCFA.

Following completion of all three phases of the low FODMAP diet, global symptom relief was reported by over two thirds of participants at 12 months. This corresponds to previous data from observational and randomised trials of continued global relief in 53-70% of individuals at 4-15 months (14, 17, 20). The mean reduction in IBS-SSS (-73) is greater than the minimally clinically important difference frequently quoted for this instrument (-50). The symptoms that improved in this long-term study (pain, bloating, flatulence) align with the symptoms reported to be improved during RCTs of short-term FODMAP restriction (30). In parallel with these symptom improvements, quality of life scores also improved, and particularly the IBS-specific quality of life outcomes.

With regard to dietary intake, FODMAP reintroduction and personalisation led to total FODMAP intake that was similar to baseline, suggesting participants were able to reintroduce considerable quantities

of FODMAPs into the diet by 12 months, potentially modifying the sources and distribution of intake to tolerance. The intake of total FODMAPs at 12 months here was higher than that reported in a recent Canadian follow-up study of patients who had received low FODMAP advice by a dietitian, although only half of patients included were currently in the personalisation phase (31) . There are a number of potential reasons to explain symptom improvement at 12-months despite FODMAP intake returning to baseline levels. Improved visceral sensitivity may have occurred over time, either due to the natural history of IBS or in response to reduced exposure to FODMAPs (during the restriction and reintroduction phase and intermittently during the personalisation phase), some of which have been shown to induce visceral hypersensitivity in animal models (32). Additionally, although the gold standard food record was used to measure dietary intake, this assessment of dietary intake may have not accurately estimated actual FODMAP intake, and the assessment at only one time point may not completely capture fluctuations in FODMAP intake over a long period.

According to user data from over 2000 individuals using a mobile app to follow a low FODMAP diet, fructans and lactose are the most commonly reintroduced FODMAPs (33). Patients may therefore place less importance on reintroducing other FODMAPs, such as sorbitol which was still restricted at 12 months. Energy intake was reduced in the long term, by approximately -100 kcal/d, resulting from lower intakes of carbohydrate, protein and fat, however, the clinical relevance of such a small reduction is questionable, especially considering that weight and BMI remained stable, although of course at the individual patient level, dietary adequacy should be considered.

This is the first report of long-term microbiota composition in individuals with IBS who have undertaken the whole low FODMAP diet including FODMAP restriction, stepwise FODMAP reintroduction, and FODMAP personalisation to tolerance. Many RCTs have reported that FODMAP restriction results in reductions in Bifidobacteria in the short term (4, 7, 10, 34, 35), however in the current study, absolute and relative abundance of Bifidobacteria spp. in the long term was not

different to baseline. Based on the well-established bifidogenic effects of fructans and galacto-oligosaccharides (GOS) (36), it is likely that this finding is due to reintroduction of prebiotic fructans and GOS during the FODMAP reintroduction and personalisation process. This may serve to reassure clinicians given the concerns surrounding the 'anti-bifidogenic' effect of a short term low FODMAP diet reported across several RCTs (4, 7, 10, 34, 35).

With regards to other genera, there was a marked reduction in relative abundance of Bacteroides spp. in the long term. The findings from the initial RCT, which the current study followed up, also reported lower relative abundance of Bacteroides spp. after four weeks of a low FODMAP diet compared with sham diet (37). Bacteroides species contain genomes encoding an array of sugar utilisation enzymes (38) and in particular flourish in the presence of soluble fibres (38) and in humans have been shown to be reduced in response to carbohydrate restriction diets (39, 40). Therefore this finding may be due to the marginally lower carbohydrate intake or more likely the alteration in types of fibre sources. Interestingly, this reduction in Bacteroides spp. (from the Bacteroidetes phylum) occurred in conjunction with an almost 2-fold increase in relative abundance of butyrate-producing Clostridium Cluster XIVa (from the Firmicutes phylum), although this change did not remain after correction for multiple testing.

At the species level, there was a significantly lower relative abundance of *F. prausnitzii* in the long term. This is of potential concern given its butyrate-producing potential and anti-inflammatory properties, and because, according to a recent systematic review, abundance is already compromised in IBS compared with healthy controls (41). One small 4-week uncontrolled trial of a gluten-free diet (which excludes wheat, the major source of fructans) in healthy individuals similarly reported >50% reduction in relative abundance of *F. prausnitzii* (42), whilst other studies report supplementation with prebiotics (e.g. inulin type fructans) enhanced abundance of *F. prausnitzii* (43). Although intakes of prebiotic fructans and GOS were not statistically significantly different between baseline and long-

term follow-up, it may be that subtle changes in the variety and source of these prebiotics may have contributed to this finding. In addition, although generally highly specific, during qPCR the primer for *F. prausnitzii* is known to also amplify some species from the Subdoligranulum genus, and therefore the effect on *F. prausnitzii* specifically is unclear and warrants further investigation.

Finally, the reductions in relative abundance of Bacteroides spp. and *F. prausnitzii* must be viewed in conjunction with data on absolute abundance, in which total abundance did not change, suggesting that there was not a specific decrease in these bacteria, but perhaps an increase in other taxa.

This is the first study to report the effects of a low FODMAP diet on stool microbial metabolite concentrations in the long term, showing reductions in total SCFA concentration and some individual SCFA concentrations including butyrate. These data are of potential concern given the role of SCFA in intestinal permeability, immunomodulation and secretory functions in the gut (44), however, the biological and clinical significance of these findings are unclear. The reduction may be due to a reduction in total carbohydrate intake (45) and the likely alteration in carbohydrate sources broadly across the diet, although it is important to note that fibre and total FODMAP intakes were maintained. Previous human studies have shown that reductions in *F. prausnitzii,* induced by reductions in carbohydrate supply, correlate with reductions in butyrate (45,46), both of which occurred here. However, the majority of stool SCFA produced by the microbiota in the gut are absorbed in the colon, and stool SCFA is affected by variable stool volume (46) and is negatively correlated with colonic transit time, suggesting slower colonic transit could lead to greater SCFA absorption (47). Lower stool SCFA concentrations might be explained by personalised low FODMAP diet increasing colonic transit time, although one trial reported no impact of a low FODMAP diet on whole gut transit time (10). Stool pH was maintained despite total SCFA concentrations being reduced. This likely reflects that stool pH is determined not just by SCFA but by the pool of colonic metabolites (e.g. lactate, ammonia, volatile

organic compounds), and that pH is a logarithmic scale and the reduction in SCFA may be reflected by the (statistically non-significant) 0.1 overall increase in pH.

The strengths of this study include the use of paired analysis over two time points, measured at baseline prior to low FODMAP dietary and again 12-months after starting the low FODMAP diet following FODMAP restriction, reintroduction and personalisation. All indepth dietary counselling was provided by a specialist dietitian, in line with evidence from research studies (31) and as widely recommended in the literature (48, 49). The limitations of this study are the relatively small sample size (n=18), compared to that of the initial RCT (n=104 patients), which was due to inability to contact participants despite multiple attempts or their unwillingness to participate in a long-term research visit. This may result in a relatively selected group of patients, and in particular that those who did not participate may have had worse symptom outcomes than those who did (non-response bias), whilst the small sample size in the study may result in type II error that would suggest null findings compared with baseline. Microbiota were analysed using qPCR which is highly accurate at measuring bacterial taxa abundance but does not provide comprehensive global microbiota composition. The microbiota and SCFA at baseline and in the long term were inevitably measured in different analytical runs which may result in analytical variations. However, between-run variations would likely be minor and were minimised by analysis in triplicate (microbiota) or duplicate (SCFA) and comparison with contemporaneous internal standards and control samples. Data for symptoms, microbiota and SCFA were not collected at the end of the restriction phase for participants originally in the sham diet group, and so in the current study tracking these across three time points (baseline, end of restriction, long term personalisation) was not possible. Finally, some of the patients in this study had been provided with four weeks of probiotic supplements to take at the start of the initial RCT. However, those continuing to supplement with any probiotic were excluded from the long-term analysis, and there is limited evidence that probiotics have a tangible impact on global bacterial abundance or composition

in the short term (50) and no evidence of probiotic persistence in the gut in the long term after cessation of supplementation.

In conclusion, this is the first ever study of clinical symptoms, dietary intake and microbiota during long-term follow-up of patients following a low FODMAP diet that includes a structured restriction, reintroduction and personalisation process, demonstrating two thirds of patients have adequate symptom relief even after 12 months. Fructan and GOS intakes and abundance of Bifidobacteria spp. were maintained over the long term, although stool SCFA were decreased. This study justifies the need for larger long-term studies to investigate these endpoints.

References

1. Sperber AD, Bangdiwala SI, Drossman DA, et al. Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome Foundation Global Study. Gastroenterology. 2021;160(1):99-114.e3.
2. Shepherd SJ, Parker FC, Muir JG, et al. Dietary triggers of abdominal symptoms in patients with irritable bowel syndrome: Randomized placebo-controlled evidence. Clin Gastroenterol Hepatol. 2008;6(7):765-71.
3. Chey WD, Keefer L, Whelan K, et al. Behavioral and diet therapies in integrated care for patients with irritable bowel syndrome. Gastroenterology. 2021;160(1):47-62.
4. Staudacher HM, Lomer MC, Anderson JL, et al. Fermentable carbohydrate restriction reduces luminal bifidobacteria and gastrointestinal symptoms in patients with irritable bowel syndrome. J Nutr. 2012;142(8):1510-8.
5. Halmos EP, Power VA, Shepherd SJ, et al. A diet low in FODMAPs reduces symptoms of irritable bowel syndrome. Gastroenterology. 2014;146(1):67-75.e5.
6. Pedersen N, Andersen NN, Vegh Z, et al. Ehealth: low FODMAP diet vs Lactobacillus rhamnosus GG in irritable bowel syndrome. World J Gastroenterol. 2014;20(43):16215-26.
7. Staudacher HM, Lomer MCE, Farquharson FM, 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.
8. Eswaran SL, Chey WD, Han-Markey T, et al. A randomized controlled trial comparing the low FODMAP diet vs. modified NICE guidelines in US adults with IBS-D. Am J Gastroenterol. 2016;111(12):1824-32.
9. Eswaran S, Chey WD, Jackson K, et al. A diet low in fermentable oligo-, di-, and monosaccharides and polyols improves quality of life and reduces activity impairment in patients with irritable bowel syndrome and diarrhea. Clin Gastroenterol Hepatol. 2017;15(12):1890-9.e3.
10. Halmos EP, Christophersen CT, Bird AR, et al. Diets that differ in their FODMAP content alter the colonic luminal microenvironment. Gut. 2015;64(1):93-100.
11. Eswaran S, Dolan RD, Ball SC, et al. The impact of a 4-week low-FODMAP and mNICE diet on nutrient intake in a sample of US adults with irritable bowel syndrome with diarrhea. J Acad Nutr Diet. 2020;120(4):641-9.
12. Staudacher H, Ralph F, Irving P, et al. Nutrient intake, diet quality and diet diversity in irritable bowel syndrome and the impact of the low FODMAP diet. J Academy Nutrition Dietetics. 2020;120(4):535-47.
13. Whelan K, Martin LD, Staudacher HM, et al. The low FODMAP diet in the management of irritable bowel syndrome: an evidence-based review of FODMAP restriction, reintroduction and personalisation in clinical practice. J Hum Nutr Diet. 2018;31(2):239-255
14. Maagaard L, Ankersen DV, Vegh Z, et al. Follow-up of patients with functional bowel symptoms treated with a low FODMAP diet. World J Gastroenterol. 2016;22(15):4009-19.
15. Weynants A, Goossens L, Genetello M, et al. The long-term effect and adherence of a low fermentable oligosaccharides disaccharides monosaccharides and polyols (FODMAP) diet in patients with irritable bowel syndrome. J Hum Nutr Diet. 2020;33(2):159-69.
16. Nawawi KNM, Belov M, Goulding C. Low FODMAP diet significantly improves IBS symptoms: an Irish retrospective cohort study. Eur J Nutr. 2019;59(5):2237-2248.
17. O'Keeffe M, Jansen C, Martin L, et al. Long-term impact of the low-FODMAP diet on gastrointestinal symptoms, dietary intake, patient acceptability, and healthcare utilization in irritable bowel syndrome. Neurogastroenterol Motil. 2018; 30(1) .
18. Peters SL, Yao CK, Philpott H, et al. Randomised clinical trial: The efficacy of gut-directed hypnotherapy is similar to that of the low FODMAP diet for the treatment of irritable bowel syndrome. Aliment Pharmacol Ther. 2016;44(5):447-59.

19. Harvie RM, Chisholm AW, Bisanz JE, et al. Long-term irritable bowel syndrome symptom control with reintroduction of selected FODMAPs. World J Gastroenterol. 2017;23(25):4632-43.
20. Goyal O, Batta S, Nohria S, et al. Low fermentable oligosaccharide, disaccharide, monosaccharide, and polyol diet in patients with diarrhea-predominant irritable bowel syndrome: A prospective, randomized trial. J Gastroenterol Hepatol. 2021; doi: 10.1111/jgh.15410.
21. Silk DB, Davis A, Vulevic J, et al. Clinical trial: The effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. Aliment Pharmacol Ther. 2009;29(5):508-18.
22. Wiklund IK, Fullerton S, Hawkey CJ, et al. An irritable bowel syndrome-specific symptom questionnaire: development and validation. Scandinavian Journal of Gastroenterology. 2003;38(9):947-54.
23. O'Donnell LJ, Virjee J, Heaton KW. Detection of pseudodiarrhoea by simple clinical assessment of intestinal transit rate. BMJ. 1990;300(6722):439-40.
24. Food Standards Agency. McCance and Widdowson's The Composition of Foods (Sixth summary edition). Cambridge: The Royal Society of Chemistry; 2002.
25. Monash University. The Monash FODMAP Calculator https://www.monashfodmapcalculator.com.au.
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. Brazier JE, Harper R, Jones NM, et al. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. BMJ. 1992;305(6846):160-4.
28. Bijkerk CJ, de Wit NJ, Muris JW, et al. Outcome measures in irritable bowel syndrome: comparison of psychometric and methodological characteristics. Am J Gastroenterol. 2003;98(1):122-7.
29. Whelan K, Judd PA, Preedy VR, et al. Fructooligosaccharides and fiber partially prevent the alterations in fecal microbiota and short-chain fatty acid concentrations caused by standard enteral formula in healthy humans. J Nutr. 2005;135(8):1896-902.
30. Staudacher HM, Whelan K. The low FODMAP diet: recent advances in understanding its mechanisms and efficacy in IBS. Gut. 2017;66(8):1517-27.
31. Tuck CJ, Reed DE, Muir JG, et al. Implementation of the low FODMAP diet in functional gastrointestinal symptoms: A real-world experience. Neurogastroenterol Motil. 2020;32(1):e13730.
32. Zhou SY, Gillilland M, Wu X, et al. FODMAP diet modulates visceral nociception by lipopolysaccharide-mediated intestinal inflammation and barrier dysfunction. J Clin Invest. 2018;128(1):267-80.
33. Dimidi E, Whelan K, Lomer M. FODMAP-specific mobile applciation: Impact on gut symptoms in 11689 people, and dietary triggers ni 2053 people. Proc Nutr Soc. 2020;79(OCE1):E8.
34. Bennet SMP, Bohn L, Storsrud S, et al. Multivariate modelling of faecal bacterial profiles of patients with IBS predicts responsiveness to a diet low in FODMAPs. Gut. 2018;67(5):872-81.
35. McIntosh K, Reed DE, Schneider T, et al. FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. Gut. 2016; 66(7):1241-1251.
36. Roberfroid M, Gibson GR, Hoyles L, et al. Prebiotic effects: metabolic and health benefits. BrJ Nutr. 2010;104 Suppl 2:S1-63.
37. Staudacher HM, Scholz M, Lomer MC, et al. Gut microbiota associations with diet in irritable bowel syndrome and the effect of low FODMAP diet and probiotics. Clin Nutr. 2021; 40(4):1861-1870.
38. Wexler HM. Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev. 2007;20(4):593-621.
39. Russell WR, Gratz SW, Duncan SH, et al. High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. Am J Clin Nutr. 2011;93(5):1062-72.

40. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559-63.
41. Pittayanon R, Lau JT, Yuan Y, et al. Gut microbiota in patients with irritable bowel syndrome-a systematic review. Gastroenterology. 2019;157(1):97-108.
42. De Palma G, Nadal I, Collado MC, et al. Effects of a gluten-free diet on gut microbiota and immune function in healthy adult human subjects. Br J Nutr. 2009;102(8):1154-60.
43. Verhoog S, Taneri PE, Roa Díaz ZM, et al. Dietary factors and modulation of bacteria strains of *Akkermansia muciniphila* and Faecalibacterium prausnitzii: A Systematic Review. Nutrients. 2019;11(7).
44. Shin A, Preidis GA, Shulman R, et al. The gut microbiome in adult and pediatric functional gastrointestinal disorders. Clin Gastroenterol Hepatol. 2019;17(2):256-74.
45. Duncan SH, Belenguer A, Holtrop G, et al. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol. 2007;73(4):1073-8.
46. Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol. 1991;70(6):443-59.
47. Ringel-Kulka T, Choi CH, Temas D, et al. altered colonic bacterial fermentation as a potential pathophysiological factor in irritable bowel syndrome. Am J Gastroenterol. 2015;110(9):1339-46.
48. Wilson B, Cox SR, Whelan K. Challenges of the low FODMAP diet for managing irritable bowel syndrome and approaches to their minimisation and mitigation. Proc Nutr Soc. 2021;80(1):19-28.
49. O'Keeffe M, Lomer MC. Who should deliver the low FODMAP diet and what educational methods are optimal: A review. J Gastroenterol Hepatol. 2017;32 Suppl 1:23-6.
50. Kristensen NB, Bryrup T, Allin KH, et al. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: A systematic review of randomized controlled trials. Genome Med. 2016;8(1):52.

Table 1: Baseline demographic and clinical data in 18 people with IBS followed up after 12-months of low FODMAP diet (restriction, reintroduction, personalisation)

Variable	Baseline
Age, yrs, median (IQR)	33 (20)
Female n (%)	11 (61)
IBS subtype, n (%)	
IBS-D	14 (78)
IBS-M	2 (11)
IBS-U	2 (11)
Ethnicity, white n (%)	12 (66)
Weight, kg, median (IQR)	72.7 (22.4)
BMI ,kg/m^2, median (IQR)	24.3 (6.7)

Table 2: FODMAP, energy and nutrient intake at baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 18 people with IBS

Dietary variable, median (IQR)	Baseline (n=18)	Long-term personalised low FODMAP diet (n=18)	p*
Total FODMAPs, g/d	16.9 (14.4)	18.4 (9.7)	0.679
Fructans, g/d	4.8 (2.7)	4.0 (4.5)	0.557
GOS, g/d	0.6 (0.5)	0.6 (0.6)	0.112
Lactose, g/d	9.0 (11.8)	9.9 (8.4)	0.983
Excess fructose, g/d	1.3 (1.9)	1.0 (1.3)	0.500
Sorbitol, g/d	0.7 (1.1)	0.3 (0.4)	**0.028**
Mannitol, g/d	0.3 (0.4)	0.3 (0.3)	0.327
Energy, kcal/d	2052 (812)	1948 (603)	**0.043**
Carbohydrate, g/d	218 (85)	196 (79)	**0.039**
Total sugar, g/d	79 (27)	78 (62)	0.879
Starch, g/d	128 (71)	116 (48)	0.085
Total fibre, g/d	17.0 (5.6)	16.6 (5.7)	0.349
Protein, g/d	78 (47)	74 (27)	**0.011**
Fat, g/d	86 (31)	77 (41)	**0.048**
Calcium, mg/d	806 (308)	819 (424)	0.267
Iron, mg/d	11.4 (5.9)	9.4 (5.7)	**0.005**

*Wilcoxon signed-rank test

Values in bold are statistically significant ($p<0.05$)

Table 3: Gastrointestinal symptom scores at baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 18 people with IBS

Median (IQR)	**Baseline (n=18)**	**Long-term personalised low FODMAP diet (n=18)**	**p***
Irritable bowel severity scoring system (IBS-SSS)[#]			
IBS-SSS total, points	227 (99)	154 (89)	**<0.001**
Pain severity	45 (28)	25 (31)	**0.006**
Days of pain (days)	55 (70)	20 (20)	**0.005**
Distension severity	39 (37)	27 (36)	**0.030**
Satisfaction with bowels	61 (27)	40 (24)	**0.017**
Affecting life	48 (29)	36 (36)	**0.011**
Gastrointestinal Symptom Rating Scale Severity[##]			
Overall symptoms	1.3 (0.4)	0.8 (1.0)	**0.005**
Abdominal pain	1.2 (0.8)	0.7 (0.6)	**<0.001**
Heartburn	0.1 (0.8)	0.0 (0.2)	0.495
Acid reflux	0.1 (0.5)	0.0 (0.1)	0.414
Nausea	0.1 (0.6)	0.1 (0.2)	0.077
Borborygmi	1.1 (1.1)	0.6 (1.2)	**0.006**
Bloating	1.2 (1.3)	0.8 (1.2)	**0.006**
Belching	0.4 (1.1)	0.1 (0.7)	0.844
Flatulence	1.3 (1.2)	0.9 (1.4)	**0.004**
Constipation	0.0 (0.0)	0.0 (0.0)	0.269
Diarrhoea	0.1 (0.8)	0 (0.2)	0.369
Loose stool	0.9 (0.8)	0.4 (1.1)	0.053
Hard stool	0.0 (0.1)	0.0 (0.1)	0.682
Urgency	0.9 (0.9)	0.5 (1.1)	0.157
Incomplete evacuation	0.5 (0.8)	0.1 (0.6)	**0.014**
Tiredness	1.0 (1.2)	1.1 (1.4)	0.267

*Wilcoxon signed-rank test

[#] IBS-SSS total score based upon seven items where worst severity is 500 points

[##]GSRS where each symptom was rated daily for severity over 7 days on a scale of 0 (absent), 1 (mild), 2 (moderate), 3 (severe)

Values in bold are statistically significant ($p<0.05$)

Table 4: Quality of life scores at baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 18 people with IBS

Quality of life, median (IQR)	Baseline (n=18)	Long-term personalised low FODMAP diet (n=18)	p*
SF-36			
Physical functioning	95 (5)	98 (26)	0.876
Role limitations due to physical health	75 (75)	100 (56)	0.214
Role limitations due to emotional problems	83 (67)	67 (75)	0.501
Energy/fatigue	50 (30)	60 (48)	0.314
Emotional wellbeing	74 (34)	74(41)	0.599
Social functioning	75 (41)	94 (38)	0.887
Pain	68 (40)	80 (33)	**0.011**
General Health	58 (23)	60 (21)	0.493
IBS-QOL			
Overall	70 (24)	85 (20)	**<0.001**
Dysphoria	75 (24)	91 (22)	**0.001**
Interference with activity	64 (24)	86 (15)	**0.001**
Body Image	69 (39)	88 (31)	**0.004**
Healthy worry	75 (33)	83 (25)	**0.018**
Food avoidance	67 (25)	50 (38)	0.377
Social reaction	66 (33)	88 (33)	**0.001**
Sexual	94 (28)	100 (25)	0.384
Relationships	83 (27)	92 (17)	**0.006**

*Wilcoxon signed-rank test
Values in bold are statistically significant ($p<0.05$)

Table 5: Absolute and relative abundance of microbiota at baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 18 people with IBS

Median (IQR)	Absolute abundance (log_{10} rRNA genes/g) (n=18)				Relative abundance (% of total) (n=18)			
	Baseline	Long-term personalised low FODMAP diet	p*	q**	Baseline	Long-term personalised low FODMAP diet	p*	q**
Total bacteria	11.13 (0.04)	11.33 (0.42)	0.054	0.108	-	-	-	-
Bacteroides spp.	10.41 (0.37)	10.48 (0.45)	0.865	0.906	30.33 (30.71)	13.34 (16.22)	**0.004**	**0.022**
Prevotella spp.	6.34 (9.81)	0.00 (8.96)	0.154	0.263	0.00 (4.03)	0.00 (0.72)	1.000	1.000
Bifidobacteria spp.	9.29 (1.45)	9.20 (1.41)	0.766	0.906	1.34 (5.77)	1.24 (3.30)	0.766	0.997
Bifidobacterium longum	8.60 (1.44)	8.70 (1.34)	0.580	0.773	0.54 (2.76)	0.30 (1.64)	0.442	0.695
Bifidobacterium adolescentis	3.26 (8.62)	0.00 (8.78)	0.906	0.906	0.00 (0.37)	0.00 (0.42)	0.906	0.997
Clostridium Cluster XIVa	10.04 (0.62)	10.70 (0.93)	**0.043**	0.104	8.79 (10.32)	19.67 (25.24)	**0.027**	0.074
Roseburia spp. & *E. rectale*	9.99 (0.55)	10.19 (0.33)	**0.043**	0.104	8.18 (11.00)	7.68 (8.01)	0.865	0.997
Faecalibacterium prausnitzii	10.10 (0.51)	9.85 (0.64)	0.433	0.650	7.11 (4.32)	2.73 (1.78)	**<0.001**	**0.001**
Ruminococcus bromii	8.79 (1.05)	9.55 (0.78)	**0.009**	0.087	0.77 (1.62)	1.75 (1.96)	**0.007**	**0.024**
Akkermansia muciniphila	0.00 (7.69)	8.05 (6.99)	**0.041**	0.104	0.00 (0.05)	0.04 (0.39)	0.187	0.343
Lactobacillus spp.	0.00 (4.88)	7.48 (7.92)	**0.014**	0.087	0.00 (0.00)	0.02 (0.04)	0.093	0.206

*Wilcoxon signed-rank test

**Adjustment for false detection rate

Values in bold are statistically significant ($p<0.05$ or $q<0.05$)

Table 6: Faecal short chain fatty acid concentration (μmol/g wet weight) and pH at baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 17 people with IBS

Median (IQR)	Baseline (n=17)	Long-term personalised low FODMAP diet (n=17)	P*
Total SCFA, μmol/g	140 (63)	93 (50)	**0.004**
Acetate	85 (40)	50 (28)	**0.002**
Propionate	24 (11)	15 (10)	**0.001**
Butyrate	22 (10)	15 (11)	**0.035**
Valerate	3 (3)	2 (2)	0.062
Isobutyrate	2 (1)	1 (1)	0.093
Isovalerate	3 (2)	2 (2)	0.435
pH	6.7 (0.5)	6.8 (0.8)	1.000

*Wilcoxon signed-rank test
Values in bold are statistically significant ($p<0.05$)

Figure 1 Relative abundances at baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 18 people with IBS

Bacteroides spp. (p=0.004, q=0.022), Bifidobacteria spp. (p=0.766, q=0.997), *Faecalibacterium prausnitzii* (p<0.001, q=0.001) and *Ruminococcus bromii* (p=0.007, q=0.024)

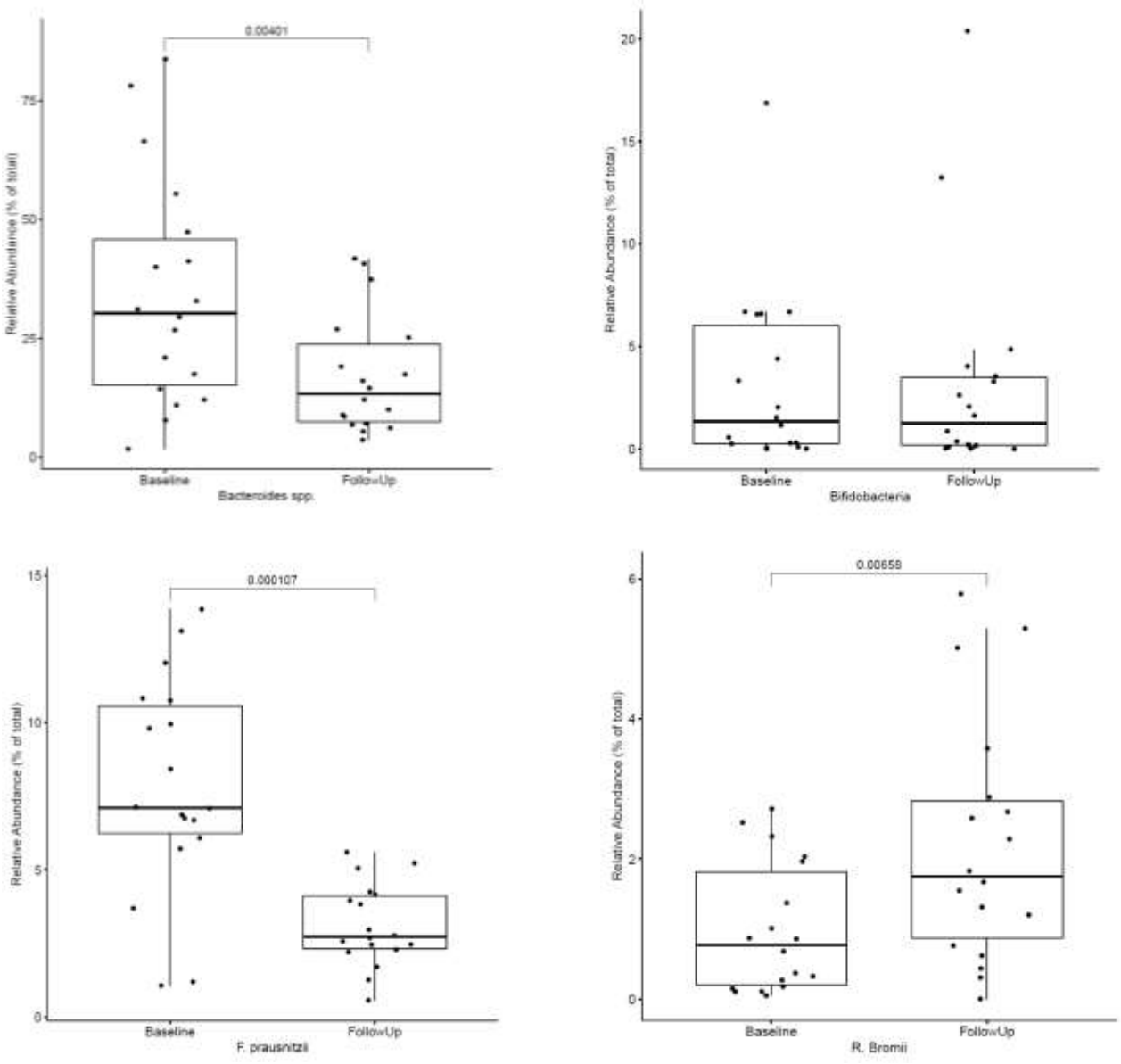
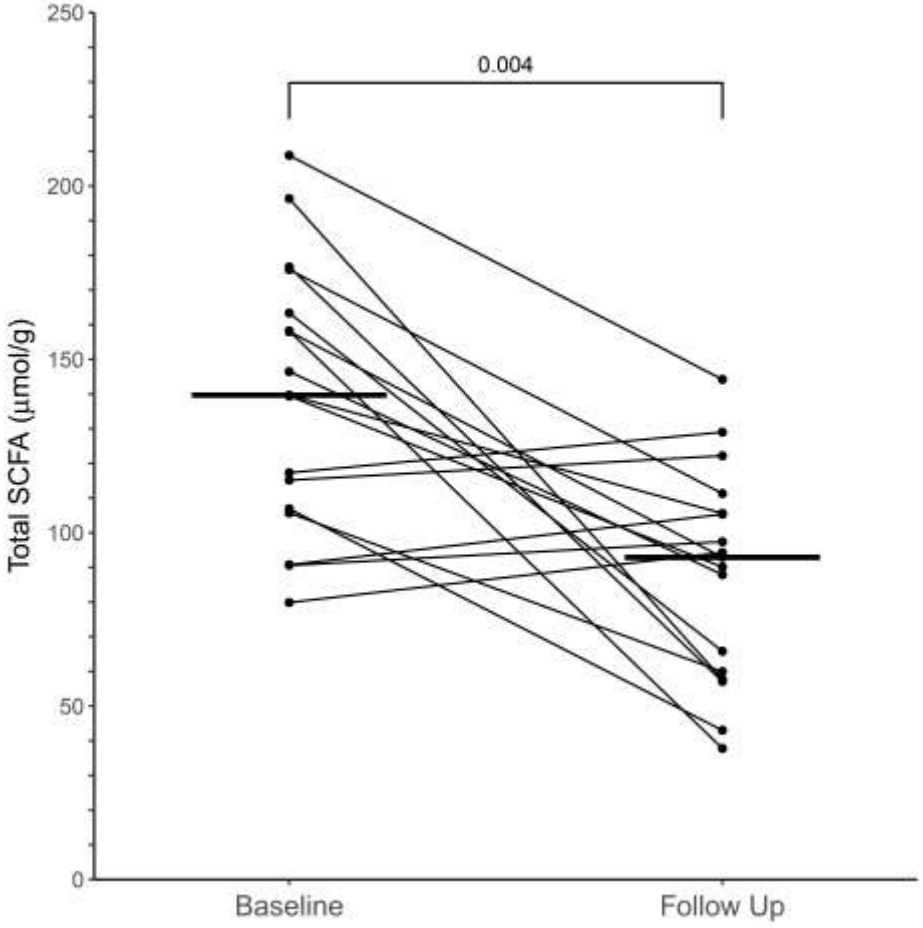


Figure 2 Total short-chain fatty acid concentrations between baseline and after 12-months of low FODMAP diet (restriction, reintroduction, personalisation) in 17 people with IBS



Online supplementary material

Microbiota analysis, quantitative PCR

DNA was extracted from 3-5g stool aliquots using FastDNA™ SPIN kit for soil (MP Biomedicals Europe, Illkirch-Graffenstaden, France) for quantitative polymerase chain reaction (qPCR) according to manufacturer instructions. After extraction, the sample was centrifuged at 14,000 x g for 1 minute to transfer eluted DNA into the clean catch tube. The sample was transferred to a sterile microcentrifuge tube and centrifuged at 14,000 x g for 1 minute. DNA concentration was measured using a NanoDrop ND1000 instrument (Thermo Scientific, Waltham, MA, USA) and then stored at 4°C for qPCR. Samples were diluted to 1.25 ng/µl by an automated pipetting system (Biomek FXP Laboratory Automation Workstation, Beckman Coulter, Brea, CA, USA) in 5 µg/ml herring perm DNA (Promega). Standard template DNA was prepared from a 16S rRNA gene of Bifidobacterium adolescentis DMS 20083. Standard curves were prepared with five standard concentrations of 10^7 to 10^3 gene copies/2µl or 10^6 to 10^2 gene copies/2µl in herring sperm DNA, depending on the expected concentration of target in the samples. Amplification was performed using primers for the quantification of 11 bacterial groups.

Samples and standards were examined in triplicate per PCR run (6µl per well) and mixed with SYBR Green Supermix (Bio-Rad) (5µl per well) and forward and reverse primers at 10µM (0.5µl per well each; total volume 10µl per well) in clear 384 MicroAmp Optical 384-well reaction plates (Applied Biosystems) sealed with optical adhesive tape. If the plate required storage prior to thermal cycling, it was covered with opaque foil and refrigerated at 4°C for no more than 2 hours. Amplification was performed with a 7900HT fast qPCR system (Applied Biosystems) using the following protocol: one cycle at 95 °C for 3 minutes, 40 cycles of 95 °C and 60 °C for 30 seconds each, one cycle at 95 °C for 10 seconds and a final cycle of 65 °C increasing to 95 °C for 5 seconds at each 0.5 °C increment to obtain melt curve data. Data were analysed using SDS 2.4.1 (Applied Biosystems).

Samples at 12 months were analysed in a different analytical run than the baseline samples. To account for differences in efficiencies between batches, the detection threshold for each bacterial group was calculated as a 10-fold higher transcript number than the highest mean no template control transcript number and applied across both baseline and 12-month data.

Short-chain fatty acid analysis, gas liquid chromatography and stool pH

Faecal samples for analysis of SCFAs were immediately frozen at 80°C. SCFAs were extracted from defrosted faeces using an extraction buffer (1% H3PO4; 0.1% HgCl2) containing 2,2-dimethylbutyric acid as an internal standard. Extracted SCFAs were injected splitless into a Hewlett Packard 6890 series GLC system (Agilent Technologies, Santa Clara, CA) equipped with a BP21 25-m capillary column with internal diameter of 0.22 mm and film thickness of 0.25 mm. Initial oven temperature was 80°C, which increased by 10°C/min up to 145°C, and then 100°C/min up to 200°C to ensure complete elution. Extracted SCFA underwent chromatography alongside contemporaneous control pure SCFA of known concentration to enable calculation of standard elution curves for calculating unknown concentrations.

Stool pH was measured on fresh stool, which was diluted 1:4 (vol: vol) in pH buffer ($1x10^{-5}$mol/L Na_2HPO_4, KH_2PO_4, 0.1 g $HgCl_2$), homogenised, and incubated at room temperature for 1 h. Stool pH was measured using a pH meter and a pH electrode specifically designed for slurries (VWR, Pennsylvania, US).