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Diet Low in FODMAPs Reduces Symptoms in Patients with Irritable Bowel Syndrome and Probiotic Restores Bifidobacterium Species: a Randomized Controlled Trial

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Short title: low FODMAP diet and probiotics in IBS

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Abbreviations:

ANCOVA	analysis of covariance
CI	confidence interval
FODMAP	fermentable oligosaccharides, monosaccharides, disaccharides and polyols
GI	gastrointestinal
GSRS	Gastrointestinal Symptom Rating Scale
HRQOL	health related quality of life
IBS	irritable bowel syndrome
IBS-D	diarrhoea-predominant irritable bowel syndrome
IBS-M	mixed subtype irritable bowel syndrome
IBS-SSS	IBS Symptom Scoring System
IBS-U	unsubtyped irritable bowel syndrome
MCID	minimal clinically important difference
OTU	operational taxonomic units
qPCR	quantitative polymerase chain reactions
RCT	randomised controlled trial
SD	standard deviation

Abstract

Background & Aims: Dietary restriction of fermentable carbohydrates (a low FODMAP diet) has been reported to reduce symptoms in some patients with irritable bowel syndrome (IBS). We performed a randomized, placebo-controlled study to determine its effects on symptoms and the fecal microbiota in patients with IBS.

Methods: We performed a 2x2 factorial trial of 104 patients with IBS (18–65 years old), based on the Rome III criteria, at 2 hospitals in the United Kingdom. Patients were randomly assigned (blinded) to groups given counselling to follow a sham diet or diet low in FODMAPs for 4 weeks, along with a placebo or probiotic supplement (VSL#3), resulting in 4 groups (27 receiving sham diet/placebo, 26 receiving sham diet/probiotic, 24 receiving low FODMAP diet /placebo, and 27 receiving low FODMAP diet /probiotic). The sham diet restricted a similar number of staple and non-staple foods as the low FODMAP diet; the diets had similar degrees of difficulty to follow. Dietary counselling was given to patients in all groups and data on foods eaten and compliance were collected. The incidence and severity of 15 gastrointestinal symptoms and overall symptoms were measured daily for 7 days before the study period; along with stool frequency and consistency. At baseline, global and individual symptoms were measured, along with generic and disease-specific health-related quality of life, using standard scoring systems. All data were collected again at 4 weeks, and patients answered questions about adequate symptom relief. Fecal samples were collected at baseline and after 4 weeks and analyzed by quantitative PCR and 16S rRNA sequencing. The co-primary endpoints were adequate relief of symptoms and stool Bifidobacterium species abundance at 4 weeks.

Results: There was no significant interaction between the interventions in adequate relief of symptoms (P=.52) or Bifidobacterium species (P=.68). In the intention-to-treat analysis, a higher proportion of patients in the low FODMAP diet had adequate symptom relief (57%) vs than in the sham diet group (38%), although the difference was not statistically significant (P=.051). In the per-protocol analysis, a significantly higher proportion of patients on the low FODMAP diet had adequate symptom relief (61%) than in the sham diet group (39%)

(P=.043). Total mean IBS- Severity Scoring System score was significantly lower for patients on the low FODMAP diet (173±95) than the sham diet (224 ± 89)(P=.001), but not different between those given probiotic (207 ± 98) or placebo (192 ± 93)(P=.721) Abundance of Bifidobacterium species was lower in fecal samples from patients on the low FODMAP diet (8.8 rRNA genes/g) than patients on the sham diet (9.2 rRNA genes/g) (P=.008), but higher in patients given probiotic (9.1 rRNA genes/g) than patients given placebo (8.8 rRNA genes/g) (P=.019). There was no effect of the low FODMAP diet on microbiota diversity in fecal samples.

Conclusions: In a placebo-controlled study of patients with IBS, a low FODMAP diet associates with adequate symptom relief and significantly reduced symptom scores compared with placebo. It is not clear whether changes resulted from collective FODMAP restriction or removal of a single component, such as lactose. Co-administration of the probiotic VSL#3 increased numbers of Bifidobacterium species, compared with placebo, and might be given to restore these bacteria to patients on a low FODMAP diet. Trial registration no: ISRCTN02275221.

KEY WORDS: food sensitivity; fructans; galacto-oligosaccharides; lactose

Introduction

Irritable bowel syndrome (IBS) is a functional bowel disorder with a global prevalence of 11%[1] and contributes up to 60% of gastroenterology outpatient appointments. [2] Diagnosis requires the presence of abdominal pain and disordered bowel habit, and although IBS has no impact on mortality it profoundly affects health-related quality of life (HRQOL)[3] likely due to its chronic nature and the co-existence of gastrointestinal (GI) and extra-intestinal symptoms. It also results in significant economic healthcare burden. Factors that are recognised as important in the aetiology of IBS include visceral hypersensitivity, immune dysregulation, the GI microbiota, altered regulation of the gut-brain axis, and psychosocial factors.[4] An incomplete understanding of the pathophysiology of IBS and its phenotypic heterogeneity has led to symptom-directed treatment approaches including anti-spasmodics and anti-diarrheals. However, less than 40% of patients are satisfied with their current treatments.[5]

Most patients with IBS believe their symptoms are diet-related. Dietary restriction of fermentable carbohydrates (low FODMAP diet) is now widely used in the management of IBS. These carbohydrates increase small intestinal water and colonic gas.[6] Their dietary restriction has been investigated in a number of trials with up to 70% of patients reporting symptomatic benefit.[6] Only one placebo-controlled trial of the low FODMAP diet has been undertaken in IBS, however this was a feeding study in which all dietary intake was provided to participants.[7] Trials of dietary advice replicate how dietary manipulation is undertaken in practice and therefore better reflect effectiveness in the clinical setting. Placebo-controlled trials are especially important in IBS where the placebo response is known to be considerable.[8] A placebo-controlled dietary advice trial of the low FODMAP diet has yet to be performed, in part due to the difficulty of designing a blinded placebo diet that modifies dietary intake without altering intakes of nutrients or fermentable carbohydrates.

The low FODMAP diet has been shown to induce alterations in some genera of the GI microbiota, including bifidobacteria[9,10] and *Faecalibacterium prausnitzii*.[9] Reductions in bifidobacteria are particularly relevant to IBS symptomatology given their inverse association with abdominal pain.[11] There is also evidence of reduced microbiome diversity

in IBS[12], as well as in other disorders (e.g. inflammatory bowel disease, obesity and diabetes).[13,14] There is reason to suspect that the low FODMAP diet may impact global microbial community structure, however the effect has never been compared with placebo.[15] It also remains to be demonstrated whether any potential clinical benefit of the low FODMAP diet in IBS is offset by impacts on specific microbiota (e.g. bifidobacteria) or on community structure, known to be a key factor influencing gut health and systemic physiology.

Therefore, approaches that prevent the microbiota-modifying effect of the low FODMAP diet are required. Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host, and some probiotic species are effective in the management of IBS.[16] However, no trials have investigated whether probiotics can modify the effect of the low FODMAP diet on the microbiota.

We designed a placebo-controlled trial to address these important questions. Firstly, we aimed to investigate the effect of the low FODMAP diet compared with a placebo (sham) diet, and secondly, to investigate whether the low FODMAP diet-induced alterations in the microbiota could be prevented through concomitant probiotic therapy compared with placebo.

Methods

Study design and participants

We performed a 2x2 factorial design, multicentre, randomised, placebo-controlled trial from clinics at two hospitals in London, UK. Patients aged 18-65 years of age with diarrhoea-predominant (IBS-D), mixed subtype (IBS-M) or unsubtyped irritable bowel syndrome (IBS-U), according to Rome III were recruited. Major medical conditions such as inflammatory bowel disease and diabetes were excluded, and coeliac disease was excluded by evaluation of endomysial IgA, tissue transglutaminase IgA serology or endoscopic biopsy. Patients with IBS-C were excluded due to the potential for exacerbation of symptoms on a low FODMAP diet based on the aforementioned impact of FODMAPs on small intestinal water. Only patients naïve to the low FODMAP diet were recruited (to improve blinding), which was

assessed by interview regarding any previous or current dietary restrictions and a diet history without reference to FODMAPs. Exclusion criteria included abdominal pain or discomfort for less than 2 days during the screening week and patients already following a restrictive exclusion diet for IBS were excluded. Lactose intolerance was not tested for and was not an exclusion criterion *per se*, although those with a pre-determined diagnosis who had symptom resolution during a low lactose diet would not be eligible due to symptom severity criteria. Patients with IBS already following a low lactose diet were eligible as long as they agreed to maintain consistent lactose intake during the study period, irrespective of diet randomisation. Other exclusion criteria included bowel preparation for investigative procedures, antibiotic therapy, prebiotics or probiotics, and change to IBS medication during the previous four weeks. Where this occurred during the trial it was considered a protocol violation and the patient was withdrawn and excluded from the per protocol analysis. Research ethics committee approval was received from the London Fulham Research Ethics Committee (Reference 12/LO/1402) and patients gave informed consent prior to participation.

Randomisation and masking

A computerised random allocation sequence was prepared by a researcher not involved in screening or recruitment. Patients were randomised in a 1:1 ratio to both diet (sham vs low FODMAP diet) and supplement (placebo vs probiotic) resulting in allocation to one of four treatment groups (sham diet/placebo, sham diet/probiotic, low FODMAP diet /placebo, low FODMAP diet /probiotic). Randomisation was stratified by gender and diagnosis of IBS-D. The diet allocation was concealed in an opaque envelope that was only opened after all baseline data had been collected.

Patients were masked to both diet and supplement allocations. The two diets were described to patients as both altering carbohydrate intake, but one designed as a placebo diet and the other being the true diet under investigation. The researcher who conducted the trial visits, a registered dietitian, provided the dietary advice and was not able to be masked to diet allocation but was masked to supplement allocation. The placebo and probiotic supplements were identical in appearance, taste and presentation. Labelling of

supplements was performed by researchers not involved in patient screening or recruitment such that boxes of supplement sachets were identifiable only by participant randomisation numbers. Allocation to diet and supplement was masked throughout data collection, laboratory analysis, data input and data analysis.

Procedures

The low FODMAP diet involves restricting dietary intake of fructans, galacto-oligosaccharides (GOS), lactose, fructose in excess of glucose, and polyols, and is described elsewhere.[17] Blinding dietary advice trials is notoriously challenging and therefore the sham diet was designed bespoke for this trial to fulfil the following criteria: [18] [1] to be an exclusion diet that restricts a similar number of staple and non-staple foods and requires similar difficulty of dietary change to the low FODMAP diet; [2] to require similar intensity and duration of dietary counselling as the low FODMAP diet; and [3] not to impact on intakes of nutrients, fibre and FODMAPs. For example, suitable carbohydrates on the sham diet included fruits such as apple, banana and pear, whereas orange, raspberry, and strawberry were not allowed, and allowed grains included wheat but not rice.

The sham diet was field tested to examine fulfilment of these criteria in a pilot study and an interim analysis of fibre and FODMAP intake was performed in the current trial following completion of 20 patients. Dietary counselling for the sham diet and low FODMAP diet lasted for similar duration (approximately 10 minutes) and detailed written food lists were provided. Dietary compliance to both sham diet and low FODMAP diet was self-reported weekly during telephone calls ('in the last week I have followed the diet…') using compliance categories adapted from previous work: [19] never/rarely (<25% of the time), sometimes (25-50% of the time), frequently (51-75% of the time) or always (76-100% of the time). Patients were considered compliant if they reported following the diet frequently or always (i.e. >50% of the time) on at least two of the four weekly assessments.

The probiotic was a multi-strain preparation containing *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *B. longum* DSM 24736, *B. infantis* DSM 24737, *Lactobacillus acidophilus* DSM 24735, *L. plantarum* DSM 24730, *L. paracasei* DSM 24733, *L.*

delbrueckii subsp. *bulgaricus* DSM 24734 (VSL#3, CD Investments VSL Pharmaceuticals Inc, Rome, Italy) and was provided in sachets in freeze dried form with maltose and silicon dioxide as inactive excipients. The placebo sachets were prepared by the same manufacturer as the probiotic product. The placebo sachets contained the same inactive excipients but no bacteria. Participants received two sachets per day (11.95 $\log_{10}$ bacteria in the intervention group) to be taken in the morning with cold food or fluid. Patients were considered compliant to the supplement if 80% of sachets were taken based on return of all unused sachets. A greater threshold for compliance was used for the probiotic/placebo, in line with those used in other probiotics trials and because compliance once per day is easier than extensive dietary modification at every meal, snack and drink.

Assessments

The incidence and severity of 15 gastrointestinal symptoms and overall symptoms were measured daily for seven days prior to baseline using the Gastrointestinal Symptom Rating Scale (GSRS),[20] and stool frequency and consistency was recorded using the Bristol Stool Form Scale. A 7-day food diary was used to measure dietary intake and FODMAP intake was quantified using dietary analysis software containing the most comprehensively analysed FODMAP food composition database available.[21-23] At the baseline visit, global and individual symptoms were also measured using the IBS Symptom Scoring System (IBS-SSS).[24] Generic and disease-specific HRQOL were measured using the SF-36[25] and IBS-QOL[26], respectively. Then, after all data had been collected, patients were randomised to dietary advice (sham, low FODMAP diet) and supplement (placebo, probiotic). Patients were telephoned weekly for assessment of dietary compliance, to address dietary questions and to ensure IBS medication remained stable. All outcomes were then repeated at four weeks (follow-up) as well as 'adequate symptom relief' (did you have adequate relief of your symptoms over the past seven days).[27]

A whole fresh stool sample was collected within one hour of passage and stored immediately on ice at baseline and follow-up. It was homogenized in a stomacher for four minutes and multiple aliquots were taken and stored at -80°C until analysis. Quantitative polymerase chain reaction (qPCR) was performed to quantify abundance of *Bifidobacterium*

species. 16S rRNA sequencing was performed to evaluate α-diversity (number of operational taxonomic units [OTU] i.e. number of species, or richness) and β-diversity (differences in species composition between baseline and follow-up) and to confirm the abundance of *Bifidobacterium* species from qPCR analysis (supplementary Information).

Outcomes

As the outcomes of interest were the impact of diet and supplement on symptoms and microbiota, the co-primary outcomes were IBS symptoms ('adequate symptom relief') and stool *Bifidobacterium* species concentration at follow-up, measured using qPCR. Pre-specified secondary outcomes were individual GI symptoms (IBS-SSS and GSRS), stool output, HRQOL, microbiota diversity and nutrient intake. Adverse events were recorded at weekly telephone calls and at the follow-up visit.

Statistical analysis

The sample size calculation was based on the co-primary endpoints assuming no interaction between the two interventions. For the first co-primary outcome (symptoms), the estimated response to low FODMAP diet advice was based upon 'adequate symptom relief' from the only randomised controlled trial (RCT) available at the time (68%)[10] and the estimated response to sham advice was based on the response rate of controls in a meta-analysis of IBS trials and two previous trials of the low FODMAP diet (36%).[10,28,29] The estimated response to the probiotic and placebo was based upon the combined global response data from previous studies of the probiotic supplement.[30,31] Based on logistic regression and assuming a power of 80% and a 2-sided significance level of 5%, the main effects of diet (sham vs low FODMAP diet) could be estimated with 88 patients. Based on previous research from our centre[10], attrition of at least 12% was anticipated, leading to an overall sample size of at least 100 such that the per protocol analysis would be adequately powered. For the second co-primary outcome (*Bifidobacterium*), the expected abundance of stool *Bifidobacterium* species in response to the low FODMAP diet was taken from the only RCT at that time[10] and the expected abundance in response to probiotic was taken from a VSL#3 RCT. [32] The sham and placebo groups were expected to have no impact on abundance of *Bifidobacterium* species and therefore values from control groups were used. Based on

linear regression, assumed power of 80% and overall 2-sided significance of 5%, the main effects for the *Bifidobacterium* outcome could be estimated with 28 patients, which ensured the trial was powered for both clinical and microbiological outcomes.

The primary analysis was by intention-to-treat. A subsequent per protocol analysis was performed for the symptom primary outcome using data from completed patients who did not violate the protocol and were compliant with the interventions. The qPCR and 16S rRNA sequencing analysis is presented for the per protocol population. Data are presented as summary data e.g. mean (SD) for continuous variables or number (%) for categorical data with estimates and 95% confidence intervals (CI). Differences were considered significant where $p \leq 0.05$.

Linear regression and ANOVA was performed to evaluate the effect of diet and supplement on continuous variables (microbiota according to qPCR, IBS-SSS, GSRS symptom severity, stool output, HRQOL). The Chi-squared test and logistic regression was performed to evaluate the effect on categorical variables ('adequate symptom relief', proportion meeting minimal clinically important difference [MCID] for IBS-SSS, IBS-QOL). Adjusted regression models were performed to account for differences between groups at baseline and bootstrapping was computed due to non-normal data. Interaction terms were added into regression models to check for interactions between the two independent variables (diet, supplement). The Kruskal-Wallis test and Wilcoxon rank sum test were used to evaluate differences in microbiota diversity and abundance according to 16S rRNA sequencing between groups and over time within groups, respectively. A correction was applied for multiple comparisons where required. Intake of FODMAPs, energy and nutrients were compared between diet groups using analysis of covariance (ANCOVA), adjusting for baseline. Statistical analysis was performed using IBM SPSS Statistics Version 22.0.

All authors had access to the trial data and reviewed and approved the final manuscript.

Results

Patients were recruited between 28 January 2013 and 21 November 2014. A total of 162 patients were screened of whom 58 were ineligible. Therefore, 104 patients were randomised to the sham (n=53) or low FODMAP diet (n=51) and placebo (n=51) or probiotic (n=53) as follows: sham/placebo (n=27), sham/probiotic (n=26), low FODMAP diet/placebo (n=24) and low FODMAP diet /probiotic (n=27) (Figure 1). Nine patients were withdrawn from the trial (six commenced antibiotics [two sham, four low FODMAP diet], one violated the protocol by following an alternative diet [sham], one was lost to follow-up [sham], one withdrew for personal reasons [sham]), five were non-compliant with placebo [two sham, three low FODMAP diet] and three were non-compliant with probiotic [two sham, one low FODMAP diet]). All 104 randomised patients were included in the intention-to-treat analysis and 87 were included in the per protocol analysis. Baseline characteristics of patients were similar for both diet and supplement interventions (Table 1). Medications used included antidiarrheals (loperamide), analgesics (acetaminophen, non-steroidal anti-inflammatories) and antispasmodics (butylscopolamine).

All patients reported following the diet >50% of the time on at least two of the four weeks and were defined as compliant to the dietary interventions. Of the 95 patients who completed the study, 88 (93%) patients reported always following the diet (76-100% of the time) over the four weeks, 7 (7%) reported frequently following the diet (51-75% of the time) and no patients reported following the diet sometimes (25-50% of the time) or never. At follow-up, dietary advice resulted in lower total FODMAP intake in the low FODMAP diet group (9·9 g/d) compared with the sham diet group (17.4 g/d, $p<0.001$), with significantly lower intakes of a number of individual FODMAPs ($p<0.05$). There was no difference between low FODMAP diet and sham diet for intakes of total energy, macronutrients or fibre (non-starch polysaccharide) (Table 2). Of the 95 that completed the study, 87 (92%) patients were defined as compliant to the probiotic/placebo, defined as consuming at least 80% of sachets (based upon returned sachets; 88 (93%) patients consumed 76-100% of sachets, 6 (6%) consumed 51-75% of sachets, 1 (1%) consumed 26-50%, and 1 (1%) consumed 0-25% of sachets.

There was no interaction between the interventions for either co-primary outcome (interaction term for adequate relief OR 0.60 (95% CI 0.12 to 2.97, p=0.52) and *Bifidobacterium* species mean difference -0.10 (95% CI -0.59 to 0.38, p=0.68). Therefore the results are presented separately for diet (sham vs low FODMAP diet) and supplement (placebo vs probiotic) interventions.

Clinical endpoints

At follow-up, in the intention-to-treat analysis there was a higher proportion of patients reporting 'adequate symptom relief' for the low FODMAP diet (29/51, 57%) compared with sham diet (20/53, 38%), although the difference was not statistically significant (χ^2=3.816, p=0.051), with an odds of symptom relief of 2·18 (95% CI 0.99 to 4.77, p=0.052). However, in the per protocol analysis more patients reported 'adequate symptom relief' for the low FODMAP diet (26/43, 61%) than for sham diet (17/44, 39%) (χ^2=4.146, p=0.042), with an odds of symptom relief of 2.43 (95% CI 1.03 to 5.75, p=0.043).

At follow-up, the proportion of patients reporting 'adequate symptom relief' for the probiotic (30/53, 57%) compared with placebo (19/51, 37%) achieved statistical significance (χ^2=3.905, p=0.048), with an odds of symptom relief of 2·20 (95% CI 1.00 to 4.82, p=0.050). However, in the per protocol analysis the proportion of patients reporting 'adequate symptom relief' for probiotic (27/47, 57%) compared with placebo (16/40, 40%) was not significant (χ^2=2.631, p=0.105), and nor were the odds of symptom relief (OR 2.03, 95% CI 0.86 to 4.77, p=0.107).

In the intention-to-treat population at follow-up, the total IBS-SSS score was significantly lower for the low FODMAP diet (173 ± 95) compared with sham diet (224 ± 89, p=0.001), and there were lower subscores for days of pain (p=0.001), distension severity (p=0.002), satisfaction with bowels (p=0.002) and the impact of IBS symptoms on life (p=0.022, Table 3). A higher proportion of patients achieved the MCID (reduction in total IBS-SSS score of ≥50) during the low FODMAP diet (37/51, 73%) compared with the sham diet (n=22/53, 42%) (OR 3.72, 95% CI 1.64 to 8·48, p=0.0017). There were also lower severity scores for a number of symptoms including abdominal pain (p=0.010) and overall symptoms (p=0.020)

according to the GSRS, and a lower stool consistency score (p=0.008) in patients following the low FODMAP diet compared with the sham diet. There was no difference in the proportion of patients achieving the MCID for probiotic (32/53, 60%) versus placebo (27/51, 53%) (OR 1.49 95% CI 0.63 to 3.52, p=0.363), or for GSRS or stool output outcomes, except for lower severity for flatulence (p=0.033, Table 3).

Although there were no differences between groups in total score for SF-36 and IBS-QOL (Table 4), there were higher scores for role limitations due to physical health (p=0.033) and energy/fatigue (p=0.016) for SF-36 and higher scores for body image (p=0.001), social reaction (p=0.026) and relationships (p=0.041) of IBS-QOL in patients on the low FODMAP diet compared with sham diet, indicating better quality of life in these domains. There was no effect of probiotic on HRQOL compared with placebo (Table 4).

Microbiota endpoints

Regarding the microbiota co-primary endpoint using the qPCR analysis, at follow-up there was a lower absolute *Bifidobacterium* species abundance following the low FODMAP diet (8.8 16S rRNA genes/g, SD 0·6) compared with sham diet (9.2 rRNA genes/g, SD 0·9) (mean difference -0.39 rRNA genes/g, 95% CI -0.64 to -0.13, p=0.008). At follow-up there was greater absolute abundance of *Bifidobacterium* species for probiotic (9.1 rRNA genes/g, SD 0·6) compared with placebo (8.8 rRNA genes/g, SD 1.0) (mean difference +0.34 rRNA genes/g, 95% CI 0.05 to 0.61, p= 0.019).

16S rRNA sequencing confirmed the low FODMAP diet led to a significant reduction in relative abundance of *Bifidobacterium* species between baseline and follow-up (1.70% vs 0.79%) that did not occur in sham group (1.57% vs 1.93%), representing a mean change in the low FODMAP diet of -0.91% compared with sham 0.36% (95% CI for differences in change 0.45% to 2.09%, p=0.0027, Figure 2A). In contrast, there were no differences in the change in relative abundance of *Bifidobacterium* species between baseline and follow-up between the probiotic group (1.60% vs 1.50%) compared with the placebo group (1.68% vs 1.18%), with a mean change in the low FODMAP diet of -0.99% vs sham -0.50% (95% CI for differences in change -0.46% to 1.20%, p=0.3549, Figure 2A). However, 16S rRNA

sequencing demonstrated a significant increase in relative abundance of *Streptococcus* species in the probiotic group (0.45% vs 1.90%) compared with placebo (0.38% vs 0.55%), representing a mean change in the probiotic group of 1.45% compared with placebo 0.17% (95% CI for differences in change 0.63% to 1.90%, p=0.00017, Figure 2B). There was no difference in relative abundance of *Streptococcus* species for the low FODMAP diet compared with sham diet (p=0.1141, Figure 2B). There were also no differences in change in relative abundance of *Lactobacillus* species between baseline and follow-up between the low FODMAP diet and sham diet (p=0.5782) or between the probiotic and placebo group (p= 0.9521, Figure 2C).

There were significant differences in absolute abundance of *Bifidobacterium* species measured using qPCR between the four randomised groups at follow-up, i.e. sham diet/placebo (8.9 rRNA genes/g, SD 1.2), low FODMAP diet/placebo (8.6 rRNA genes/g, SD 0.5), sham diet/probiotic (9.2 rRNA genes/g, SD 0.5), low FODMAP diet/probiotic (8.9 rRNA genes/g, SD 0.6) (ANOVA p=0.037), with a significant difference specifically between low FODMAP diet/placebo versus sham diet/probiotic (mean difference -0.67, 95% CI -1.25 to -0.08, p=0.020; Games-Howell *post hoc* correction).

16S rRNA sequencing analysis revealed no significant differences in global microbiota α-diversity (Chao index) following a low FODMAP diet (mean change 824.0) compared with sham diet (286.6) (95% CI for differences in change -840.2 to 1915.1, p=0.401) or between probiotic (mean change 298.1) and placebo (867.7) (95% CI for differences in change -815.5 to 1954.7, p=0.212). Neither were there differences in the change in α-diversity between the four randomised groups (p=0.473).

There was no difference in β-diversity (Bray Curtis dissimilarity index) for the low FODMAP diet compared with sham diet (mean difference -0.010, 95% CI -0.044 to 0.024, p=0.575) or for probiotic compared with placebo (mean difference -0.004, 95% CI -0.039 to 0.032, p=0.833).

Adverse events

Overall, the number of adverse events reported was small. Six patients reported worsened GI symptoms (four sham, two low FODMAP diet; four placebo, two probiotic). Other adverse events not thought to be related to the diet or supplement were reported in 40% of patients (e.g. headache, cold, toothache) and this was not different between diet (p=0.516) or supplement groups (p=0.388). No serious adverse events were reported.

Discussion

This RCT demonstrates that low FODMAP dietary advice leads to adequate relief of GI symptoms in 57% of patients compared with 38% of patients receiving sham dietary advice (and 61% and 39% in the per protocol analysis). Although this finding did not reach statistical significance, the totality of the clinical outcomes reported here point toward the clinical effectiveness of the low FODMAP diet over and above placebo, the first to do so using dietary advice, making it directly relevant to clinical practice. Low FODMAP dietary advice was shown to restrict FODMAP intake whilst not altering energy and macronutrient intake compared with sham diet advice, indicating that the observed effects are likely the result of FODMAP restriction rather than changes in nutrient intake. Furthermore, this RCT evaluated whether the low FODMAP diet-induced decline in bifidobacteria[9,10] can be modified by probiotic supplementation.

Despite the low FODMAP diet resulting in significantly lower composite and individual symptom scores according to a range of validated instruments, the proportion reporting the co-primary endpoint of 'adequate symptom relief' during the low FODMAP diet compared with sham diet was only of borderline statistical significance. There are several issues that explain this discrepancy. Firstly, the proportion of patients achieving 'adequate symptom relief' after the low FODMAP diet (57%) was lower than reported in a previous low FODMAP dietary advice trial (68%)[10]; dietary allocation in the previous study was unblinded and therefore the response may have been artificially enhanced. Secondly, in the current RCT, the blinded low FODMAP dietary advice was delivered without explanation of how the diet affects GI physiology, as is done in routine clinical practice and which may be important for enhancing patient 'buy in'. This also led to the patient education process being of shorter duration than that which would normally be applied in clinical practice. Thirdly, analysing

only those who completed the study and complied with the intervention resulted in statistically significant differences between groups in favour of the low FODMAP diet.

Although the 'adequate symptom relief' endpoint is currently considered an important outcome measure in IBS trials[4] and has been used as the primary endpoint in drug trials (e.g. alosetron, tegaserod) it has several limitations. First, a binary response requires synthesis of multiple symptoms into a single response and does not allow detail regarding individual symptoms, which is better captured by a multi-item instrument (e.g. IBS-SSS). Second, it is not possible to quantify the magnitude of symptom improvement. Finally, it requires subjective assessment of whether symptoms are adequately controlled, which is likely to have high inter-individual variation. Conversely, the presence of a minimal clinically important difference for the IBS-SSS allows for meaningful interpretation of the score.[24]

Previous non-placebo controlled low FODMAP dietary advice trials have reported similar changes in IBS-SSS score over time (-78 to -133 points) to that reported here (mean -117 points).[33,34] Moreover, the impact of the low FODMAP diet on abdominal pain, bloating, flatulence, urgency, and stool consistency are supportive of results from previous RCTs[7,10], and are likely explained by the effect of fermentable carbohydrates on small intestinal water and colonic gas production. This trial also reported a significant benefit of the low FODMAP diet on aspects of HRQOL in a blinded RCT, an endpoint which is particularly pertinent in IBS. Whether the positive impact on some domains of HRQOL translates into reduced healthcare utilisation or has wider economic implications requires evaluation.

'Adequate symptom relief' was reported in more patients receiving probiotic than the placebo. Although this was statistically significant this may represent a type I error; the trial was not powered to detect differences in this outcome for the probiotic and yet the effect size was much higher (57%) compared with previous studies of VSL#3 (33%-46%)[30,31], and no differences were found in the per protocol analysis. Furthermore, the 'adequate symptom relief' outcome distinctly deviates from other clinical endpoints in this trial.

As hypothesised, the low FODMAP diet led to a reduction in stool *Bifidobacterium* species according to qPCR and 16S rRNA sequencing. These findings are likely the result of restriction of prebiotic carbohydrates, essentially leading to the opposite effect to that which occurs during prebiotic supplementation (an 'anti-prebiotic' effect). Bifidobacteria have established immunomodulatory effects and have been inversely associated with clinical symptoms in IBS.[11] The impact of the reduction of *Bifidobacterium* species by the low FODMAP diet on clinical symptoms in IBS, or on colonic function is unknown. However, the probiotic, which contained bifidobacteria strains, resulted in a greater abundance of *Bifodobacterium* species according to qPCR analysis. This finding was not replicated in the sequencing analysis, although this discrepancy is not uncommon and can be attributed to the fact that qPCR is a quantitative method using specific primer sets to enumerate the population of interest whereas 16S rRNA sequencing targets the whole bacterial community, sometimes failing to provide extensive coverage for specific genera. Given the lack of interaction between diet and probiotic interventions in this study, the effects of each can be considered additive. We have therefore shown that the low FODMAP diet-induced reduction in *Bifidobacterium* can be modified with the addition of a specific bifidobacteria-containing probiotic.

Reduced microbiome species richness is evident in IBS, as well as in inflammatory bowel disease, obesity and diabetes.[13,14] Two previous studies report no within-group change in microbiota diversity in response to the low FODMAP diet,[15,35] and another has demonstrated increased richness in *Clostridial* Cluster XIV compared with habitual diet.[9] However, the effect of the low FODMAP diet on microbiota diversity compared with a placebo diet has not been examined. Here we have shown that the low FODMAP diet does not affect α-diversity or β-diversity compared with a sham control diet. Given the reduction of carbohydrate available for colonic fermentation during the low FODMAP diet, the absence of an impact on diversity is reassuring, particularly in view of its negative associations with gastrointestinal and extra-intestinal disease.

This RCT evaluated the therapeutic benefit of low FODMAP dietary advice, as utilised in clinical practice, above the effect of placebo, which is known to have a powerful effect in

IBS.[8] In dietary intervention trials, the problem of designing a control group is often resolved through feeding studies that deviate considerably from clinical practice. Therefore an important strength of the current study is the implementation of a placebo sham diet that required equivalent dietary counselling, disruption to daily life, and which changed dietary intake but not FODMAP intake.

Limitations of this RCT include the difficulty of maintaining blinding. Blinding is challenging in dietary intervention trials compared with nutrient or drug trials. However, the best attempts were made to blind patients to their allocated diet through a range of approaches: the sham diet also focused on modifying intake of carbohydrate-rich foods, required modification of a similar number of foods and specialist foods compared with the low FODMAP diet, and the burden of teaching and following the sham diet was equivalent to the low FODMAP diet. Second, as the study was performed in secondary care and excluded patients with constipation-predominant IBS, it is not possible to say if the results are applicable beyond this setting. Third, although there was a measurable reduction in total FODMAP intake, it is unknown whether symptom response was due to collective FODMAP restriction or the removal of one (e.g. lactose, fructans) or several individual FODMAPs. Fourth, there is the problem of collinearity, which is unavoidable in dietary exclusion studies (i.e. changing one component of the diet leads to compensatory changes in other components). Although macronutrient and fibre intake was maintained in this study, there may have been changes in unmeasured dietary substrates (e.g. polyphenols, gluten) that could contribute to the observed findings. This limitation is also applicable to the sham group; there may have been alteration in unmeasured dietary components that impacted the findings. However, we carefully measured intake of numerous dietary constituents with an established potential to impact IBS symptoms (e.g. fiber, fat) and these did not change, suggesting it fulfilled its purpose as a sham diet. Fifth, changing dietary intake may impact on other physiological parameters (e.g. transit time) that might independently affect microbiota composition. Finally, this study raises questions regarding the use of a dichotomous endpoint as a primary outcome measure given the disparity between this outcome and the various non-dichotomous endpoints used (e.g. IBS-SSS, GSRS). Trials in IBS-

C have also reported clinically important changes in individual symptoms in patients that did not meet a dichotomous endpoint.[36]

Further research should prospectively evaluate longer term durability of the low FODMAP diet, which has been associated with symptom benefit in retrospective studies.[19,38] It should also address the low FODMAP diet-induced microbiota disruption to determine whether the microbiota changes persist over time, and whether co-administration of probiotic should be considered in the long term. Cost-benefit analysis of both interventions will confirm their utility for long term use. Furthermore, whether the mucosal microbiota compartment is affected, whether changes in microbiome function occur (dependent or independent of the compositional shift) and whether this leads to short-term or long-term consequences require evaluation. Furthermore, the identification of biomarkers that predict response to the low FODMAP diet would be extremely valuable. IBS is a heterogeneous syndrome with inter-individual differences in the contribution of physiological, cognitive and behavioral components to the manifestation of symptoms. It is currently unclear to what extent each of these will respond to dietary and probiotic intervention.

In conclusion, we have demonstrated that low FODMAP dietary advice leads to improvement in overall and specific GI symptoms in IBS. In fact, 73% of patients reported a global clinical response based on the IBS-SSS, and 57% report response based on the dichotomous primary outcome, although the limitations of the latter endpoint are acknowledged. At a conservative estimate, this corresponds to a 2-3 greater odds of response to low FODMAP dietary advice compared with placebo dietary advice, which is equivalent to several pharmaceutical treatments (e.g. antispasmodics, antidepressants).[37] We also present findings related to the impact of the low FODMAP diet on the microbiota, and the first evidence that the effect of the low FODMAP diet on bifidobacteria can be modified by adjunctive probiotic therapy.

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TABLE AND FIGURE LEGENDS

Table 1: Demographic data for the intention-to-treat population
Data are mean (SD) or n (%). BMI= body mass index

Table 2: Dietary intake at follow-up in patients receiving sham dietary advice and low FODMAP dietary advice
Data are mean (SD) at follow up for total and individual FODMAPs (g/d), energy (kcal/d) and nutrient intake (g/d). *Total FODMAPs are calculated as the sum of individual carbohydrates including excess fructose (not total fructose). NSP=non-starch polysaccharides. Data were log transformed for analysis.

Table 3: Symptom and stool output outcomes at follow-up in patients receiving sham dietary advice, low FODMAP dietary advice, placebo and probiotic
Data are mean (SD) at follow up. *IBS Severity Scoring System (IBS-SSS) instrument scores symptom severity on five visual analogue scale items, where worst severity is 500 points. †Gastrointestinal Symptom Rating Scale (GSRS) instrument, where severity was rated daily over seven days on a scale of 0 (absent), 1 (mild), 2 (moderate), 3 (severe). ‡Mean consistency based on Bristol Stool Form Scale type over the 7-day period. **Mean number of stools over the 7-day period. †† Proportion of stools that were Bristol Stool Form types 3-5 over the 7-day period.

Table 4: Health-related quality of life outcomes at follow-up in patients sham dietary advice, low FODMAP dietary advice, placebo and probiotic
Data are mean (SD) at follow up.

Figure 1: CONSORT flow diagram

Figure 2: Changes in relative abundance of selected bacterial genera according to 16S rRNA sequencing.
(A) Change in relative abundance of Bifidobacteria for low FODMAP diet (LFD) vs sham diet (p=0·0027) and probiotic vs placebo (p=0·3549). (B) Change in relative abundance of *Streptococcus* species for low FODMAP diet vs sham diet (p=0·1141) and probiotic vs placebo (p=0·00017). (C) Change in relative abundance of *Lactobacillus* species for low FODMAP diet vs sham diet (p=0·5782) and probiotic vs placebo (p=0·9521) (Wilcoxon rank-sum test, FDR correction). The central line indicates the median, the box indicates the 25th and 75th percentiles and the whiskers indicate 1.5x interquartile range. LFD, low FODMAP diet.

Tables

Table 1:

	Sham diet and placebo (n=27)	Sham diet and probiotic (n=26)	Low FODMAP diet and placebo (n=24)	low FODMAP diet and probiotic (n=27)
Age (years)	33 (12)	35 (11)	36 (11)	38 (13)
Female	18 (67)	17 (65)	17 (71)	18 (67)
Symptom duration (months)	59 (58)	70 (95)	63 (81)	104 (131)
IBS subtype				
IBS-D	18 (67)	16 (61)	16 (67)	19 (70)
IBS-M	5 (18)	7 (27)	5 (21)	7 (26)
IBS-U	4 (15)	3 (12)	3 (13)	1(4)
Current medications				
Antidiarrheal	1 (4)	1 (4)	1 (4)	2 (7)
Analgesic	0 (0)	0 (0)	3 (13)	1 (4)
Antispasmodic	3 (11)	3 (12)	5 (21)	0 (0)
Ethnicity (white)	22 (82)	23 (89)	19 (79)	22 (82)
Smoker	1 (4)	2 (8)	0 (0)	5 (19)
Vegetarian	0 (0)	0 (0)	1 (4)	1 (4)
Weight (kg)	69 (13)	75 (22)	72 (17)	69 (13)
BMI (kg/m^2)	24 (4)	25 (6)	25 (5)	24 (4)

Table 2:

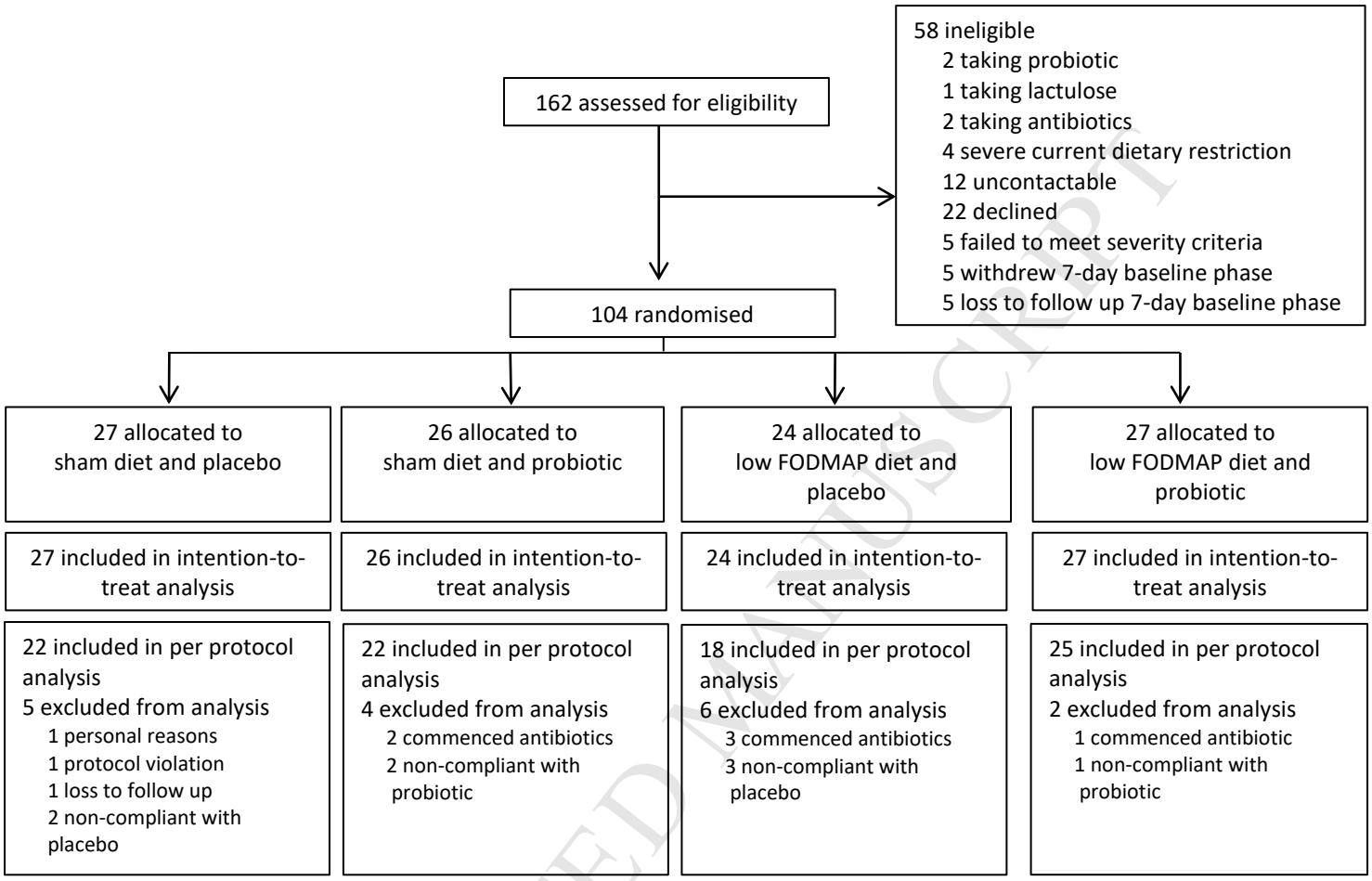
	Sham diet n=53	**Low FODMAP diet n=51**	**p**
Energy	1891 (599)	1861 (465)	0.517
Protein	75 (21)	78 (22)	0.100
Fat	80 (28)	78 (25)	0.787
Carbohydrate	206 (62)	198 (58)	0.678
Starch	115 (39)	110 (39)	0.916
Sugars	84 (33)	75 (30)	0.457
Non-starch polysaccharide	13.3 (5.1)	12.8 (4.8)	0.578
Total FODMAPs *	17.4 (10.5)	9.9 (6.4)	<0.001
Fructans	5.0 (2.9)	2.5 (1.9)	<0.001
GOS	0.9 (0.5)	0.8 (0.6)	0.080
Lactose	8.9 (9.1)	4.3 (4.3)	<0.001
Total fructose	15.5 (8.7)	12.7 (5.9)	0.112
Excess fructose	1.4 (1.4)	1.9 (2.8)	0.821
Sorbitol	1.0 (1.0)	0.3 (0.5)	<0.001
Mannitol	0.3 (0.3)	0.1 (0.2)	0.041

Table 3:

	Diet			Supplement		
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**p**
IBS-SSS*						
Total score	224 (89)	173 (95)	**0.001**	207 (98)	192 (93)	0.721
Pain severity	40 (23)	33 (24)	0.062	38 (24)	35 (24)	0.892
Days of pain	44 (29)	30 (27)	**0.001**	39 (28)	35 (30)	0.690
Distension severity	40 (24)	29 (25)	**0.002**	34 (24)	35 (26)	0.766
Satisfaction with bowels	53 (17)	42 (23)	**0.002**	49 (22)	46 (20)	0.459
Affecting life	47 (21)	40 (20)	**0.022**	46 (21)	41 (20)	0.322
Change in IBS-SSS	-44 (72)	-117 (86)	**0.001**	-78 (96)	-82 (78)	0.750
†Gastrointestinal Symptom Rating Scale Severity Scores						
Abdominal pain	1.1 (0.6)	0.9 (0.7)	**0.010**	1.0 (0.7)	0.9 (0.6)	0.753
Heartburn	0.2 (0.3)	0.2 (0.5)	0.872	0.2 (0.3)	0.2 (0.5)	0.128
Acid reflux	0.2 (0.5)	0.2 (0.4)	0.515	0.2 (0.3)	0.2 (0.4)	0.276
Nausea	0.3 (0.5)	0.3 (0.4)	0.535	0.3 (0.6)	0.2 (0.3)	0.191
Borborygmi	1.0 (0.7)	0.7 (0.6)	**0.003**	0.9 (0.7)	0.8 (0.7)	0.913
Bloating	1.1 (0.7)	0.8 (0.7)	**0.001**	1.0 (0.7)	1.0 (0.7)	0.780
Belching	0.6 (0.7)	0.5 (0.6)	**0.031**	0.6 (0.7)	0.5 (0.6)	0.084
Flatulence	1.3 (0.7)	0.9 (0.6)	**0.001**	1.2 (0.7)	1.0 (0.6)	**0.033**
Constipation	0.3 (0.4)	0.2 (0.4)	0.559	0.2 (0.4)	0.3 (0.4)	0.452
Diarrhoea	0.3 (0.5)	0.2 (0.5)	0.257	0.3 (0.5)	0.2 (0.4)	0.505
Loose stool	0.7 (0.7)	0.5 (0.6)	0.080	0.7 (0.7)	0.5 (0.6)	0.542
Hard stool	0.2 (0.2)	0.2 (0.3)	0.166	0.2 (0.2)	0.2 (0.3)	0.203
Urgency	0.7 (0.6)	0.6 (0.7)	**0.001**	0.7 (0.7)	0.6 (0.6)	0.610
Incomplete evacuation	0.7 (0.7)	0.5 (0.6)	**0.039**	0.7 (0.7)	0.6 (0.6)	0.674
Tiredness	1.3 (0.7)	1.0 (0.8)	0.067	1.2 (0.8)	1.0 (0.8)	0.393
Overall symptoms	1.2 (0.6)	1.0 (0.6)	**0.020**	1.2 (0.6)	1.0 (0.6)	0.066
Stool output						
Stool consistency‡	4.3 (1.1)	3.9 (1.0)	**0.008**	4.2 (1.0)	4.0 (1.1)	0.544
Normal consistency (proportion) ††	61 (30)	67 (26)	0.200	64 (30)	64 (26)	0.689
Stool frequency**	12.9 (7.4)	14.0 (8.5)	0.843	13.8 (8.3)	13.1 (7.6)	0.136

	Diet			Supplement		
	Sham diet (n=53)	**Low FODMAP diet (n=51)**	**p**	**Placebo (n=51)**	**Probiotic (n=53)**	**p**
SF-36						
Physical functioning	87.3 (22.3)	86.3 (21.3)	0.357	88.9 (19.2)	84.7 (23.8)	0.278
Role limitations due to physical health	55.2 (39.6)	70.6 (39.3)	**0.033**	62.8 (39.8)	62.7 (40.6)	0.330
Role limitations due to emotional problems	65.4 (37.5)	64.1 (43.1)	0.598	71.2 (38.3)	58.5 (41.3)	0.330
Energy/fatigue	42.6 (19.9)	52.1 (23.3)	**0.016**	43.9 (19.7)	50.4 (23.8)	0.427
Emotional wellbeing	63.3 (17.3)	68.7 (17.8)	0.082	66.0 (17.8)	65.8 (17.7)	0.991
Social functioning	77.8 (20.7)	73.3 (27.3)	0.398	76.0 (25.0)	75.2 (23.6)	0.731
Pain	65.1 (20.4)	63.5 (27.0)	0.349	61.0 (23.7)	67.5 (23.6)	0.460
General Health	56.2 (19.7)	57.5 (22.4)	0.141	55.8 (20.7)	57.8 (21.4)	0.235
IBS-QOL						
Overall	70.6 (18.1)	72.4 (19.7)	0.057	68.6 (20.7)	74.3 (16.6)	0.849
Dysphoria	72.2 (20.5)	71.9 (24.7)	0.640	69.6 (24.7)	74.4 (20.3)	0.937
Interference with activity	71.2 (20.6)	72.9 (24.2)	0.120	68.9 (23.3)	75.0 (21.1)	0.640
Body Image	64.2 (22.7)	73.2 (22.7)	**0.001**	64.8 (24.2)	72.2 (21.5)	0.847
Healthy worry	71.1 (20.8)	73.0 (20.0)	0.383	69.6 (23.3)	74.4 (17.0)	0.336
Food avoidance	57.9 (29.2)	51.1 (26.7)	0.823	53.6 (28.8)	55.5 (27.5)	0.683
Social reaction	71.7 (22.2)	77.5 (22.4)	**0.026**	71.2 (23.3)	77.7 (21.1)	0.769
Sexual	76.2 (28.6)	79.7 (24.7)	0.163	73.3 (29.3)	82.3 (23.4)	0.150
Relationships	80.5 (19.9)	81.2 (18.8)	**0.041**	77.5 (20.1)	84.1 (18.1)	0.451

Table 4:



A

Bifidobacterium

B

Streptococcus

C

Lactobacillus

p = 0.0027

p = 0.00017

Relative abundance (Follow up – Baseline)

6
5
4
3
2
1
0
−1
−2
−3
−4

LFD
SHAM
PROBIOTIC
PLACEBO