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Randomized Control Trials

Gut microbiota associations with diet in irritable bowel syndrome and the effect of low FODMAP diet and probiotics☆

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SUMMARY

Background and aims: Diet is both a modulator of the gastrointestinal microbiota and an important therapy in irritable bowel syndrome (IBS).
We aimed to comprehensively (i) identify diet-microbiota associations in adults with IBS consuming habitual diet; (ii) assess the impact of two nutritional interventions on the microbiota; and (iii) determine whether baseline microbiota can predict clinical response to diet or probiotic intervention.
Methods: Data were analyzed from 95 individuals with IBS participating in a previously published 4-week 2x2 factorial design randomized controlled trial investigating the impact of the low FODMAP diet (LFD) and co-administration of a probiotic. Diet was assessed at four hierarchical levels and partial 16S rRNA gene sequencing was used to profile the microbiota.
Results: There were numerous diet-microbiota associations especially at the nutrient level, including a negative association between protein and *Bifidobacterium* abundance ($r_s = -0.358$, $p < 0.001$). After correction for multiple testing, the significance for this association ($q = 0.237$) and all others was lost. Low FODMAP diet led to changes in abundance of major saccharolytic genera compared with sham diet, including higher *Bacteroides* (LFD 34.1% (15.7%) vs sham 23.3% (15.2%), $q = 0.01$) and lower *Bifidobacterium* (0.9% (1.0%) vs 2.1%, (2.5%) $q = 0.029$). Compared with placebo, probiotic supplementation led to higher *Lactobacillus* (probiotic 0.08% (0.1%) vs placebo 0.03% (0.2%), $q < 0.001$), and *Streptococcus* abundance (2.0% (2.2%) vs 0.6% (1.2%), $q = 0.001$). The probiotic treatment buffered the impact of the low FODMAP diet on *Bifidobacterium*. Baseline microbiota did not predict clinical response to either intervention.
Conclusions: Although diet modifies the gut microbiota, bivariate correlation analysis may only provide a limited explanation of the complex diet interactions with individual gut bacteria in IBS. Some diet interventions modify the microbiota in IBS.
Trial registry: ISRCTN (http://www.isrctn.com) Registered under ISRCTN registry identifier no.ISRCTN 02275221.
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1. Introduction

Irritable bowel syndrome (IBS) is a chronic functional bowel disorder characterized by abdominal pain and altered bowel habit [1]. There is mounting evidence of an aberrant microbiota compared with healthy controls [2] and specific differences in microbial signatures are associated with symptom profile and/or severity [3–6].

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Diet is one of the major environmental determinants of gut microbiome composition in humans. This is supported by markedly differing microbiota between populations with distinct geographical locations and diets [7,8], and the effect of short term dietary changes on the microbiota in intervention studies [9–11]. The dietary restrictions undertaken by many with IBS [12,13] may be a major determinant of the altered microbiota composition. However, studies of the microbiota in IBS rarely consider diet as a covariate in statistical modelling [3,5], and where diet is measured, it is reported at the traditional granular level of energy and nutrients [4]. This does not account for the multi-dimensionality of dietary exposure, including nutrient–nutrient interactions and the cumulative or antagonistic effects of multiple nutrients and bio-active constituents on the microbiota. Similarly, whether the microbiota in IBS responds to diet in a similar manner to that of health individuals is unknown.

Therapeutic dietary interventions in IBS, such as the low FODMAP diet [14] and probiotics can also modulate microbiota composition [15]. Short-term restriction of fermentable oligosaccharides disaccharides, monosaccharides and polyols (FODMAPs) has consistently shown to reduce abundance of bifidobacteria compared with controls or baseline [16–19], however most studies are limited by sample sizes of ≤40 patients and are likely underpowered to detect diet-induced microbiota changes [16,20–22]. Also, specific species and strains of probiotics have evidence of efficacy in IBS [23], but the majority of trials investigating probiotic efficacy do not evaluate the impact on microbial composition, and compliance is neither routinely nor effectively measured and reported [24].

The gastrointestinal microbiota may predict responders and non-responders to interventions, enabling targeted treatment that avoids large numbers of patients starting dietary interventions to which they are unlikely to respond. For example, greater abundances of saccharolytic taxa [25] and lower scores on a 'dysbiosis test' have been shown to be predictive of response to a low FODMAP diet [26,27]. However, the evidence is inconsistent [19].

The aim of this study was to address current gaps in understanding to comprehensively: (i) identify diet-microbiota associations in adults with IBS consuming habitual diet; (ii) assess the impact of the low FODMAP diet and a probiotic on the microbiota for which we have published preliminary findings [18]; and (iii) determine whether baseline microbiota can predict clinical response to low FODMAP diet or probiotic intervention.

2. Methods

Clinical, dietary and microbiology data were analysed from 104 patients with IBS recruited to a 4-week 2x2 factorial, blinded, placebo-controlled RCT [18]. Dietary intake and microbiota were measured at baseline (during habitual diet) and after 4 weeks of diet (low FODMAP diet, sham diet) and/or supplement (probiotic, placebo) intervention. Full description of the clinical trial design, participants, and clinical outcome measures are provided in the previously published clinical trial paper [18] and summarised here to provide essential context to the current diet and microbiota analysis.

2.1. Participants

Patients aged 18–65 years diagnosed with IBS according to Rome III who were naive to the low FODMAP diet (assessed by previous/current dietary restrictions and diet history) were included. Exclusion criteria included abdominal pain or discomfort for <2 days during the screening week, patients already following a severely restrictive diet (e.g. vegan) and bowel preparation for investigative procedures, antibiotics, and/or prebiotic or probiotic supplementation during the previous four weeks. A comprehensive list of inclusion and exclusion criteria are detailed elsewhere [18]. Patients were recruited from two tertiary centres in London UK between January 2013 to November 2014. Informed consent was obtained prior to participation. The study was approved by the London Fulham Research Ethics Committee (Reference 12/LO/1402), and conducted in accordance with the Helsinki Declaration of 1975 as revised in 1983.

2.2. Microbiota

Microbiota profile was assessed at baseline (during habitual diet) and after the end of the 4-week intervention period using partial 16S rRNA gene sequencing. A whole fresh stool sample was collected within 1 h of passage, stored immediately on ice and homogenized in a stomacher for 4 min. Aliquots were stored at −80 °C until analysis. The primer set 341F/806R was used to amplify the V3–V4 region of bacterial 16S rRNA gene. Relative abundances of taxa at phylum, family and genus level were identified. Microbiota diversity was evaluated by alpha diversity (number of unique operational taxonomic units (OTUs), Chao index) and beta diversity (unweighted UniFrac, weighted UniFrac statistics and Bray-Curtis dissimilarity). Where there was an operational taxonomic unit (OTU) abundance lower than 0.01% or presence in less than 25% of the samples were excluded from the statistical analysis. Detailed methodology is provided in online supplementary information.

2.3. Nutrients, foods, food groups and dietary patterns

Data on dietary intake were collected at baseline (habitual diet) and during the final week of the 4-week intervention using a 7-day food diary and household measures and food photographs to assist portion estimation. Data from food diaries were entered into dietary analysis software (Diet Plan, Version 6 P3 Forestfield Software, Horsham, UK) and were analysed at four hierarchical levels (nutrients, foods, food groups, dietary patterns). Nutrient intake was calculated using the national nutrient composition database (Composition of Foods Integrated Dataset, CoFID) [28] and FODMAP intake was calculated by entry into a comprehensive FODMAP composition database provided by Monash University [29–31]. Food intake was calculated by categorising intake into 41 foods adapted from published classifications [32] (Supplemental Table 1). For example, vegetables were categorised into seven vegetable categories based on botanical family and dairy foods were categorized into six categories based on nutrient composition. A total of 39 foods were analysed due to low intakes of 'game' and 'non-soy vegetarian' foods. Food group intakes were calculated by condensing the 39 foods into 14 broader nutritionally-meaningful food groups based on national food composition data from CoFID [28] (Supplemental Table 2). Nutrient, food and food group data were reported as mean intake per day. Dietary patterns were calculated using five validated scores to measure diet quality [33,34], diet diversity [35,36] and compliance with a Mediterranean diet [37].

2.4. Interventions

Patients were randomized in a 1:1 ratio to diet (sham diet 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). Patients were blinded to both diet and supplement allocations however the dietitian conducting the trial visits and dietary counselling was unable to be blinded to diet allocation but

was blinded to supplement allocation. The development of the sham diet and methods to facilitate blinding of diet allocation are detailed elsewhere [18,38]. Patients were counselled on the dietary intervention (sham or low FODMAP) by a dietitian and personalized dietary advice was provided with comprehensive written information. Self-rated dietary compliance was assessed using categories adapted from those used previously ("in the last week I have followed the diet": never/rarely (<25% of the time), sometimes (25%–50% of the time), frequently (51%–75% of the time), always (76%–100% of the time) [39], and patients were considered compliant to diet if they reported following the diet frequently or always (i.e. >50% of the time) on at least 2 of the 4 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 (now exclusively available in Europe under the trademark Vivomixx and in the United States under the trademark Visbiome, as indicated in other recent scientific publications) and was provided in sachets in freeze-dried form. The placebo sachets were identical in appearance, taste and presentation and contained maltose and silicon dioxide as inactive agent. Patients received two sachets per day (11.95 $\log_{10}$ bacteria in the intervention group) and were considered compliant to the supplement if $\geq$ 80% of sachets were taken based on quantification of returned, unused sachets.

All assessments of clinical symptoms, dietary intake and gut microbiota were undertaken at baseline and at 4 weeks. Gastrointestinal symptoms were measured using the IBS-Symptom Scoring System (IBS-SSS), and clinical response was defined as a $\geq$50-point reduction in total score [40].

2.5. Statistical analysis

Habitual diet-microbiota relationships were assessed at baseline (prior to intervention). Spearman's correlations were performed to assess the relationship between intakes of nutrients (in addition to energy and FODMAPs), foods, food groups, and dietary patterns and the relative abundance of taxa and alpha diversity. Multiple comparisons against all taxa were corrected using false discovery rate (FDR) and associations were defined as significant if the FDR value (q) was <0.05. For habitual diet-microbiota associations, hypothesis-driven analyses were also conducted for individual nutrients (fiber, non-starch polysaccharides) and food components (fructans, galacto-oligosaccharides) previously shown to impact the microbiota in intervention studies, and these analyses were partially corrected for the number of taxa variables.

Adherence to the diet intervention was assessed objectively by comparing FODMAP intake at follow-up between diet groups using adjusted linear regression to account for baseline differences, and bootstrapping was computed because of non-normal data. Intakes are presented as estimated marginal means. Clinical response ($\geq$50-point reduction in IBS-SSS) was compared between intervention groups using the Chi-squared test, and regression analysis was used to test for interactions between the two interventions (diet, supplement). Relative abundance of microbiota and alpha diversity at follow-up was compared between diet groups (sham vs low FODMAP), supplement groups (placebo vs probiotic), and between the four randomized groups using Kruskal-Wallis test. These analyses add to previously published data from this RCT comparing change in abundance of a limited number of genera between groups [18].

In order to investigate whether baseline microbiota could discriminate responders ($\geq$50-point reduction in IBS-SSS) and non-responders (<50-point reduction in IBS-SSS) to low FODMAP diet or probiotic, a random forest supervised learning algorithm was applied to the reduced OTU set at genus level. The random forest analysis was performed with 1000 trees and 10-fold cross validation using the QIIME script supervised_learning.py. Abundance of microbiota are reported as relative abundance, mean (SD) and alpha diversity reported as Chao 1, Shannon and observed OTUs. Statistical analysis was performed using IBM SPSS Statistics Version 25.0 (IBM, Armonk, NY), QIIME 1.9.1 [41], and R version 3.4.

3. Results

In total, 95 patients completed the RCT and provided stool samples at both baseline and follow-up (48 sham diet, 47 low FODMAP diet; 45 placebo, 50 probiotic; 24 sham diet/placebo, 24 sham diet/probiotic, 21 low FODMAP diet/placebo, 26 low FODMAP diet/probiotic; Table 1). Of these, four baseline samples were excluded from analysis (3 sham diet, 1 low FODMAP diet; 4 placebo, 0 probiotic; 3 sham diet/placebo, 1 low FODMAP diet/placebo) due to insufficient sequencing quality. No follow-up samples were excluded. A consort diagram is provided in online supplementary information.

An average of 70,992 high-quality 16S rRNA gene sequences were generated per stool sample (range: 8,038–295,626), with an average of 2786 OTUs. All 95 participants completed a 7-day food diary at baseline for complete analysis of habitual intakes of nutrients, foods, food groups and dietary patterns.

3.1. Habitual diet-microbiota associations in IBS

In terms of nutrient intake, there were 193 significant associations with microbiota at genus level (Fig. 1), 135 at family level (Supplemental Fig. 1), 66 at phylum level (Supplemental Fig. 2), and 30 associations with alpha diversity. For example, at the genus level, there were many negative associations between intakes of nutrients and *Bifidobacterium*, including for protein ($r_s = -0.358$, $p < 0.001$) and numerous micronutrients, and negative associations between *Dialister* and fiber ($r_s = -0.270$, $p = 0.010$) and numerous micronutrients. There were many positive associations with an unclassified *Clostridiales*, including intakes of total sugars ($r_s = 0.290$, $p = 0.005$) and numerous micronutrients.

For food intake, there were 103 significant associations with microbiota at genus level (Fig. 1), 58 at family level (Supplemental Fig. 1) and 18 at phylum level (Supplemental Fig. 2), and 7 associations with alpha diversity. For example, at the genus level there were positive associations between intakes of tropical fruit and *Haemophilus* ($r_s = 0.406$, $p < 0.001$), liquers and spirits and *Holdemania* ($r_s = 0.393$, $p < 0.001$), and nuts and seeds and *Streptococcus* ($r_s = 0.364$, $p = 0.001$). There were positive associations between intakes of beer and cider ($r_s = 0.250$, $p = 0.017$) and allium vegetables ($r_s = 0.249$, $p = 0.017$), with alpha diversity (OTUs).

For food groups, there were 36 significant associations with microbiota at genus level (Fig. 1), 20 at family level (Supplemental Fig. 1) and 9 at phylum level (Supplemental Fig. 2). For example, at the genus level there was a positive association between intakes of nuts and *Streptococcus* ($r_s = 0.364$, $p < 0.001$) and fruit and *Haemophilus* ($r_s = 0.358$, $p < 0.001$). There were no associations between food groups and alpha diversity.

For dietary patterns, there were 16 significant associations at genus level (Fig. 1), 8 at family level (Supplemental Fig. 1) and 2 at phylum level (Supplemental Fig. 2). For example, at the genus level, there were negative associations between Mediterranean diet (MDS) and an unclassified genus within the *Clostridia* class ($r_s = -0.340$, $p = 0.001$) and healthy diet (HDS, HDI) and *Dialister*

Table 1
Baseline demographic data.

	Two-group comparisons				Four-group comparisons			
	Sham (n = 48)	Low FODMAP (n = 47)	Placebo (n = 45)	Probiotic (n = 50)	Sham + placebo (n = 24)	Sham + probiotic (n = 24)	Low FODMAP + placebo (n = 21)	Low FODMAP + probiotic (n = 26)
Age, yr	34 (12)	37 (12)	34 (12)	37 (12)	32 (12)	36 (12)	35 (11)	38 (13)
Female, n (%)	30 (63)	33 (70)	31 (69)	32 (64)	15 (63)	15 (63)	16 (76)	17 (65)
Weight, kg	73 (18)	69 (14)	70 (13)	72 (19)	70 (12)	76 (23)	70 (15)	68 (14)
BMI, kg/m^2	25 (5)	24 (4)	25 (4)	25 (5)	25 (4)	26 (6)	25 (5)	24 (4)
Symptom duration, months	64 (78)	84 (115)	56 (67)	91 (117)	54 (54)	74 (106)	58 (81)	106 (133)
IBS subtype, n (%)								
IBS-D	31 (65)	32 (68)	29 (65)	34 (68)	16 (67)	15 (63)	13 (62)	19 (73)
IBS-M	11 (23)	11 (23)	10 (22)	12 (24)	5 (21)	6 (25)	5 (24)	6 (23)
IBS-U	6 (12)	4 (9)	6 (13)	4 (8)	3 (13)	3 (13)	3 (14)	1 (4)
Ethnicity, white n (%)	34 (71)	30 (64)	33 (73)	31 (62)	18 (75)	16 (67)	15 (71)	15 (58)
Smoker, n (%)	3 (6)	5 (11)	1 (2)	7 (14)	1 (4)	2 (8)	0 (0)	5 (19)
Vegetarian, n (%)	1 (2)	1 (2)	0 (0)	2 (4)	0 (0)	1 (4)	0 (0)	1 (4)

Data are mean (SD) or n (%).
Ethnicity data was provided by self-report.
IBS, irritable bowel syndrome; IBS-D, diarrhea-predominant irritable bowel syndrome; IBS-M, mixed subtype irritable bowel syndrome; IBS-U, un-subtyped irritable bowel syndrome.

($r_s = -0.320$, $p = 0.002$). There were no associations between dietary patterns and alpha diversity.

Overall, there were more diet-microbiota associations at the nutrient level than at any other hierarchical level. Importantly, none of these gut microbiota associations with nutrients, foods, food groups and dietary patterns remained significant after adjusting for multiple testing.

In the *a priori* hypothesis-driven analyses there were 14 significant associations in total between individual nutrients (fiber, non-starch polysaccharides) and food components (fructans, galacto-oligosaccharides), previously shown to impact the microbiota in intervention studies, and the microbiota. For example, at the genus level there was a positive association between fiber and a genus within the order Clostridiales ($r_s = 0.272$, $p = 0.009$, $q = 0.059$), and a negative association between fiber and *Dialister* ($r_s = -0.270$, $p = 0.010$, $q = 0.096$) and *Bifidobacterium* (r_s = -0.228, $p = 0.030$, $q = 0.060$), all of which were not significant after adjustment for multiple testing. There were no associations between these nutrients or food components and alpha diversity. Of particular note, there were no associations between *Bifidobacterium* abundance and intakes of non-starch polysaccharides, fructans or galacto-oligosaccharides either on initial analysis or following adjustment for multiple testing.

3.2. Impact of low FODMAP diet and probiotic on microbiota in IBS

All participants reported following the low FODMAP diet frequently (50%–75%) or always (76%–100%) on at least two of the four intervention weeks. Intake of FODMAPs was lower at follow-up on the low FODMAP diet (total FODMAP intake 8.7 g/d [5.4–12.2]) compared with sham diet (16.2 g/d [10.1–22.5]) after adjusting for baseline differences (mean difference −8.9 g/d, 95% CI -5.9 to −12.0, $p < 0.001$). Of the 95 participants, 87 (92%) consumed at least 80% of the probiotic/placebo sachets provided.

After intervention, there were differences between low FODMAP diet and sham diet groups in relative abundances of 10 genera. After adjustment for multiple testing, the low FODMAP diet group had lower abundance of *Bifidobacterium* (0.9% (1.0%) vs 2.1%, (2.5%) $q = 0.029$), an unclassified genus in the *Ruminococcaceae* family (8.3% (5.1%) vs 12.8% (5.9%), q=<0.001), and higher abundance of *Bacteroides* genus (34.1% (15.7%) vs 23.3% (15.2%), $q = 0.01$; Fig. 2), compared with the sham diet group. These findings were supported at the family (lower *Bifidobacteriaceae* ($q = 0.016$) and *Ruminococcaceae* ($q = 0.008$) and higher *Bacteroidaceae* ($q = 0.008$) for low FODMAP compared with sham) and phylum level (lower Actinobacteria ($q = 0.007$) and Firmicutes ($q = 0.05$) and higher Bacteroidetes ($q = 0.05$) for low FODMAP compared with sham). Alpha diversity did not differ between low FODMAP and sham diet groups following intervention (Chao 1 $p = 0.757$, Shannon $p = 0.275$, observed OTUs $p = 0.669$; Fig. 2).

There were differences following intervention between probiotic and placebo groups in relative abundances of four genera. After adjustment for multiple testing, probiotic supplementation resulted in higher abundance of *Lactobacillus* (0.08% (0.1%) vs 0.03% (0.2%), $q < 0.001$), *Streptococcus* (2.0% (2.2%) vs 0.6% (1.2%), $q = 0.001$) and an unclassified genus within the *Lactobacillaceae* family (0.06% (0.08%) vs 0.0003% (0.001%), $q < 0.001$) compared with placebo (Fig. 3). There was no difference in abundance of *Bifidobacterium* between probiotic and placebo (1.6% (2.1%) vs 1.3% (2.0%), $q = 0.429$). Findings at the genus level were supported at the family level (higher *Lactobacillaceae* and *Streptococcaceae* ($q < 0.001$) for probiotic compared with placebo). There were no differences between groups at the phylum level. Alpha diversity was lower in the probiotic group compared with placebo when measured using observed number of OTU's (2820 vs 3318, $p = 0.034$; Fig. 3), but not with Chao 1 ($p = 0.059$) or Shannon index ($p = 0.620$).

When the 2x2 factorial trial was analysed as the four randomized groups (i.e. sham diet/placebo, sham diet/probiotic, low FODMAP diet/placebo, low FODMAP diet/probiotic) and compared following intervention, there were significant differences in relative abundance for the following genera: *Lactobacillus* ($q < 0.001$, lowest in low FODMAP diet/placebo), an unclassified genus within the family *Lactobacillaceae* ($q < 0.001$, highest in the two probiotic groups), *Streptococcus* ($q = 0.003$, highest in the two probiotic groups), an unclassified genus within *Ruminococcaceae* ($q = 0.003$, lowest in the two low FODMAP groups), *Bifidobacterium* ($q = 0.016$, lowest in low FODMAP diet/placebo) and *Bacteroides* ($q = 0.03$, highest in the two low FODMAP groups; Fig. 2). Alpha diversity did not differ between the four groups (Fig. 4).

3.3. Baseline microbial composition as a predictor of clinical response to low FODMAP diet or probiotic

Of the 91 participants included in the final sample analysis, clinical response (≥50-point reduction in IBS-SSS) was achieved in

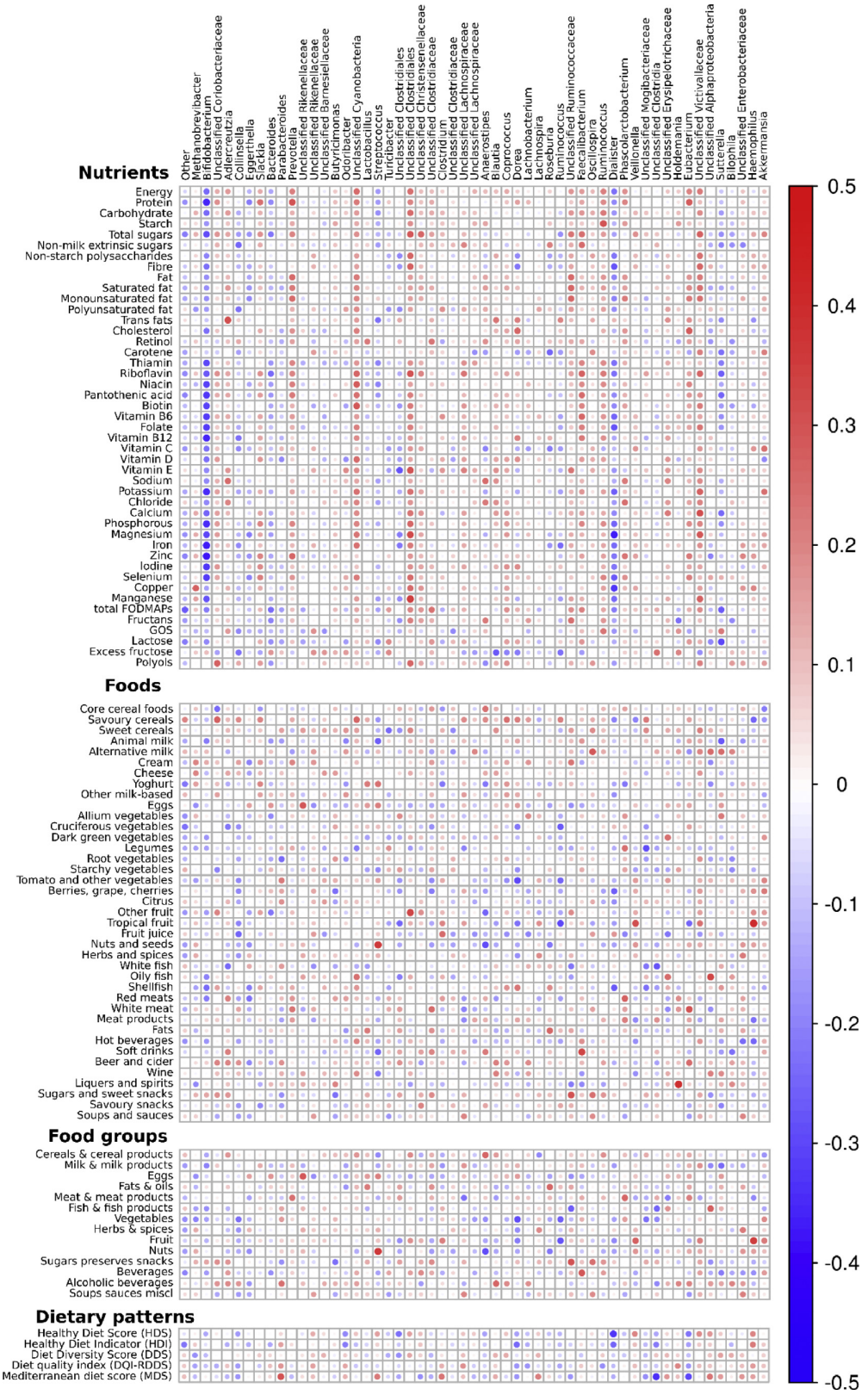


Fig. 1. Correlation between diet and microbiota in irritable bowel syndrome. Dietary intake is described at four hierarchical levels (nutrients, foods, food groups, dietary patterns). The figure shows a heat-map which correlates dietary variables (vertical) with microbial relative abundance data at genus level (horizontal). Dietary variables that correlated most positively with specific taxa are indicated in deep red, while dietary variables that correlated most negatively are indicated in deep blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

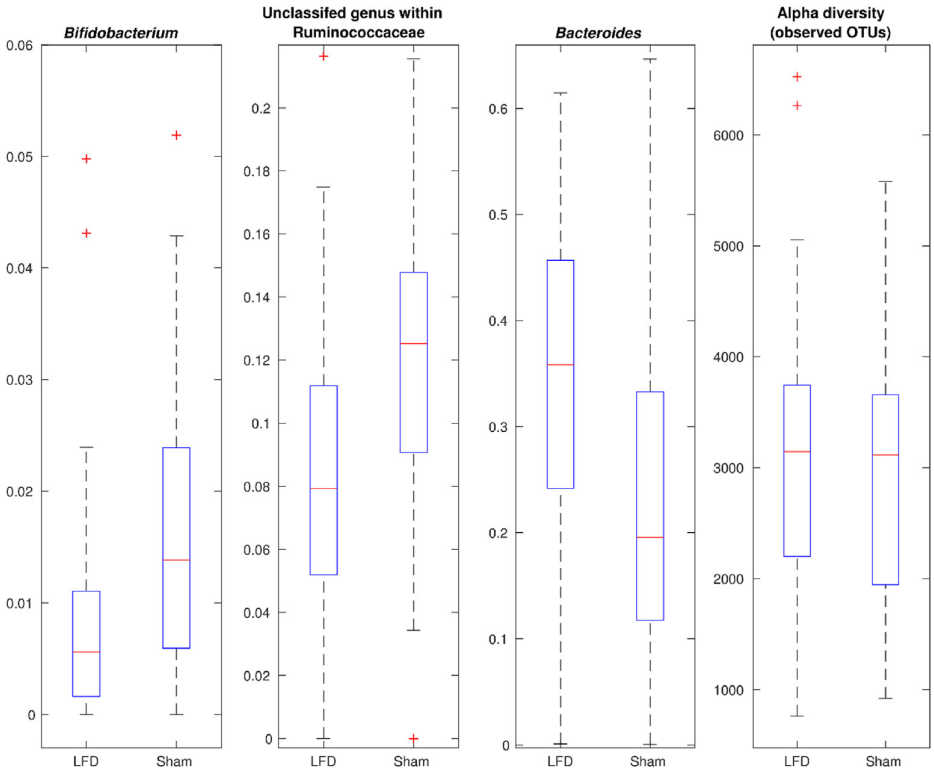


Fig. 2. Relative abundances of microbiota at genus level for low FODMAP diet (LFD) and sham diet groups after intervention. In the LFD group there was a lower abundance of *Bifidobacterium* (q = 0.029), an unclassified genus in the *Ruminococcaceae* family (q < 0.001) and a higher abundance of *Bacteroides* (q = 0.01) compared with sham diet. There was no difference in observed OTUs between groups (p = 0.669). Data presented are median (central line), 25th and 75th percentiles (box) and 1.5 interquartile range (whisker), with data falling outside these quartiles plotted as outliers.

more patients following the low FODMAP diet (36/46, 78%) than sham diet (21/45, 47%, p = 0.002). Clinical response to probiotic (32/50, 64%) was not significantly different to placebo (25/41, 61%, p = 0.468). There was no interaction between the two interventions for clinical response (interaction term [OR 0.75 95% CI 0.23 to 2.44, p = 0.632).

To investigate the predictive potential of the microbiota at baseline on clinical response, random forest analysis was applied to microbial composition at the genus level as a multivariate classifier, but showed very poor classification performance (i.e. poor predictive power). The model classified all 46 patients randomized to the low FODMAP diet as responders, meaning 36/36 responders were correctly classified as responders (0% error rate), but 0/10 non-responders were correctly classified as non-responders (100% error rate). For probiotic supplementation, 29/32 responders were correctly classified (9% error rate), but only 1/18 non-responders were correctly classified (94% error rate). Likewise, the impact of individual taxa on predicting any response was limited. The family *Clostridiaceae* was the most predictive marker, although its exclusion from the model led to only a 1% increase in prediction error (0.009 mean decrease in accuracy). Overall, there was a very limited discriminative power of baseline microbiota for predicting response to either the low FODMAP diet or probiotic.

4. Discussion

This is the most comprehensive analysis of diet-microbiota interactions in IBS, analysing diet at four hierarchical levels and evaluating microbiota composition using partial 16 S rRNA gene sequencing. Despite many diet-microbiota associations, in particular with nutrients, surprisingly, none remained significant following adjustment. Intervention with a low FODMAP diet or probiotic supplementation altered microbiota composition compared with control, but baseline microbial composition could not predict clinical response to either intervention.

There is extensive evidence of associations between habitual nutrient intake and composition of the GI microbiota in healthy individuals. For example, in a seminal studies of diet-microbiota associations, higher carbohydrate intake was associated with *Prevotella* enterotype and higher animal protein intake with *Bacteroides* enterotype [42]. These diet-microbiota associations can be explained by greater substrate availability leading to increased abundance of primary taxa but also of secondary taxa that cross-feed on metabolites produced by primary degraders. Although no nutrient-microbiota associations were evident in the present study after FDR testing, there were many associations prior to correction. These included a negative association between protein intake and *Bifidobacterium* abundance, a saccharolytic genera that may be exposed to less exogenous carbohydrate when protein intake is elevated.

However, the assessment of diet at the nutrient level alone fails to recognize the complexity of dietary exposure at numerous hierarchical levels that may better capture existing important diet-microbiota interactions. For example, intake of at least 30 unique plant foods per week has been associated with greater microbial diversity and abundance of *F. prausnitzii* in healthy individuals [43]. At the level of dietary pattern, a higher diet quality has been associated with greater alpha diversity [44], and Mediterranean diet adherence has been associated with greater abundance of

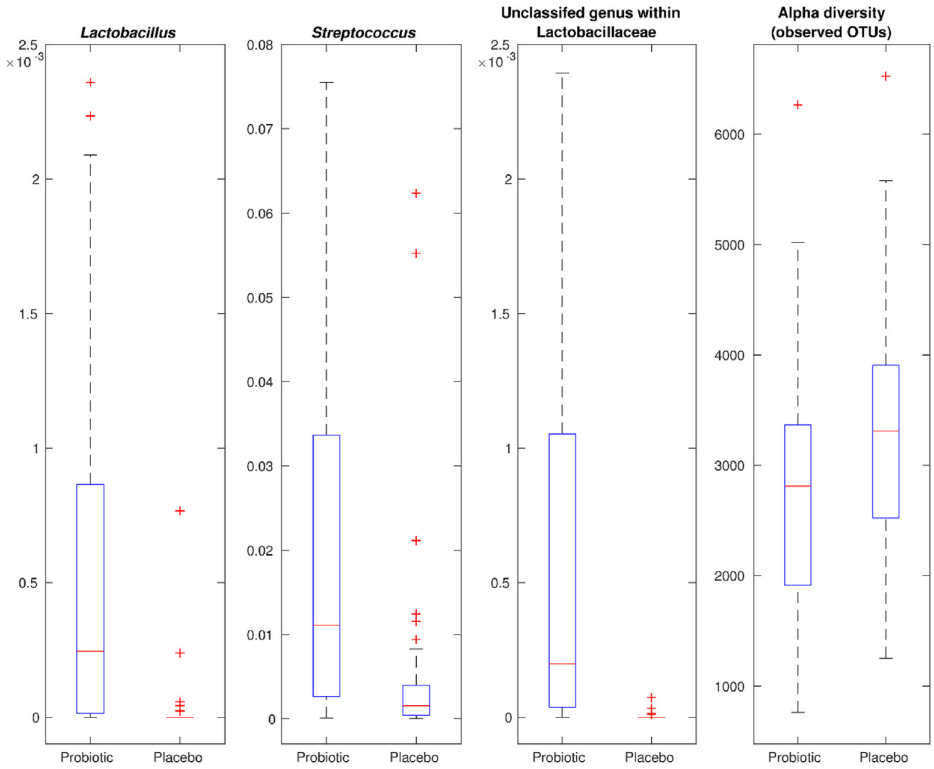


Fig. 3. Relative abundances of microbiota at genus level for probiotic and placebo groups after intervention. In the probiotic group there was higher abundance of *Lactobacillus* ($q < 0.001$), *Streptococcus* ($q = 0.001$) and an unclassified genus within the *Lactobacillaceae* family ($q < 0.001$) compared with placebo. Alpha diversity (observed OTUs) was lower in the probiotic group compared with placebo ($p = 0.034$).

Prevotella [45], although the latter did not adjust for multiple comparisons. The lack of association between foods, food groups and dietary patterns with microbiota composition in this study contrasts with these data. Most microbiota associations to date have been studied in healthy individuals. In the one existing study in IBS, no association between diet and an IBS microbial signature was detected, however diet data was limited to energy, macronutrients and FODMAPs [4]. Our findings present the first analysis of diet-microbiota associations in IBS at different levels of diet ranging from nutrients to dietary pattern scores.

The lack of diet-microbiota associations in this study may be explained in a number of ways. Firstly, both dietary intake in IBS [46] and the commensal gut microbiota [2] are altered compared with healthy controls. Evidence from a recent longitudinal sampling study suggests diet-microbiota associations in healthy individuals are strongly personalized [47]. It is plausible the diet-microbiota interaction diverges from 'normal' in IBS, given the interplay of other factors known to interact with the microbiota such as altered physiology (e.g. gut motility, intestinal permeability), psychological stressors, exposure to gut-directed medication and altered diet. Dietary homogeneity in this defined IBS patient population may have also led to a lack of extreme values that greatly impact Spearman's correlations. Secondly, comparisons across studies are difficult due to methodological differences including the technique used to measure the microbiota, the method to collect raw dietary data, the process by which dietary data is aggregated for a particular hierarchical level (e.g. food group allocations, choice of diet quality score), and statistical methodology utilized. Our analysis incorporated stringent correction to control for type 1 error, which may be responsible for our lack of findings compared with others who used conservative corrections or did not incorporate adjustment at all.

Dietary and probiotic intervention led to numerous differences in microbial composition between intervention groups compared with controls. The finding that *Bifidobacterium* abundance was lower after low FODMAP diet compared with sham diet supports previous data [16,17,19]. We also found significantly higher *Bacteroides* abundance after low FODMAP diet which has not previously been reported, a genera that have genomes encoding an array of sugar utilization enzymes [48]. This finding may represent a shift in the nature of carbohydrate sources consumed during the low FODMAP diet. The lower abundance of an unclassified genus within the *Ruminococcaceae* family in the low FODMAP group can be explained by the ability of several members to degrade carbohydrates such as inulin [49]. The lack of difference in alpha diversity between low FODMAP diet and controls aligns with previous findings [16,22].

There were distinct effects of probiotic supplementation on the microbiota. The higher abundances of *Lactobacillus* and *Streptococcus* in the probiotic group were unsurprising considering these micro-organisms were present in the supplement. However, *Bifidobacterium* abundance did not differ between groups despite the supplement containing three *Bifidobacterium* strains. Failure of 16S rRNA sequencing to provide extensive coverage for this genus, or variable colonisation between individuals may be responsible, or these strains may not survive gut transit. Lower microbial diversity in the probiotic group compared with placebo contrasted with recent data that microbial diversity is unaltered in response to probiotic supplementation in healthy individuals [50], however this may be an analytical artefact relating to influx of probiotic species and reduced ability to sequence less abundant OTUs.

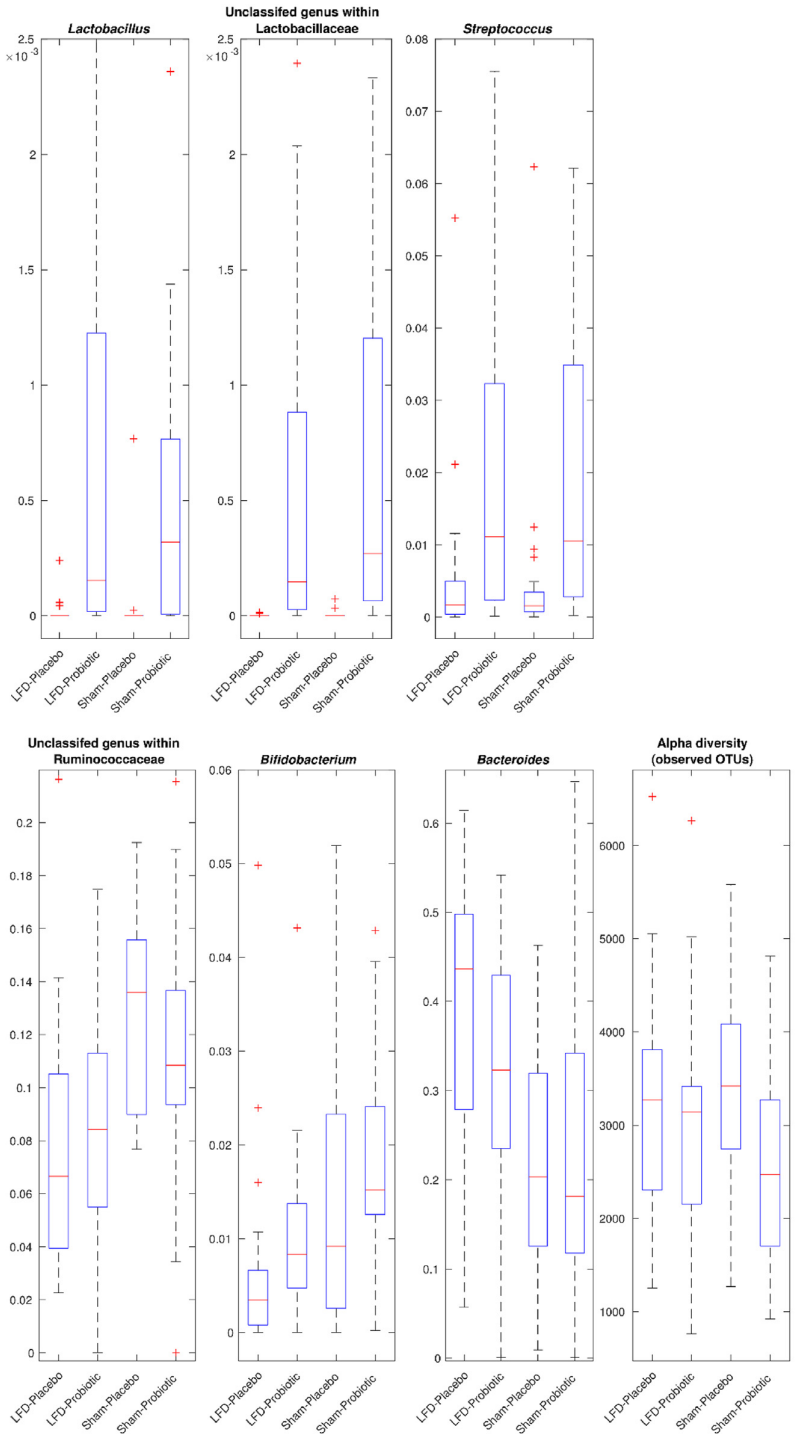


Fig. 4. Relative abundances of microbiota at genus level for the four randomised groups (sham diet/placebo, sham diet/probiotic, low FODMAP diet/placebo, low FODMAP diet/probiotic) after intervention. There were significant differences between groups for *Lactobacillus* ($q < 0.001$), an unclassified genus within the family *Lactobacillaceae* ($q < 0.001$), *Streptococcus* ($q = 0.003$), an unclassified genus within *Ruminococcaceae* ($q = 0.003$), *Bifidobacterium* ($q = 0.016$) and *Bacteroides* ($q = 0.03$). Alpha diversity did not differ between groups (observed OTUs $p = 0.669$).

The ability to personalize nutrition interventions based on demographic, clinical and/or microbiota profile may be particularly important where dietary interventions are intensive and require healthcare supervision [51]. In this study of 95 patients with IBS, in whom there was a 78% clinical response rate to a low FODMAP diet, the baseline microbiota did not predict clinical response. Previous data are equivocal [19,25–27] and varied definitions of 'response' from validated criteria to arbitrary cut-offs, differential extent of FODMAP restriction, insufficient washout in crossover studies, small sample sizes, and varying stool analysis and statistical modelling techniques may be responsible.

There are several limitations of this study. Firstly, dietary data were collected using food records considered the gold standard for dietary assessment, however a food frequency questionnaire may have better assessed chronic dietary exposure. Secondly, a strict cut off for inclusion of OTUs for analysis was used together with a stringent adjustment for multiple comparisons, sometimes not used in diet-microbiota studies, which may have resulted in a type II error and masked true diet-microbiota relationships.

These findings provide important insight into the relative absence of diet-microbiota relationships in IBS. Studies that account for symptom heterogeneity and other confounders and that carefully assess diet at multiple hierarchical levels are needed. Clear differences in the microbial community were evident after both diet and probiotic intervention compared with controls. However, it is important to establish whether critical functional changes in the microbiota occur to warrant concerns about the safety of the low FODMAP diet and to facilitate identification of mechanisms of action of probiotics in IBS. With regards to the microbiota predicting response to diet or probiotic treatment in IBS, large scale studies accounting for baseline variables known to modulate the microbiota, and use of consistent clinical response thresholds and bioinformatic approaches will be important in confirming whether this is possible in this patient group.

Guarantor of the article

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Specific author contributions

KW, HS, KT, ML, FF, PI, JL planned the research; HS, FR, ML, MS, FF, KT, KW collected and/or interpreted the data; HS and KW drafted the manuscript; All authors approved the final manuscript.

Conflict of Interest

ML and KW are co-inventors of a mobile application relating to the low FODMAP diet. KW has acted as a consultant for Danone, and received research funding from Danone. FR receives consultation fees from Lavida Food Co. that distributes low FODMAP food products in the UK. All other authors have no other relevant conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2020.10.013.

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