

Effects of varying dietary content of fermentable short-chain carbohydrates on symptoms, fecal microenvironment, and cytokine profiles in patients with irritable bowel syndrome

T. N. Hustoft¹ | T. Hausken^{1,2,3} | S. O. Ystad^{1,3} | J. Valeur⁴ | K. Brokstad⁵ |
J. G. Hatlebakk^{1,2,3} | G. A. Lied^{1,2,3}

¹Department of Clinical Medicine, University of Bergen, Bergen, Norway

²Section of Gastroenterology, Department of Medicine, Haukeland University Hospital, Bergen, Norway

³National Centre of Functional Gastrointestinal Disorders, Haukeland University Hospital, Bergen, Norway

⁴Unger-Vetlesen's Institute, Lovisenberg Diaconal Hospital, Oslo, Norway

⁵Department of Clinical Science, University of Bergen, Bergen, Norway

Correspondence

Tonje Nesvik Hustoft, Department of Clinical Medicine, University of Bergen (UiB), Bergen, Norway
Emails: tonje_hustoft@hotmail.com

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Abstract

Background: A diet low in fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) is increasingly recommended for patients with irritable bowel syndrome (IBS). We aimed to investigate the effects of a blinded low-FODMAP vs high-fructo-oligosaccharides (FOS) diet on symptoms, immune activation, gut microbiota composition, and short-chain fatty acids (SCFAs).

Methods: Twenty patients with diarrhea-predominant or mixed IBS were instructed to follow a low-FODMAP diet (LFD) throughout a 9-week study period. After 3 weeks, they were randomized and double-blindly assigned to receive a supplement of either FOS (FODMAP) or maltodextrin (placebo) for the next 10 days, followed by a 3-week washout period before crossover. Irritable bowel syndrome severity scoring system (IBS-SSS) was used to evaluate symptoms. Cytokines (interleukin [IL]-6, IL-8, and tumor necrosis factor alpha) were analyzed in blood samples, and gut microbiota composition (16S rRNA) and SCFAs were analyzed in fecal samples.

Key Results: Irritable bowel syndrome symptoms consistently improved after 3 weeks of LFD, and significantly more participants reported symptom relief in response to placebo (80%) than FOS (30%). Serum levels of proinflammatory IL-6 and IL-8, as well as levels of fecal bacteria (*Actinobacteria*, *Bifidobacterium*, and *Faecalibacterium prausnitzii*), total SCFAs, and n-butyric acid, decreased significantly on the LFD as compared to baseline. Ten days of FOS supplementation increased the level of these bacteria, whereas levels of cytokines and SCFAs remained unchanged.

Conclusions and Inferences: Our findings support the efficacy of a LFD in alleviating IBS symptoms, and show changes in inflammatory cytokines, microbiota profile, and SCFAs, which may have consequences for gut health.

KEYWORDS

FODMAPs, gut microbiota, irritable bowel syndrome, proinflammatory cytokines, short-chain fatty acids

1 | INTRODUCTION

Irritable bowel syndrome (IBS) is a functional gastrointestinal (GI) disorder, characterized by recurrent abdominal pain or discomfort and altered bowel habits, often accompanied by abdominal bloating and/or distension.¹ The etiology of IBS remains unknown, but several pathogenetic factors seem to be involved, including enhanced visceral sensitivity and altered intestinal motility. Irritable bowel syndrome is increasingly viewed as a dysfunction of the “gut-brain axis” that should be considered in a broader biopsychosocial context.^{2,3}

Although about three quarters of IBS patients state that food triggers symptoms, dietary treatments have, until recently, not been a central part of therapy. There is, however, an increasing interest in using diet in the management of IBS, and emerging evidence supports the efficacy of a diet with reduced content of fermentable, short-chain carbohydrates.⁴ The so-called low-FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet (LFD) is a pathophysiologically based and promising approach to dietary management in IBS.^{5–7} The general strategy is to avoid foods high in FODMAPs with the intention of limiting the delivery of osmotically active and readily fermentable substrates to the intestine, thereby minimizing luminal distension.^{8,9} In addition, it has been proposed that the mechanisms by which symptom response is mediated, may partly be through inducing changes in the gut microbiota, in GI endocrine cells, in immune function, and/or in the intestinal barrier.^{5,10–12} However, due to a lack of placebo-controlled studies and studies conducted over longer periods of time, there is a current uncertainty regarding sustained effects on symptom relief and long-term health consequences.¹³ Fermentable carbohydrates provide an energy source for the GI bacteria, and fermentation products such as short-chain fatty acids (SCFAs) provide nutrition for the colonic mucosa, and interact with the immune system. Hence, a LFD might elicit alterations in the GI microbiota and its fermentation products.¹⁴

In the present randomized, double-blinded, placebo-controlled crossover study, we aimed to investigate the effects of a low-FODMAP vs high-fructo-oligosaccharides (FOS) diet on symptoms, immune activation, gut microbiota composition, and SCFAs in patients with IBS.

2 | MATERIAL AND METHODS

2.1 | Participants

Twenty consecutive patients with diarrhea-predominant IBS (IBS-D) or mixed IBS (IBS-M) according to Rome III criteria¹ and moderate to severe symptoms (IBS Severity Scoring System [IBS-SSS]¹⁵ score of >175) were recruited in the trial. The recruitment took place in the autumn of 2015 via the outpatient clinic at the section of gastroenterology at Haukeland University Hospital, Bergen, and through a list of patients waiting for “IBS school” participation (a 2-day interdisciplinary approach about living with IBS). Exclusion criteria comprised pregnant or lactating women, patients with constipation-predominant IBS (IBS-C) or an IBS-SSS score of <175, patients using probiotics or antibiotics, and

Key Points

- A low-FODMAP diet is a widespread management strategy in irritable bowel syndrome, but biological consequences are only partly known, and an important placebo effect on symptom relief has not been excluded.
- In a double-blinded interventional study, we found that restricting the ingestion of fermentable carbohydrates significantly reduced the severity of IBS symptoms, as well as additional gastrointestinal and comorbidity symptoms.
- A low-FODMAP diet reduced the levels of selected pro-inflammatory cytokines, altered gut bacterial profile, and reduced fecal levels of short-chain fatty acids, which may have consequences for gut health.

patients presenting with organic diseases explaining their GI symptoms. Laxatives and antidiarrheal agents were not allowed during the trial.

2.2 | Study protocol

The study used a randomized, double-blinded, placebo-controlled, crossover design to compare a low-FODMAP vs high-FOS diet. Participants were instructed by Hustoft TN, both in oral and written form, to implement and follow a LFD for 9 weeks. After 3 weeks they were randomized according to a computer-generated list to receive a supplement of either A (16 g of FOS [FODMAP] per day for 10 days), a 3-week washout, and then B (16 g of maltodextrin [placebo] per day for 10 days), or the reverse sequence (hence, AB or BA). The allocation sequence was double blinded to minimize placebo and nocebo effects on symptom alterations. Test substances were carbohydrates given as powders, identical in appearance, color, and taste. Participants were instructed to reconstitute the powder in 500 mL water and distribute the drink into three meals daily (morning, midday, and evening). It was stressed that the drinks should be taken with meals rather than drinking on empty stomachs. The latter was important as the physiological response to FODMAPs delivered in liquid might be different from their delivery within foods, and we wanted to reflect a realistic intake of FODMAPs (ie, mainly through the ingestion of foods).¹⁶ Preliminary testing on FOS was performed on six healthy subjects (five women and one man) to ensure that the amount of 16 g was not too high. This was of importance due to the risk of producing a biased result showing benefits of a LFD, as most people experience uncomfortable GI symptoms when ingesting large quantities of undigested carbohydrates.⁵ The test period lasted for 2 days and the drinks were well-tolerated by all except for the experience of mild to moderate bloating and increased flatulence (well-known and often accepted side effects of fermentation¹⁷). The respective times for data and sample collection were as the following: at baseline, after 3 weeks of LFD, after 10 days with supplementation (A/B), after the 3-week washout period, and finally, after 10 last days with supplementation (A/B).

The study protocol was approved by the Regional Ethic Committee of Northern Norway (2015/594), and performed according to the Declaration of Helsinki and the BMJ guidelines. All participants provided written informed consent prior to study commencement.

2.3 | Symptom assessment

Changes in symptoms were assessed using the validated IBS-SSS questionnaire.¹⁵ Irritable bowel syndrome-SSS consists of five items that measure, on a 100-point visual analog scale (VAS), the severity of abdominal pain, frequency of abdominal pain, severity of abdominal distension, dissatisfaction with bowel habits, and interference with life in general, over the preceding 10 days.¹⁵ Each question generates a maximum score of 100, leading to a possible overall score of 0–500 with a higher score indicating more severe symptoms.¹⁸ The overall score is used to classify IBS severity and to evaluate response to treatment, of which a reduction of at least 50 points is considered as a significant treatment response.^{15,18} The questionnaire was also supplemented with a “global” question about satisfaction with symptom relief with regard to the last 7 days.

In addition to IBS-SSS, participants were asked about selected additional GI complaints and comorbidity symptoms. These included nausea and/or vomiting, early satiety, headache, backache, tiredness, belching and/or passing gas, heartburn, frequent or sudden urge to urinate, pain in the thighs, and pain in the muscles and joints. These respective questions were also measured using a 100-point VAS.

2.4 | Blood samples

Blood samples were centrifuged for at least 30 minutes within 2 hours after phlebotomy and serum was frozen for later analyses at -80°C . The Cytokine Human Ultrasensitive Magnetic Luminex[®] platform (Thermo Fisher Scientific, Life Technologies AS, Oslo, Norway) was used for quantifying proinflammatory cytokines, interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNF- α) in serum.

2.5 | Fecal samples

At each visit, the participants were given a sample container for feces collection prior to the next appointment, and were told to store it in a refrigerator for a maximum of 3 days before delivery. Afterward, the fecal samples were kept in a freezer at -80°C until analysis for microbiota composition and metabolites (SCFAs).

2.6 | Gut microbiota

Microbiota analysis was performed using the GA-map[™] DysbiosisTest (Genetic Analysis AS, Oslo, Norway). The GA test is based on regular molecular biology techniques comprising fecal sample homogenization and mechanical bacterial cell disruption, combined with chemical cell lysis, automated total bacterial genomic DNA extraction using magnetic beads, 16S rRNA PCR DNA amplification covering the variable regions V3–V9, probe labeling by single nucleotide extension,

hybridization to complementary probes coupled to magnetic beads, and signal detection using BioCode 1000A 128-Plex Analyzer (Applied BioCode, Santa Fe Springs, CA, USA).¹⁹ A dysbiosis index (DI) above 2 (maximum 5) indicate a microbiota that differs from the reference group (DI 1–2: non-dysbiosis, DI 3: moderate, DI 4–5: severe dysbiosis).¹⁹

2.7 | Short-chain fatty acids

The fecal material (0.5 g) was homogenized after addition of distilled water containing 3 mmol/L of 2-ethylbutyric acid (as internal standard) and 0.5 mmol/L of H_2SO_4 ; 2.5 mL of the homogenate was vacuum distilled, according to the method of Zijlstra et al.,²⁰ as modified by Hoverstad et al.²¹ The distillate was analyzed with gas chromatography (Agilent 7890 A; Agilent, CA, USA) using a capillary column (serial no. USE400345H, Agilent J&W GC columns; Agilent, CA, USA) and quantified using internal standardization. Flame ionization detection was employed. The following SCFAs were analyzed: major SCFAs (acetic, propionic, and n-butyric acids) and minor SCFAs (i-butyric, n-valeric, i-valeric, n-caproic, and i-caproic acids). The results were expressed in mmol/kg wet weight.

2.8 | Statistical analyses

Statistical analyses were performed using SPSS Statistics version 23.0 (IBM, Armonk, NY, USA) and GraphPad Prism version 6.0 (GraphPad Software, Inc., San Diego, CA, USA). Comparisons were assessed by the use of Student's *t* test (mean) or Wilcoxon's signed rank test (median) in the cases of non-parametric distributions. McNemar's test was used for categorical data and correlations were assessed with Pearson's and Spearman's correlation coefficients. Cluster analysis and principal component analyses (PCA) were used to visualize the microbiota data, showing the extent to which microbial communities share branch length. *P* values of less than or equal to 5% were regarded as statistically significant. Reported *P* values and 95% CI are based on two-sided tests unless otherwise specified.

3 | RESULTS

3.1 | Demographic and clinical data

Baseline characteristics are presented in Table 1. Twenty patients were included of whom 75% were women. Mean age was 34.6 years (range 18–52 years). Eleven patients presented with IBS-D (55%) and nine with IBS-M (45%). Duration of IBS symptoms was from 1.5 to 40 years (mean 15.8 years). Four patients had moderate IBS (IBS-SSS score of 175–300) and 16 had severe IBS (IBS-SSS score of >300). Eleven patients were overweight (BMI: $>25\text{ kg/m}^2$) and nine were normal weight (BMI: $18.5\text{--}25\text{ kg/m}^2$). One patient was in need of several pharmacological agents with potential GI side effects, and another used loperamide. However, both patients held their respective dosages constant throughout the study period. All 20 study participants completed the trial.

TABLE 1 Demographic and clinical data in the study population

Participants	n=20
Male/Female	5/15
Mean age (range), years	34.6 (18–52)
Mean duration of symptoms (range), years	15.8 (1.5–40)
IBS severity	
Mild	n=0
Moderate	n=4
Severe	n=16
IBS subtype	
IBS diarrhea predominant	n=11
IBS mixed	n=9
Median Bristol Stool Form (range)	4.8 (1.5–6.5)
Mean BMI (range)	26.9 (19.5–37.2)
IBS-associated disorders	
Migraine	n=6
Fibromyalgia	n=2
Reflux	n=3
Depressed mood, depression, and/or anxiety (present or history)	n=6
Other disorders	n=9
A history of gastroenteritis	n=8

3.2 | Symptoms

There was a significant improvement in all IBS symptoms after 3 weeks of LFD (Figure 1) with a mean reduction in IBS-SSS total score of 163.8 (one-sided 95% CI: 135.7–500). All participants had an overall reduction of at least 50 (range 57–275). When supplementing the LFD with FOS or placebo, significantly more participants reported symptom relief in response to placebo (80%) than FOS (30%; $P=.013$). The mean overall IBS-SSS score and 4 of 5 subscores were significantly higher in response to FOS compared with placebo (Figure 1), with no evidence of a carryover or a period effect. There was a large intersubject variability in the responses to FODMAP provocation (FOS vs placebo) as compared to FODMAP reduction (baseline vs LFD; Figure 1).

Seven of the 10 additional GI complaints and comorbidity symptoms were significantly improved from baseline to 3 weeks of LFD, with the greatest mean VAS reduction observed for belching and/or passing gas (39.4; $P<.001$), followed by nausea and/or vomiting (24.3; $P<.001$) and tiredness (21.2; $P=.001$). Early satiety, heartburn, and pain in the thighs were not improved at this time, but a significant relief was found for the two first symptoms after 7 weeks dietary adherence. When comparing symptom severity between the FOS and placebo periods, VAS scores of nausea and/or vomiting, headache, and belching and/or passing gas were significantly higher in response to FOS ($P=.002$, $P=.04$, and $P<.001$, respectively).

3.3 | Proinflammatory cytokines

Baseline median (range) values of IL-6, IL-8, and TNF- α were 0.11 pg/mL (0.00–1.87), 3.30 pg/mL (0.00–15.70), and 0 pg/mL (0.00–1.15), respectively. Levels of IL-6 and IL-8, but not TNF- α , decreased significantly after 3 weeks of LFD, with a median reduction in 0.065 pg/mL ($P=.001$) and of 2.95 pg/mL ($P<.001$), respectively (Figure 2). The levels did, however, not change in response to FOS supplementation.

3.4 | Gut microbiota composition

At baseline, 50% of the patients were dysbiotic, and this was increased to 60% after LFD and decreased to 55% after FOS supplementation. Dysbiosis index was mainly 3 (moderately dysbiotic; $n=6$ at baseline and $n=9$ after LFD). A PCA scores plot of the microbiota profiles corrected for sample differences indicates that the LFD treatment systematically shifts the microbiota of a number of patients. The following FOS treatment again changes this shift and takes the microbiota back to levels more similar to the baseline measurement (Figure 3).

Significant reductions from baseline to 3 weeks of LFD were found for *Clostridium* ($P=.002$), *Faecalibacterium prausnitzii* ($P=.009$), *Bifidobacterium* ($P=.008$), *Megasphaera* ($P=.044$), *Pediococcus* ($P=.024$), and *Actinobacteria* ($P=.015$), while a significant increase was found for *Dorea* ($P=.027$). Moreover, significant increases from LFD to FOS were found for *Bacteroides* ($P=.019$), *Bifidobacterium* ($P=.009$), *Actinobacteria* ($P=.004$), *F. prausnitzii* ($P=.030$), and *Firmicutes* ($P=.048$), while a significant reduction was found for *Proteobacteria* ($P=.036$) and *Mycoplasma hominis* ($P=.048$). Hence, *F. prausnitzii*, *Actinobacteria*, and *Bifidobacterium* abundance were significantly altered in both dietary interventions (Figure 4).

3.5 | Short-chain fatty acids

Levels of total SCFAs and n-butyric acid decreased following a LFD as compared to baseline ($P=.035$ and $P=.014$, respectively), but SCFA levels were otherwise not significantly altered when comparing values from samples obtained at baseline, following LFD, and after FOS supplementation (Figure 5).

3.6 | Correlations

There was no correlation between reductions in the levels of proinflammatory cytokines, *Bifidobacterium*, *Actinobacteria*, *F. prausnitzii*, or SCFAs with symptom relief from baseline to LFD, nor was there any correlation between the levels of *F. prausnitzii* and n-butyric acid.

4 | DISCUSSION

In the present randomized, double-blinded, placebo-controlled cross-over study, we observed that dietary FODMAP content was related to symptom severity, as well as levels of proinflammatory cytokines and

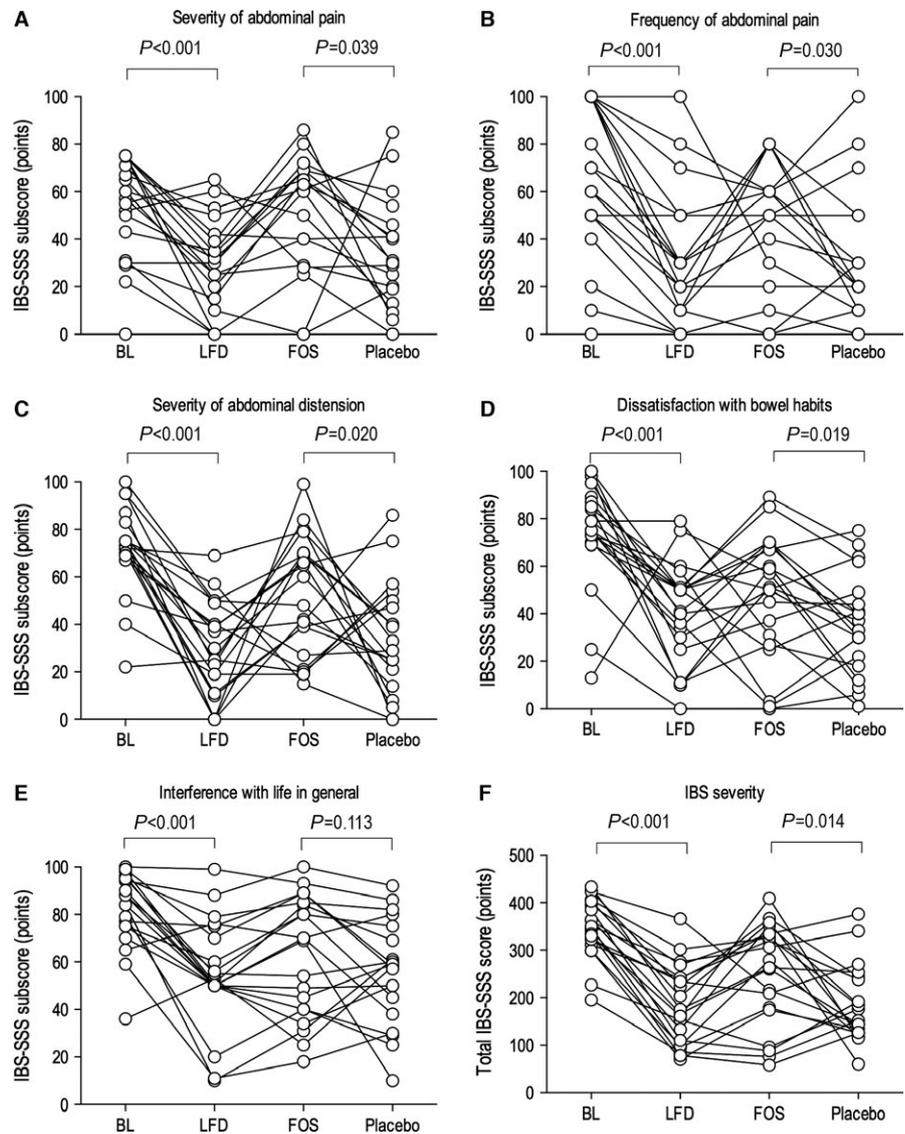


FIGURE 1 Individual responses in IBS-SSS subscores (A–E) and total score (F) registered at baseline (BL), following a 3-week low-FODMAP diet (LFD), and after a 10-day fructo-oligosaccharides (FOS) and placebo intervention in patients with irritable bowel syndrome ($n=20$). There was a significant improvement in all symptoms after implementing the LFD, and four of the five symptoms (A–D) were significantly worsened in response to FOS compared with placebo. The responses to a global restriction of FODMAPs (BL vs LFD) were much more consistent among participants compared to a 10-day provocation of a single FODMAP

microbiota composition and function. Although a small study population, all IBS-SSS symptoms significantly improved on the LFD, and four of the five symptoms were significantly worsened in response to FOS compared with placebo. The differences observed were irrespective of the order and time the interventions were given. Hence, the efficacy of a LFD seems to be beyond placebo effects, and symptom relief is thus likely to be sustainable. In the following, we will discuss possible implications of these findings.

After 3 weeks of restricting high-FODMAP foods, there was an average reduction in the overall IBS-SSS score of 164, considerably greater than the 50 points reduction considered to be a meaningful treatment response. Visual analog scale scores were reduced to a level that arguably is considered good symptom control, confirmed through an overall patient satisfaction of 85%. These results are consistent with those reported in the current literature, albeit somewhat higher.^{5,12,22} Although IBS symptoms were significantly worsened in response to FOS, the severity was not comparable to the symptom level observed at baseline. As participants were following a LFD when taking the supplement, their ingestion of the other respective FODMAPs were kept

low. The symptom worsening observed was therefore an effect related to a high-FOS content in the diet, while baseline measurements reflected a broader high-FODMAP diet. The different response to these two diets supports the notion that a collective restriction has greater and more consistent effect than a limited restriction.²² Despite a statistically significant difference in symptoms between the FOS and placebo periods, the mean differences were not very large numerically, probably explaining the lack of a significant difference in symptoms' interference with life in general. There was also a large intersubject variability in response to the two supplements, in contrast to a much more consistent response from baseline to LFD. These observations may support the view of performing a reintroduction phase to individualize the diet.

Baseline severity of symptoms not included in the diagnostic criteria but frequently present in IBS patients were surprisingly high. Interestingly, restricting high-FODMAP foods improved several of these symptoms. An improvement in belching and/or passing of gas was not a surprising finding as a restriction of FODMAPs leads to a reduced production of intestinal gases. However, the explanations

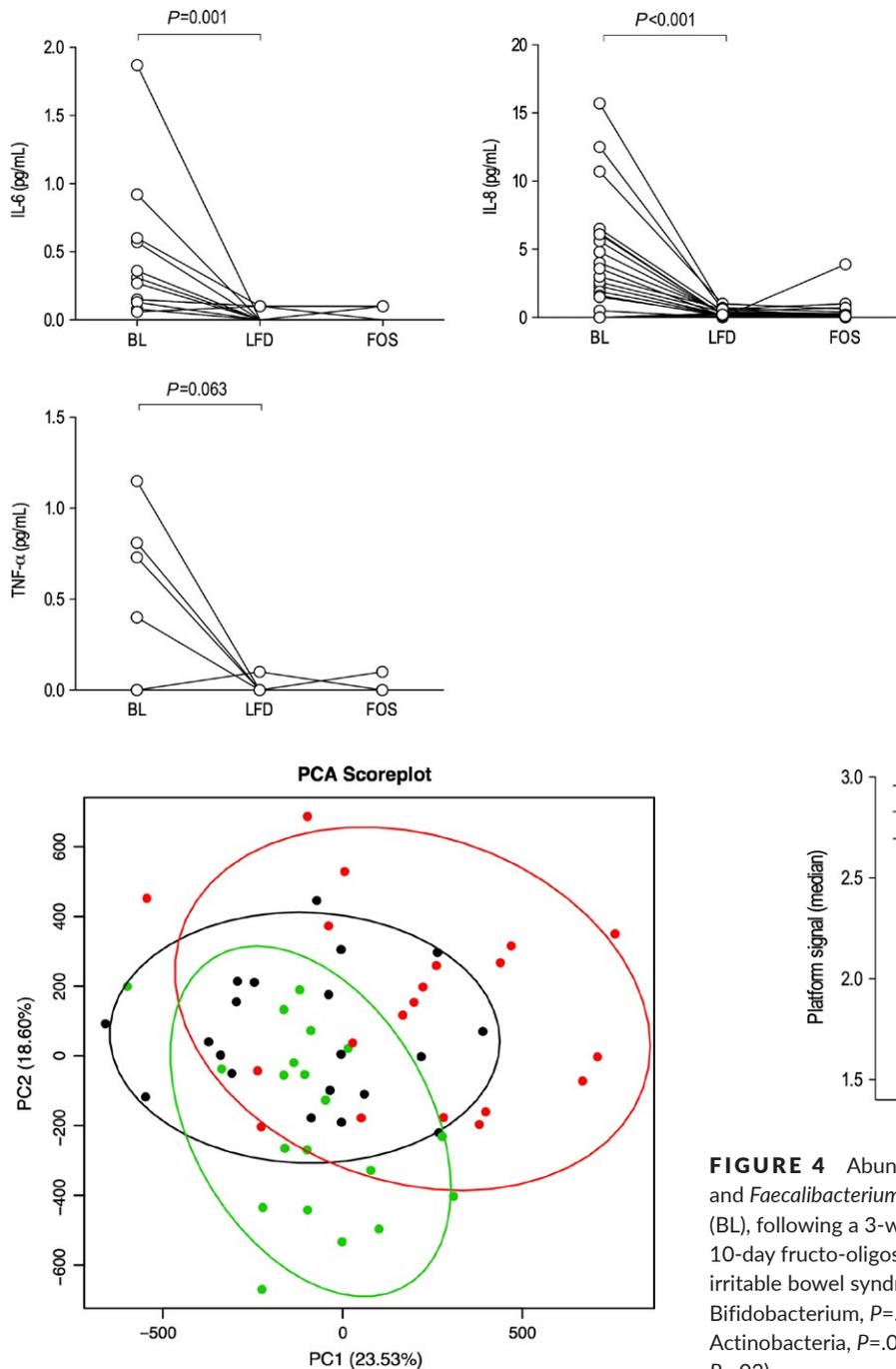


FIGURE 2 Concentrations (pg/mL) of proinflammatory cytokines in blood samples collected at baseline (BL), following a 3-week low-FODMAP diet (LFD), and after a 10-day fructo-oligosaccharides (FOS) intervention in patients with irritable bowel syndrome ($n=20$). There was a significant decrease in IL-6 and IL-8, but not TNF- α , after 3 weeks of LFD

FIGURE 3 Scores for the first two principal components analyses (PCA) of fecal microbiota in a group of patients with irritable bowel syndrome ($n=20$). Data are shown at baseline (BL; black dots), after 3 weeks on a low-FODMAP diet (LFD; green dots), and after a 10-day fructo-oligosaccharides (FOS) provocation (red dots). Each dot represents data from one patient. The first two PCs account for 42.1% (sum of PC1 and PC2) of the variation. The colored ellipses demonstrate the 95% confidence interval for PC1 and PC2. The PCA scores show that both LFD and FOS supplementation seem to have an effect on the gut microbiota; the cohort shifts down during LFD and back up after FOS intervention.

behind the effects observed in upper GI symptoms such as heartburn and nausea, as well as comorbidities such as headache and tiredness, remain unknown. Earlier studies have also evaluated benefits of a

FIGURE 4 Abundance of luminal Actinobacteria, Bifidobacterium, and *Faecalibacterium prausnitzii* in stool samples collected at baseline (BL), following a 3-week low-FODMAP diet (LFD), and after a 10-day fructo-oligosaccharides (FOS) provocation in patients with irritable bowel syndrome ($n=20$; BL vs LFD: Actinobacteria, $P=.015$; Bifidobacterium, $P=.008$; and *F. prausnitzii*, $P=.009$; and LFD vs FOS: Actinobacteria, $P=.004$; Bifidobacterium, $P=.009$; and *F. prausnitzii*, $P=.03$)

LFD beyond the relief in GI IBS symptoms, but the results have been inconsistent.^{6,16,23}

With respect to the gut microbiota, we observed that most patients were moderately dysbiotic at baseline, as consistent with a previous study.¹⁹ Furthermore, it was observed that patients tended to become more dysbiotic on the LFD. The phylum Firmicutes is known to contain several fermenting bacteria, and it was expected to find a reduction in fermenting bacteria after implementing the LFD as these are being starved. An increase in other bacteria might also be expected because when some species are reduced, other bacteria may occupy their metabolic niches. In our study *Clostridium*, *F. prausnitzii*, *Megasphaera*, and *Pediococcus*, which are all members of the phylum

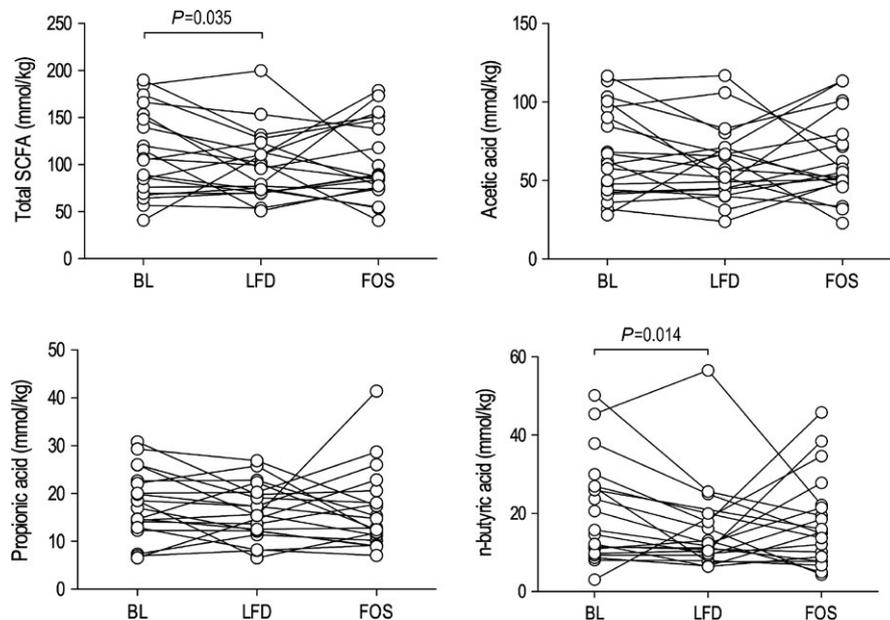


FIGURE 5 Concentrations (mmol/kg) of major short-chain fatty acids (SCFAs) in fecal samples collected at baseline (BL), following a 3-week low-FODMAP diet (LFD), and after a 10-day fructo-oligosaccharides (FOS) provocation in patients with irritable bowel syndrome ($n=20$)

Firmicutes, decreased significantly from baseline to 3 weeks of LFD. Interestingly, Dorea, another member of this phylum increased significantly. Moreover, a significant reduction in Bifidobacterium was observed from baseline to LFD, with a subsequent increase in response to FOS supplementation. The latter was, however, accompanied by symptom relief and worsening, respectively, a paradoxical finding considering studies showing beneficial effects of Bifidobacteria probiotics.^{24,25} This observation has already been seen in other studies.^{14,26} However, despite a simultaneous reduction in levels of Bifidobacterium and symptom severity, this does not establish a relationship between cause and effect. In fact, levels of Bifidobacterium did not correlate with symptom severity, and whether a Bifidobacteria probiotic in addition to LFD enhances symptom response is yet to be determined.¹⁴ Reduced levels of Bifidobacterium and *F. prausnitzii* after 3 weeks of LFD are the effects most likely due to a reduced availability of prebiotic fructans (including FOS) and galacto-oligosaccharides.¹⁴ Increased levels of these bacteria in response to FOS supplementation support this supposition. Considering promising beneficial effects of both Bifidobacterium and *F. prausnitzii*,^{27,28} consequences of possible reduced levels when following a LFD need to be investigated. Moreover, as diet-induced changes in the microbiota are known to be rapidly reversed, it should be evaluated if potential changes persist once FODMAPs are reintroduced to tolerance.¹⁴ In contrast to the reduced level of Actinobacteria observed in the present trial, McIntosh et al.²⁶ reported a higher richness and diversity of this phylum when comparing low- and high-FODMAP diets. Interestingly, the latter was observed despite a decrease in Bifidobacteria relative abundance.

We observed significantly reduced levels of total SCFAs and n-butyric acid after 3 weeks of LFD. The latter may indicate a reduced level and/or activity of butyrate-producing bacteria. *Faecalibacterium prausnitzii* is a major commensal butyrate producer, and has been labeled as a biomarker of intestinal health in adults.²⁸ Despite a simultaneous reduction from baseline to LFD in the levels of n-butyric acid and this bacterium, the changes were not significantly correlated. In

contrast to the present study, neither Halmos et al.²⁹ nor Staudacher et al.¹⁴ observed reduced levels of SCFAs when restricting FODMAPs. When supplementing LFD with FOS, n-butyric acid did not increase significantly. This finding contrasts with a recently published study on healthy adults by Clarke et al.,³⁰ using fructan supplementation in a similar amount as in the present study. However, different duration of supplementation (10 days vs 28 days) may explain this discrepancy. Interestingly, animal studies have shown that rectal instillation of n-butyric acid may induce visceral hypersensitivity,³¹ whereas studies in humans are conflicting.^{32,33} It remains to be evaluated if a restriction of FODMAPs leads to a reduction in the level of n-butyric acid, and whether this plays a part in the symptom relieving effect by reducing visceral sensitivity. More important, it should be evaluated if a reduced level of n-butyric acid is detrimental considering putative beneficial effects on colonic health and diseases.^{33,34}

Several studies have suggested imbalanced cytokine signaling as an important pathogenetic factor of IBS, but the alterations found have not been consistent.^{35,36} Prior research has especially suggested elevated systemic IL-6, IL-8, and TNF- α in IBS patients relative to healthy controls.³⁶⁻⁴¹ Our data do not support this finding, as baseline values did not seem to deviate from those measured in the general population. Interestingly, 3 weeks of LFD reduced serum levels of proinflammatory IL-6 and IL-8. To our knowledge, no previous studies have evaluated effects of a LFD on cytokine profiles. However, McIntosh et al.²⁶ also reported changes in immune activation in response to a LFD, using histamine as a measurement of inflammatory state. Proinflammatory cytokines and histamine are both synthesized and secreted by inflammatory cells, suggesting a reduced level and/or activity of these cells when restricting FODMAPs. Paradoxically, in the present study, both n-butyric acid and selected bacteria associated with anti-inflammatory properties were reduced concomitantly with a reduced level of immune activation.^{34,42}

We acknowledge that there are limitations in the present study design. Due to the lack of a control group when comparing baseline

diets with LFD, we cannot conclude that the effects observed were exclusively due to dietary changes. Despite the use of a double-blinded design, it was difficult to ensure a 100% successful blinding, as participants were likely to be influenced by changes in symptoms. Participants were initially told that they were about to receive powders both high and low in FODMAPs, an informative message likely to create expectations. With regard to the low-FODMAP powder, there was no significant placebo response as only minimal differences, in both directions, were observed between the LFD and placebo periods. However, as discussed by Shepherd et al.,¹⁶ the placebo response rate is typically higher when reduction rather than induction of symptoms is the endpoint of interest. While a LFD restricts all FODMAPs collectively, we chose only one of them (FOS) to represent a high-FODMAP diet. However, using only one FODMAP enabled us to evaluate FOS effects alone on the measured parameters and demonstrated differences in FOS sensitivity among IBS patients. It would have been interesting to do the same provocation with other isolated FODMAPs to consider which of them matter most for the individual patient. With regard to the performed correlation analyses, one should be aware of potential unreliable results due to small sample size.

In conclusion, the results from the present controlled study support the efficacy of a LFD in reducing functional GI symptoms in patients with IBS-D or IBS-M, as significantly more participants reported symptom relief in response to placebo (80%) than FOS (30%). The changes observed in proinflammatory cytokines IL-6 and IL-8, microbiota profile with alterations in Actinobacteria, Bifidobacteria, and *F. prausnitzii*, as well as decreased fecal levels of SCFAs may have consequences for gut health. Gastroenterologists should consider the LFD as a strategy to alleviate IBS symptoms, although the implications of long-term changes in microbiota composition and function require further elucidation.

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DISCLOSURE

None.

AUTHOR CONTRIBUTION

TH, JGH, TNH, GAL, and SOY designed the project; TNH conducted the research; KAB, TNH, JV, GAL, and Genetic Analysis AS, Oslo, analyzed the data; JGH, TH, TNH, GAL, and JV wrote the paper. All authors read and approved the final manuscript.

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