Human milk oligosaccharide supplementation in irritable bowel syndrome patients: A parallel, randomized, double-blind, placebo-controlled study

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Abstract

Objectives: Human milk oligosaccharides safely and beneficially impact bifidobacteria abundance in healthy adults, while their effects in patients with irritable bowel syndrome (IBS) are unknown. Hence, we aimed to determine the dose of 4:1 mix of 2'-O-fucosyllactose and Lacto-N-neotetraose (2'FL/LNnT) that increases fecal bifidobacteria abundance without aggravating overall gastrointestinal symptoms in IBS patients in a randomized, double-blind, controlled study. Additionally, the impact of 2'FL/LNnT on the fecal bacterial profile was assessed.

Methods: Irritable bowel syndrome patients diagnosed according to the Rome IV criteria received placebo (glucose), or 5 g or 10 g 2'FL/LNnT for 4 weeks followed by a four-week follow-up period. Gastrointestinal Symptom Rating Scale-IBS was used to assess gastrointestinal symptom severity; fecal microbiota composition was evaluated by GA-map™ Dysbiosis Test.

Results: Of the included 60 patients, two (one placebo and one 10 g) discontinued prematurely. Fecal bifidobacteria abundance was increased at week 4, but not at week 8, in the 10 g group compared to the other groups. Severity of overall or individual gastrointestinal symptoms did not differ between the groups at week 4 or 8, and no symptom deterioration was seen in any of the groups. The 10 g dose influenced overall fecal microbiota composition, and responders—defined as bifidobacteria increase ≥50%—could be discriminated from non-responders based on fecal microbiota modulation.

Conclusions: The 10 g dose of 2'FL/LNnT induced an increase in the beneficial Bifidobacterium spp. without aggravating gastrointestinal symptoms in patients with IBS. This approach may be worthwhile to modulate gut microbiota of IBS patients toward a healthier profile.
1 | INTRODUCTION

The pathophysiology of irritable bowel syndrome (IBS), a functional bowel disorder characterized by altered bowel habits and abdominal pain, remains ambiguous. However, subgroups of IBS patients show an unbalanced intestinal microbiota profile associated with severity of symptoms. Moreover, a recent systematic review of gut microbiota in IBS described a decrease in the genus Bifidobacterium in some patients. Potentially, the gut microbiota composition may be modulated by diet, prebiotics, probiotics, synbiotics, and even non-absorbable antibiotics. To date, only a few intervention studies have explored the effect of prebiotics, non-digestible compounds that stimulate specific bacterial growth, in IBS patients, with diverse study outcomes. Trans-galactooligosaccharides have been shown to promote bifidobacteria growth, in IBS patients, with diverse study outcomes. Furthermore, it was demonstrated that short-chain fructo-oligosaccharides improved digestive comfort. In contrast, another study demonstrated that short-chain fructo-oligosaccharides had no therapeutic value for IBS patients, and a recent meta-analysis of 11 randomized controlled studies concluded that prebiotics did not improve GI symptoms but increased bifidobacteria.

Human milk oligosaccharides (HMO) are unique, complex glycans found in high concentrations (5 - 25 g/L HMO) in human breast milk. They are selective substrates for specific intestinal bacteria, protect against infection by blocking pathogen attachment to epithelial cells, promote immunomodulatory activity, and improve gut barrier function. The most abundant structural classes are fucosylated HMO (35%-50%) and neutral core HMO (42%-55%). 2'-O-Fucosyllactose (2'FL) and Lacto-N-neotetraose (LNnT), respectively, are major component of each class. HMO reach the colon intact and undergo fermentation by specific bacteria, such as bifidobacteria. A 2:1 mix of 2'FL and LNnT has been demonstrated to influence the fecal microbiota composition, including an increase in bifidobacteria in infants. Furthermore, supplementation with either 2'FL or LNnT alone or in a mix was safe and promoted fecal bifidobacteria in healthy adults. However, there are no published studies of the effects of HMO in IBS patients.

We hypothesized that a 4:1 mix of 2'FL/LNnT will be well tolerated and able to beneficially modulate gut microbiota in IBS patients, in particular inducing an increase in Bifidobacterium spp. abundance. Hence, we aimed to determine the dose of a 4:1 mix of 2'FL/LNnT which increases fecal bifidobacteria abundance, without negatively influencing GI symptoms. Additionally, we assessed the effects on other IBS-related symptoms and on the general fecal microbiota profile.

2 | MATERIAL AND METHODS

2.1 | Study design and population

This was a phase II, parallel, double-blind, randomized, placebo-controlled study in adult male and female IBS patients (n = 61 at randomization). The patients were randomized (1:1:1) into three groups, consuming either active product (two groups) or a placebo product (one group) for four weeks with a four-week follow-up period after the end of the intervention. The design of the study is graphically presented in Figure 1.

The study population comprised patients (18-75 years of age) with IBS symptoms of at least moderate severity (IBS Symptom Severity Scale (IBS-SSS) score ≥175) and diagnosed according to the Rome IV criteria (See Appendix S1 for further description of the main selection criteria). The patients were characterized based on the predominant bowel habit: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), or IBS with mixed bowel habits (IBS-M) (See Appendix S2.1). Patients were recruited from the outpatient clinic specialized in functional GI disorders at Sahlgrenska University Hospital (Gothenburg, Sweden) or by advertising in the local newspaper. The study followed the principles of Declaration of Helsinki II and was approved by the Regional Ethical Review Board in Gothenburg (Reg. No. 548-16). The study was registered at www.ClinicalTrials.gov (NCT02875847). Written informed consent was obtained from all patients at the screening visit before any study procedures. No major amendments were made after study commencement. The study was funded by Glycom A/S (Hersholm, Denmark).
2.2 | Study intervention

Patients were randomized to receive one of the three different interventions for 4 weeks: placebo, 5 g or 10 g doses of 2'FL/LNnT (1:1 ratio) stratified on IBS subtypes (IBS-C, IBS-D or IBS-M), using a web-based program developed by Gothia Forum (Gothenburg, Sweden). The active product, a mix of 2'FL/LNnT in a 4:1 mass ratio, was supplied by Glycom A/S and provided as a 5 g or 10 g white powder in single-serve sachets and distributed in boxes containing 30 sachets, packaged by HB Medical (Hørsholm, Denmark). As placebo, sachets containing 5 g of powdered glucose (Dextropur, Dextro Energy GmbH & Co.) were provided. Both, active product and placebo were similar with regard to appearance, smell, and taste (sweet). All packages were identical except from a unique ID-number. Patients were instructed to dissolve the sachet contents in a minimum of 100 mL of liquid and consume it daily with breakfast. Furthermore, all subjects were advised to keep to their usual diet and medication regimen throughout the study.

After inclusion, patients visited the clinic three times. At visits, the patients completed validated clinical questionnaires electronically to assess the severity of GI and psychological symptoms, including the Gastrointestinal Symptom Rating Scale for IBS (GSRS-IBS),23 the IBS-SSS,21 a stool diary based on the Bristol Stool Form scale,24 and the Hospital Anxiety and Depression Scale (HADS)25 (See Appendix S2 for more detailed description). Bodyweight (kg), height (cm), adverse events, changes in medication or diet, and compliance were recorded. Patients taking the full dose for ≥24 days during the 28-day supplementation period were considered fully compliant. A physical examination was done by a study physician. Study subjects, investigators, and sponsor were all blinded to treatment allocation until the end of the study.

The patients received equipment for fecal sampling including cooling kits. Samples were collected and kept at −20°C freezers maximum 4 days prior to a visit (visits 2, 3, and 4). Fecal samples were stored at −80°C after each visit.

2.3 | Bifidobacteria and microbiota profiling

The fecal samples were analyzed using a commercially available genome-based microbiota test (GA-map™ Dysbiosis Test, Genetic Analysis AS). Briefly, fecal samples were homogenized prior to mechanical disruption of bacterial cells, total bacterial genomic DNA isolated with magnetic beads, and hypervariable regions V3-V9 amplified by 16S rRNA polymerase chain reaction (PCR), using fifty-four probes, validated to be GI disorder-specific, targeting more than 300 bacterial strains.26 Probe intensity signal correspondent to fecal bacterial abundance was detected and measured by BioCode 1000A 128-Plex Analyzer (Applied BioCode).

2.4 | Data analyses; primary, secondary, and exploratory endpoints

The primary endpoint was to determine the dose of 2'FL/LNnT that increases Bifidobacterium spp. abundance without aggravating GI symptoms, measured by GSRS-IBS. As secondary efficacy endpoints, IBS severity, measured by IBS-SSS, bowel habits (stool consistency), and anxiety and depression were assessed. Moreover, the effect on fecal microbiota and the proportion of responders, defined as a patient with a bifidobacteria abundance increase ≥50% at the end of the intervention period, relative to baseline were explored. All eligible and randomized patients were included in the analysis of the comparisons of clinical questionnaires (intention-to-treat analysis, ITT; n = 60). Statistical analyses were chosen based on normality of distribution determined by boxplots. For the effect on the fecal microbiota profile, patients who completed the intervention were included (per-protocol analysis, PP; n = 58). All analyses were performed after unblinding.

2.5 | Statistical analyses

This study was explorative, and no formal sample size calculation was performed. Instead, it was based on the sample size of a previous study in healthy adults which showed microbiota modulation after supplementation with HMO.20 For this study, the sample size was slightly increased to address the inherent background noise of microbiota results and the statistical analysis significance. Natural logarithmic transformation was used for the microbiota data to obtain a more homogeneous variance. All tests used an alpha of .05 as cutoff for significance and two-sided confidence intervals.
Each primary endpoint of logarithmic Bifidobacterium spp. abundance and global GSRS-IBS was analyzed using restricted maximum-likelihood mixed modeling for repeated measurements (SAS, Proc Mixed) with SAS version 9.4, SAS Institute Inc. The model included fixed categorical effects of group dose, visit, and group dose-by-visit interaction. A compound symmetry covariance structure was used to model the within-subject errors. Baseline (visit 2) was included as continued fixed covariate for between-group comparisons and in the categorical effect of visit for within-group comparisons. For the exploratory endpoint, the microbiota profile was assessed by multivariate analysis by using SIMCA® software (version 15.0.2, MKS Umetrics AB). Orthogonal partial least-squares-discriminant analyses (OPLS-DA) were performed within placebo, 5 g, and 10 g 2’FL/LnNt groups to identify microbiota profile differences between baseline and week 4 and fold change (week 4/baseline) of responders and non-responders.

Additional information about statistical methodology can be found in Appendix S3.

Demographic information at baseline was analyzed based on normality of distribution and compared between the groups. Categorical variables were analyzed with chi-square test, while continuous variables were analyzed by using one-way ANOVA with Bonferroni’s correction or Kruskal-Wallis test.

3 | RESULTS

3.1 | Demographics and clinical characteristics

In total, 73 IBS patients were screened for eligibility between January 2017 and April 2018, and of these, 61 patients (41 women and 20 men) were randomized into one of the three intervention groups:
placebo (n = 21), 5 g (n = 20), and 10 g (n = 20) 2’FL/LNnT. Fifty-nine out of 61 patients completed the study to the follow-up visit (week 8). There were no major changes in diet. Fifty-eight patients reported consumption of the study product for ≥24 of the 28 days (Table S1). One subject did not fulfill the compliance criteria but took full dose for 23 days and was compliant to all other study procedures, and was therefore included in all the analysis. Two patients, one from the placebo group and one from the 10 g 2’FL/LNnT group, discontinued prematurely after 2 weeks of intervention due to increased IBS symptoms. Additionally, after completion of the intervention, one patient in the placebo group was diagnosed with Crohn’s disease. This patient was excluded from the ITT and PP analyses (Figure 2). No other major protocol violations were reported. Therefore, 40 women and 20 men (mean age 45 (range 19-73) years) of all IBS subtypes (14 IBS-C, 26 IBS-D, 20 IBS-M) were included in the ITT analysis (Table 1). The exploratory analysis (PP analysis) excluded the patients who discontinued the intervention (n = 58). Sex, age, body mass index, and IBS subtype classification did not differ between the three groups at baseline. However, patients in the 5 g 2’FL/LNnT group demonstrated a lower GSRS-IBS total score at baseline compared to the placebo and 10 g 2’FL/LNnT groups (P = .03) (Table 1).

### 3.2 Dose effect of 2’FL/LNnT on Bifidobacterium spp

The 10 g 2’FL/LNnT group presented with a higher abundance of fecal bifidobacteria after four weeks intervention as compared to placebo

| TABLE 1 Demographic data of the randomized patients at baseline |
|---------------------------------------------------------------|
| **Placebo (n = 20)** | **5 g 2’FL/LNnT (n = 20)** | **10 g 2’FL/LNnT (n = 20)** | **P value** |
| Sex (Female: Male) | 14:6 | 11:9 | 15:5 | .38 |
| Age, y | 46 (21-71) | 42 (19-67) | 46 (26-73) | .55 |
| Body mass index, kg/m² | 24.9 (23.3-26.5) | 24.0 (21.7-26.3) | 24.3 (22.6-26.0) | .38 |
| IBS subtype, number of patients | | | | |
| IBS-C | 5 | 5 | 4 | .93 |
| IBS-D | 8 | 9 | 9 | .95 |
| IBS-M | 7 | 6 | 7 | .95 |
| GSRS-IBS total score | 50.2 (10.1) | 45.2 (8.0) | 52.6 (8.4) | .03 |

*Abbreviations: 2’FL/LNnT, 4:1 HMO mix of 2’-O-fucosyllactose and Lacto-N-neotetraose; GSRS-IBS, Gastrointestinal Symptoms Rating Score for IBS patients; IBS-C, constipation-predominant; IBS-D, diarrhea-predominant IBS; IBS-M, IBS with mixed loose and hard stools.

*Age shown as mean and range (min-max).

*Data shown as mean (25th-75th percentile).

*Data shown as mean (SD). Differences between groups shown in bold (P < .05).
and 5 g 2’FL/LNnT groups (Figure 3A). However, no differences in fecal bifidobacteria abundance between groups were detected at week 8 (Figure 3B). Within-group comparisons of changes at week 4 and week 8, respectively, relative to baseline, demonstrated an increase in *Bifidobacterium* spp. abundance in the 10 g 2’FL/LNnT group after the intervention but not after the follow-up (Figure 3C). The 5 g 2’FL/LNnT group did not show a change at week 4 but had decreased bifidobacteria abundance at week 8 compared to baseline. No changes were seen in the placebo group at any time point (Figure 3C).

### 3.3 Dose effect of 2’FL/LNnT on IBS-related symptoms

The overall mean of GRSRS–IBS total score at baseline was 49.29 (9.42), implying that on average patients reported “mild to moderately severe discomfort” on the five main domains of GI symptoms. No differences in overall GI symptom severity (GRSRS–IBS total score) between groups were identified at week 4 (Figure 4A) or week 8 (Figure 4B). Within-group comparisons at week 4 and week 8 relative to baseline demonstrated no aggravation of symptoms in any of the groups with the active product. In fact, GI symptoms were improved at week 8, but not week 4, in the 10 g 2’FL/LNnT group (Figure 4C). GI symptoms were improved at week 4 and week 8 in the placebo group, but no changes were seen in the 5 g 2’FL/LNnT group. None of the individual domains of GRSRS, that is, abdominal pain, bloating, constipation, diarrhea, and satiety differed between groups at baseline or week 4. Within-groups comparisons detected a decrease in the severity of bloating and diarrhea in the placebo group at week 4, but no differences in the other groups. Additionally, no significant changes were observed at week 8 in the groups, except for a decrease in bloating in the 5 g 2’FL/LNnT group and in abdominal pain in the 10 g 2’FL/LNnT group (Table 2). Furthermore, there were no differences between groups or within groups at week 4 or week 8 regarding IBS symptom severity (IBS–SSS). However, the placebo and 5 g groups demonstrated a tendency toward milder symptoms (See Table S2, Figure S1). Regarding bowel habits, placebo showed a decrease in the proportion of abnormal stool consistency (BSFS 1-2 and BSFS 6-7) at week 4 compared with baseline, which was not seen in the active groups. However, all intervention groups demonstrated improved bowel habits after eight weeks compared to
baseline (See Table S3). Lastly, anxiety and depression subscales did not show any differences between groups at baseline, week 4, or week 8 (See Table S4).

3.4 | Effect of 2’FL/LNnT on microbiota profile

According to multivariate factor discriminant analysis by OPLS-DA, the microbiota profile of patients in the placebo (Figure 5A) and 5 g 2’FL/LNnT (Figure 5B) groups could not differentiate between baseline and week 4, as demonstrated by poor fitness ($R^2 Y < 0.5$) and predictability ($Q^2 < 0$). In contrast, the microbiota profile of the 10 g 2’FL/LNnT group was able to differentiate week 4 from baseline ($R^2 Y = 0.55$), although the predictability was poor ($Q^2 = -0.80$) (Figure 5C). The bacterial taxa most important for driving the separation in the 10 g 2’FL/LNnT group were Firmicutes A, Clostridia, Actinobacteria, Bifidobacterium spp., Alistipes, Bacteroides spp., Prevotella spp., and Parabacteroides spp., which were all increased
**FIGURE 6** Microbiota profile fold change in responders and non-responders. (A, B, C) Orthogonal partial least squares-discriminant analysis (OPLS-DA) scatter plot showing the difference between responders (green) and non-responders (yellow) based on the fold change (week 4/baseline) of the 29 most discriminatory genera (VIP ≥ 0.9) of responders and non-responders in (A) placebo, (B) 5 g 2’FL/LNnT, and (C) 10 g 2’FL/LNnT groups. (D) OPLS-DA loading column plot showing the 29 most discriminatory microbiota genera fold change (week 4/baseline) differentiating the microbiota composition profiles of responders and non-responders in the 10 g 2’FL/LNnT group. *Bifidobacterium* spp. excluded. *Firmicutes* A and B, *Lactobacillus* spp. A, and *Streptococcus* spp. A and B probes target specific families within *Firmicutes* phylum, and species the genus of *Lactobacillus* and *Streptococcus*, respectively. A and B probes do not overlap. Error bars correspond to 95% confidence range. Asterisks identify statistically significant bacteria taxa *P < .05; **P < .01; ***P < .001*. 

**Responders**  
**Non-responders**
at week 4 as compared to baseline (Figure 5D). Some bacterial taxa showed a significant change during the intervention within the placebo or 5 g group (See Table S5), although these changes were not reflected in modulation of the overall microbiota profile.

3.5 | Microbiota profile in 2’FL/LNnT responders and non-responders

In the full study cohort, 24 patients (41%) met the criteria for response, that is, ≥50% increase of bifidobacteria abundance, whereas 34 patients (59%) were non-responders. The number of responders increased with the dose of 2’FL/LNnT, resulting in five responders in the placebo group (26%), seven responders in the 5 g 2’FL/LNnT (35%), and 12 responders in the 10 g 2’FL/LNnT group (63%). Differentiation of responders and non-responders was determined based on the modulation of the microbiota profile during the 4 weeks of intervention period (fold change; week 4/baseline). Bifidobacterium spp. was removed from this analysis to reduce skewness. For the placebo and 5 g 2’FL/LNnT groups, good fitness was achieved, although with poor predictability (Figure 6A,B).

However, the 10 g 2’FL/LNnT group demonstrated good fitness and predictability, that is, good discrimination between responders and non-responders (Figure 6C). The bacterial taxa most important for differentiating responders from non-responders in the 10 g 2’FL/LNnT group were high abundance of Actinobacteria, Eubacterium hallii, Eubacterium biforme, Lactobacillus spp. A, and Coprobacillus cateniformis (Figure 6D). However, the microbiota profile before the start of the intervention could not predict the treatment response in any of the groups (See Table S6).

4 | DISCUSSION

In this study, daily intake of 10 g 2’FL/LNnT increased abundance of fecal Bifidobacterium spp. without negatively influencing GI symptoms in patients with IBS. Further, the product was well tolerated as no worsening of IBS symptoms, bowel habits, anxiety, or depression were detected. Moreover, the dose of 10 g 2’FL/LNnT influenced overall fecal microbiota composition, and responders, defined by bifidobacteria increase ≥50%, could be discriminated from non-responders based on the microbiota composition.

Several factors are considered to influence the pathophysiology of IBS, and growing evidence shows that the intestinal microbiota plays a key role. A well-balanced intestinal microbiota, comprising beneficial members such as bifidobacteria, is crucial to ensure the production of important compounds that improve intestinal health. HMO have been shown to increase bifidobacteria abundance in infants and healthy adults, but their ability to alleviate symptoms in IBS patients is unknown. The study was designed to determine the dose of a 4:1 mix of 2’FL/LNnT that could increase fecal bifidobacteria abundance, without aggravating GI symptoms. The ratio used aimed to reflect the proportion of these two oligosaccharides in human breast milk. The study was not designed to detect improvement and included only lower doses than 20 g as IBS patients are considered more sensitive than healthy subjects. Similar to healthy adults, the four-week intake of 5 and 10 g 2’FL/LNnT in adult IBS patients was well tolerated and did not deteriorate GI symptoms, as assessed by GSRS-IBS total score and individual items of GSRS-IBS. Surprisingly, the placebo group showed a modest tendency to improve GI symptoms. Importantly, the intervention did not increase gas production, a potential risk previously raised due to the non-absorbable properties of other prebiotics. Only two patients, one from the placebo group and one from the 10 g 2’FL/LNnT group, discontinued the study due to worsening of GI symptoms. Thus, the low dropout rate together with lack of GI symptom deterioration in IBS patients completing the intervention suggests that the product was well tolerated, as the study was expected to demonstrate.

2’FL and LNnT have been identified as substrates for Bifidobacterium spp., in both formula-fed infants and healthy adults. Higher levels of bifidobacteria in elderly seem to correlate with healthy status and longevity, whereas reduction in this genus has been associated with several conditions, including IBS, inflammatory bowel diseases, and metabolic disorders. This study showed, similar to a previous study in a healthy cohort, that a daily intake for four weeks of 10 g, but not 5 g, 2’FL/LNnT increased fecal bifidobacteria abundance in IBS patients. The lack of persistent effects during the washout period may indicate the necessity of continuously supplementation for maintaining increased bifidobacteria abundance.

Consumption of prebiotics induces growth of specific bacteria, but has also been shown to modulate the overall gut microbiota composition. In this line with this, the results from the current trial suggest that consumption of 10 g 2’FL/LNnT not only increased Bifidobacterium spp. abundance but also modulated the overall gut microbiota profile. In the 10 g 2’FL/LNnT group, increased abundance of Actinobacteria (class that includes Bifidobacterium spp., Alistipes, Bacteroides spp., and Prevotella spp. was seen after four weeks. Interestingly, these bacterial taxa have previously been demonstrated to be in lower abundance in IBS patients relative to healthy individuals, so restoring the abundance of these bacteria may be associated with improved health. However, the study design did not allow for investigating whether these specific bacteria were directly metabolizing 2’FL/LNnT or influenced by cross-feeding. In parallel, despite significant modulation of some bacterial taxa within the placebo and the 5 g 2’FL/LNnT group was observed, these populations might not have been relevant enough to impact the overall gut microbiota profile.

As part of the secondary endpoints, microbiota modulation in responders and non-responders was compared. According to previous experience from determining fecal microbiota composition over time in ulcerative colitis patients in remission (manuscript in preparation), the normal individual monthly variation of Bifidobacterium
spp. abundance is <30%. We therefore chose to define response to the intervention as ≥50% increase (after the intervention period) of relative abundance of Bifidobacterium spp. The overall microbiota composition was differentially modulated by the HMO mix in responders and non-responders, and the difference was most evident in the 10 g 2’FL/LNnT group. The modulation of microbiota profiles might reflect higher abundance of HMO utilizing bifidobacteria in responders compared to non-responders at the start of the intervention. Despite this, the fecal microbiota could not predict response to the intervention. Although bifidobacteria was removed from the analysis, the model can have been influenced by Actinobacteria class, including Bifidobacterium spp., but also other bacterial taxa that utilize 2’FL/LNnT as substrate, directly or indirectly. In fact, Eubacterium spp. has been shown to have feeding interactions with Bifidobacterium spp. during HMO degradation. Unexpectedly, a small proportion (five out of 14) of patients fulfilled the responder definition in the placebo group. Studies have suggested that diet impacts on gut microbiota composition, which may account for a bifidogenic effect within some patients within the placebo group. Moreover, since healthy subjects were not included in this study, we cannot evaluate if the microbiota profile of IBS patients responding to the HMO mix changes toward a profile resembling the healthy population. Nevertheless, increased abundance of Bifidobacterium, as well as Eubacterium spp. and Lactobacillus spp., as seen among responders in this study, is often associated with saccharolytic processes and improved gut health. Hence, HMO could potentially be used for IBS patients with unbalanced gut microbiota, to re-establish a healthy microbiota profile.

To our knowledge, this is the first study aiming to determine the daily dose of 2’FL/LNnT that increases abundance of Bifidobacterium spp. without negatively influencing GI symptoms in IBS patients. Even with its strengths and promising results, there are limitations with the study. Being a truly exploratory study, the size of the study population was small and did not allow for subgroup analyses regarding IBS subtypes, which likely also influenced the fit and predictive ability of microbiota profile analysis models. Moreover, the study did not intend to assess a clinical effect of the intervention but to evaluate the tolerability of the study product. The inclusion of patients of all IBS subtypes, potentially with different disease driving mechanisms, may also have influenced the study outcome. However, since this is the first study exploring effects of the 2’FL/LNnT on IBS patients, we did not want to exclude any IBS subtype. Further, the relatively short intervention period did not allow to study the long-term effect, and this should be considered for future studies, since longer treatment periods may lead to an effect on both bifidobacteria and clinically relevant endpoints. Moreover, the use of GA-map technology might have excluded some relevant taxa that could be detected by other techniques, as 16S rRNA gene sequencing. However, the commercially available test used in the study simplified the establishment of gut bacterial profiles and may also be applied in a clinical setting.

In conclusion, four-week daily intake of 10 g 2’FL/LNnT increased the abundance of Bifidobacterium spp. without aggravating GI symptoms in IBS patients. This dose was well tolerated and did not induce worsening of IBS symptoms, bowel habits, anxiety, or depression, and most patients completed the four weeks of intervention without significant side effects. Moreover, the 10 g 2’FL/LNnT modulated overall fecal microbiota composition, and responders, defined by bifidobacteria increase ≥50%, could be discriminated from non-responders based on fecal microbiota modulation. Thus, this intervention might be favorable in order to restore gut microbiota of IBS patients toward a healthier profile.

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CONFLICT OF INTEREST

Declaration of personal interests: CI, IA, MKM, and JS do not have conflict of interest to declare. LKV, IDA, BM, and DS are employed at Glycom A/S, Denmark. HT has served as Consultant/Advisory Board member for Almirall, Allergan, Danone, and Shire. LÖ has served as Consultant/Advisory Board member for Genetic Analysis AS, and as a speaker for Ferring Pharmaceuticals, Takeda, AbbVie, and Meda. MS has served as a Consultant/Advisory Board member for AstraZeneca, Danone Nutricia Research, Nestlé, Almirall, Allergan, Albireo, Genetic Analysis AS, Biocodex, Glycom, Arena and Shire, and as a speaker for Tillotts, Takeda, Menarini, Kyowa Kirin, Allergan, Shire, Biocodex, Alimentary Health, AlfaSigma, and Almirall; and has received unrestricted research grants from Danone, and Ferring Pharmaceuticals.

AUTHOR CONTRIBUTIONS

Guarantor of article: MS.

Specific author contributions: CI involved in data acquisition, assembling of database, analyses and interpretation of microbiota database, and drafting the manuscript. HT and IA collected the study subject materials and finalized the manuscript. JS and IDA involved in project planning and finalizing the manuscript. MKM interpreted the data, drafted and finalized the manuscript. LKV, DS, and BM involved in project planning, interpretation of data, and finalizing the manuscript. LÖ involved in project planning, interpretation of data, drafting, and finalizing the manuscript. MS involved in project planning, interpretation of data, material acquisition, drafting, and finalizing of manuscript. All authors have approved the final draft submitted.

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**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.