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Efficacy and safety of galacto-oligosaccharide in the treatment of functional constipation: randomized clinical trial†

Jae-Hwan Lee,[a] Geun-Bae Kim,[a] Kisoo Han,[b] Eun-Jin Jung,[c] Hyung Joo Suh [d,e] and Kyungae Jo *[e]

The efficacy and safety of galacto-oligosaccharides (GOS) in treating functional constipation were evaluated in a four-week randomized, double-blind clinical trial on 63 patients who met Rome IV criteria (34 GOS, 29 placebo group). The number of bowel movements per day and changes in the shape of bowel movements in the treatment group significantly improved compared to those in the control group after four weeks. The Patient Assessment Constipation Quality of Life questionnaire showed that satisfaction with constipation significantly increased in the treatment group. The levels of *Bifidobacterium* sp. and *Lactobacillus* sp. significantly increased after four weeks of GOS treatment compared to those measured at baseline. No significant adverse drug reactions were identified in any indicator except for pulse rate. Thus, the prebiotic GOS can be safely used in foods and pharmaceuticals to alleviate symptoms of functional constipation by improving the intestinal flora.

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1. Introduction

Constipation is a common digestive tract symptom. Although the reported prevalence varies depending on the specific definition used, constipation is estimated to affect 5–20% of the total population, with a higher incidence in women and the older population.[1,2] Constipation can be defined as an intestinal disorder that includes various symptoms such as a decrease in the frequency of defecation, feeling that the anus is blocked or exerting excessive force during defecation, and requiring manipulations such as pressing the anus with a finger for defecation.[3] Constipation itself can be secondary to a disease and medications.[1] According to the Rome IV criteria, functional disorders associated with constipation are classified as functional constipation, opioid-induced constipation, functional defecation disorder, and irritable bowel syndrome (IBS) with predominant constipation.[4] Constipation and its associated symptoms are often intermittent and mild, but in some cases can be chronic, difficult to treat, and debilitating.[3]

With the development of various digestive tract function tests, many studies have recently been conducted to explore the pathophysiology of constipation, resulting in the identification of mechanisms of action of constipation medications. However, many patients with constipation continue to prefer treatments with unknown mechanisms or self-treatment regimens. In general, in cases of chronic constipation, the recommended treatment is to increase the amount of fiber in the diet or consume a fiber preparation as a supplement.[5] The primary categories of drug therapy for constipation include osmotic laxatives such as magnesia, sorbitol, and polyethylene glycol; stimulant laxatives such as bisacodyl or senna; and gastrointestinal motility promoters such as tegaserod.[6] However, in many cases, laxatives are associated with various serious side effects such as water and electrolyte loss, secondary aldosteronism, protein-losing gastroenteritis, osteomalacia, allergic reactions, renal failure, liver failure, lipid pneumonia, and malabsorption; therefore, their long-term use is not recommended.[7,8]

To improve constipation, the use of food to induce changes in the intestinal microbiota and prevent or alleviate symptoms is a safe method that can be maintained over the long term. Changes in the gut microbiota can be induced by the direct intake of probiotics, with *Bifidobacterium* and *Lactobacillus* being the most widely used probiotics in humans.[9,10] In particular, galacto-oligosaccharide (GOS) is a growth-promoting

[a]*Department of Animal Science and Technology, Chung-Ang University, Anseong 17546, Republic of Korea*
[b]*NeoCremar Co. Ltd, Seoul 05702, Republic of Korea*
[c]*Department of Food and Biotechnology, Korea University, Sejong 30019, Republic of Korea*
[d]*Transdisciplinary Major in Learning Health Systems, Graduate School, Korea University, Seoul 02841, Republic of Korea*
[e]*Department of Integrated Biomedical and Life Science, Graduate School, Korea University, Seoul 02841, Republic of Korea. E-mail: kyungae11@korea.ac.kr; Fax: +82-2-921-7207; Tel: +82-2-940-2764*

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substance for *Bifidobacterium* that inhibits the proliferation of harmful bacteria such as *Escherichia coli*, *Enterococcus*, and putrefactive bacteria in the intestine, and is therefore classified as a prebiotic.[11,12] GOS is a non-digestible functional oligosaccharide produced by the β-galactosidase enzyme using lactose as the raw material.[13] Intake of these prebiotics increases the composition of the intestinal microbiota relative to the growth of *Bifidobacterium* and *Lactobacillus*. Changes in the intestinal microbiota have been reported to reduce the occurrence of intestinal diseases, improve mineral absorption, and suppress the development of colorectal cancer, thereby improving overall health.[14,15] Therefore, in this study, the efficacy and safety of GOS were evaluated in adults with functional constipation.

2. Materials and methods

2.1 Participants and study design

The subjects of this study were male and female adults aged between 19 and 75 years who satisfied the Rome IV diagnostic criteria for functional constipation (Table S1†). Patients who met the Rome IV diagnostic criteria for IBS (Table S2†) were excluded. This clinical trial was approved by the Institutional Bioethics Committee of Woosuk University Hospital of Oriental Medicine (IRB No. H1910-01). All subjects signed a consent form for the clinical trial after receiving a detailed explanation of the purpose and content of the study.

This clinical trial aimed to evaluate the clinical efficacy and safety of GOS for chronic constipation. Seventy participants were randomly assigned to the treatment and control groups, with 35 participants in each group. Double blinding was performed until all subjects completed the trial. The treatment group orally took three capsules of GOS twice a day for four weeks within 30 min after meals; the components per capsule were 333.33 mg GOS, 58.67 mg maltodextrin, 4 mg silicon dioxide, and 4 mg magnesium stearate. The control group received three capsules of the control drug (placebo) twice daily for four weeks; the components per placebo capsule were 392 mg maltodextrin, 4 mg silicon dioxide, and 4 mg magnesium stearate.

2.2 Symptom assessment

Two weeks before drug administration, at the first (baseline) visit, written informed consent, body measurements [weight and body mass index (BMI)], dietary survey, lifestyle survey, vital signs (blood pressure, pulse, and body temperature), physical examination, and constipation symptom survey (Rome IV criteria) data were obtained.

Bowel habits were assessed using the Bowel-Related Symptom Questionnaire. The number of bowel movements was calculated as the average number of bowel movements per day and the bowel movement time was calculated as the time required for one bowel movement. The degree of strain during defecation, sensation of incomplete evacuation, sensation of anorectal obstruction or blockage, and abdominal discomfort were scored on a scale of 0–10 (0 = "very mild" and 10 = "very severe"). Satisfaction with defecation was scored from 0 (very dissatisfied) to 10 (very satisfied).[16] Stool morphology was measured using the Bristol Stool Form Scale (BSF) developed by Heaton *et al.*[17] The BSF is a tool that can determine the hardness of feces by visually observing the fecal shape and characteristics. Scores of 1–2 represent constipation, scores of 3–4 represent normal stools, and scores of 5–7 represent a tendency toward diarrhea.

Health-related quality of life was assessed using the Short-Form Health Survey-36 (SF-36) tool developed by Ware and Sherbourne.[18] The SF-36 consists of 36 questions from the following eight domains: physical functioning, role limitation-physical, role-emotional, vitality, mental health, social functioning, bodily pain, and general health. Each scale was calculated by converting to 100 points; the higher the score, the higher the level of health-related quality of life.

Constipation-related quality of life was measured using the Patient Assessment Constipation Quality of Life (PAC-QOL) questionnaire developed by Marquis *et al.*[19] The PAC-QOL consists of self-reported items examining the effect of constipation on quality of life in the last two weeks, comprising 28 questions encompassing the following seven domains: symptoms related to constipation, frequency of influence on daily life, degree of influence on daily life, emotional functioning, mood, social functioning, and satisfaction felt in relation to constipation. Scores are given on a scale of 1–5, with 1 indicating "not at all", 2 indicating "a little bit", 3 indicating "moderately", 4 indicating "quite a bit", and 5 indicating "extremely". A lower total score indicates a higher constipation-related quality of life.

2.3 Radiographic evaluation of colon transit time

Colonic transit time was examined using a radiopaque pellet in all participants before and after the start of the study. Plain abdominal radiography was performed 72 h after the patient ingested the 20 radiographic markers (M.I.Tech, Seoul, Korea).[20]

2.4 Gut microbiome analysis

Approximately 10–20 g of feces was collected from two or more different areas before and on the last day of test drug intake. The sample was placed in a collection container provided by the research team and delivered to the research team in a refrigerated state. Total DNA was extracted from the feces using a QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer instructions. Fecal microbiota were analyzed using pyrosequencing, a high-throughput analysis technique, according to amplification of the hypervariable V1–V3 region of the 16S rRNA gene using the Roche 454 GS FLX Titanium instrument (454 Life Sciences, Branford, CT, USA).[21] Microbiota analysis was conducted at the Korea Research & Institute of Biomedical Science (KRIBS; Daejeon, Korea).

2.5 Safety evaluation

For the safety evaluation, all adverse reactions that occurred after administration of the clinical trial drugs were recorded, and vital signs measurements (blood pressure, pulse, and body temperature), physical measurements (weight and BMI), hematology, blood chemistry assessment, urinalysis, and thyroid function tests were performed. The type and incidence

of adverse reactions and their relevance to clinical trial drugs were evaluated using statistical analyses.

2.6 Statistical analysis

All statistical analyses were performed using SAS Version 9.4 (SAS Institute). A two-sample *t*-test was conducted for comparisons of continuous variables, and Fisher's exact test for categorical variables. Statistical significance was set at $p < 0.05$, and all results are expressed as mean ± standard deviation.

3. Results

3.1 Characteristics of test subjects

A total of 7 out of 70 subjects dropped out of the study or terminated the trial early, leaving measurement results of a total of 63 subjects for statistical analysis. The treatment group consisted of 34 subjects, including 5 males and 29 females, with an average age of 36.41 ± 14.90 years (Table S3†). The control group consisted of 29 subjects, 4 males and 25 females, with an average age of 34.55 ± 13.55 years. There were no significant differences in the pre-trial characteristics such as demographic information, dietary intake, alcohol consumption, smoking status, and caffeinated drink intake, of the test subjects between the two groups.

3.2 Symptom evaluation

In the treatment group, the average number of defecations observed for one week increased by 0.36 times per day from 0.42 ± 0.20 at baseline to 0.78 ± 0.31 after taking GOS for four weeks (Fig. 1A). In addition, the treatment group that received GOS for four weeks showed a significant increase of 0.15 bowel movements per day compared to that of the control group (p = 0.0480). The time taken for one bowel movement was lower in the treatment group (6.29 ± 4.26 min) than that of the control group (7.47 ± 4.74 min) after four weeks, although the difference was not statistically significant (p = 0.3054; Fig. 1B). The Bristol Stool Scale score was significantly higher in the treatment group than in the control group after four weeks (4.06 ± 1.32 *vs.* 3.34 ± 1.17, p = 0.0283; Fig. 1C). Straining during defecation, sensation of incomplete evacuation, sensation of anorectal obstruction or blockage, and abdominal discomfort were all decreased in the treatment group compared to those of the control group, although the differences were not statistically significant (Table 1).

The results of the SF-36 survey showed no significant differences between the control and GOS groups in any of the eight domains (Table 2). Physical functioning and role-physical, role-emotional, and vitality scores increased in the GOS group compared to those of the control group, although the differences were not statistically significant.

In the PAC-QOL questionnaire, the score for the satisfaction felt in relation to constipation was significantly improved in the treatment group (2.86 ± 1.13) compared to that of the control group (3.49 ± 0.97) after 4 weeks (Table 3). Scores for symptoms related to constipation, frequency of influence on daily life, degree of influence on daily life, emotional functioning, mood, and social functioning all decreased in the treatment group compared to those in the control group, although the differences were not statistically significant.

3.3 Colon transit time

Colonic transit time was reduced by 21.41 h in the treatment group from 57.99 ± 12.37 h at baseline to 36.59 ± 18.99 h after four weeks of taking GOS (Fig. 2). In addition, the colonic

Table 1 Results of bowel habit survey

		Treatment (n = 34), mean ± SD	Control (n = 29), mean ± SD	p-Value
Straining during defecation	Baseline	6.24 ± 2.02	6.66 ± 2.21	0.4334
	4 weeks	4.74 ± 2.65	5.69 ± 2.44	0.1449
Sensation of incomplete evacuation	Baseline	4.79 ± 2.97	5.86 ± 2.64	0.1400
	4 weeks	3.59 ± 2.74	4.86 ± 2.68	0.0683
Sensation of anorectal obstruction or blockage	Baseline	4.15 ± 2.80	4.97 ± 2.31	0.2149
	4 weeks	3.38 ± 2.84	3.83 ± 2.85	0.5383
Abdominal discomfort	Baseline	4.50 ± 2.85	4.31 ± 2.79	0.7914
	4 weeks	3.41 ± 2.68	4.24 ± 2.23	0.1909
Satisfaction of defecation	Baseline	4.62 ± 2.75	3.21 ± 2.24	0.0312*
	4 weeks	5.91 ± 2.77	5.21 ± 2.53	0.2986

SD, standard deviation. p-Values are based on Fisher's exact test (*$p < 0.05$).

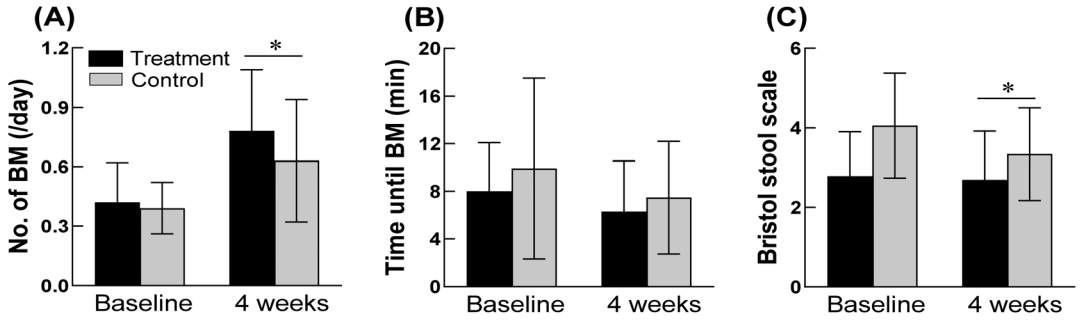

Fig. 1 Effects of galacto-oligosaccharides on number of bowel movements (BM) (A), time until BM (B), and Bristol stool scale (C). Values are presented as the mean ± standard deviation; p-values were calculated with Fisher's exact test comparing data between the treatment and control groups after four weeks (*$p < 0.05$).

Table 2 Results of the short-form 36 survey

		Treatment (n = 34), mean ± SD	Control (n = 29), mean ± SD	p-Value
Physical functioning	Baseline	85.15 ± 16.67	84.31 ± 20.95	0.2084
	4 weeks	90.57 ± 21.45	89.66 ± 15.06	0.4137
Role-physical	Baseline	86.03 ± 31.50	86.21 ± 24.63	0.9805
	4 weeks	93.81 ± 26.12	92.24 ± 22.26	0.9456
Role-emotional	Baseline	82.35 ± 34.07	85.06 ± 30.32	0.7424
	4 weeks	92.24 ± 36.76	91.95 ± 26.21	0.9072
Vitality	Baseline	56.03 ± 15.85	52.93 ± 18.00	0.4703
	4 weeks	60.85 ± 12.14	56.03 ± 14.78	0.3545
Mental health	Baseline	68.47 ± 17.86	71.31 ± 18.24	0.5356
	4 weeks	72.65 ± 20.85	74.07 ± 13.76	0.7259
Social functioning	Baseline	82.72 ± 18.97	89.66 ± 18.01	0.1441
	4 weeks	92.49 ± 20.12	93.53 ± 11.39	0.8559
Bodily pain	Baseline	76.76 ± 22.03	80.95 ± 21.98	0.4550
	4 weeks	84.98 ± 32.48	86.64 ± 19.62	0.9848
General health	Baseline	55.88 ± 18.44	59.14 ± 20.92	0.5140
	4 weeks	61.57 ± 22.96	62.59 ± 21.28	0.7574

SD, standard deviation. p-Values are based on Fisher's exact test.

Table 3 Results of the patient assessment constipation quality of life survey

		Treatment (n = 34), mean ± SD	Control (n = 29), mean ± SD	p-Value
Symptoms related to constipation	Baseline	3.03 ± 0.98	3.17 ± 0.68	0.5107
	4 weeks	2.00 ± 0.81	2.33 ± 0.86	0.7284
Frequency of influence on daily life	Baseline	2.88 ± 0.76	2.64 ± 0.65	0.1922
	4 weeks	1.74 ± 0.74	1.85 ± 0.66	0.5343
Degree of influence on daily life	Baseline	2.48 ± 0.81	2.37 ± 0.89	0.6163
	4 weeks	1.55 ± 0.67	1.61 ± 0.75	0.7144
Emotional functioning	Baseline	3.12 ± 0.87	3.06 ± 0.86	0.7669
	4 weeks	2.03 ± 0.79	2.39 ± 0.75	0.0685
Mood	Baseline	3.30 ± 0.85	3.32 ± 0.82	0.9331
	4 weeks	1.81 ± 0.85	2.18 ± 0.94	0.1068
Social functioning	Baseline	3.17 ± 0.99	3.23 ± 0.92	0.7947
	4 weeks	1.84 ± 0.81	2.11 ± 1.07	0.2561
Satisfaction felt in relation to constipation	Baseline	4.10 ± 0.76	4.15 ± 0.74	0.7984
	4 weeks	2.86 ± 1.13	3.49 ± 0.97	0.0216*

SD, standard deviation. p-Values are based on Fisher's exact test (*p < 0.05).

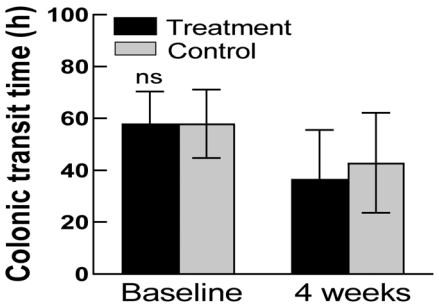

Fig. 2 Effects of galacto-oligosaccharides on the colonic transit time. Values are presented as the mean ± standard deviation; p-values were calculated with Fisher's exact test comparing data between the treatment and control groups after four weeks. ns, not significant (p > 0.05).

transit time was decreased in the treatment group compared to that in the control group at the end of the study, although the difference was not statistically significant (p = 0.2181).

3.4 Fecal evaluation

3.4.1 Changes in alpha-diversity of intestinal microbiota. Alpha-diversity was measured to compare and analyze the diversity index of intestinal microbial communities in the two groups. The Shannon index, which represents species diversity (Fig. 3A), showed no significant difference pre- and post-intake in the control and GOS groups. After 4 weeks, the control group showed significantly higher species diversity than the GOS group (p = 0.0248). There was no significant difference in the abundance coverage estimate, which represents species richness (Fig. 3B), between the control and GOS groups or pre- and post-intake in each group.

3.4.2 Composition of intestinal microbiota. Analysis of the changes in intestinal flora at the phylum level (Fig. 4) showed that the main strains in both the control and GOS groups were Firmicutes, with a relative abundance above 60% (Fig. 4). Actinobacteria and Bacteroidetes had a relative abundance of 16.59–17.51% and 13.33–16.92%, respectively. At baseline, Firmicutes levels in the control group were significantly lower than those in the GOS group (p = 0.0410). After intake, there was no significant difference in the relative abundance of Firmicutes between the two groups. In the control group, the relative abundance of Proteobacteria before ingestion was 1.10%, which was significantly lower than that detected after ingestion (1.74%; p = 0.0382). The F/B ratio, representing the ratio of Firmicutes (harmful bacteria in the intestine) to Bacteroidetes (beneficial bacteria), increased slightly in the placebo group after intake compared to that before intake, but the difference was not statistically significant. The F/B ratio in the GOS group decreased from 8.38 before intake to 5.45 after 4 weeks; however, the difference was not statistically significant. There was also no significant difference in the F/B ratio between the control and GOS groups after intake. However, GOS administration decreased the relative abundance of Firmicutes and increased that of Bacteroidetes.

Changes in the intestinal flora were measured at the genus level (Fig. 5). In the control group, *Bifidobacterium* sp., as beneficial bacteria in the intestine, showed a tendency to increase after 4 weeks compared with that measured before intake, although the difference was not statistically significant. However, in the GOS group, *Bifidobacterium* increased significantly after intake compared to that measured before intake (p = 0.0047; Fig. 5A). In addition, *Lactobacillus* showed a significant increase in the GOS group after 4 weeks compared to that measured before intake (p = 0.0182, Fig. 5B). The levels of *Bifidobacterium* and *Lactobacillus* did not differ significantly between the control and GOS groups either before or after intake. There was also no significant difference in *Lactococcus* and *Leuconostoc* levels before and after intake between the control and GOS groups (Fig. 5C and D). The level of *Clostridium*, a harmful bacterial genus, was significantly

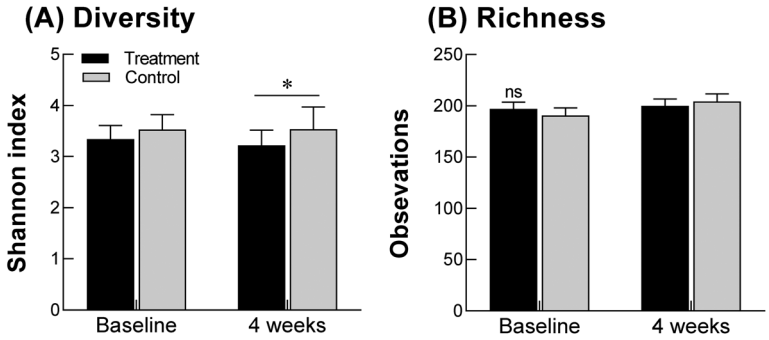

Fig. 3 Alpha-diversity analysis of fecal microbiota between control and GOS group. Data are expressed as the mean ± standard deviation. *$p < 0.05$ and ***$p < 0.001$ (Student's *t*-test); ns, not significant ($p > 0.05$).

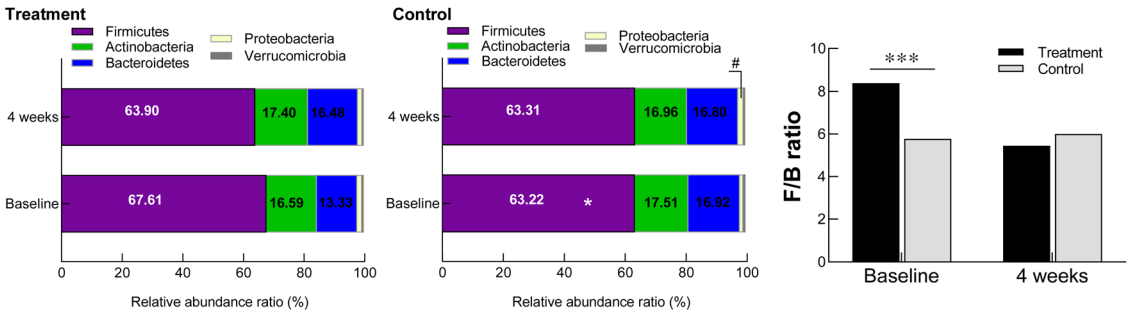

Fig. 4 Changes in intestinal microbiota at the phylum level. Data are expressed as the mean ± standard deviation. F/B ratio, Firmicutes/Bacteroidetes ratio. *$p < 0.05$ and ***$p < 0.001$ indicate significant differences between groups (Student's *t*-test); #$p < 0.05$ indicates a significant difference pre- and post-intake within groups (Student's *t*-test).

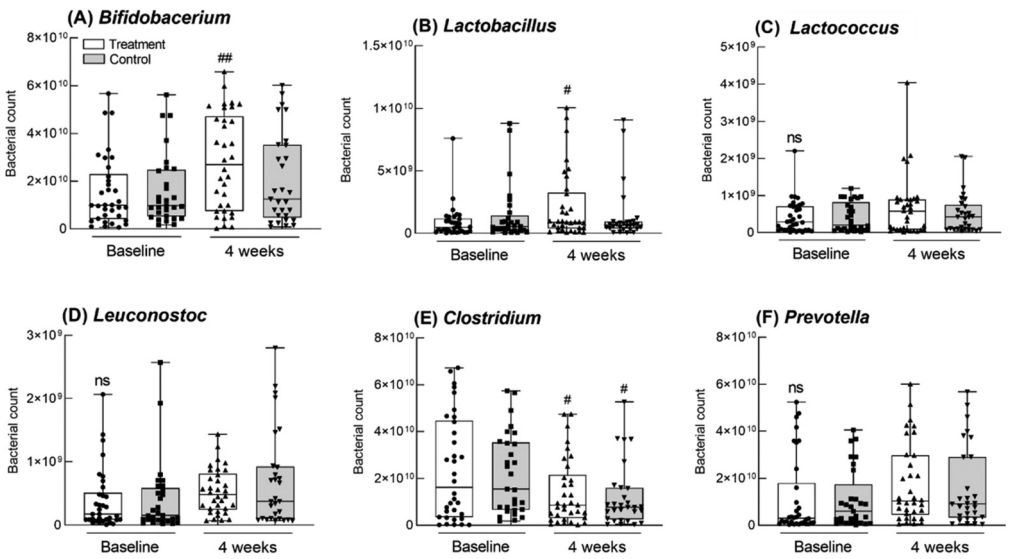

Fig. 5 Changes in intestinal microbiota at the genus level. Data are expressed as the mean ± standard deviation. #$p < 0.05$ and ##$p < 0.01$ indicate significant differences pre- and post-intake within groups (Student's *t*-test); ns, not significant ($p > 0.05$).

reduced in both the control and GOS groups after intake compared to that measured before intake ($p = 0.0210$ and $p = 0.0355$, respectively; Fig. 5E); however, there was no significant difference between the two groups after four weeks of drug intake. The level of *Prevotella*, another harmful bacterium, did not differ significantly between the groups (Fig. 5F). Therefore, at the genus level, GOS administration significantly increased *Bifidobacterium* and *Lactobacillus*, suggesting that GOS may

contribute to an increase in beneficial bacteria in the intestine that could help to improve constipation.

3.5 Safety evaluation

Subjects who were randomly assigned to the clinical trial and took the sample at least once were established as the safety set; a total of 63 subjects (34 in the treatment group and 29 in the control group) were included for the safety evaluation. Overall, two patients (5.9%) in the treatment group and three patients (10.3%) in the control group reported mild or moderate adverse effects; however, no severe adverse reactions were reported and there were no dropouts due to adverse reactions (Table S4†). Among the vital signs, pulse was significantly decreased in the treatment group (77.38 ± 11.66 beats per min) compared to that of the control group (83.59 ± 12.94 beats per min, p = 0.0498), whereas no significant differences were found between the groups in other vital signs, including systolic and diastolic blood pressure, body temperature, body weight, and BMI (Table S5†), or hematology (Table S6†) and blood chemistry (Table S7†) parameters.

4. Discussion

A total of 63 adult men and women were included in this double-blind study to evaluate the efficacy and safety of GOS or placebo intake for four weeks to improve constipation. Although the participants were recruited regardless of sex, there was a high proportion of women (85%) in each group. This distribution represents a common demographic feature in other chronic constipation studies.[22,23] Chronic constipation can be subdivided into various types depending on its mechanism or etiology, including slow-transit constipation, rectal outlet atresia, and constipation-type IBS.[1] However, in this study, the subjects were limited to those with functional constipation according to the Rome IV criteria. According to these diagnostic criteria, functional constipation includes various causes other than organic diseases and constipation-type IBS, and thus represents various types of constipation.[24] If these are classified pathophysiologically, in addition to physiological causes such as lack of fiber or water intake, bowel transit time delay-type constipation and pelvic outlet obstruction-type constipation may be mentioned. These types may overlap with each other and show various symptoms depending on the pattern or degree of colonic transit time and the type and degree of underlying pelvic exit disorders.

Various methods have been proposed and several drugs have been developed to treat constipation. Since GOS is a pH-stable compound, it is not decomposed by stomach acid or enzymes secreted in the human digestive tract; therefore, most of the intake is not absorbed by the human body and is instead used by beneficial bacteria present in the small and large intestines. In addition, GOS has proven to be safe, even with long-term administration, and has shown positive effects in infants, adults, older individuals, and patients with IBS.[25–31] However, the mechanism through which GOS improves functional constipation remains unclear.

In this study, GOS intake was associated with the improvement in various symptoms of constipation such as the number of bowel movements, shape of the stool, and change in the time required for one bowel movement. We further evaluated the degree of symptoms used as criteria for diagnosing functional constipation, such as excessive straining during defecation, feeling of anal blockage during defecation, feeling of residual defecation after defecation, abdominal discomfort, and satisfaction with defecation. After taking GOS for four weeks, there was a significant improvement in the number of bowel movements per day and the shape of the stool. In addition, the SF-36 and the PAC-QOL, a questionnaire specific to constipation, were used to measure the quality of life of patients with constipation.[32–34] According to previous studies, pain, general health, social functioning, and mental health scores are significantly lower in individuals with constipation.34 As such, quality of life tends to decrease when symptoms of constipation are present, but then improve when the symptoms are alleviated with treatment.[35] Consistent with these previous findings, we found that after taking GOS for 4 weeks, there was a significant improvement in satisfaction related to constipation and the detailed areas of the PAC-QOL questionnaire. The quality of life in patients with constipation is related to disease severity, particularly the number of bowel movements.[36,37] Therefore, our results suggest that GOS can improve the frequency of defecation to result in an overall improvement of satisfaction related to constipation.

Additionally, colon transit time and pH were evaluated as indicators of constipation improvement. Previous studies have shown that colonic transit time is improved in patients with constipation following the administration of specific probiotic strains (*e.g.* Bifidobacterium lactis DN-173 010).[38] Additionally, probiotics alone or in combination with prebiotics can decrease the intestinal pH as the levels of bacterial short-chain fatty acids (SCFAs) increase.[39] In particular, GOS is known to inhibit the growth of pathogenic bacteria and to promote the growth of beneficial bacteria in the intestine because GOS is fermented by beneficial intestinal bacteria such as bifidobacteria to produce SCFAs that lower intestinal pH.[25,26,28] In this study, the colonic transit time decreased by 36.9% in the GOS group compared to that measured at baseline and decreased by 14.7% in the GOS group compared to that of the control group at the end of the study, although no statistically significant difference was confirmed. In addition, fecal pH decreased in the treatment group, but this difference was also not statistically significant.

The intestinal microbiota is composed of various bacterial strains that interact with the host,[40] and an imbalance in the intestinal microbiota affects intestinal homeostasis to play a role in the occurrence of constipation.[41] Indigestible prebiotics such as GOS act as energy sources for beneficial bacteria in the gut and contribute to changes in the gut microbial community,[42] resulting in an overall improvement in the gut microbiota composition. We found that GOS ingestion decreased

the relative abundance of Firmicutes at the phylum level and the F/B ratio. An increase in Firmicutes is a major feature observed in patients with constipation,[42] and an increase in the F/B ratio has also been associated with constipation.[43,44]

GOS intake significantly increased the abundances of *Bifidobacterium* sp. and *Lactobacillus* sp. at the genus level. Although the level of *Clostridium* sp., a harmful bacterium, tended to decrease after four weeks of intake, this effect was found in both the GOS and placebo groups. Lactobacilli and bifidobacteria are important components of the human gut microbiota at all ages.[45] Lactobacilli are an important part of the native microbiome of humans and animals,[46] which help to improve or promote lactose intolerance, intestinal peristalsis, and fecal excretion.[45] Bifidobacteria are primarily found in the intestine, which help to maintain a proper balance in the human intestine and play a role in protecting against pathogens and decaying bacteria.[47] The genus *Bifidobacterium* plays an important role in maintaining intestinal homeostasis and is closely associated with IBS, inflammatory bowel disease, and constipation.[48] *Bifidobacterium* spp. break down and ferment carbohydrates to produce SCFAs that improve the intestinal environment.[49] A decrease in *Bifidobacterium* spp. in the intestinal microbiota has been reported among patients with constipation.[50,51] Previous studies also showed that GOS intake of 1.0 to 10.0 g per day improved the intestinal environment, increased the relative abundance of bifidobacteria, and suppressed the production of harmful substances by intestinal bacteria.[52–56] The intestinal microbiota of patients with constipation is unique and can be distinguished from that of healthy adults.[57] Therefore, GOS, which contributes to an increase in *Bifidobacterium* spp. and *Lactobacillus* spp. in the intestinal microbiota, will help to improve constipation.

In the safety evaluation, two mild adverse events occurred in the treatment group and two mild and one moderate adverse event occurred in the control group. All of these events were confirmed to be unrelated to the sample ingestion and were completely cured. In the treatment group, the pulse rate decreased after four weeks, but there were no statistically significant changes in blood pressure, body temperature, body weight, BMI, hematology, blood chemistry, urine, and thyroid function tests for either the treatment or control group. Therefore, based on the results of this study and numerous previous studies evaluating GOS safety, GOS can be safely used in patients with constipation.[58,59]

In conclusion, in this clinical trial, when GOS was ingested for four weeks by patients with functional constipation, statistically significant improvements were observed in the number of bowel movements per day, shape of stool, and satisfaction with constipation. In addition, GOS showed a trend toward improving the colonic transit time and bowel habits. This functional constipation improvement was confirmed to be due to the significant promotion in the proliferation of beneficial intestinal bacteria, *Bifidobacterium* sp. and *Lactobacillus* sp., and GOS showed excellent results in terms of safety. Overall, these results suggest that GOS can be used to improve functional constipation. To verify this possibility clearly, further investigations on the correlation between GOS intake and the change in intestinal microbiota will be performed in our future research.

Author contributions

Conceptualization: Suh HJ. Data curation: Kim G. Formal analysis: Han K, Jung E. Methodology: K Jo, Lee J. Software: K Jo, Suh HJ. Validation: Lee J, Kim G. Investigation: Han K, Jung E. Writing – original draft: Lee J, Suh HJ, Jo K. Writing-review & editing: Lee J, Kim G, Han K, Jung E, Suh HJ, Jo K.

Conflicts of interest

There are no conflicts of interest to declare.

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