Note

Increased Fecal Frequency and Gastrointestinal Symptoms Following Ingestion of Galacto-Oligosaccharide-Containing Yogurt

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Summary Gastrointestinal symptoms and fecal frequency following ingestion of yogurt containing 15 g of galacto-oligosaccharides (GOS) per d were observed in 12 healthy volunteers. The effect of GOS on intestinal microflora was also studied in six volunteers. Defecation frequency increased during the administration period, but gastrointestinal symptoms, especially flatulence, also increased. The level of fecal bifidobacteria did not increase by the yogurt intake, but a significant increase was observed in the fecal bacteria growing on MRS media. The results indicate that dietary GOS increase gastrointestinal symptoms and fecal frequency in normal adults and have an effect on intestinal microecosystem.

Key Words galacto-oligosaccharides, gastrointestinal symptoms, bifidobacteria, defecation frequency

Dietary fiber, as it passes to the colon, improves the colonic function (1). Fructo-oligosaccharides (FOS) are recently characterized components of the diet that are now considered in many countries as prebiotics and as a dietary fiber (2). A prebiotic is a “non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that have the potential to improve host health” (3). The prebiotics that have been identified today and which have served to introduce this concept are carbohydrates that resist autoenzymic digestion and that are quantitatively fermented in the colon. FOS have been studied intensively over the past decade, and many positive health effects of FOS have been found (4). There is evidence that FOS relieve constipation, reduce the production of toxic substances

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in the colon, and possibly reduce serum total cholesterol, blood pressure, and blood glucose in diabetic subjects (5). The principal effect of FOS is believed to be that they increase the number of bifidobacteria in the colon (6). Bifidobacteria have many potential benefits to human health (7).

Galacto-oligosaccharides (GOS) are composed of lactose and galactose units (8). They have been less well studied than FOS and are found naturally in human milk (9) and to a smaller extent in cow’s milk (10). They are commercially produced from lactose in enzymatic reactions in which galactose residues are attached to lactose (8). GOS are used in Japan, being expected to help maintain a good gastrointestinal condition with increased numbers of bifidobacteria (11, 12). Increases in the number of bifidobacteria were observed when 10 g GOS were ingested daily by young (13) and middle-aged subjects (11), and in older subjects even at a dose of 2.5 g GOS per d (12). The overgrowth of bifidobacteria accompanies a reduction in the number of other bacteria such as Bacteroides, Clostridium, and Fusobacterium, thus leading to a major modification in the composition of the colonic microflora (3).

Since GOS are fermented in the colon (14), symptoms such as abdominal bloating and flatulence may occur when large quantities of GOS are ingested. In one study, the daily ingestion of 10 g GOS increased the sensation of fullness, and there was a tendency also for flatulence and abdominal pain to increase (11). In another study, the subjects experienced no symptoms after 10 g GOS per day (13). The aim of this study was to investigate gastrointestinal symptoms and fecal frequency following ingestion of a yogurt containing 15 g of galacto-oligosaccharides per day. The effect of GOS on the number of bifidobacteria in the feces was also studied.

Materials and methods

The study population consisted of 12 volunteers (9 female, 3 male), whose mean age was 38 y (25–55 y range). Prerequisites included normal bowel function (more than 3 bowel movements weekly and no feelings of discomfort or constipation) and no antimicrobial therapy for two months before the study. All volunteers gave their written informed consent to participate in the present study, which lasted for three weeks. The first week served as a control period, after which there was a two-week administration period. Throughout the study, the subjects daily recorded the frequency of defecation and their subjective assessments (+/−) of good appetite, flatulence, loose stools, diarrhea, hard stools, abdominal distension, and any other abdominal symptoms.

During the administration period, 200 mL of drinking yogurt containing 38 g/L GOS was ingested twice a day (15 g GOS per d). The yogurt was produced at Valio Ltd. (Helsinki, Finland). The yogurt used in the present study was manufactured by mixing, 1:1, a syrup containing GOS and a lactose-hydrolyzed fermented milk. The syrup, which contained a large quantity of GOS, was manufactured by hydrolyzing lactose in a high-lactose syrup with lactase (15). The final
fermented drinking yogurt was orange-flavored. The GOS content was determined as the difference between free glucose and free galactose content in yogurt (16). The difference was multiplied by 2.8 to obtain the GOS content. Lactose was not included in the GOS in the present study. The yogurt was produced 2–4 d before the subjects started consuming it and was refrigerated constantly from production to consumption. The yogurt was pasteurized, but not sterilized, and it contained no live bifidobacteria.

Fecal samples were obtained from 6 subjects during the control period, during the administration period, and one week after the administration period. The subjects took a subsample of their feces on the previous day or on the morning of the transportation day; the sample was analyzed within 24 h after the sampling. Immediately after defecation, these subjects measured the pH of their feces by pressing pH test paper (Merck Spezialindikator pH 4.0–7.0, Merck, Darmstadt, Germany) onto their fecal samples. The samples were refrigerated until analysis, except for the transportation time of less than 2 h. Fecal samples were transported to the laboratory in anaerobic bags (Merck), and all the samples were handled in an anaerobic chamber. Specimens (1 g) were suspended in 9 mL of anaerobic diluent, and the serial dilutions were plated on the appropriate plates. The numbers of total lactic acid bacteria and bifidobacteria were counted on MRS agar (Difco, Detroit, USA). Bifidobacteria were selected on a selective medium containing 62 g MRS-broth, 15 g agar, and 1,000 mL H₂O, supplemented with 50 mg gentamicin, 20 mg nalidixin acid, 3 g lithium chloride, 25 mg sodium iodoacetate, and 25 mg 2,3,5-triphenyltetrazoliumchloride. The plates were incubated anaerobically at 37°C for 3 d, and colony-forming units per gram fecal wet weight were calculated.

The study was approved by the local Ethics Committee (Foundation for Nutrition Research, Helsinki, Finland).

Results and discussion

The median frequency of defecation (interquartile range) increased significantly ($p = 0.02$, Friedman's ANOVA) from 8 (6.5–9) times/week during the control week to 10 (8–13.5) and 9 (7.5–11) times/week during the first and second administration weeks, respectively. This increase in the frequency of defecation was not found in the study of Ito et al (11), in which 10 g GOS were ingested. The difference in the results between our study and that of Ito et al may be explained by the different amounts of GOS or the different consistencies of the GOS products used. The different ways of ingesting GOS (in yogurt in our study and in 115 mL of apple juice in the Ito study) may have influenced the result. The overall diets of the subjects in these two studies were different (Japanese vs. Finnish). In some Japanese studies, however, GOS were found to relieve constipation (17, 18). The promotion of bowel movements has also been observed with other slowly absorbable carbohydrate components in the diet (5, 19). According to our study, GOS in the yogurt may be suitable for relieving constipation, since defecation frequency was significantly increased.
Table 1. Number of subjects having symptoms and number of symptomatic days during the control week and during the first and the second administration weeks (15 g/d galacto-oligosaccharides).

| Symptom                  | Control week | Administration period | Friedman test |
|--------------------------|--------------|-----------------------|---------------|
|                          | n*           | Median (range)**      | n             | Median (range) | n         | Median (range) | p-value |
| Poor appetite            | 2            | 0 (0-1)               | 0             | 0 (0-0)       | 0         | 0 (0-1)       |        |
| Flatulence               | 8            | 1 (0-4)               | 11            | 7 (0-7)c      | 11        | 7 (1-7)c      | 0.0008 |
| Loose stools             | 4            | 0 (0-2)               | 7             | 1 (0-5)       | 7         | 1 (0-7)       | 0.06   |
| Diarrhea                 | 0            | 0 (0-0)               | 0             | 0 (0-0)       | 0         | 0 (0-1)       |        |
| Hard stools              | 7            | 1 (0-4)               | 5             | 0 (0-3)       | 5         | 0 (0-3)       | 0.38   |
| Abdominal distention     | 1            | 0 (0-4)               | 5             | 0 (0-7)       | 5         | 0 (0-7)       | 0.07   |
| Other abdominal symptoms | 1            | 0 (0-1)               | 2             | 0 (0-7)       | 2         | 0 (0-2)       | 0.67   |
| Sum of symptoms          | 3            | 0 (0-12)              | 9             | 2 (16) c      | 10        | 2 (15)c       | 0.003  |

* Number of symptomatic subjects (n=12).
** Median and range of the number of symptomatic days per week.
* Significantly (p<0.05) different from the control week.

The number of days per week during which flatulence was experienced increased during the administration period, compared with the control period (p<0.05, Wilcoxon’s matched pairs test) (Table 1). The flatulence during the administration period is likely to be due to efficient fermentation of GOS in the colon (14). GOS are not absorbed or hydrolyzed in the small intestine, as evidenced by the increase in breath hydrogen concentration after GOS ingestion in humans (14) and by the lack of hydrolases in rat mucosal homogenate (20).

When all the days on which any of the symptoms were experienced were pooled, overall gastrointestinal symptoms appeared more frequently in the administration period than in the control period (p<0.05, Wilcoxon’s matched pairs test). The overall extent of gastrointestinal symptoms increased mainly because of flatulence and abdominal distention. These side effects were also found in an earlier study, besides abdominal pain (11). In another study, however, 10 g GOS caused no symptoms in healthy males (13). In the present study, the higher GOS dose (15 g/d) may be the reason for the more severe gastrointestinal symptoms. It is obvious that gastrointestinal symptoms limit the use of large quantities of GOS. This is also true for many other indigestible carbohydrates such as sorbitol (21), which is well tolerated when the intake is below 15 g/d (19).

There was no significant difference (Wilcoxon’s matched pairs test) in the pH of the feces between the control and the second administration week (6.9 ± 0.2 vs. 6.9 ± 0.3). No significant changes in the fecal concentrations of bifidobacteria were
observed (geometric mean $1.87 \times 10^7$ during the control period vs. $0.42 \times 10^7$ during the administration period, $p=0.60$, Wilcoxon's matched pairs test) (Fig. 1). GOS have been reported to have bifidogenic properties \(11-13, 17, 22\). However, the ingestion of GOS caused no increase in the amount of bifidobacteria in this study. Some bifidobacteria might have been inhibited by the selective factors used, although they were tested on 10 different strains and were shown to be only slightly inhibitory to three of them. The bifidobacteria are heterogeneous, and no ideal selective medium exists for their isolation. In this study, fecal frequency increased, although the number of bifidobacteria did not. Therefore an increase in the number of bifidobacteria is not necessary for fecal frequency to increase when oligosaccharides are ingested.

The total number of anaerobic bacteria growing on MRS plates increased significantly during the administration period (geometric mean $0.8 \times 10^{10}$ cfu/g wet weight during the control period vs. $1.81 \times 10^{10}$ cfu/g wet weight during the administration period, $p=0.03$, Wilcoxon's matched pairs test). We observed that the MRS medium used to count total lactic acid bacteria was not sufficiently selective, since some of the colonies were not bifidobacteria or lactic acid bacteria. Based on microscopy, they might have been \textit{Bacteroides}, but no further identification was carried out. The increase in bacteria growing on MRS medium shows that the intake of 15 g of GOS in fermented milk does have an influence on fecal microflora. The change in the microflora, and thus the different microbial metabolites in the colon, may have increased fecal frequency.

In conclusion, the results show that ingesting yogurt containing 15 g GOS per day increases fecal frequency and changes fecal microecosystem in healthy adults. However, the consumption of such a product also increases gastrointestinal symptoms.
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