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Note

Influence of Galactooligosaccharides on the Human Fecal Microflora

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Summary The effects of galactooligosaccharides intake on fecal microflora and their metabolism were investigated in twelve healthy volunteers, in whom the numbers of indigenous bifidobacteria are comparatively low. The galactooligosaccharides ingestion increased the number of bifidobacteria, but remarkable changes of other organisms were not observed. This sugar also lowered fecal nitroreductase activity, the concentrations of indole and isovaleric acid.

Key Words galactooligosaccharides, fecal microflora, bifidobacteria, nitroreductase, indole

The indigenous intestinal micorflora and their metabolites are thought to play important roles in preserving human health (*1,2*). Among the human colonic microflora, the bifidobacteria are the most common anaerobic bacteria and may exert beneficial effects on their host (*3,4*). It has been shown that this bacteria is dominant in the neonatal gut microflora; however, the number of bifidobacteria decreases by advanced-age in human (*3,5*). We have already reported that galactooligosaccharides increase the indigenous bifidobacteria in human subjects (*6,7*). In this study, we determined the effect of galactooligosaccharides on the human fecal microflora in middle-aged or elderly persons, in whom the numbers of indigenous bifidobacteria are comparatively low.

Galactooligosaccharides (Oligomate-50®) prepared from lactose through the transgalactosylation reaction of β-galactosidases from *Aspergillus oryzae* and *Streptococcus thermophilus* were used (*8*). This sugar syrup contains 25% of water, and is composed of 52% galactooligosaccharides, 38% monosaccharides, and 10% lactose on a dry basis (Table 1). The main component of the galactooligosaccharides is trisaccahride, Galβ(1-6)Galβ(1-4)Glc (Gal, galactose; Glc, glucose). From among twenty-eight healthy Japanese male volunteers (35–67 years old; mean age, 50 years old), in whom the mean number of indigenous bifidobacteria was 9.81 ± 0.40 (values are mean counts of bacteria and SD expressed as $\log_{10}$ of the

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Table 1. Composition (% dry base) of galactooligosaccharides.

Component		Composition (%)
Galactooligosaccharides		52
Disaccharides	Gal-Glc, Gal-Gal	16
Tri-	Gal-Gal-Glc	24
Tetra-	Gal-$(Gal)_2$-Glc	10
Penta- and hexa-	Gal-$(Gal)_3$-Glc, Gal-$(Gal)_4$-Glc	2
Monosaccharide	Gal, Glc	38
Lactose	Gal-Glc	10

Gal, galactose; Glc, glucose.

number of organisms per gram of feces (wet weight)), twelve subjects who had lower numbers of indigenous bifidobacteria were selected. The mean number of bifidobacteria in the selected subjects (35–55 years old; mean age, 49 years old) was 9.46±0.30. These subjects were given 180 ml of apple juice which included 2.5 g of galactooligosaccharides (6.7 g of Oligomate-50®), each day before lunch for three weeks. All the subjects usually consumed the Japanese styles of food composition. None of the subjects took lactose-containing food, fermentation products, or antimicrobial drugs during the experimental period. Freshly passed fecal samples were obtained on day 6 of each experimental week. Fecal microflora analysis, pH measurement, fecal enzyme analysis (β-glucuronidase and nitroreductase), and fecal chemical analysis (ammonia, *p*-cresol and indole) were carried out immediately (*7, 9*). Bonferroni test was used for comparison of the mean values before, during, and after galactooligosaccharides ingestion. This study was performed in accordance with the Helsinki Declaration as updated in Tokyo, Japan, 1975.

The changes in the fecal microflora composition after the consumption of galactooligosaccharides are shown in Table 2. The numbers of bifidobacteria was significantly increased during the galactooligosaccharides ingestion. After the discontinuation of galactooligosaccharides intake, the level of this bacteria returned to the pretreatment levels. We previously observed that indigenous bifidobacteria increased significantly from 9.78 to 10.08 on the ingestion of 10 g of galactooligosaccharides (25.6 g of Oligomate-50®) in middle-aged subjects (26–48 years old; mean age, 40 years old) (*6*). At that time, 2.5 g galactooligosaccharides ingestion caused a slight increase in bifidobacteria, but the increase was not statistically significant (these data were tested for significance by means of Latin square design analysis of variance and Williams' multiple-range test). Furthermore, a slight increase in bifidobacteria was also observed when 3 g of galactooligosaccharides was fed to the subjects (25–35 years old; mean age, 30 years old) (*10*). In this study, we showed that ingestion of 2.5 g galactooligosaccharides per day was enough to increase the indigenous bifidobacteria in middle-aged or elderly persons (mean age, 49 years old), in whom the numbers of indigenous bifidobacteria are comparatively low. Many researchers have already reported that very low doses of

Table 2. The influence of galactooligosaccharides intake on the human fecal microflora composition.

Organisms	Before feeding	During feeding			After feeding
		1 w	2 w	3 w	
Total bacteria	10.30±0.27	10.42±0.27	10.47±0.30	10.46±0.29	10.41±0.21
Bacteroidaceae	9.84±0.37	9.84±0.36	9.94±0.28	9.94±0.32	9.85±0.33
Bifidobacteria	9.42±0.22	9.69±0.23**	9.72±0.27**	9.65±0.21*	9.49±0.32
Clostridium perfringens	3.04±1.55	3.44±1.93	3.33±1.52	3.25±1.82	3.34±1.87
Enterobacteriaceae	7.34±1.31	6.73±0.69	6.96±0.96	7.01±1.03	7.01±1.02
Enterococci	6.98±1.28	6.65±1.55	6.90±1.08	6.96±0.93	7.32±1.16
Lactobacilli	5.56±1.50	5.16±1.70	5.18±1.54	5.57±1.58	5.27±1.81
Bacilli	3.08±1.22	3.72±1.54	3.12±1.64	2.89±0.83	2.78±0.83
Staphylococci	3.19±0.87	3.32±1.00	3.36±1.02	3.38±0.96	3.55±0.96
Candida spp.	2.46±0.42	2.57±0.42	2.60±0.60	2.65±0.38	2.44±0.53

Values are mean counts of bacteria and SD expressed as $\log_{10}$ of the number of organisms per gram of feces (wet weight). * and ** denote a statistically significant difference, respectively, at the $p<0.05$ level and the $p<0.01$ level, when compared with the numbers obtained before galactooligosaccharides intake.

a non-absorbable sugar induced a selective increase in indigenous bifidobacteria in the human intestine, e.g., lactulose 3 g/day (*11*), soybean oligosaccharides 3 g/day (*12*), xylooligosaccharides 2 g/day (*13*), galactooligosaccharides 2 g/day (*14*), and fructooligosaccharides 1 g/day (*15*).

A significant reduction in the activity of nitroreductase was also observed on the ingestion of galactooligosaccharides (Table 3). Bacterial nitroreductase catalyzes the metabolic activation of nitrated polycyclic aromatic hydrocarbons, e.g., 1-nitropyrene and 1,6-dinitropyrene, DNA- and tRNA-bound adducts being produced (*16*). These compounds and their intermediates have been implicated as environmental mutagens and carcinogens. Therefore, such a change in metabolic activity of the fecal flora may have a beneficial influence on colonic health (*17,18*).

Stool weight, fecal pH, water content, β-glucuronidase, ammonia, and *p*-cresol were not influenced by the administration of galactooligosaccharides (Table 3). However, indole and isovaleric acid, which are good markers of bacterial proteolysis and subsequent deamination (*19*) respectively, were decreased during and after the test sugar feeding (Tables 3 and 4). This suggested that the active fermentation of carbohydrate occurs in the large intestine and that the putrefaction was depressed by galactooligosaccharides feeding.

In this study we found that the administration of galactooligosaccharides at a dosage of 2.5 g/day significantly affected the fecal microflora and its metabolic activities in favor of health preservation in humans.

Table 3. The influence of galactooligosaccharides intake on fecal weight, pH, water content, β-glucuronidase, nitroreductase, ammonia, *p*-cresol, and indole.

	Before feeding	During feeding			After feeding
		1 w	2 w	3 w	
Stool weight (g)	203.6±112.9	182.6±65.2	175.6±61.9	140.8±54.3	160.2±72.6
pH	6.2±0.5	6.1±0.4	6.2±0.4	6.3±0.5	6.2±0.5
Water content (%)	83.1±4.7	83.1±4.9	82.3±3.4	80.9±6.3	81.3±6.1
β-Glucuronidase[1]	1.32±0.54	1.10±0.77	1.11±0.48	1.45±0.85	1.37±0.61
Nitroreductase[2]	0.09±0.06	0.04±0.02***	0.04±0.02***	0.03±0.03***	0.03±0.03***
Ammonia (mM)	27.6±11.5	26.0±14.9	33.6±16.5	29.5±11.0	22.6±10.9
p-Cresol (mM)	0.45±0.43	0.49±0.48	0.44±0.37	0.38±0.25	0.38±0.34
Indole (mM)	0.43±0.22	0.30±0.23	0.32±0.19	0.27±0.14*	0.24±0.14**

[1] *p*-Nitrophenol μmol/h/g wet feces. [2] *p*-Aminobenzoic acid μmol/h/g wet faces. *, **, and *** denote a statistically significant difference, respectively, at the $p<0.05$ level, the $p<0.01$ level, and the $p<0.001$ level, when compared with the numbers obtained before galactooligosaccharides intake.

Table 4. The influence of galactooligosaccharides intake on fecal organic acids.

	Before feeding	During feeding			After feeding
		1 w	2 w	3 w	
Succinic acid	1.2±4.2	0.3±1.2	ND	0.2±0.4	1.1±2.7
Formic acid	ND	0.5±1.6	0.2±0.7	ND	ND
Acetic acid	67.8±28.5	65.8±22.0	76.6±24.2	74.4±32.7	77.3±34.5
Propionic acid	23.9±8.5	24.4±7.8	29.5±10.4	27.3±12.6	25.4±11.9
Isobutyric acid	ND	ND	ND	ND	ND
Butyric acid	19.4±9.5	17.8±6.4	18.5±8.5	19.0±9.1	19.2±10.7
Isovaleric acid	22.4±11.7	13.2±10.3	7.1±10.2***	5.1±3.5***	2.1±4.0***
Valeric acid	ND	ND	ND	ND	ND
Total organic acids	134.5±44.7	122.1±25.1	131.9±39.7	126.1±51.6	125.1±55.0

All values are means and SD expressed as mmol/liter per supernatant of feces. ND: not detected. ***denote a statistically significant difference, at the $p<0.001$ level, when compared with the numbers obtained before galactooligosaccharides intake.

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