Effects of Transgalactosylated Disaccharides on the Human Intestinal Microflora and Their Metabolism

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(Received February 8, 1993)

Summary The effects of transgalactosylated disaccharide (TD) intake on human fecal microflora and their metabolism were investigated in 12 Japanese males. TD is a mixture of sugars, galactosyl galactose, and galactosyl glucose, synthesized from lactose through the transgalactosylation reaction of Streptococcus thermophilus β-galactosidase. Volunteers took 15 g of the test sugar daily for 6 days. The TD ingestion increased the number of bifidobacteria and lactobacilli, but decreased the number of Bacteroidaceae and Candida spp. in the feces. The ratio of bifidobacteria to total bacteria increased from 0.28 to 0.51. TD decreased the fecal concentrations of propionic acid, isobutyric acid, isovaleric acid, and valeric acid. This sugar also lowered the fecal pH, and the concentrations of fecal ammonia, p-cresol, and indole. Moreover, a positive correlation was found between the concentration of ammonia, and that of branched-chain fatty acids (isobutyric acid and isovaleric acid), p-cresol, and indole. All of these compounds are produced from amino acids through deamination by the intestinal bacteria. The depression of amino acid fermentation by intestinal bacteria may be involved in the reduction of fecal ammonia. These results suggest that a part of the transgalactosylated disaccharides passes into the colon, inducing changes in the colonic microflora composition, hastening carbohydrate fermentation, and depressing amino acid fermentation in the human gut.

Key Words transgalactosylated disaccharides, intestinal microflora, bifidobacteria, lactobacilli, ammonia, p-cresol, indole, branched-chain fatty acids

The purpose of this study was to investigate the effects of transgalactosylated disaccharide supplementation on fecal microflora and their metabolism in human subjects.

β-Galactosidase (EC 3.2.1.23, β-D-galactoside galactohydrolase) is generally

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known as an enzyme which catalyzes the hydrolysis of lactose (1). This enzyme also catalyzes the transgalactosylation reaction (2). The hydrolysis to transgalactosylation reaction ratio varies depending on the source of the enzyme (3). β-Galactosidase from Streptococcus thermophilus produces disaccharides in large quantities (4). These disaccharides, including Galβ(1-6)Glc, Galβ(1-3)Glc, Galβ(1-2)Glc, and Galβ(1-6)Gal, are collectively named transgalactosylated disaccharides (TD) (Gal, galactose; Glc, glucose). Galβ(1-6)Glc and Galβ(1-6)Gal are already known to be present in yogurt (5) and milk hydrolyzed with lactase (6). Burvall et al. (7) reported that galactooligosaccharides having β(1-6) linkages were much less susceptible to lactase (β-galactosidase) derived from the human small intestine, as compared to lactose (Galβ(1-4)Glc). Therefore, ingested TD can be expected to pass through the small intestine without being digested by endogenous enzymes and so is fermented by the bacteria in the large intestine.

The indigenous intestinal microflora and their metabolites (in particular, short-chain fatty acids) are thought to play an important role in preserving human health. They provide protection against infection (8, 9) and facilitate the normal functions of the gut: absorption of Na and water (10), epithelial cell proliferation (11), and motor activity (12). The microflora composition and activity are determined by a great variety of factors (13). Because it can be controlled artificially, the diet might be the most important regulating factor. Foods such as resistant starches, dietary fibers, and dietary proteins (whole seeds and grains) which are not digested in the small intestine and which are the main nutrients for intestinal bacteria may be particularly important (14, 15). It is known that non-digestible and fermentable carbohydrates including dietary fiber and lactulose have beneficial effects on the host (16–18). However, in contrast to carbohydrates, the fermentation of proteins is assumed to result in the formation of a number of potentially toxic metabolites, such as ammonia, volatile phenols, and indoles (19–21). Therefore, modulation of the rate of formation of harmful microbial metabolites from foods such as non-absorbable and rapidly-fermentable sugars might be of considerable importance in both the prevention of several serious diseases and in the improvement of colon health.

MATERIALS AND METHODS

Transgalactosylated disaccharides. The methods used for the preparation of TD have been described elsewhere (3, 4). A lactose solution of high concentration was allowed to react with β-galactosidase from S. thermophilus. The reaction mixture was then purified by fractionation by column chromatography, and crystalline disaccharides (galactosyl galactose and galactosyl glucose) were obtained. The sugar composition of the test sample thus produced is shown in Table 1.

Experimental design. Twelve healthy Japanese male volunteers (27–48 years old; mean age, 39) were given 15 g of transgalactosylated disaccharide in 115 ml of iced tea, once daily for 6 days. None had taken lactose-containing foods, ferme-
TOS is a generic term for transgalactosylated oligosaccharides, which consist of tri, tetra-, penta-, and hexasaccharides, and are formed from lactose through the transgalactosylation reaction of β-galactosidase (22).

Freshly passed fecal samples were obtained in the morning before TD ingestion, on the sixth day of TD ingestion, and the sixth day after cessation of TD ingestion. pH measurements, and fecal microflora and fecal chemical analyses were carried out immediately. This project was approved by the Human Subject Ethical Committee of the Yakult Central Institute and each subject gave informed consent.

Analysis of fecal microflora. Investigation of the fecal microflora was performed using previously described techniques (23). Bacterial counts are expressed in log_{10} c.f.u./g feces (wet weight).

Chemical measurements. Fecal pH values were determined directly by inserting a needle-shaped glass electrode (Cat. 6028; Horiba, Tokyo) into five different locations of the fecal specimens. Fecal moisture was measured using approximately 5 g samples that were weighed before and after drying in a microwave oven for 30 min. We have determined the reliability of this method by comparing it to ordinary vacuum drying methods using rat feces (data not shown). Fecal organic acids were measured by HPLC, as previously described by Kikuchi and Yajima (24). Fecal ammonia was measured using a commercial kit (Ammonia-Test Wako; Wako Pure Chemical, Osaka, Japan). Phenolic compounds (p-cresol) and indoles were analyzed by GC using the methods of Spoelstra (25).

Statistical analysis. The paired Student’s t-test was used for comparison of mean values before and during TD ingestion. The relationships among ammonia, and indole, p-cresol, isobutyric acid, and isovaleric acid were investigated by using a coefficient of correlation. Statistical analysis was performed using the package Statistical Library I (Yukms Corp. Tokyo, Japan).
RESULTS

**Fecal microflora**

The changes in fecal microflora composition after the consumption of TD are shown in Table 2. The numbers of bifidobacteria \((p < 0.01)\) and lactobacilli \((p < 0.05)\) significantly increased during the TD period, whereas Bacteroidaceae \((p < 0.05)\) and *Candida* spp. \((p < 0.05)\) decreased. Additionally, the percentage of bifidobacteria \((p < 0.05)\) increased from 28 to 51% of the total bacteria during TD intake \((p < 0.05)\), whereas that of Bacteroidaceae decreased from 31 to 22% of the total bacteria (Fig. 1). After discontinuation of TD intake, the number of these bacteria returned to pretreatment levels.

Table 2. The influence of TD intake on fecal microflora composition.

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Before TD intake</th>
<th>During TD intake</th>
<th>After TD intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bacteria</td>
<td>10.67±0.15 (100)</td>
<td>10.68±0.16 (100)</td>
<td>10.58±0.27 (100)</td>
</tr>
<tr>
<td>Bacteroidaceae</td>
<td>10.10±0.23 (100)</td>
<td>9.86±0.51 (100)</td>
<td>10.08±0.22 (100)</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>10.06±0.25 (100)</td>
<td>10.31±0.17 (100)</td>
<td>10.00±0.32 (100)</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>3.99±2.19 (58)</td>
<td>3.16±2.01 (42)</td>
<td>4.24±1.89 (83)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>6.60±1.48 (100)</td>
<td>6.81±0.71 (100)</td>
<td>7.18±0.79 (100)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>6.78±1.18 (100)</td>
<td>7.12±1.34 (100)</td>
<td>7.16±1.12 (100)</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>4.97±1.65 (83)</td>
<td>5.68±2.09 (92)*</td>
<td>5.08±1.97 (75)</td>
</tr>
<tr>
<td>Staphylococci</td>
<td>2.85±0.71 (58)</td>
<td>2.90±0.56 (67)</td>
<td>2.90±0.62 (75)</td>
</tr>
<tr>
<td>Bacilli</td>
<td>3.28±0.77 (100)</td>
<td>3.53±2.12 (83)</td>
<td>3.50±1.45 (83)</td>
</tr>
<tr>
<td><em>Candida</em> spp.</td>
<td>3.00±0.92 (58)</td>
<td>2.66±0.88 (25)*</td>
<td>3.31±1.02 (67)</td>
</tr>
</tbody>
</table>

Values are mean counts of bacteria and SD expressed as log_{10} of the number of organisms per g of feces (wet weight). Numbers in parentheses indicate the frequency of occurrence: (number of subjects with organisms detected/number of subjects examined) × 100. *\(p < 0.05\), **\(p < 0.01\).

![Fig. 1. The ratios of the numbers of bifidobacteria and Bacteroidaceae to the total number of bacteria. The data are presented as percentages which correspond to the mean value of the ratios for individuals.](image-url)
Table 3. Influence of TD intake on fecal pH, water content, ammonia, and volatile phenols.

<table>
<thead>
<tr>
<th>Chemical parameters</th>
<th>Before TD intake</th>
<th>During TD intake</th>
<th>After TD intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.35±0.50</td>
<td>6.10±0.42*</td>
<td>6.21±0.42</td>
</tr>
<tr>
<td>Water content (%)</td>
<td>80.8±2.8</td>
<td>82.0±4.3</td>
<td>80.2±4.6</td>
</tr>
<tr>
<td>Ammonia (µmol/g)</td>
<td>36.4±20.3</td>
<td>20.4±18.2**</td>
<td>27.9±11.1</td>
</tr>
<tr>
<td>p-Cresol (nmol/g)</td>
<td>90.8±106.0</td>
<td>30.7±43.4*</td>
<td>81.9±63.1</td>
</tr>
<tr>
<td>Indole (nmol/g)</td>
<td>89.5±81.5</td>
<td>62.8±80.2**</td>
<td>71.0±25.2</td>
</tr>
</tbody>
</table>

Values are M±SD expressed per g wet weight of fecal contents. * p<0.05, ** p<0.01.

DISCUSSION

In this study, TD-feeding resulted in selective increases in bifidobacteria and lactobacilli in the fecal microflora. These bacteria are considered beneficial to their maintenance of the equilibrium of the intestinal microflora (26, 27). We have previously observed that several kinds of intestinal bacteria change on lactose (β-1, 4-linked galactosyl-glucose) loading (28). TD was utilized as well as lactose by bifidobacteria and lactobacilli. However, it was practically unutilized by fusobacteria, peptococci, and propionibacteria, and was utilized less by Bacteroidaceae (4). It is generally considered that the composition and growth of the predominant polysaccharide-utilizing bacteria in the large intestine are mainly dependent upon...
substrates which they can use as energy sources (29). In particular, dietary carbohydrate sources play a main role in the proliferation of bifidobacteria (30). Therefore, these selective changes in the microflora may be caused by substrate competition or a difference in the availability of the test sugar for intestinal bacteria. However, indirect influences through alteration of the pH and other factors must be considered. For instance, the optimum pH for the growth of both bifidobacteria and lactobacilli is lower than that for other intestinal bacteria, Bacteroidaceae (31; Bergey's Manual, 1986).

A decrease in the number of Bacteroidaceae may be accompanied by an increase in the number of bifidobacteria, although the relationship between bifidobacteria and Bacteroidaceae was not accepted statistically (data not shown). Such
suppression of the Gram-negative anaerobes, Bacteroidaceae, was also observed when non-absorbable sugars such as TOS (22), lactulose (32), and raffinose (33) were administered to humans.

In the human large intestine, protein breakdown and amino acid fermentation give rise to organic acids including branched-chain fatty acids (isobutyric acid, isovaleric acid, and 2-methyl-butyric acid), ammonia, and a range of phenols, indoles, and amines. In this study a significant positive correlation between the concentration of fecal ammonia, and the concentrations of fecal p-cresol, indole, and branched-chain fatty acids was established. These compounds are good markers of bacterial proteolysis and subsequent deamination (34). Isobutyric acid was formed from valine; isovaleric acid from leucine, and isoleucine by reductive deamination (35). Phenol and indole are produced through deamination of the aromatic amino acids, tyrosine, phenylalanine, and tryptophan (36). These amino acid metabolisms by gut bacteria follow the production of ammonia. Wrong (37) indicated that deamination from the amino nitrogen of dietary and endogenous proteins was the major source of ammonia in the large intestine. Therefore, it is evident that the reduction of fecal ammonia on TD ingestion participates in the depression of the deamination of amino acids produced through the breakdown of proteins. Similar results were obtained when lactulose was tested in fecal incubation systems (35).

Ammonia is also the preferred source of nitrogen for many intestinal bacteria. When suitable energy sources such as lactulose or dietary fibers were provided, the bacteria assimilated ammonia for protein synthesis. Bacterial growth is stimulated and fecal nitrogen excretion is induced (38, 39). We believe that the same mechanisms are involved in the decrease in ammonia on TD ingestion. This effect of TD on the reduction of ammonia makes TD a potential alternative to lactulose in the treatment of hepatic encephalopathy.

The fecal concentrations of acetic acid and total organic acids did not increase

Table 4.  Influence of TD intake on fecal organic acids (µmol/g).

<table>
<thead>
<tr>
<th>Organic acid</th>
<th>Before TD intake</th>
<th>During TD intake</th>
<th>After TD intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinic acid</td>
<td>2.0±5.4</td>
<td>1.6±2.2</td>
<td>3.8±12.6</td>
</tr>
<tr>
<td>Lactic acid</td>
<td>0.5±1.1</td>
<td>1.9±4.9</td>
<td>0.9±2.4</td>
</tr>
<tr>
<td>Formic acid</td>
<td>0.7±1.3</td>
<td>1.2±2.0</td>
<td>0.9±2.5</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>106.9±33.7</td>
<td>105.3±32.5</td>
<td>101.7±29.9</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>34.4±8.4</td>
<td>27.5±9.1*</td>
<td>32.6±11.3</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>1.7±1.8</td>
<td>0.5±1.1**</td>
<td>1.1±0.8</td>
</tr>
<tr>
<td>Butyric acid</td>
<td>32.4±14.4</td>
<td>27.1±11.3</td>
<td>28.8±21.1</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>3.9±2.4</td>
<td>1.3±2.2**</td>
<td>1.4±1.2</td>
</tr>
<tr>
<td>Valeric acid</td>
<td>4.7±3.7</td>
<td>3.1±4.1*</td>
<td>3.5±2.4</td>
</tr>
<tr>
<td>Total</td>
<td>187.1±47.8</td>
<td>169.3±44.5</td>
<td>174.7±54.6</td>
</tr>
</tbody>
</table>

Values are M±SD expressed per g wet weight of fecal contents. * p < 0.05, **p < 0.01.
with TD supplementation, although the proportion of acetic acid increased. TD is rapidly fermented by intestinal bacteria and a large amount of acetic acid is produced. Furthermore, propionic acid and butyric acid are also formed in substantial amounts in vitro (unpublished observation). This difference in SCFA (short-chain fatty acid) production between in vivo and in vitro is thought to be due to the fecal SCFA concentration, which does not reflect the environment in the colon, as SCFAs are rapidly absorbed from the human gut (15).

The administration of TD leads to a decrease in fecal pH. This shows that active fermentation occurs in the large intestine. Such a decrease in colonic pH may reduce the risk of developing large bowel cancer, because an inverse correlation between stool pH and colon cancer risk was observed (40, 41).

Further studies are needed to determine the roles of rapid and active fermentable carbohydrates in vivo. However, our results suggest that TD can alter human intestinal microflora and their metabolism.

We wish to thank Dr. Tsuneya Ohno for critically reviewing the manuscript. This study was presented in part at the 1990 Annual Meeting of the Japan Society of Nutrition and Food Science.

REFERENCES


