Administration of Transgalacto-Oligosaccharides Increases Fecal Bifidobacteria and Modifies Colonic Fermentation Metabolism in Healthy Humans[1]

Yoram Bouhnik,[2] Bernard Flourié, Laurence D'Agay-Abensour, Philippe Pochart, Geneviève Gramet,* Michéle Durand* and Jean-Claude Rambaud

*INSERM U290, Hôpital Saint-Lazare, 75475 Paris Cédex 10 and *INRA, Laboratoire de Nutrition et Sécurité Alimentaire, Domaine de Vilvert, 78352 Jouy-en-Josas Cédex, France*

ABSTRACT Transgalacto-oligosaccharides are a mixture of oligosaccharides consisting of glucose and galactose; they are not digested in the human small intestine. In vitro, they specifically stimulate the growth of bifidobacteria. The aim of the present work was to assess tolerance of transgalacto-oligosaccharides and the effects of their prolonged administration on bifidobacteria and fermentative activity of colonic flora. Eight healthy subjects were given 10 g of transgalacto-oligosaccharides per day for 21 d in two daily doses. A breath test and stool sample collection were carried out on d 1, 7, 14 and 21 of transgalacto-oligosaccharides ingestion. The stools of three subjects were collected and mixed before the study, and then inoculated in vitro into a fermentor to which 10 g transgalacto-oligosaccharides was added daily for 14 d. In the eight volunteers, administration of transgalacto-oligosaccharides led to a significant decrease in breath hydrogen excretion ($P < 0.01$) and a significant increase in fecal concentrations of bifidobacteria from (means ± SEM) 8.6 ± 0.6 to 9.7 ± 0.5, 9.7 ± 0.6 and 9.5 ± 0.6 log colony-forming units (CFU)/g on d 1, 7, 14 and 21, respectively ($P < 0.05$). Fecal concentrations of enterobacteria, as well as stool weight, fecal water and pH did not change during the study. In vitro, transgalacto-oligosaccharides fermentation became more efficient and faster with time. In addition, metabolic alterations such as a rise in acetate proportion and lactate formation after 7 d of fermentation were observed, indicating the transformation of the inoculated fecal flora into an acid-resistant lactic flora. Prolonged administration of transgalacto-oligosaccharides, at a dose which does not induce digestive symptoms, increases the number of bifidobacteria and alters the fermentative activity of colonic flora in humans. J. Nutr. 127: 444–448, 1997.

KEY WORDS: • *bifidobacteria* • *fermentation* • *prebiotics* • *short-chain fatty acids* • *transgalacto-oligosaccharides* • *humans*

Although the resident bacteria of the colon depend to a large extent on exogenous substrates for energy and growth, it has proved experimentally difficult to produce consistent changes by dietary manipulation (Keush and Gorbach 1985). However, it could be of interest to manipulate colonic flora because it is supposed that some intestinal bacteria could be beneficial to health whereas others coud be harmful (Metchnikoff 1908). Prebiotics are mainly oligosaccharides which, on oral ingestion, could lead to changes in the colonic bacterial ecosystem. In healthy humans, these oligosaccharides are virtually not digested by the intestinal enzymes and reach the colon, where they are fermented by the bacterial flora (Gibson et al. 1995). Unlike other di- or oligosaccharides not digested in the small intestine such as lactose or inulin (Bouhnik et al. 1996, Ito and Kimura 1993), they are usually fermented by only a limited number of colonic bacteria and for this reason might specifically stimulate the growth of certain microorganisms (Rowland 1992, Tanaka et al. 1983). They might therefore alter the intracolonic bacterial balance and foster microorganisms such as bifidobacteria, which might have beneficial effects on the host (Gibson et al. 1995, Mitsuoka 1984).

Transgalacto-oligosaccharides consist of a mixture of indigestible oligosides obtained by biosynthesis from lactose, by the action of a transfer enzyme β-galactosidase from *Aspergillus orizae* (Kan et al. 1989). In vitro experiments in continuous cultures showed that transgalacto-oligosaccharides were extensively converted to short-chain fatty acids with a large acetate molar proportion (Durand et al. 1992). Other in vitro investigations showed that transgalacto-oligosaccharides were preferentially consumed by most of the bifidobacterial species and by a few strains of enterobacteria (Minami et al. 1985, Sumihara 1987, Tanaka et al. 1983). In fact, galactose could represent an optimal substrate because the enzymes of galactose metabolism are constitutive in cells of *Bifidobacterium* (Lee et al. 1980). In addition, transgalacto-oligosaccharides have useful technological properties and are already used for animal feeds. In Japan, they are also used to make food for human consumption, chiefly with the claim of bifidobacteria promoters (Ito et al. 1990, Sumihara 1987).

[1] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

[2] To whom correspondence should be addressed.

0022-3166/97 $3.00
Manuscript received 31 October 1995. Initial review completed 27 November 1995. Revision accepted 7 November 1996.

The aim of the present work was to assess, in healthy humans, clinical tolerance of transgalacto-oligosaccharides and the effects of prolonged administration of 10 g/d on fecal concentrations of bifidobacteria and respiratory excretion of fermentation gases. Because of the difficulty of studying intracolonic fermentation products in vivo, an additional study was conducted in vitro to examine the consequences of prolonged administration of transgalacto-oligosaccharides on the bacterial metabolism.

SUBJECTS AND METHODS

Subjects. Eight healthy volunteers (4 men and 4 women, aged 20–32 y) participated in the study. They had no history of gastrointestinal disease and had not taken antibiotics or laxatives during the month before the study. They had no lactase deficiency [negative breath hydrogen (H_2) test after administration of 50 g of lactose]. The subjects were methane producers, i.e., the concentrations of methane (CH_4) in expired gases exceeded the atmospheric concentrations by at least 1 mL/L (Bond and Levitt 1976). Subjects were not permitted any medication during the period of investigation, but their diet was not restricted. They gave written informed consent to participate in the study, which was approved by the Consultative Committee for the Protection of Persons in Biomedical Research of Hôpital Saint-Louis.

In vivo study. The transgalacto-oligosaccharides used comprised a mixture of oligosaccharides whose formula was $(Galactose)_n$-Galactose-Glucose, $1 < n < 4$ (trisaccharides: 48%, tetrasaccharides: 37%, pentasaccharides and hexasaccharides: 15%, Yakult Institute, Tokyo, Japan). The study lasted for 21 d; during that time period, subjects ingested 10 g transgalacto-oligosaccharides per day in two 5-g powder doses, taken morning and evening after meals. Breath and feces were sampled on d 1, 7, 14 and 21. The evening before sampling, subjects ingested a residue-free meal, i.e., steak, rice and rusk. On the day of sampling, they fasted and at 0800 h were given 10 g of transgalacto-oligosaccharides diluted in 100 mL of water; they continued to fast for 12 h and did not receive the evening dose. Gases were collected in a syringe at the end of expiration every 30 min, using a Haldane-Priestley tube (Bond and Levitt 1972). Stools (24-h) were collected in plastic containers in which an anaerobic atmosphere was maintained by adding a gas pack (Anaerocult A, Merck, Darmstadt, Germany) and were stored at 4°C. Stools were weighed and analyzed within 12 h after they were passed. During preliminary experiments, we had determined that this period was sufficiently brief to avoid any marked alterations in bacterial counts.

To assess tolerance of transgalacto-oligosaccharides, subjects filled in a diary sheet on which the following symptoms were graded from 0 to 3: excess rectal gases, bloating, borborygmi and abdominal pains. Stool frequency and consistency were also noted.

In vitro study. To simulate fermentation in the human colon, we used a system of semi-continuous culture, similar to that previously used to study fermentation in rumen (Blanchart et al. 1989) and pig large intestine (Denis et al. 1990). This system used to be inoculated with the combined digesta taken from three different animals in order to minimize the variations. Thus, we applied the same technique to human inocula. Before the consumption of transgalacto-oligosaccharides, stool specimens were collected from three of the volunteers who took part in the in vivo study, mixed, homogenized and diluted (100 g/L) in mineral phosphate-bicarbonate buffer (2 g/L Na_2HPO_4 and 9 g/L $NaHCO_3$). All of these operations were done under nitrogen. Two fermentors were inoculated with 1 L of the above dilution. These fermentors were continuously perfused with a solution of a nutritive medium (1 L/24 h) which simulated the composition of ileal chyme and contained a mixture of mineral buffers and trace elements (Denis et al. 1990), a mixture of organic compounds: (g/L) porcine stomach mucin (Sigma type II), 2; pancreatin, 1; casein, 2; cystein, 0.2; hemin, 0.1; peptone, 0.01; bile salts, 0.05; urea, 0.18; xylan, 0.6; pectin, 0.6; amylopectin, 0.6; arabinogalactan, 0.6; lintner starch, 2.0; and a vitamin mixture. After 7 d of adaptation to the nutritive medium, one fermentor was inoculated with a supplement of 10 g of transgalacto-oligosaccharides in one daily dose for 14 d; the other served as control and was given only nutritive medium.

The experimental fermentor was sampled at given times after inoculation of transgalacto-oligosaccharides on d 1, 2, 7 and 14. The control fermentor was also sampled at the same times. The pH and the concentrations of adenosine triphosphate were measured at 0, 1, 2, 3, 5 and 8 h after inoculation. Short-chain fatty acids and D- and L-lactic acids were measured at 0, 2 and 5 h after inoculation. The volume of gases produced was measured hourly for 5 h and at h 8. To measure residual transgalacto-oligosaccharides, 24-h effluents were collected at the fermentor outlet on the first 6 d after the addition of transgalacto-oligosaccharides, and then on d 12 and 13.

TABLE 1

Fecal data in healthy volunteers during ingestion of 10 g transgalacto-oligosaccharides per day for 21 d[1]

	Day 1	Day 7	Day 14	Day 21
Stool weight, *g/d*	105 ± 25	67 ± 14	96 ± 19	80 ± 15
Fecal water, *g/100 g*	74 ± 18	73 ± 14	73 ± 14	78 ± 16
Fecal pH	6.8 ± 0.2	6.6 ± 0.1	6.6 ± 0.2	6.8 ± 0.2

[1] Values are means ± SEM, $n = 8$.

Sample analysis. *In vivo study.* In expired gases, H_2 and CH_4 concentrations were measured by gas chromatography using a Quintron analyzer (Model DP, ABS, Saint Dié, France) because the sensitivity of this method is <1 μL/L. In stools, bifidobacteria were counted on Beerens medium (Beerens 1990) and enterobacteria on Drigalsky medium. Bifidobacteria were left in culture for 5 d at 37°C in anaerobic jars (Gas-pack system, BBL, Cockeysville, MD) containing Anaerocult A. The enterobacteria were incubated for 24 h at 37°C under aerobiosis. The fecal pH was measured with a pH meter (Radiometer, Copenhagen, Denmark) and a stool aliquot was lyophilized to measure dry matter content.

In vitro study. Short-chain fatty acid concentrations in the fermentors were determined by gas chromatography (Ottenstein and Bartley 1971), and adenosine triphosphate concentrations by bioluminescence (Komisarczuk et al. 1984). Concentrations of residual transgalacto-oligosaccharides in the effluents were measured by colorimetry after hexose hydrolysis with phenolsulfuric acid (Dubois et al. 1956). A sample of effluent was first deproteinized with HClO4; then 0.5 mL was mixed with 0.5 mL phenol (50 g/L) and 2.5 mL concentrated sulphuric acid. The mixture was left at 20°C for 30 min before shaking and the absorbance was determined at 490 nm.

Expression of results and statistical analysis. The areas under curves for the H_2 and CH_4 concentrations were converted into milliliters, using the formula of Solomons et al. (1977). Fecal concentrations of bacteria were expressed as logarithmic coordinates of colony-forming units (log CFU)/g of stool. Values were expressed as means ± SEM. One-way ANOVA was used to compare the bacterial concentrations and metabolism products. After a significant F test ($P < 0.05$), the Newman-Keuls test was used to identify differences between individual means (Linton and Gallo 1975).

RESULTS

In vivo study. Subjects did not experience any symptoms after ingesting transgalacto-oligosaccharides. Stool weight (24-h), the percentage of fecal water and stool pH were not modified by transgalacto-oligosaccharides administration (**Table 1**).

Bifidobacteria concentrations were significantly greater on d 7, 14 and 21 after transgalacto-oligosaccharides ingestion compared with d 1 (**Fig. 1**). Before ingestion, these concentrations were 8.6 ± 0.6 log CFU/g vs. 9.7 ± 0.5, 9.7 ± 0.6 and 9.5 ± 0.6 on d 7, 14 and 21, respectively ($P < 0.05$). However, the concentrations of enterobacteria were not affected during transgalacto-oligosaccharides ingestion.

Breath H_2 excretion was significantly lower on d 7, 14 and 21 than on d 1 ($P < 0.01$) and methane excretion was unchanged (**Table 2**).

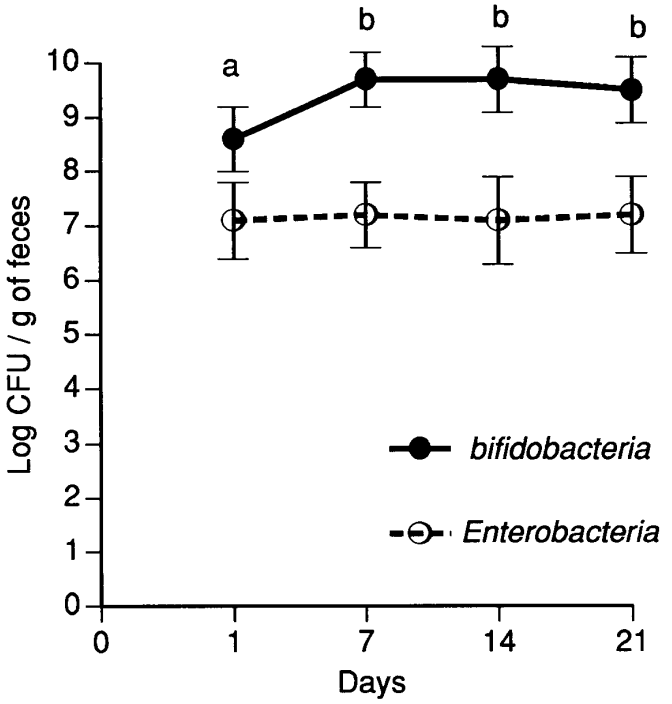

FIGURE 1 Fecal concentrations of bifidobacteria and enterobacteria in healthy volunteers after ingestion of transgalacto-oligosaccharides for 21 d. Each value represents the mean ± SEM, $n = 8$. Results are expressed as log colony-forming units (CFU)/g of feces. Values with different letters are significantly different, $P < 0.05$.

In vitro study. *Measurements at the fermentor outlet.* During the first day after the introduction of transgalacto-oligosaccharides, 87% were degraded (13% were measured in the effluent). The maximum degradation rate (95%) was reached on d 4 and then was unchanged.

Kinetic study in the fermentors. In the control fermentor, the concentrations of ATP, which reflect fermenting activity, remained stable, at a mean of 2.8 mmol/L. After the addition of transgalacto-oligosaccharides, ATP concentrations rose to a peak of nearly 12 mmol/L (**Fig. 2**). However, the peak was maximal at h 5, 3 and 1 on d 1, 2 and 7, respectively. On d 14, it was again observed at the first hour.

The pH of the control fermentor remained stable and was close to neutral (6.9). After the addition of transgalacto-oligosaccharides, the acidification kinetics showed that as early as d 2, the pH dropped to 6.0 at h 3, and by d 7 fell to 5.2 (**Fig. 3**). At d 14, its level was close to that of d 7.

The concentrations of total short-chain fatty acids in the control fermentor were 50 mmol/L, with proportions of acetate, propionate and butyrate of 50, 24 and 12%, respectively. After the addition of transgalacto-oligosaccharides, the initial total short-chain fatty acid concentrations (time 0) gradually rose, between d 1 and 7, from 50 to 100 mmol/L (**Fig. 4**). On d 1 and 2, the rise in these concentrations occurred between h 2 and 5. On d 7 and 14, the concentrations measured at h 2 were close to those measured at h 5. The mean proportion of acetate rose from 51 to 65% between d 1 and 7 at all of the times measured. This rise occurred at the expense of the proportion of propionate, which dropped from 25 to 17% between d 1 and 7.

TABLE 2

Volume of H_2 and CH_4 in gases expired by healthy volunteers after ingestion of 10 g transgalacto-oligosaccharides per day for 21 d[1]

	Day 1	Day 7	Day 14	Day 21
	mL/12 h			
H_2	476 ± 110[a]	164 ± 32[b]	267 ± 65[b]	206 ± 60[b]
CH_4	811 ± 249	806 ± 133	789 ± 123	846 ± 182

[1] Values are means ± SEM, $n = 8$. Values in a row with different superscript letters are significantly different, $P < 0.01$.

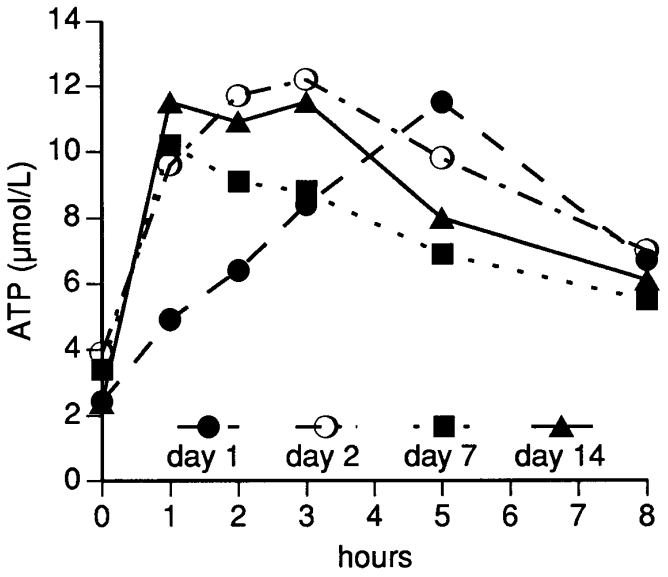

FIGURE 2 Effect of adding 10 g of transgalacto-oligosaccharides on adenosine-triphosphate (ATP) concentrations in the fermentor, measured before and at different times (1, 2, 3, 5 and 8 h) after feeding on d 1, 2, 7 and 14 after adaptation. The values are means of three determinations and the variation coefficients are within 10% of the means.

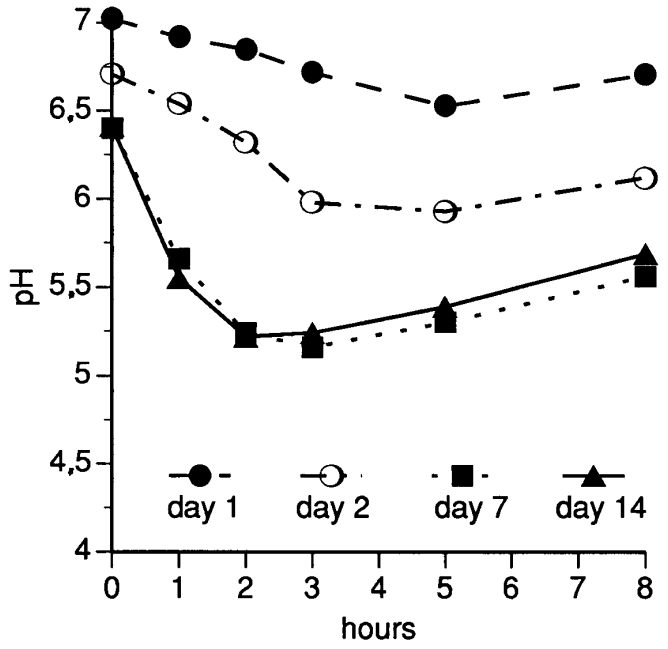

FIGURE 3 Effect of adding 10 g of transgalacto-oligosaccharides on fermentor pH. The conditions were similar to those for Figure 2. Values are ± 0.1 pH unit.

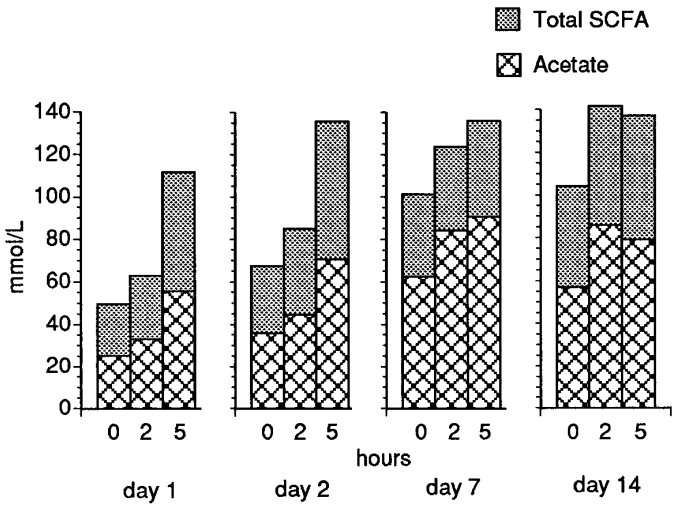

FIGURE 4 Effect of adding 10 g of transgalacto-oligosaccharides on total short-chain fatty acid (SCFA) and acetate concentrations in the fermentor, before and at times 2 and 5 h after feeding on d 1, 2, 7 and 14 after adaptation. The values are means of three determinations and the variation coefficients are within 10% of the means.

In the control fermentor, and also during d 1 and 2 after the addition of transgalacto-oligosaccharides, no lactate was detected. On the other hand, it was detected on d 7 when its concentration at h 0, 2 and 5 ranged from 1.1 to 1.7 mmol/L for L-lactate, and from 1 to 1.2 mmol/L for D-lactate. At d 14, L- and D-lactate (1 mmol/L) were observed only in the sample taken 2 h after transgalacto-oligosaccharides inoculation.

The volumes of total gases produced during the 8 h after transgalacto-oligosaccharides inoculation (**Fig. 5**) were close on d 1, 2, 7 and 14 (about 2500 mL compared with 340 mL in the control fermentor). However, the initial rate of gas production, calculated from the values for the first 2 h, rose from 187 mL/h on d 1 post-inoculation to 580 mL/h on d 7 and 14.

DISCUSSION

The subjects studied experienced no symptoms after daily ingestion of 10 g of transgalacto-oligosaccharides. None complained of diarrhea, and 24-hour stool weight, as measured on d 1, 2, 14 and 21, was unchanged. Low molecular weight carbohydrates which are not absorbed by the human small intestine exert osmotic activity and cause diarrhea when the amount ingested in one dose is large enough for some of it to escape colonic fermentation and be eliminated in the stools (Rambaud and Flourié 1994, Saunders and Wiggins 1991). We therefore chose to give a low dose of 5 g after the morning and evening meals, so as to reduce the osmotic flow through the small intestine. Under these conditions, we hoped that digestive tolerance would be good and that there would be no diarrhea. Although we did not specifically measure fecal excretion of transgalacto-oligosaccharides, the absence of diarrhea and constant fecal weight suggest that all of the ingested transgalacto-oligosaccharides was fermented in the colon.

Although the colonic flora depend on substrates from food for energy, it is very difficult to demonstrate alterations in the flora equilibrium that occur after ingestion of food supplements in the form of indigestible carbohydrates such as dietary fibers (Drasar et al. 1976, Woods and Gorbach 1993). In our study, a slight alteration in diet led to a 10-fold rise in the concentration of bifidobacteria in the colonic flora. In vitro, transgalacto-oligosaccharides proved to be good substrates for enterobacteria (Minami et al. 1985, Sumihara 1987). In contrast, after transgalacto-oligosaccharides consumption, numbers of enterobacteria were significantly decreased in rats, results obtained by inoculating germ-free rats with a suspension of fresh human feces (Rowland and Tanaka 1993). In our study, no modifications were observed in the fecal concentrations of enterobacteria. This is a good illustration of the discrepancies obtained from in vitro and in vivo studies, which can be explained by the extreme complexity of colonic flora in humans (Ducluzeau 1989, Keush and Gorbach 1985). In our study, in vivo transgalacto-oligosaccharides may have had no effect on the enterobacteria because these microorganisms belong to the subdominant flora and are therefore 1000 to 10000 times less numerous than the bacteria of the predominant flora such as the bifidobacteria, which preferentially consume most or all of the substrate (Keush and Gorbach 1985). Ito et al. (1990) also showed a significant although moderate, increase in fecal bifidobacteria in volunteers who had ingested 10 g of transgalacto-oligosaccharides per day for a week. In their study, ingestion of transgalacto-oligosaccharides did not alter either the fecal concentrations of enterobacteria or those of total anaerobes, which also suggests that transgalacto-oligosaccharides might act specifically on the growth of bifidobacteria. In a subsequent study of rats harboring a human colonic flora, administration of transgalacto-oligosaccharides (5 g/100 g diet) for 4 wk led to a significant rise in cecal concentrations of bifidobacteria and also of total anaerobes (Rowland and Tanaka 1993). However, the proportion of bifidobacteria in the total bacterial mass diminished, suggesting that transgalacto-oligosaccharides also stimulated the growth of other bacterial anaerobes. This effect of transgalacto-oligosaccharides, which was not confined to bifidobacteria, might be attributable to the doses used in different studies because, for an equal body weight, the doses given to rats (Rowland and Tanaka 1993) were 20 to 30 times larger than in our study and that of Ito et al. (1990), both conducted in humans.

Prolonged administration of transgalacto-oligosaccharides led

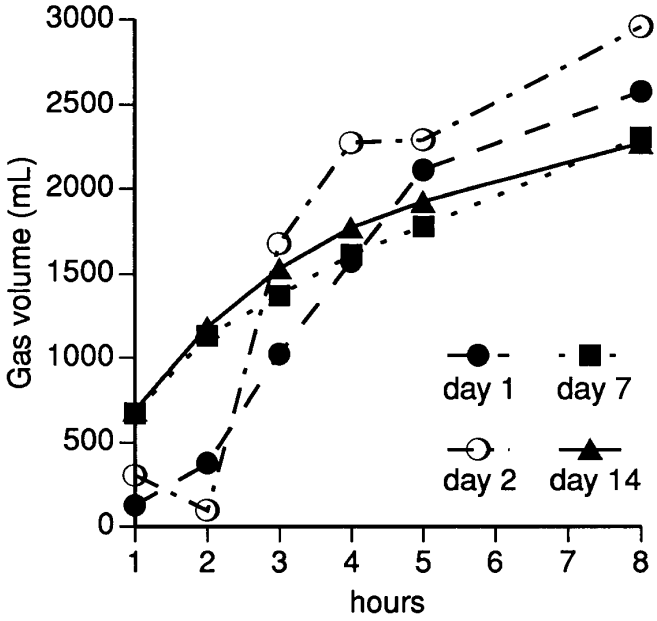

FIGURE 5 Effect of adding 10 g of transgalacto-oligosaccharides on total gas production, measured before and at different times (1, 2, 3, 5 and 8 h) after feeding on d 1, 2, 7 and 14 after adaptation. The values are means of three determinations and the variation coefficients are within 10% of the means.

to a change in their fermentation metabolism. In vivo in humans, the composition of the fermentation metabolites in stools is a poor reflection of what really occurs in the colonic lumen in clinical situations in which there is no diarrhea. For instance, when volunteers ingest 20 g of lactulose in the morning and evening for a week, the cecal metabolism of this sugar and the resulting metabolites undergo great changes between the first and last doses, whereas stool composition remains largely unaffected (Florent et al. 1985). Fermentation metabolites are efficiently absorbed in the human colon, and if the time allowed for this process is not shortened, the fraction of metabolites eliminated in the stools does not correspond to their intracolonic production. On the other hand, in the lung emunctory, the fraction of breath H_2 expired is roughly proportional to the amount produced in the colon (Christl et al. 1992). In our study, prolonged transgalacto-oligosaccharides ingestion led to a significant drop in expired breath H_2 without altering expired breath methane. In view of the inaccessibility of the human colon, and for better understanding of what was happening in the colonic contents, we used an in vitro model. In view of its principle and the levels of acidification reached, the model does simulate fairly well what may occur in the human proximal colon, but it does have certain limits: it consists of only one compartment and can simulate neither motor activity nor the absorption and secretion processes which occur in the human colon. The results we obtained in vitro showed that transgalacto-oligosaccharides were fermented more efficiently and faster from d 7 after the beginning of daily transgalacto-oligosaccharides introduction. However, the changes that already appeared on d 2 suggested that the adaptation of bacterial metabolism to transgalacto-oligosaccharides occurred earlier, between d 2 and 7 of administration. After that, the degree of acidification, the evolution of the ATP and short-chain fatty acid concentrations, and gas production were stable between d 7 and 14 of transgalacto-oligosaccharides administration. In addition to more efficient and faster fermentation of transgalacto-oligosaccharides, the fermentation pathways were altered, exhibiting lactic acid production and a greater molar proportion of acetic acid among the short-chain fatty acids. These two metabolic changes are evidence that lactic acid–resistant flora developed in the fermentor to which transgalacto-oligosaccharides were added (Rasic and Kurmann 1983). These flora consist of bacteria known as lactic, which ferment hexoses via a heterolactic pathway, which does not produce H_2 and leads to the formation of acetate and lactate. Indeed, in our in vivo human experiments, there was a drop of H_2 production with maintained ingestion of transgalacto-oligosaccharides. Methane is produced from H_2 in the distal part of the human colon (Pochart et al. 1993). Consequently, although the concentrations of bifidobacteria increased throughout the entire colon, the absence of reduction in breath methane and in fecal pH suggests that the heterolactic metabolism of the bifidobacteria occurs only in the proximal part of the human colon.

In conclusion, our results show that the addition to the diet of small amounts of indigestible oligosaccharides, which do not induce digestive symptoms, alters the concentrations of bifidobacteria and the intracolonic fermentation metabolism. Such metabolic changes are those expected, but their real beneficial effects on the host have yet to be proved.

ACKNOWLEDGMENT

The authors thank the Yakult Institute (Tokyo, Japan) for kindly supplying them with transgalacto-oligosaccharides.

LITERATURE CITED

Beerens, H. (1990) An elective and selective medium for *Bifidobacterium* spp. Lett. Appl. Microbiol. 11: 155–157.

Blanchart, G., Durand, M., Barry, J. L., Bouiller-Oudot, M. & Jouany, J. P. (1989) Intérêts et limites des fermenteurs à flux semi-continu de type Rusitec dans l'étude des fermentations du rumen. Ann. Zootech. 38: 285–314.

Bond, J. H. & Levitt, M. D. (1972) Use of pulmonary hydrogen measurements to quantitate carbohydrate absorption. J. Clin. Invest. 51: 1219–1225.

Bond, J. H. & Levitt, M. D. (1976) Quantitative measurement of lactose absorption. Gastroenterology 70: 1058–1062.

Bouhnik, Y., Flourié, B., Andrieux, C., Bisetti, N., Briet, F. & Rambaud, J. C. (1996). Effects of *Bifidobacterium* sp. fermented milk with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans. Eur. J. Clin. Nutr. 50: 269–273.

Christl, S. U., Murgatroyd, P. R., Gibson, G. R. & Cummings, J. H. (1992) Production, metabolism, and excretion of hydrogen in the large intestine. Gastroenterology 102: 1269–1277.

Denis, I., Durand, M., Stévani, J., Hannequart, G. & Dumay, C. (1990) Fermentation of different types of purified pectins by pig hindgut microflora in a semi-continuous culture system. Sci. Alim. 10: 265–274.

Drasar, B. S., Jenkins, D.J.A. & Cummings, J. H. (1976) The influence of a diet rich in wheat fibre on the human faecal flora. J. Med. Microbiol. 9: 423–431.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956) Colorimetric method for determination of sugars and related substances. Anal. Chem. 28: 350.

Ducluzeau, R. (1989) Role of experimental microbial ecology in gastroenterology. In: Microbial Ecology and Intestinal Infections (Bergogne-Berezin, E., ed.), pp. 7–26. Springer Verlag, Paris, France.

Durand, M., Cordelet, C., Hannequart, G., Beaumatin, P. & Grivet, J. P. (1992) *In vitro* fermentation of a galacto-oligosaccharide by human bacteria in continuous culture. Proc. Nutr. Soc. 51: 6A.

Florent, C., Flourié, B., Leblond, A., Rautureau, M., Bernier, J. J. & Rambaud, J. C. (1985) Influence of chronic lactulose ingestion on the colonic metabolism of lactulose in man (an in vivo study). J. Clin. Invest. 75: 608–613.

Gibson, G. R., Beatty, E. R., Wang, X. & Cummings, J. H. (1995) Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108: 975–982.

Ito, M., Degushi, Y., Miyamori, A., Matsumoto, K., Kikuchi, H., Matsumoto, K., Kobayachi, Y., Yauma, T. & Kan, T. (1990) Effects of administration of galactooligosaccharides on the human faecal microflora, stool weight and abdominal sensation. Microb. Ecol. Health Dis. 3: 285–292.

Ito, M. & Kimura, M. (1993) Influence of lactose on faecal microflora in lactose maldigestors. Microb. Ecol. Health Dis. 6: 73–76.

Kan, T., Kobayashi, Y. & Matsumoto, K. (1989) Character of galacto-oligosaccharides and application for food. New Food Industry 31: 25–30.

Keush, G. T. & Gorbach, S. L. (1985) Ecology of the gastrointestinal tract. In: Gastroenterology (Berk, J. E., ed.), 4th ed., pp. 1632–1650. Saunders, Philadelphia, PA.

Komisarczuk, S., Durand, M. & Hannequart, G. (1984) ATP measurement in sheep rumen digesta using dimethyl sulfoxide as an extraction reagent. Reprod. Nutr. Develop. 24 : 903–913.

Lee, L., Kinoshita, S., Kumagai, H. & Tochikura, T. (1980) Galactokinase of *Bifidobacterium bifidum.* Agric. Biol. Chem. 40: 2961–2966.

Linton, M. & Gallo, P. S. (1975) The Practical Statistician Simplified Handbook of Statistics. Wadsworth, Belmont, CA.

Metchnikoff, E. (1908) The Prolongation of Life, pp. 1–38. Putman and Sons, New York, NY.

Minami, Y., Yazawa, K., Nakamura, K. & Tamura, Z. (1985) Selectivity and efficiency of utilization of galactosyl-oligosaccharides by bifidobacteria. Chem. Pharm. Bull. 33: 710–714.

Mitsuoka, T. (1984) Taxonomy and ecology of bifidobacteria. Bifidobacteria Microflora 28: 619–625.

Ottenstein, D. M. & Bartley, D. A. (1971) Improved gas chromatography separation of free acids C_2-C_5 in dilute solution. Anal. Chem. 43: 952–955.

Pochart, P., Lémann, F., Flourié, B., Pellier, P., Goderel, I. & Rambaud, J. C. (1993) Pyxigraphic sampling to enumerate methanogens and anaerobes in the right colon of healthy humans. Gastroenterology 105: 1281–1285.

Rambaud, J. C. & Flourié, B. (1994) Mechanisms of carbohydrate-induced diarrhoea. In: Short Chain Fatty Acids (Binder, H. J., Cummings, J. & Soergel, K., eds.), pp. 232–239. Kluwer Academic, Dordrecht, The Netherlands.

Rasic, J. L. & Kurmann, J. A. (1983) Bifidobacteria and Their Role. Birkhauser Verlag, Basel, Switzerland.

Rowland, I. R. (1992) Metabolic interactions in the gut. In: Probiotics. The Scientific Basis (Fuller, R., ed.), pp. 29–54. Chapman & Hall, London, UK.

Rowland, I. R. & Tanaka, R. (1993) The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with a human faecal microflora. J. Appl. Bacteriol. 14: 667–674.

Saunders, D. R. & Wiggins, H. S. (1981) Conservation of mannitol, lactulose, and raffinose by the human colon. Am. J. Physiol. 241: G397–G402.

Solomons, N. W., Viteri, S. E. & Hamilton, L. H. (1977) Application of a simple gas chromatography technic for measuring breath hydrogen. J. Lab. Clin. Med. 90: 856–862.

Sumihara, Y. (1987) The function of galactooligosaccharides and their application for food. Food Chem. 6: 87–94.

Tanaka, R., Takayama, H., Morotomi, M., Kuroshima, T., Ueyama, S., Matsumoto, K., Kuroda, A. & Mutai, M. (1983) Effects of administration of transgalacto-oligosaccharides and *Bifidobacterium breve* 4006 on the human fecal flora. Bifidobacteria Microflora 2: 17–24.

Woods, M. N. & Gorbach, S. L. (1993) Influences of fiber on the ecology of the intestinal flora. In: CRC Handbook of Dietary Fiber in Human Nutrition (Spiller, G. A., ed.), 2nd ed., pp. 362–370. CRC Press, Boca Raton, FL.