Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons

Brigitta Kleessen, Bernd Sykura, Hans-Joachim Zunft, and Michael Blaut

ABSTRACT Constipation is an ailment encountered often in elderly people. A study was initiated to test the effects of lactose or inulin on the bowel habits of constipated elderly patients and to correlate these effects with several variables measured in feces such as microflora composition, concentration of lactate and short-chain fatty acids (SCFAs), pH, and the activities of β-glucosidase and β-glucuronidase. Groups of 15 and 10 patients received lactose and inulin, respectively, for a period of 19 d. The dose, 20 g/d from days 1 to 8, was gradually increased to 40 g/d from days 9 to 11 and was kept at this dose from days 12 to 19. There was considerable interindividual variations with this kind of dietary intervention. Inulin increased bifidobacteria significantly from 7.9 to 9.2 log10/g dry feces, but decreased enterococci in number and enterobacteria in frequency. In individuals consuming lactose, a noticeable increase in fecal counts of enterococci and a decrease in lactobacilli and clostridia was detected. Total bacterial counts remained unchanged. No changes in the concentrations of fecal SCFAs and lactate were observed. SCFAs showed a slight trend toward higher molar ratios of acetate to butyrate in response to the intake of lactose or inulin. The fecal pH and the β-glucosidase and β-glucuronidase activities were not influenced by sugar intake. Inulin showed a better laxative effect than lactose and reduced functional constipation with only mild discomfort. Am J Clin Nutr 1997;65:1397–1402.

KEY WORDS Bifidobacteria, constipation, elderly subjects, fecal microflora, inulin, lactose, stool analysis, short-chain fatty acids

INTRODUCTION

Many societies in the Western world have had a considerable increase in the number of elderly people. Therefore, knowledge of age-related alterations in the gastrointestinal tract is important in the treatment and prophylaxis of diseases and in maintenance of health and the quality of life in elderly populations. Impairment of several gastrointestinal functions with clinically relevant effects may be attributed to aging, such as the following: 1) loss of teeth, which prevents thorough chewing and thus diminishes digestibility of food; 2) reduced olfactory and gustatory sensitivity, which restricts food selection; 3) recurrent atrophic gastritis, which results in hypochlorhydria; and 4) reduced intestinal motility, which retards digestion and causes constipation (1, 2). Furthermore, earlier findings indicate a distinct alteration in the composition of intestinal microflora with age (3). In elderly persons, bifidobacteria decrease or disappear, while lactobacilli, enterococci, enterobacteria, and clostridia increase. This in turn may lead to increased pathogenic and toxic burdens, cancer, and disorders of liver function (4, 5).

With a view toward improving colonic function in the elderly, the influence of nutrition on the intestinal ecosystem has been a subject of great interest. Nutrition can influence the microflora and its activity in two ways (6, 7): by consumption of fermented milk products containing viable microorganisms that are resistant to digestion and become metabolically active in the colon (probiotics), and by intake of nondigestible substrates that become available for colonic fermentation of health-promoting bacteria (prebiotics). Because intestinal bifidobacteria are considered to be beneficial to the host (8), many attempts have been made to increase the proportion of bifidobacteria in the intestinal microflora. Various substrates, including fructooligosaccharides, galactooligosaccharides, and inulin have been tested for their bifidogenic effect (9–12).

Moreover, these carbohydrates behave as dietary fiber (13). The objective of this study was to find out whether it is possible to reestablish a stable bifidobacterial flora in elderly subjects. We investigated the effect of consumption of either lactose or inulin on the composition of the fecal microflora and on selected indicators of microbial activity [concentration of short-chain fatty acids (SCFAs) and lactate, fecal pH, and β-glucosidase and β-glucuronidase activities] in elderly constipated persons. The possible laxative properties of these carbohydrates were of particular interest.

SUBJECTS AND METHODS

Subjects

In total, 35 female subjects suffering from constipation (symptoms: abdominal discomfort, only one or two bowel movements per week, and hard stool consistency) with a mean age of 76.4 y (range: 68–89 y) were admitted to the Department of Internal Medicine of the Medical Center of Oranienburg, 1 From the German Institute of Human Nutrition, Potsdam-Rehbrücke, and the Internal Medicine Medical Center of Oranienburg, Germany.
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Germany. They underwent a full medical examination and were confirmed to have not been taking antibiotics for \( \geq 4 \) wk. The Ethical Committee of Brandenburg approved the protocol and all persons were asked to give their informed consent before beginning the study.

**Experimental design**

The study lasted 19 d. Participants consumed their regular hospital diet and were encouraged to eat portions similar in size to those of the other patients. Their diet was supplemented with lactose (EDELWEISS-MILCHWERKE, K Hoefelmayer GmbH/Kempten, Germany) or chicory inulin (Rafinose; Raffinerie Tirlemontoise, Tienen, Belgium). The latter is a polydispersed \( \beta(2\rightarrow1) \)-fructan that contains a significant amount of oligofructose with an average degree of polymerization of 10 (14). The 35 subjects were randomly assigned to receive inulin \((n = 17)\) or lactose \((n = 18)\). They received 20 g lactose or inulin per day for 8 d (first study period) followed by an adaptation period of 3 d, during which the daily dose was increased stepwise to 40 g/d. The latter dose was maintained from day 12 to 19 (second study period). At the 20-g/d dose the sugar was given at once in 250 mL water at 0800; at the 40-g/d dose the sugar was given in 20-g portions at 0800 and 1600. Ten of the 35 patients had to be excluded from the study because of the occurrence of infectious illness, antibiotic treatment, use of a laxative, or lack of samples. Thus, for data evaluation, 15 subjects were included in the lactose group and 10 patients in the inulin group.

The dietary supplement (lactose or inulin) was blinded so that the patients, the personnel delivering the diet to the patients, and the laboratory staff involved had no knowledge of the key of the study. Daily interviews were conducted throughout the study periods to obtain a subjective evaluation of the patients' well-being. The patients were questioned about their gastrointestinal responses. The following items were recorded: 1) number of bowel movements per day, 2) abdominal pain, 3) stool consistency (eg, diarrhea), 4) episodes of flatulence, and 5) well-being (eg, nausea and headaches). Severity of symptoms 2–5 was rated by the subjects as absent, mild, moderate, or severe.

**Sampling**

Stool samples were collected within 1–5 d before the beginning of the study (baseline conditions), on day 7 or 8 in the first study period, and on day 18 or 19 in the second study period within 30 min after defection. For the microbiological investigations, \(-0.5\) g fresh specimen was immediately placed into a preweighed tube with 2.0 mL cryoprotective broth (preduced brain-heart infusion broth containing 20% glycerol; DIFCO Laboratories, Detroit), which maintains the viability of fecal bacteria (15). The remaining sample material was placed in plastic vials for determination of dry weight. SCFAs, L- and D-lactate, \( \mathrm{pH} \), and \( \beta \)-glucosidase and \( \beta \)-glucuronidase activities. All specimens were stored immediately at \(-20^\circ \mathrm{C}\). They were analyzed within 3 wk after collection. We confirmed that the cell counts of the bacterial groups determined in our study and \( \beta \)-glucosidase and \( \beta \)-glucuronidase activities were not significantly affected by freezing for a period of 3 wk when samples were prepared as described above.

**Microbiological studies**

Investigation of the fecal microflora in the blinded probes was performed by using techniques described previously (16). Bacterial counts are expressed as log_{10} colony-forming units (CFUs)/g dry mass of feces. Bacterial reference strains present in the laboratory strain collection or obtained from the American Type Culture Collection (Rockville, MD) were used for typing *Bifidobacterium* species. The isolated organisms from the fecal samples were identified biochemically by using API 50 CHL chemical strips (BioMérieux, France) and by comparing the profiles with those of reference species.

**Analytic procedures**

Stool samples were thawed for 30 min and homogenized. SCFAs were extracted as described previously by Pomare et al (17). Samples of 1.0 mL were injected into a gas chromatograph (HP 5890 A; Hewlett Packard GmbH, Waldbronn, Germany) equipped with a flame-ionization detector and a capillary column (25 m \( \times 0.23 \) mm) impregnated with 20 M Carbowax (Hewlett Packard GmbH). Helium was used as carrier gas at a column flow rate of 12 mL/min with a split ratio of 1:10. The column temperature was 125 \( ^\circ \mathrm{C} \). Isobutyric acid served as an internal standard. L- and D-Lactate were determined enzymatically as described previously (18).

Fecal \( \beta \)-glucosidase and \( \beta \)-glucuronidase activities were measured by following the hydrolysis of the chromogenic substrates \( \beta \)-nitrophenyl-\( \beta \)-glucopyranoside and \( \beta \)-nitrophenyl-\( \beta \)-glucuronide photometrically, respectively (19). Enzyme activities are expressed as the amount of enzyme that hydrolyzes 1 \( \mu \)mol substrate \( \cdot \) min \(^{-1} \) \( \cdot \) mg fecal dry mass \(^{-1} \) at \( 37^\circ \mathrm{C} \).

Stool \( \mathrm{pH} \) was determined in each sample on a threefold dilution with deionized, distilled water. \( \mathrm{pH} \) was measured with a standard \( \mathrm{pH} \) meter (pH 537; WTW GmbH, Germany) with a bioelectrode (Hamilton AG, Bonaduz, Switzerland). Wet and dry masses were obtained by weighing before and after freeze-drying the samples with a Gamma IA apparatus (Christ, Osterode, Germany).

**Statistical analysis**

The statistical software package SYSTAT, version 5.2, was used (Systat, Inc, Chicago). Data are expressed as \( x \pm SD \). The normality of data was checked by using the Kolmogorov-Smirnov test. Two-factor repeated-measures analysis of variance (ANOVA) was performed. Groups were compared statistically by using the nonparametric Mann-Whitney \( U \) test. Within groups, time comparison was done with the Wilcoxon matched-pairs signed-ranks test.

**RESULTS**

**Bowel habit**

Before the study began all patients had only one or two bowel movements per week. Individuals differed widely in their response to ingested lactose. In 4 of 15 subjects 20 g lactose/d increased the stool frequency. In these patients, the average number of bowel movements per week was now between three and four and the higher dose of 40 g/d led to a stool frequency of 7.5 per week. The patients reported easier defection without diarrhea, mild passing of rectal gas, and no other
discomfort. In seven other subjects only the lower dose of 20 g lactose/d induced a higher stool frequency (one bowel movement per day). In these patients, administration of 40 g/d reduced the average frequency of bowel movements to two to three per week, resulting in firmer stools and defecation problems. Four of the seven patients complained about moderate abdominal pain. Four other subjects experienced only minor laxative effects independent of the dose of lactose offered (two to three bowel movements per week) and complained about severe flatus.

Inulin increased the stool frequency in 7 of 10 patients to eight and nine per week, independent of the amount of inulin ingested. Stools were soft, but diarrhea was not observed. Only mild-to-moderate flatulence, which did not cause discomfort, was reported. In two other subjects, the laxative effect of inulin was dependent on the amount taken. In one subject, intake of 40 g inulin/d increased the stool frequency (seven bowel movements per week compared with five bowel movements per week with intake of 20 g inulin/d), whereas the other subject noted a slightly constipating effect at this dosage (four bowel movements per week compared with six bowel movements per week at 20 g/d). In 1 of the 10 patients, stool frequency did not change distinctly after ingestion of inulin (three bowel movements per week, but the stool was softer and mild flatulence was observed. All 25 patients reported the absence of nausea and headaches. The percentage of dry fecal matter decreased significantly in comparison with the baseline measurements in response to feeding 20 g lactose or inulin per day, corresponding to an increase in the water content (Table 1). This decrease was also observed at 40 g lactose/d.

Fecal microflora

There was no difference in the fecal flora between constipated patients before inulin or lactose intake (Table 1). After sugar administration, total bacterial numbers were constant throughout the study, but there were differences with respect to counts and frequency of certain bacterial groups (Table 1). We observed a significant decrease in lactobacilli and clostridia ($P < 0.05$) and an increase in enterococci ($P < 0.01$) during lactose intake. In 5 of 15 patients who received 40 g lactose/d, Bacteroides species increased by $\approx 2$ logs. In persons fed inulin, in contrast, a steady increase in bifidobacteria from $\log_{10} 7.9$ to $\log_{10} 9.2$ was observed. At the same time, entero-coccal counts decreased significantly ($P < 0.01$) and enterobacteria were less frequently isolated. Counts of hydrogen sulfide-producing bacteria were unchanged. Hydrogen sulfide is an undesirable toxic product of proteolytic and sulfate-reducing bacteria.

Considerable interindividual variations were observed for all bacterial groups both before and after administration of lactose or inulin. Bifidobacterium adolescentis, B. longum, and B. bifidum were the predominant species whereas B. catenulatum, B. angulatum, and B. infantis were detected only occasionally.

Microbial activity

As shown in Table 2, neither the ingestion of lactose nor inulin affected the concentrations of SCFAs and L- and D-lactate in the feces of elderly patients. Lactate was detectable in only small amounts ranging from 4.2 to 8.0 $\mu$mol/g dry feces. Few changes were observed in the relative proportions of SCFAs (Table 3). The acetate:butyrate ratio showed a slight increase in response to the administration of lactose or inulin. Fecal pH was similar before and after sugar intake (Table 2), as were the activities of $\beta$-glucosidase and $\beta$-glucuronidase (Table 4).

DISCUSSION

Constipation in the elderly is a considerable problem in developed Western countries (20, 21). Many factors may contribute to the development of constipation with aging, such as

TABLE 1

<table>
<thead>
<tr>
<th>Fecal variable</th>
<th>Lactose ($n = 15$)</th>
<th>Inulin ($n = 10$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before administration</td>
<td>20 g/d</td>
</tr>
<tr>
<td>Total counts</td>
<td>9.3 ± 0.7$^1$</td>
<td>9.7 ± 0.8</td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>8.2 ± 0.7</td>
<td>8.6 ± 0.9</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>9.0 ± 1.1</td>
<td>9.3 ± 0.9</td>
</tr>
<tr>
<td>Clostridia</td>
<td>6.4 ± 1.2</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>7.9 ± 0.9</td>
<td>7.1 ± 1.1$^5$</td>
</tr>
<tr>
<td>Enterococci</td>
<td>7.1 ± 1.1</td>
<td>8.0 ± 0.6$^6$</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>7.1 ± 1.6</td>
<td>6.8 ± 1.6</td>
</tr>
<tr>
<td>H$_2$S-forming bacteria</td>
<td>6.9 ± 1.7</td>
<td>6.4 ± 1.7</td>
</tr>
<tr>
<td>Percentage of dry matter (%)</td>
<td>32.3 ± 6.3</td>
<td>28.4 ± 6.4$^8$</td>
</tr>
</tbody>
</table>

$^1$ Bacterial counts expressed as $x \pm SD \log_{10}\mu$g dry feces. Counts of organism based exclusively on positive cultures.

$^2$ Frequency of occurrence in brackets.

$^3,^4$ Significantly different from before administration: $^1 P < 0.05$, $^4 P < 0.01$. 

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Concentrations of short-chain fatty acids (SCFAs) and lactate in feces of elderly patients before and after administration of lactose or inulin

<table>
<thead>
<tr>
<th>Fermentation products</th>
<th>Lactose (n = 15) Before administration 20 g/d 40 g/d</th>
<th>Inulin (n = 10) Before administration 20 g/d 40 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCFAs (μmol/g dry feces)</td>
<td>Total 251.8 ± 152.6 250.4 ± 104.5 226.7 ± 111.4</td>
<td>Total 218.9 ± 56.1 267.3 ± 103.3 213.0 ± 92.9</td>
</tr>
<tr>
<td>Acetate</td>
<td>126.6 ± 64.0 137.6 ± 61.8 133.4 ± 48.0</td>
<td>98.9 ± 25.8 137.5 ± 64.0 114.3 ± 43.1</td>
</tr>
<tr>
<td>Propionate</td>
<td>49.2 ± 42.6 45.4 ± 23.0 39.0 ± 30.4</td>
<td>50.6 ± 28.5 60.5 ± 25.5 42.3 ± 23.5</td>
</tr>
<tr>
<td>Butyrate</td>
<td>50.2 ± 45.7 49.0 ± 35.9 34.7 ± 29.1</td>
<td>36.6 ± 11.6 33.1 ± 20.3 31.6 ± 26.0</td>
</tr>
<tr>
<td>Isovalerate</td>
<td>16.7 ± 17.0 16.7 ± 5.1 13.1 ± 6.6</td>
<td>20.4 ± 12.8 22.1 ± 10.3 14.6 ± 7.4</td>
</tr>
<tr>
<td>Valerate</td>
<td>10.0 ± 6.0 8.4 ± 4.1 9.0 ± 5.1</td>
<td>12.5 ± 3.4 14.1 ± 5.8 10.2 ± 4.9</td>
</tr>
<tr>
<td>Lactate (μmol/g dry feces)</td>
<td>4.2 ± 3.5 5.7 ± 6.2 6.0 ± 6.2</td>
<td>5.6 ± 4.8 6.0 ± 4.3 8.0 ± 6.9</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.7 7.3 ± 0.6 7.5 ± 0.6</td>
<td>7.4 ± 0.5 7.5 ± 0.4 7.3 ± 0.6</td>
</tr>
</tbody>
</table>

Note: There were no significant differences among treatments or doses.

TABLE 3
Molar ratios of acetate, propionate, and butyrate in feces of elderly patients before and after administration of lactose or inulin

<table>
<thead>
<tr>
<th>Fermentation products</th>
<th>Lactose (n = 15) Before administration 20 g/d 40 g/d</th>
<th>Inulin (n = 10) Before administration 20 g/d 40 g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>61.1 ± 14.1 60.8 ± 10.5 68.7 ± 12.1</td>
<td>54.0 ± 6.3 59.1 ± 7.6 62.7 ± 8.8</td>
</tr>
<tr>
<td>Propionate</td>
<td>19.4 ± 6.1 19.6 ± 5.5 17.1 ± 6.3</td>
<td>26.0 ± 7.7 26.4 ± 5.3 22.1 ± 6.4</td>
</tr>
<tr>
<td>Butyrate</td>
<td>19.5 ± 9.7 19.6 ± 9.9 14.2 ± 6.9</td>
<td>20.0 ± 5.1 14.5 ± 5.2 15.3 ± 5.4</td>
</tr>
</tbody>
</table>

Note: Percentage of total acetate + propionate + butyrate ± SD.
the differences in their rate of production by the colonic flora. Absorption alters the SCFA concentration during the passage through the colon (36). Therefore, the fermentative processes in the upper colon can be characterized only by measuring SCFAs and lactate in the cecal and colonic chyme. To what extent metabolism in the bowel content is reflected in the feces depends on numerous variables, such as gut motility, total intake of dietary fiber, intestinal secretions, and duration of dietary interventions. Similarly, we could not find any differences in fecal pH during the test periods. However, because the fecal pH is the net sum of the degree of SCFA absorption and bicarbonate secretion during passage through the colon, fecal pH may not accurately reflect the pH in the colon (37).

Our data indicate no changes in the fecal activities of β-glucosidase and β-glucuronidase in response to the feeding of lactose or inulin (Table 4). These enzymes may play a role in the metabolic activation of prostaglandins and deconjugation processes in the colonic lumen (5, 38). The lack of alterations in the enzyme activities in the feces may be due to the same reasons mentioned for the SCFAs.

Several studies in humans suggest that fermentation of carbohydrates stimulates colonic motility (13). Our study confirms that clinical signs of constipation may be improved by the intake of unabsorbed carbohydrates such as lactose or inulin. In the past, the intake of lactulose (39) or lactose (40) was widely recommended for the treatment of a variety of gastrointestinal disorders, although it is difficult to find adequate scientific support for the effectiveness of these sugars in treating constipation. Hidaka et al. (27) observed that the administration of fructooligosaccharides relieved constipation. In our study, the patients were highly variable in their response to lactose and inulin. The data indicate a better laxative effect of lactose at 20 g/d than at 40 g/d. This positive effect, however, was sometimes accompanied by side effects such as flatulence, intestinal pressure, and abdominal pain. The ingestion of inulin improved the metabolic activation of procarcinogens and deconjugation of enzymes mentioned for the SCFAs.

Further studies are needed to clarify whether long-term administration of these carbohydrates alters the colonic microbial activity to a measurable degree and whether alterations in colonic function and microflora may be detectable in patients after the dietary intervention has ended.

REFERENCES


