Constipation Is Relieved More by Rye Bread Than Wheat Bread or Laxatives without Increased Adverse Gastrointestinal Effects

Reetta Holma,1,4 Sanna-Maria Hongisto,5 Maija Saxelin,6 and Riitta Korpela3,6

Abstract
Rye bread and lactobacilli modify the colonic environment and have the potential to relieve constipation and could be a safe and convenient alternative to laxatives. The effects of rye bread and cultured buttermilk with Lactobacillus rhamnosus GG (LGG) on bowel function and colon metabolism were investigated and compared with laxatives in 51 constipated adults. They were randomized to receive whole-grain rye bread (minimum 240 g/d), LGG (2 x 10^10 colony-forming units/d), whole-grain rye bread (minimum 240 g/d) + LGG (2 x 10^10 colony-forming units/d), white wheat bread (maximum 192 g/d), or laxatives (as usual for a participant) for 3 wk. Participants recorded their dietary habits, bowel function, and gastrointestinal symptoms. Fecal weight, pH, SCFA and bacterial enzyme activities, total intestinal transit time (TITT), and breath hydrogen were determined. Rye bread, compared with white wheat bread, shortened TITT by 23% (P = 0.040), increased weekly defecations by 1.4 (P = 0.014), softened feces (odds ratio [OR] 3.98; P = 0.037), eased defecation (OR 5.08; P = 0.018), increased fecal acetic acid and butyric acid contents by 24% (P = 0.044) and 63% (P < 0.001), respectively, and reduced fecal β-glucuronidase activity by 23% (P = 0.014). Compared with laxatives, rye bread reduced TITT by 41% (P = 0.006), fecal β-glucuronidase activity by 38% (P = 0.033), and fecal pH by 0.31 units (P = 0.006). LGG did not relieve constipation or significantly affect colonic metabolism. Gastrointestinal adverse effects did not significantly differ among the study groups. In conclusion, rye bread relieves mild constipation and improves colonic metabolism compared with white wheat bread and commonly used laxatives without increasing gastrointestinal adverse effects. J. Nutr. 140: 534–541, 2010.

Introduction
Chronic constipation affects up to 27% of the population of Western countries (1). Symptoms of constipation, such as infrequent bowel movements, hard stools, straining on defecation, cramps, abdominal bloating, flatulence, and sometimes severe abdominal pain, may affect patients’ overall quality of life (1). Slow colonic transit has been connected to the development of colon cancer (2).

The management of constipation is commonly begun by increasing fiber intake (3,4). Daily intake of 25 g of fiber is considered effective (3). Soluble fiber produces viscous solutions and is fermented, in the proximal colon, by bacteria to a greater extent than insoluble fiber (5). SCFA and gas produced during fermentation reduce colonic transit time. Insoluble fiber, on the other hand, undergoes minimal change in the digestive tract and increases fecal mass by retaining water, leading to the shortening of colonic transit time (5). Although the use of fiber supplementation for treatment of constipation is common, evidence from clinical trials is limited (3). Rye is a traditionally used cereal in Northern and Eastern Europe. It is rich in dietary fiber comprising mainly arabinoxylans, fructans, and β-glucans (6,7). Arabinoxylans appear to be preferred substrates for the fermentative production of SCFA and, in particular, butyrate (8).

Constipation has been associated with abnormal fecal flora, such as decreased concentrations of bifidobacteria and lactobacilli, and increased concentrations of methanogens as well as potentially pathogenic bacteria and fungi (9–11). Two double-blind, randomized trials produced promising results on the effect of single strains of lactobacilli on chronic constipation (12,13). It has been suggested that lactobacilli exert their effects by increasing SCFA production. On the other hand, increased intake of dietary fiber often causes abdominal discomfort (such as flatulence and abdominal bloating) (14), which has been shown to be alleviated by Lactobacillus rhamnosus GG (LGG)

1 Supported by the Finnish National Technology Agency TEKES program "Innovation in Foods," Valio Ltd, and Fazer Bakeries Ltd.
2 Author disclosures: R. Korpela is the professor of medical nutrition physiology at the Institute of Biomedicine, University of Helsinki, as well as the president of research at Valio Research Centre, Helsinki, Finland. S-M. Hongisto is an employee of Fazer Bakeries Ltd, Lahti, Finland. M. Saxelin is a former employee of Valio Research Centre. R. Holma has received compensation from Valio Research Centre.
3 Institute of Biomedicine, FIN-00014 University of Helsinki, Finland; "Navida Ltd, Kalliollantie 7, FIN-00140 Helsinki, Finland;
4 Fazer Bakeries Ltd, FIN-15101 Lahti, Finland; and "Valio Research Centre, FIN-00039 Helsinki, Finland
5 Abbreviations used: LGG, Lactobacillus rhamnosus GG; OR, odds ratio; TITT, total intestinal transit time.
supplementation during colorectal cancer chemotherapy (15). A recent study showed that transient flatulence and abdominal bloating caused by increased rye bread consumption were ameliorated by yogurt containing LGG (16).

We undertook this study to investigate the effects of rye bread and cultured buttermilk with LGG and their interaction on bowel function, colon metabolism, and gastrointestinal symptoms and to compare their efficacy to that of laxative agents in constipated adults. Dietary habits and laxative use were carefully monitored.

Methods

Participants. Fifty-one adults (47 women, 4 men) aged 22–78 y with self-reported constipation and use of laxatives were recruited for the study. The inclusion criteria were <5 defecations/wk without laxatives or a maximum of 7 defecations/wk with laxatives, and constipation according to the participant’s own report. Exclusion criteria included gastrointestinal diseases (such as diverticulosis, peptic or duodenal ulcer, and celiac disease), antibiotics within the 3 wk preceding the study, regular medication affecting gastrointestinal function, pregnancy, and lactation. The study protocol was approved by the Ethics Committee of the Foundation for Nutrition Research, Helsinki, Finland, and was carefully explained to the participants, who then gave their written informed consent.

Study design. The study was carried out in the Helsinki metropolitan area, where the average daily intake of dietary fiber for both women and men is 20 g (17), which is less than the Nordic recommendation of 25–35 g/d (18). The study had a randomized, unblinded, 2 × 2 factorial design with a 1-wk baseline period and a 3-wk intervention period. The laxative group served as an additional control. After the baseline period, the participants were randomized into 1 of the following 5 groups: rye bread (minimum 6 × 40 g/d whole-grain rye bread (=30 g fiber/d)), LGG [2 × 200 g/d cultured buttermilk with LGG, maximum 8 × 24 g/d white wheat bread (=8.6 g fiber/d), rye bread + LGG [minimum 6 × 40 g/d whole-grain rye bread (=30 g fiber/d), 2 × 200 g/d cultured buttermilk with LGG], control [maximum 8 × 24 g/d white wheat bread (=8.6 g fiber/d)], and laxative [laxative use as usual, maximum 8 × 24 g/d white wheat bread (=8.6 g fiber/d)]. Additional bread was allowed for every group, but it was restricted to low fiber bread in groups receiving white wheat bread (LGG, control, and laxative groups).

The whole-grain rye bread was 100% rye bread and contained 12.3 g fiber/100 g (Real, Fazer Bakeries). The white wheat bread contained 4.5 g fiber/100 g (Suomi Paahtis, Fazer Bakeries). The cultured buttermilk with LGG (Lactobacillus GG, ATCC 53103, LGG) contained at least 5 × 10^8 colony-forming units LGG/g (Gefilus, Valio). In addition to laxative drugs, foods generally considered to have a laxative effect, including prunes, flax, and fiber products, were considered laxatives in this study and were only allowed for the laxative group. However, all patients were allowed to use laxatives if several days passed without defecation, but only after first contacting the principal investigator. During the baseline period, no participants in any group were allowed to consume the intervention foods (Real, Suomi Paahtis, and Gefilus buttermilk) or laxative drugs and foods, but other rye bread was allowed if it was included in the participant’s normal diet.

Before the beginning of the study, participants were instructed not to use LGG-containing products for 3 wk preceding the study. No other LGG products or products containing any other lactic acid bacteria considered probiotic were allowed during the study, nor was candy sweetened with xylitol, sorbitol, mannitol, or maltitol. At the beginning of the study, the participants were instructed to not change their ordinary diet other than according to the interventions and to not change their ordinary level of physical exercise, smoking habits, or alcohol consumption during the study.

Questionnaires. Before the beginning of the study, the participants were questioned about their bowel function, normal rye bread consumption, laxative use, diseases, medication (including recent antibiotics), physical activity, and smoking habits. Dietary habits were controlled at baseline and during wk 1 and 3 with 1-wk dietary records that put special emphasis on fiber-rich foods and fluid intake. Compliance with the study interventions was checked in a separate section. The records were checked by a nutritionist when they were returned and the intake of fiber and fluid was calculated using Micro-Nutrica software based on data files for Finnish foods (19). Participants were instructed to record the use of any temporary medication, with special attention to laxative use.

During the baseline week and wk 1 and 3, the participants kept a daily diary of their bowel functions. They reported the number and consistency of stools (−1 = loose, 0 = normal, 1 = hard), difficulty of defecation (−1 = easy, 0 = normal, 1 = straining at defecation), and abdominal symptoms (abdominal pain, flatulence, borborygmi, abdominal bloating, constipation, and diarrhea: 0 = no symptoms, 1 = mild, 2 = moderate, 3 = severe symptoms).

Fecal sample collection and analysis. All participants were instructed to collect all feces for 5 d during the baseline period and wk 3. In addition, participants collected all feces for 1 d during wk 1. The participants were asked to freeze the samples immediately and to keep them frozen until they took them to the study centre. In the laboratory, the fecal samples were stored at −20°C until determination of the wet and dry weights, pH, SCFA concentrations, bacterial enzyme activities, and total intestinal transit time (TITT).

TITT was determined twice: during the baseline week and during wk 3 and was measured using radiopaque Sitzmarks capsules (Konsyl), each containing 24 polyvinyl chloride rings, which can be seen on X-ray. At the beginning of each TITT measurement, the participants ingested 1 Sitzmarks capsule and then collected all their feces for the subsequent 5 d. The frozen samples were X-rayed and TITT was calculated as the mean time for the rings to pass through the gastrointestinal tract, as previously described (16).

Frozen feces were weighed then thawed at 4°C and the weight per day was calculated. Thawed fecal samples were pooled by adding 20 g/100 g distilled water and homogenizing with a Stomacher laboratory blender before determining fecal pH, fecal dry weight, enzyme activities, and fecal metabolites. Bacterial enzyme activities were determined twice: during the baseline week and during wk 3. The thawed and pooled fecal samples were homogenized, sonicated (in an ice bath), and centrifuged, and the activities of fecal bacterial enzymes were determined from the supernatant as described by Ling et al. (20) and Freeman (21). Enzyme activities are expressed as nmol min^-1 mg protein^-1. Fecal dry weight was determined from the homogenized fecal samples (1 g) by oven drying at 105°C for 17 h and was expressed as percent of original wet weight.

Samples of the homogenized fecal samples (1 g) were analyzed for SCFA by GC (HP5890; Hewlett Packard) using a DB-WAX column (30-m, 0.3-μm film) by the method of Schooley et al. (22) and as previously described (23). All fecal analyses were performed at VTT Technical Research Centre of Finland.

Breath hydrogen. After an overnight fast, exhaled breath hydrogen concentrations were measured with a Micro H2 monitor (Micro Medical) (24). Measurements were made in triplicate early in the morning. No smoking or food was allowed before testing. No beer, other alcohol, or foods that are slowly ingested or that cause flatulence (except for the intervention foods), such as beans, peas, cabbage, onions, apples, and dried prunes, were allowed on the preceding day.

Statistical analysis. The analyses were performed in 2 parts. First, the 5 study groups (rye bread, LGG, rye bread + LGG, control, and laxative) were compared and in the event of a significant global P-value, pairwise comparisons were performed using the control group and the laxative group as reference groups. The pairwise comparisons were Bonferroni-corrected, except in analysis of TITT and bowel function variables. Secondly, the 2 × 2 factorial study design with 2 factors (with and without rye bread; with and without LGG) was applied and the extra control group with laxatives was not included. Data were analyzed with SPSS statistical software (version 15.0). All tests were carried out as 2-sided and P < 0.05 was considered significant.

Rye bread relieves constipation 535
were logarithmically transformed before analyses. The 5 study groups were compared using ANCOVA, in which the baseline TITT was included as a covariate. Using ANCOVA with 2 grouping factors (rye bread and LGG), the effects of the factors were estimated as ratios (with vs. without rye bread; with vs. without LGG) with 95% CI. The results for groups and factors are given as baseline-adjusted geometric means with 95% CI.

**Number of bowel movements.** The 5 study groups were compared using ANCOVA for repeated measures, where the number of bowel movements at the baseline was included as a covariate. The effects of factors were estimated using ANCOVA for repeated measures with 2 grouping factors. The results for groups and factors are given as baseline-adjusted marginal means with 95% CI.

**Softening of feces and easing of defecation.** Hardness of feces and ease of defecation during wk 1 and 3 were compared with the baseline period. The individual changes were dichotomized to the variables “softening of feces” and “easing of defecation” (yes vs. no). The effects of groups and both factors were estimated using univariable logistic regression analysis. The results are given as odds ratio (OR) with 95% CI.

**Fecal weight.** The 5 study groups were compared using ANCOVA for repeated measures, where the figure for fecal weight at the baseline was included as a covariate. The significance of the factors and interaction was estimated using ANCOVA for repeated measures with 2 grouping factors. Subgroup analyses were performed because of the significant interaction between rye bread and LGG. The effect of rye bread was estimated (using ANCOVA for repeated measures) in subgroups with and without LGG. Accordingly, the effect of LGG was estimated in subgroups with and without rye bread. The results for groups and factors are given as baseline-adjusted marginal means with 95% CI.

**Fecal pH and SCFA.** In terms of fecal pH, there was an almost significant ($P = 0.085$) interaction between rye bread and LGG. Thus, the analyses were performed using the same methods as described for fecal weight. Fecal SCFA variables were analyzed using the same methods as for the number of bowel movements.

**Fecal enzyme activities.** The variables were skewed to the right and were logarithmically transformed before analysis. The analyses were performed using the same methods as for TITT.

### TABLE 1
Characteristics of the study participants

<table>
<thead>
<tr>
<th></th>
<th>Rye bread</th>
<th>LGG</th>
<th>Rye bread + LGG</th>
<th>Control</th>
<th>Laxative</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>12</td>
<td>11</td>
<td>10</td>
<td>8</td>
<td>51</td>
</tr>
<tr>
<td>Age, y</td>
<td>51 (31-78)</td>
<td>44  (28-63)</td>
<td>48 (24-67)</td>
<td>43 (24-55)</td>
<td>42 (22-61)</td>
<td>46 (22-78)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28 (22-34)</td>
<td>24 (20-30)</td>
<td>24 (20-33)</td>
<td>24 (22-29)</td>
<td>25 (20-31)</td>
<td>25 (20-34)</td>
</tr>
<tr>
<td>Rye bread, slices/d</td>
<td>2.8 (0-6.5)</td>
<td>3.3 (0-10.0)</td>
<td>3.3 (0.5-6.5)</td>
<td>3.4 (1.0-6.0)</td>
<td>3.6 (2.0-6.0)</td>
<td>3.4 (0-10.0)</td>
</tr>
<tr>
<td>Fiber intake, g/d</td>
<td>20.0 (12.5-30.6)</td>
<td>19.7 (7.4-29.3)</td>
<td>16.6 (10.3-29.3)</td>
<td>17.1 (6.9-23.3)</td>
<td>19.0 (13.4-25.4)</td>
<td>18.5 (6.9-30.6)</td>
</tr>
<tr>
<td>Fluid intake, L/d</td>
<td>1.49 (0.83-2.57)</td>
<td>1.74 (0.80-3.53)</td>
<td>1.72 (0.59-3.53)</td>
<td>1.63 (0.54-3.21)</td>
<td>1.85 (1.41-2.60)</td>
<td>1.68 (0.54-3.53)</td>
</tr>
<tr>
<td>Daily physical exercise, n</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>None, very little, or moderate</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Considerable</td>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

1 Values are means (ranges) unless otherwise specified. Participants consisted of 47 women and 4 men (one man in each group except for the rye bread group). Three participants were current smokers (2 in the LGG group, 1 in the laxative group). Due to missing information in some of the characteristics, the number of participants varied: $n = 11–12$ in the LGG, 9–10 in the rye bread, and 9–10 in the control groups. There were no statistical differences among the groups.

2 Slice = 35 g.

---

**Gastrointestinal symptoms.** The daily gastrointestinal symptoms (abdominal pain, abdominal bloating, flatulence, borborygmi, constipation, and diarrhea) were each coded 0–3. First, the daily score (sum of all symptoms, 0–18) and then the weekly sum were calculated. The total score per week was analyzed using the same methods as for the number of bowel movements.

The Pearson correlation coefficient was calculated to study the association between breath hydrogen and total symptom score at baseline and during wk 1 and 3. In addition, applying the method given by Bland and Altman (25), the correlation was calculated using the results of repeated measurements made during the study.

**Results**

**Participant characteristics and compliance.** The characteristics of the participants are presented in Table 1. All 51 participants completed the study. Compliance with the intervention foods was excellent, with an average 96% and 85% of the rye bread and 96 and 89% of the buttermilk consumed according to the instructions during the wk 1 and 3 of intervention, respectively. The approximately double daily fiber intake during the intervention in groups receiving rye bread compared with baseline provides additional support for the compliance with the rye bread consumption. Intakes during wk 1 and 3 were [mean (range)]: rye bread 40.4 (34.4–55.2) g/d, LGG 14.0 (11.3–17.8) g/d, rye bread + LGG 38.2 (31.6–46.6) g/d, control 15.8 (9.8–23.4) g/d, and laxative 14.9 (10.9–21.8) g/d.

Each participant in the laxative group used laxatives during wk 1 and 3 [3 used lactulose (Duphalac, 667 g/L or 950 mg/g, or Levolac, 670 g/L, resulting in 10.0–16.7 g lactulose/d); 2 used bran (All-Bran Regular 20 g/d or oatbran 4 g/d), 1 used sodium picosulphate (Laxoberon, 7.5 g/L, 6 drops/d); 1 used senna (Senade, 13.5 mg/tablet, 1.5 tablets/d); and 1 used Testa ispaghula (Vi-Siblin S, 880 mg/g, 5 g/d)]. Compliance was good with avoiding laxative use in the other groups [rye bread group: 1 used bisacodyl (Metalax, 5 mg/tablet, 0.5 tablets/d) during each period; rye bread + LGG group: 1 used sodium picosulphate (Laxoberon, 7.5 g/L, 2 drops/d) during wk 1; LGG
There tended to be an interaction (butyric acid, and propionic acid compared with wheat bread with rye bread. Likewise, consumption of cultured buttermilk with LGG significantly increased fecal pH only when consumed with rye bread.

On the whole, the provision of samples for analysis and the response rates to the questionnaires by the study participants were excellent. Transit time calculation was not possible in the case of 2 participants in the rye bread + LGG group, 2 in the LGG group, and 1 in the rye bread group (erythromycin for bronchitis, started after fecal sampling). Their exclusion from the main variables did not affect the results obtained, so the results are presented for all the participants.

TITT and bowel function. Rye bread consumption reduced TITT by 23% compared with wheat bread (Table 2). In the rye bread group, TITT was 65% of that in the control group and 59% of that in the laxative group. Bowel function was significantly improved by rye bread consumption. Rye bread consumption increased the number of bowel movements compared with wheat bread (Table 2). Feces were more frequently softened and defecation was eased by rye bread compared with wheat bread during wk 3 (Table 3). This effect was already seen during wk 1 but was not significant (OR 2.51; 0.67–9.46; P = 0.173 for softening of feces and OR 3.19; 0.84–12.14; P = 0.089 for easing of defecation). Cultured buttermilk with LGG did not significantly affect TITT, bowel movements (Table 2), softening of feces, or easing of defecation (Table 3) compared with groups not consuming cultured buttermilk.

Fecal weight. There was a significant interaction between rye bread and cultured buttermilk with LGG (P = 0.011), and subgroup analyses therefore were conducted. Rye bread significantly increased fecal weight only when consumed with cultured buttermilk with LGG (Table 4).

Fecal dry weight (percent of wet weight) was slightly reduced by rye bread compared with wheat bread (effect –2.67; −4.92 to −0.42; P = 0.021). There was no significant difference in fecal dry weight between groups receiving or not receiving cultured buttermilk with LGG (effect 0.35; −1.91 to 2.61; P = 0.758).

Fecal pH and SCFA. Fecal pH was lower in the rye bread group than in both the control and laxative groups (Table 4; Fig. 1). There tended to be an interaction (P = 0.085) between rye bread and cultured buttermilk with LGG. Rye bread significantly reduced fecal pH only when consumed without cultured buttermilk with LGG. Likewise, consumption of cultured buttermilk with LGG significantly increased fecal pH only when consumed with rye bread.

Rye bread increased the concentrations of fecal acetic acid, butyric acid, and propionic acid compared with wheat bread

<table>
<thead>
<tr>
<th>Study groups</th>
<th>TITT</th>
<th>Bowel movements</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>h</td>
<td>P</td>
</tr>
<tr>
<td>Rye bread</td>
<td>9</td>
<td>42.5* (33.2–54.5)</td>
</tr>
<tr>
<td>LGG</td>
<td>10</td>
<td>67.0 (63.1–84.6)</td>
</tr>
<tr>
<td>Rye bread + LGG</td>
<td>9</td>
<td>60.5 (47.3–77.3)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>65.5 (51.9–82.7)</td>
</tr>
<tr>
<td>Laxative</td>
<td>8</td>
<td>72.5 (65.6–94.6)</td>
</tr>
</tbody>
</table>

2x2 factorial design

| Rye bread given | 18   | 52.2 (43.4–62.6) | 18   | 6.7 (5.9–7.5) |
| Rye bread not given | 20  | 68.1 (72.8–81.0) | 21   | 5.3 (4.6–6.1) |
| Effect of rye bread | 0.77 (0.59–0.99) | 0.040 | 1.4 (0.3–2.5) | 0.014 |
| LGG given | 19   | 65.5 (54.8–78.2)  | 20   | 6.2 (5.4–7.0) |
| LGG not given | 19   | 54.3 (43.4–64.8)  | 19   | 5.8 (5.0–6.6) |
| Effect of LGG | 1.21 (0.94–1.55) | 0.139 | 0.3 (0.8 to 1.5) | 0.558 |

TABLE 2 TITT and weekly bowel movements in 5 study groups and the estimated effects of the interventions with rye bread and LGG (2 × 2 factorial design)1

1 TITT: Values are baseline-adjusted geometric means (95% CI) during wk 3; values were logarithmically (ln) transformed before analysis. Weekly bowel movements: Values are baseline-adjusted marginal means (95% CI).

2 Baseline-adjusted ANCOVA for repeated measurements in case of bowel movements (wk 1 and 3). P-value refers to the global test. *Rye bread vs. control, ratio 0.65; 95% CI 0.46–0.91; P = 0.014 and rye bread vs. laxative, ratio 0.59; 95% CI 0.41–0.85; P = 0.060.

3 Baseline-adjusted ANCOVA for repeated measurements in case of bowel movements for 2 × 2 factorial design. No significant interaction between rye bread and LGG in TITT (P = 0.196) or in the number of bowel movements (P = 0.200).

4 The effect for TITT is given as the ratios rye bread given:not given and LGG given:not given with 95% CI.

TABLE 3 Softening of feces and easing of defecation during wk 3 in 5 study groups and the estimated effects of the interventions with rye bread and LGG (2 × 2 factorial design)1

1 Values are percents of participants.

2 Logistic regression analysis. P-value refers to the global test.

3 Univariable logistic regression analysis for 2 × 2 factorial design. No significant interaction between rye bread and LGG in softening of feces (P = 0.849) or in easing of defecation (P = 0.581).

4 The effects of factors are given as OR with 95% CI.
Fecal enzyme activities. Rye bread reduced fecal β-glucuronidase activity by 23% compared with wheat bread (Table 5). In the rye bread group, fecal β-glucuronidase activity was only 62% of that in the laxative group. Activities of urease and β-glucosidase were not significantly affected by rye bread consumption compared with wheat bread. Cultured buttermilk with LGG had no significant effect on fecal enzyme activities compared with groups not consuming the cultured buttermilk.

Gastrointestinal symptoms. Gastrointestinal side effects (abdominal pain, flatulence, borborygmi, abdominal bloating, constipation, and diarrhea) did not significantly differ between the study groups. The baseline-adjusted marginal means for the total symptom score were 27.3 (18.9–35.7) in the rye bread + LGG group, 22.9 (14.9–30.9) in the LGG group, 25.2 (16.8–33.6) in the rye bread group, 20.1 (12.1–28.1) in the control group, and 26.8 (16.5–37.2) in the laxative group (P = 0.724). The effects of rye bread (4.7; 2.3–13.2; P = 0.265) and LGG (2.5; 1.6–11.1; P = 0.560) were not significant. The global effect of rye bread consumption on the total symptom score was small (and nonsignificant), as the effect was reduced from wk 1, during which it was 6.8 (2.1–15.3; P = 0.118), to wk 3, during which it was 3.2 (5.6 to 11.9; P = 0.469).

Breath hydrogen. There was a correlation between breath hydrogen and the total gastrointestinal symptom score (R = 0.30; 95% CI 0.25 to 0.36; P = 0.008 and rye bread vs. laxative, effect 0.31; 95% CI 0.26 to 0.06; P = 0.006 (Bonferroni corrected). The effects were estimated in subgroups due to interaction between rye bread and LGG in fecal weight (P = 0.011) and in fecal pH (P = 0.085).

### TABLE 4 Fecal weight and pH during the intervention in 5 study groups and the estimated effects of the interventions with rye bread and LGG (2 × 2 factorial design)1

<table>
<thead>
<tr>
<th>Study groups2</th>
<th>n</th>
<th>Fecal weight, g/d</th>
<th>Fecal pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye bread</td>
<td>9</td>
<td>159 (128–189)</td>
<td>6.70* (6.58–6.81)</td>
</tr>
<tr>
<td>LGG</td>
<td>11</td>
<td>107 (80–135)</td>
<td>7.15 (7.05–7.25)</td>
</tr>
<tr>
<td>Rye bread + LGG</td>
<td>9</td>
<td>198 (169–226)</td>
<td>7.04 (6.94–7.14)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>145 (114–175)</td>
<td>7.00 (6.88–7.11)</td>
</tr>
<tr>
<td>Laxative</td>
<td>8</td>
<td>148 (116–180)</td>
<td>7.01 (6.89–7.13)</td>
</tr>
<tr>
<td>P</td>
<td>0.002</td>
<td></td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

2x2 factorial design3

Effect of rye bread

- If LGG given: 20 90.3 (53.2–127.4) P < 0.001
- If LGG not given: 19 14.4 (6.3–35.5) P = 0.548

Effect of LGG

- If rye bread given: 18 37.7 (8.9–72.9) P = 0.072
- If rye bread not given: 21 38.3 (8.9–43.3) P = 0.075

1 Baseline-adjusted marginal means (95% CI).
2 Baseline-adjusted ANCOVA for repeated measurements (wk 1 and 3). P-value refers to the global test. *Rye bread vs. control, effect −0.30; 95% CI −0.55 to −0.06; P = 0.008 and rye bread vs. laxative, effect −0.31; −0.56 to −0.06; P = 0.006 (Bonferroni corrected).
3 The effects were estimated in subgroups due to interaction between rye bread and LGG in fecal weight (P = 0.011) and in fecal pH (P = 0.085).

### FIGURE 1 Fecal pH (A) and fecal acetic acid (B), butyric acid (C), and propionic acid (D) concentrations at baseline and during wk 1 and 3 in the rye bread, LGG, rye bread + LGG, control, and laxative groups. Values are means ± SEM, n = 8–12. (A) Rye bread vs. control, effect −0.30, P = 0.008 and rye bread vs. laxative, effect −0.31, P = 0.006 (baseline-adjusted repeated-measures ANOVA with Bonferroni corrections). Interaction between rye bread and LGG (P = 0.085, see text for details). (B–D) Effect (mmol/kg) of rye bread on acetic acid 7.72 (0.21–15.22; P = 0.044), butyric acid 4.98 (2.37–7.58; P <0.001), and propionic acid 2.17 (−0.04 to 4.37; P = 0.054) compared with wheat bread (baseline-adjusted repeated-measures ANOVA with 2 grouping factors). Effects of LGG were not significant. The estimated effects (mmol/kg) were −2.85 (−10.25 to 4.56; P = 0.441) for acetic acid, −0.50 (−3.09 to 2.09; P = 0.698) for butyric acid, and 1.03 (−1.17 to 3.24; P = 0.347) for propionic acid. Differences between the laxative group and the other groups were not significant.
TABLE 5  Fecal enzyme activities during wk 3 in 5 study groups and the estimated effects of the interventions with rye bread and LGG (2 × 2 factorial design)1

<table>
<thead>
<tr>
<th>Study groups2</th>
<th>n</th>
<th>Urease</th>
<th>β-Glucosidase</th>
<th>β-Glucuronidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rye bread</td>
<td>10</td>
<td>23.8 (17.4–32.7)</td>
<td>7.96 (6.66–9.51)</td>
<td>2.23* (1.82–2.74)</td>
</tr>
<tr>
<td>LGG</td>
<td>12</td>
<td>21.5 (15.6–29.0)</td>
<td>8.12 (6.89–9.57)</td>
<td>3.44 (2.86–4.14)</td>
</tr>
<tr>
<td>Rye bread + LGG</td>
<td>11</td>
<td>21.0 (15.6–28.4)</td>
<td>8.52 (7.18–10.10)</td>
<td>2.68 (2.21–3.26)</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>18.7 (13.6–25.5)</td>
<td>7.69 (6.43–9.18)</td>
<td>2.96 (2.42–3.63)</td>
</tr>
<tr>
<td>Laxative</td>
<td>8</td>
<td>28.6 (20.2–40.6)</td>
<td>7.72 (6.33–9.43)</td>
<td>3.58 (2.85–4.49)</td>
</tr>
</tbody>
</table>

| P             | 0.431 | 0.921 | 0.014 |

2x2 factorial design3

- Rye bread given: 21 (23.2 (18.4–29.3))
- Rye bread not given: 22 (20.5 (16.5–25.8))
- Effect of rye bread4: 1.13 (0.82–1.56)
- P: 0.453
- LGG given: 23 (21.8 (17.4–27.4))
- LGG not given: 21 (21.8 (17.1–27.8))
- Effect of LGG4: 1.00 (0.71–1.40)
- P: 0.999

1 Baseline-adjusted geometric means (95% CI). Variables were logarithmically (ln) transformed before analysis.
2 Baseline-adjusted ANCOVA. P-value refers to the global test. *Rye bread vs. laxative, ratio 0.62, 0.40 to 0.98, P = 0.033 (Bonferroni corrected).
3 Baseline-adjusted ANCOVA for 2 × 2 factorial design. There were no interactions between rye bread and LGG, P = 0.452–0.917.
4 The effect is given as the ratios rye bread given:not given and LGG given:not given with 95% CI.

0.424; P = 0.010; n = 36) during wk 3. An increase in breath hydrogen concentration appeared to be associated with an increase in the total gastrointestinal symptom score during the whole study period (R = 0.215; P = 0.063; n = 116). Breath hydrogen concentrations did not differ among the groups (P > 0.1; data not shown).

**Discussion**

Our purpose in this study was to determine the effects of rye bread and cultured buttermilk with LGG, separately and in combination, on bowel function, colon metabolism, and gastrointestinal symptoms, and to compare their efficacy to that of commonly used laxatives in constipated adults. Diet and laxative use were carefully monitored. The degree of constipation was investigated by several methods: TITT, fecal weight, defecation frequency, stool consistency, and difficulty of defecation. Whole-grain rye bread significantly reduced constipation compared with white wheat bread as assessed by all of these methods. Rye bread lowered fecal pH and bacterial β-glucuronidase activity and increased fecal SCFA content compared with wheat bread. The favorable effects of rye bread on TITT, fecal pH, and β-glucuronidase activity were significantly different from those of laxative agents, which were not effective in relieving constipation in the present study, as was also the case with cultured buttermilk with LGG.

The result that constipation is alleviated by rye bread consumption is supported by the results of previous investigations, in which rye bread had positive effects on bowel function in healthy (23,26) as well as in constipated adults (16). The active component of rye bread in the treatment of constipation is most probably fiber, which increases fecal mass and serves as a substrate for bacterial fermentation in the colon, leading to an increased content of colonic SCFA. Both increases were shown in the present study. Arabinoyxylan, which is abundant in rye, appears to be a preferred substrate for fermentative generation of SCFA (8). SCFA may induce propulsive contractions, leading to accelerated transit and relief of constipation (27,28). The decrease in fecal pH caused by rye bread consumption, which was found in the present study, is a natural consequence of increased fecal SCFA and also decreased intestinal transit time (2,29). The importance of maintaining a slightly acidic environment is critical, because the majority of harmful bacterial enzymes operate optimally at a neutral to slightly basic pH (30).

The bacterial enzymes β-glucuronidase and β-glucosidase are capable of hydrolyzing glycosidic linkages, which occur naturally in foods or are formed in the liver and excreted via the bile, leading to the release of potentially carcinogenic compounds (31). Urease catalyzes the hydrolysis of urea to yield ammonia and carbon dioxide (32). Ammonia derivatives are highly toxic and can induce mutagenic DNA damage in the host (32). In the present study, fecal β-glucosidase and urease activities were not affected by rye bread, but β-glucuronidase activity was clearly reduced. In postmenopausal women, rye bread consumption increased fecal urease and β-glucosidase activities, but did not affect β-glucuronidase activity (26). Fecal β-glucuronidase activity, expressed per wet fecal weight, has previously been shown to be reduced by whole-grain rye foods in overweight men (33). However, this reduction could not be shown to be due to altered balance of microbiota, because of the possible influence of dilution of microbiota by the increased bulk effect. Rye bran enhances the growth of bifidobacteria in mice (34) and rye arabinoxylans support the growth of bifidobacteria in vitro (35). Bifidobacteria appear to decrease β-glucuronidase activity (36,37) and also to reduce intestinal transit time and relieve constipation (37–39). Therefore, rye bread may possibly exert its effects on β-glucuronidase activity and intestinal transit partly through enhancing the growth of colonic bifidobacteria. This possible prebiotic effect of rye bread should be confirmed in an intervention trial in humans.

Cultured buttermilk with LGG did not relieve constipation in the present study, consistent with previous studies of yogurt containing LGG for relieving constipation in women (16) and LGG given as an adjunct to lactulose for the treatment of constipation in children (40). Cultured buttermilk with LGG increased fecal pH in the present study, which is inconsistent with a previous small study that observed no significant alterations in fecal pH (although fecal pH appeared to decrease from the baseline) after LGG-fermented whey drink administration in elderly nursing home residents (41). We are unable to explain the reason for the increase in fecal pH, but considering the evidence that distal colonic pH directly correlates with whole gut transit time (2,29), the increase may be associated with the slight, although not significant, constipating effect of cultured buttermilk with LGG according to the several variables in the present study. Cultured buttermilk with LGG did not increase SCFA unless consumed together with rye bread, suggesting either that LGG does not modify the production of SCFA or that LGG in a fiber-poor diet is not able to affect SCFA production in detectable amounts because of the shortage of available carbohydrate. Cultured buttermilk with LGG did not modify the fecal bacterial activities of β-glucosidase, urease, or β-glucuronidase. Yogurt containing LGG has previously been reported not to alter β-glucosidase activity but to slightly decrease β-glucuronidase activity (20). Studies on the effects of LGG on fecal urease activity have demonstrated varying results (20,42,43). Unlike bifidobacteria, lactobacilli do not belong to the major genera of...
colonic bacteria (44) and might thus lack a strong influence on fecal pH and bacterial activities.

The primary goal in the treatment of constipation is to improve the patient’s quality of life by relieving symptoms and normalizing gastrointestinal motility (1). Nearly one-half of constipated patients are unsatisfied with their treatment, mostly because they consider it is ineffective (1). Rye bread consumption alleviated symptoms of mild constipation by easing defecation and softening feces. It also resulted in an average increase of 1.4 bowel movements/wk in the present study, the same as generally achieved by fecal mass-increasing laxatives (1), whereas in this study, commonly used laxatives were not effective in relieving constipation. The fact that one-half of the participants in the laxative group used laxatives at the baseline might account for some of the ineffectiveness. To the best of our knowledge, this study is the first to evaluate the effects of rye bread in treating constipation as compared with laxatives and to simultaneously investigate the changes in the colonic metabolism. The results are encouraging.

In conclusion, rye bread alleviated mild constipation compared both to white wheat bread and to laxatives without significantly increasing gastrointestinal adverse effects. It also favorably modified the colonic environment by reducing fecal pH and bacterial β-glucuronidase activity and by increasing fecal SCFA content.

Acknowledgements
We thank Tuia Poussa for the statistical analyses and Kirs-Helena Liukkonen and Teija Jokila for the laboratory analyses. R.H. analyzed the data, wrote the paper, and had primary responsibility for final content; R.K., M.S., and S-M.H. designed the research; S-M.H. conducted the research; and M.S. and R.K. directed the research. All authors read and approved the final manuscript.

Literature Cited


