A Probiotic Mixture Including Galactooligosaccharides Decreases Fecal \( \beta \)-Glucosidase Activity but Does Not Affect Serum Enterolactone Concentration in Men during a Two-Week Intervention\(^1\)\(^-\)\(^3\)

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Abstract

A high serum concentration of enterolactone, an enterolignan produced by colonic microbiota from precursors in cereals, vegetables, and fruits, is associated with reduced risk of acute coronary events. Probiotics and prebiotics modify colonic metabolism and may affect the serum enterolactone concentration. The effects of a probiotic mixture alone and with galactooligosaccharides (GOS) on serum enterolactone concentration and fecal metabolism were investigated in 18 healthy men. Participants received 3 interventions, each for 2 wk: 1) probiotics [Lactobacillus rhamnosus strains GG (LGG) and LC705, Propionibacterium freudenreichii ssp. shermanii JS, and Bifidobacterium breve Bb99, for a total amount of 2 \times 10^{10} \text{CFU/d}]; 2) probiotics and GOS 3.8 g/d; 3) probiotics, GOS, and rye bread (minimum 120 g/d). Serum enterolactone and fecal dry weight, enzyme activities, pH, SCFA, lactic acid bacteria, bifidobacteria, propionibacteria, and the strains LGG and LC705 were determined. The serum enterolactone concentration (nmol/L) tended to be decreased from baseline [mean (95% CI) 18.6 (10.8–26.4)] by probiotics alone [15.2 (7.8–22.7); \(P = 0.095\)], was not significantly affected by probiotics with GOS [21.5 (12.2–29.8)], and was increased by probiotics with GOS and rye bread [24.6 (15.4–33.7); \(P < 0.05\)]. Probiotics alone did not affect fecal \( \beta \)-glucosidase activity and bifidobacteria, but probiotics with GOS decreased \( \beta \)-glucosidase activity and increased bifidobacteria compared with baseline (\(P < 0.05\)) and with probiotics alone (\(P < 0.01\)). In conclusion, this probiotic mixture with or without GOS does not significantly affect serum enterolactone concentration. Because probiotics with GOS decreased fecal \( \beta \)-glucosidase activity but not serum enterolactone, the reduced fecal \( \beta \)-glucosidase, within the range of activities measured, does not seem to limit the formation of enterolactone. J. Nutr. 141: 870–876, 2011.

Introduction

Increased serum enterolactone concentrations have been associated with reduced risk of acute coronary events and death from coronary heart disease and from cardiovascular disease (1–3). Enterolactone is produced by colonic bacteria from plant-derived precursor lignans, such as matairesinol, secoisolariciresinol, pinnoresinol, and lariciresinol (4,5), and possesses various biological activities (6). Though consumption of lignan-containing foods such as whole-grain cereals, beans, other vegetables, and some fruits and berries (6) and constipation appear to be among the most important determinants of serum enterolactone concentration, they account for only a small part of the differences in serum enterolactone concentrations between individuals (7,8). This suggests, along with the effects of oral antimicrobials in decreasing serum enterolactone concentration (9), that intestinal microbiota is very important in the metabolism of lignans. The bacteria species that produce enterolactone are beginning to be clarified. Formation of enterolactone from one of the most abundant dietary lignans, secoisolariciresinol diglucoside, involves phylogenetically di-
verse bacteria, most of which belong to the dominant human intestinal microbiota (10).

The intestinal microbiota of humans predominantly consist of the phyla Bacteroidetes and Firmicutes, the latter including genera Dorea, Eubacterium, Ruminococcus, Clostridium, Lactobacillus, and Streptococcus as well as the species Faecalibacterium prausnitzii (11). Also, members of Actinobacteria, including the genus Bifidobacterium, are abundant in the intestine. Several species of lactobacilli and bifidobacteria fulfill the criteria of probiotics (12), “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (13). They may modify fecal enzyme activity (14–20). Galactooligosaccharides (GOS) are nondigestible carbohydrates that stimulate the growth of colonic bifidobacteria and, to a lesser extent, lactobacilli (21) and are considered prebiotics. They have a history of safe commercial use (21). Theoretically, a combination of probiotics and prebiotics may enhance survival and function of probiotics in addition to resident beneficial microbes (12).

Our primary aim in this study was to investigate the effects of a probiotic mixture alone and together with GOS on serum enterolactone concentration. The secondary aim was to investigate their effects on fecal metabolic activity. We used a probiotic combination of Lactobacillus rhamnosus GG (LLG), L. rhamnosus LC705 (LC705), Propionibacterium freudenreichii ssp. shermanii JS, and Bifidobacterium breve Bb99 (Bb99). This combination alleviates symptoms in irritable bowel syndrome (IBS) and during Helicobacter pylori eradication therapy (22,23). Also, when administered together with GOS, it reduces eczema and increases resistance to respiratory infections in infants (24,25). Rye bread was included in this study, because rye has the highest concentration of total lignans among cereal species (26) and rye foods are known to increase the serum enterolactone concentrations between individuals (7), we chose a study design with sequential interventions, where each participant served as his own control. Wash-out periods were not included because of the “additive” sequential interventions. During the study, the participants were not allowed to eat seeds, nuts, and products containing probiotic bacteria. The participants were instructed not to change their ordinary diet during the study other than according to the interventions.

The probiotic juice (Valio) contained 2 Lactobacillus rhamnosus strains, LLG (ATCC 53103, 5.9 × 10⁸ CFU/L) and LC705 (DSM7061, 1.3 × 10⁸ CFU/L), 1 bifidobacterium strain, Bb99 (DSM 13692, 5.7 × 10⁷ CFU/L), and 1 propionibacterium strain, Propionibacterium freudenreichii ssp. shermanii JS (DSM7067, 1.1 × 10¹¹ CFU/L). After the probiotics period, 10.3 g GOS syrup (Valio) was added to the probiotic juice. The syrup contained 37% pure GOS, resulting in a daily amount of 3.8 g GOS/participant. The extra rye bread (Reissumies, Fazer Bakeries) specially delivered to the participants for this study contained 62% rye flour and 12% fiber. The mean daily amount of the extra rye bread consumed during the probiotics+GOS+rye bread period was 143 g (range 78–210 g), resulting in a mean daily total (extra + usual) amount of 211 g (range 78–351 g).

Questionnaires
Participants estimated the severity of gastrointestinal symptoms (stomachache, abdominal distension, flatulence, heartburn, loose stools, hard stools) during the previous 2 wk by using a visual analogue scale (100 mm, 0–100) at the end of each intervention period and at the end of follow-up. During the probiotics+GOS+rye bread period, the participants recorded each day the amount of rye bread they consumed. Use of nonpermitted food products (seeds, nuts, and products containing probiotic bacteria) and medication during the previous 2 wk was evaluated by the participants at the end of each intervention period and at the end of follow-up. In addition, such use during the study was controlled by a frequency questionnaire conducted at the end of the follow-up period. Finally, the participants were asked at the end of the follow-up period if there were changes in their body weight, alcohol use, intake of fat, and exercise habits during the study.

Serum sample analysis
Blood samples were collected 5 times during the experiment at the end of each period. The sampling was performed in the morning and always on the same day of the week. Serum was separated from the blood samples not more than 2 h after sampling. The samples were centrifuged and stored at −20°C until analysis. Enterolactone analyses were made by time-resolved fluoroimmunoassay (29,30). At baseline, the blood sample was taken after an overnight fast, and serum cholesterol and TG as well as blood glucose were analyzed by a biochemical analyzer (Refractometer IV, Boehringer Mannheim). LDL-cholesterol was calculated according to the following formula: total cholesterol − HDL-cholesterol − (0.45 × TG).

Fecal sample analysis
Fecal samples were collected 5 times during the experiment, at the end of every period. The participants were asked to freeze the samples immediately and to keep them frozen until taken in a cooler with ice packs to the study center. In the laboratory, the fecal samples were stored at −70°C until further analysis.

Fecal pH and dry weight. The pH of the fecal samples was measured with the Mettler Toledo InLab 427 electrode. For dry matter determination, 1 g of fecal sample was weighed and dried in the oven (105°C 17 h), cooled down in an excitor to room temperature, and reweighed. Fecal dry weight was expressed as a percent of the wet weight.

Fecal enzyme activities. Fecal activities of β-glucosidase and β-glucuronidase were analyzed by a method described by Goldin et al.
SCFA. For the SCFA and enzyme analyses, fecal samples were diluted 1:10 with 0.1 mol/L phosphate buffer and homogenized with a stomacher blender, filtered, sonicated 1 min at 4°C, and centrifuged at 450 × g for 15 min at 4°C. The supernatant fraction was used for analysis. The samples were stored at −70°C. The concentrations of acetic acid, propionic acid, butyric acid, valeric acid, isovaleric acid, caproic acid, and isocapronic acid were determined with the capillary gas chromatograph (HP-6890, Hewlett-Packard) by adapting the method of Hoverstad et al. (32).

Microbiological analyses. Fecal samples were homogenized 1:10 in Wilkins-Chalgren broth (Oxoid Ltd) in an anaerobic chamber and serial dilutions were plated on MRS-agar (LAB M, International Diagnostics Group) for total lactic acid bacteria, raffinose agar (33) for bifidobacteria, and yeast extract lactose agar (34) supplemented with 1% (wt/vol) of β-glycerophosphate (Merck) for propionibacteria. MRS plates were incubated at 37°C for 3 d, raffinose agar plates at 37°C for 2 d, and yeast extract lactose agar plates at 30°C for 7 d, all anaerobically. LGG and LC705 strains were analyzed by plating the diluted samples on MRS-vancomycin (50 mg/L) agar and anaerobically cultivating for 2–3 d at 37°C. In LGG and LC705 analyses, 20 isolates of every sample were purified further on MRS agar with vancomycin and tested for lactose utilization by anaerobically incubating overnight at 37°C on MRS agar (pH 7.2) containing bromocresol purple 0.005% and 2% lactose, resulting in a yellow (LC705) or white (LGG) color due to lactose utilization.

Statistical analysis
Serum enterolactone was the primary outcome variable studied. Fecal enzyme activities, fecal bacterial counts, fecal pH and dry weight, SCFA, protein test kit (Bio-Rad Protein Assay, Bio-Rad Laboratories). Statistical analysis
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Results
Participant characteristics and compliance. At baseline, the serum concentration in fasting participants of total cholesterol was 5.29 ± 1.15 mmol/L, of HDL cholesterol, 1.15 ± 0.32 mmol/L, of LDL cholesterol, 3.53 ± 0.92 mmol/L, of TG, 1.39 ± 0.77 mmol/L, and of fasting glucose, 5.34 ± 0.70 mmol/L. None of the participants used antibiotics during the study. Three used forbidden probiotic products once and 1 ate nuts once during the study. The rest of the participants did not consume any of the excluded products. One participant lost 1.5 kg during the study, and 1 participant reported that his intake of fat and alcohol decreased during the study. All the participants completed the study.

Gastrointestinal symptoms. Of individual symptoms, only flatulence and abdominal distension changed during the study (P < 0.02). These symptom scores were higher during the period of probiotics+GOS+rye bread [median (IQR)] [49.5 (17.3–73.3) and 23.0 (13.0–49.9)] than during the follow-up period [23.0 (10.8–41.3) and 12.5 (9.0–27.0)] (P < 0.01). Consequently, the total symptom score changed during the study (P = 0.027), being significantly higher during the period of probiotics+GOS+rye bread [mean (95% CI)] [153.6 (108.1–199.1)] than during the follow-up [96.5 (65.8–127.2)] (P = 0.025). There were no significant changes in other gastrointestinal symptoms.

Serum enterolactone. The serum enterolactone concentration tended to be lower during the probiotics period than at baseline [mean difference (95% CI) −3.4 nmol/L (−7.4–0.7)] (P = 0.095) but did not differ from baseline during the probiotics+GOS period [mean difference (95% CI) 2.9 nmol/L (−4.1–9.9)] (P = 0.39) (Table 1). On the other hand, during the period of probiotics+GOS+rye bread, the serum enterolactone concentration was higher than at baseline [mean difference (95% CI)]

<p>| TABLE 1 | Men’s serum enterolactone concentrations during a run-in period, sequential 2-wk interventions with probiotics, probiotics+GOS, and probiotics+GOS+rye bread, and a follow-up period |
|------------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Serum enterolactone, nmol/L</th>
<th>n</th>
<th>Run-in</th>
<th>Probiotics</th>
<th>Probiotics+GOS</th>
<th>Probiotics+GOS+rye bread</th>
<th>Follow-up</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>18</td>
<td>15.2 (7.8–22.7)</td>
<td>21.5 (13.2–29.8)</td>
<td>24.6 (15.4–33.7)</td>
<td>23.2 (13.5–32.8)</td>
<td>0.045</td>
<td></td>
</tr>
<tr>
<td>Subgroups by run-in enterolactone</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 nmol/L</td>
<td>9</td>
<td>5.0 (0.4–9.5)</td>
<td>3.2 (1.0–5.5)</td>
<td>10.5 (1.8–19.2)</td>
<td>9.6 (2.3–16.9)</td>
<td>6.8 (0.4–13.1)</td>
<td>0.19</td>
</tr>
<tr>
<td>≥20 nmol/L</td>
<td>9</td>
<td>32.3 (25.9–38.8)</td>
<td>27.3 (17.9–36.8)</td>
<td>32.6 (21.9–43.2)</td>
<td>39.5 (30.1–48.1)</td>
<td>39.5 (30.9–48.2)</td>
<td>0.096</td>
</tr>
</tbody>
</table>

*Values are means (95% CI). *Different from run-in, P < 0.05.
1 ANOVA for repeated measures. *P-value refers to the global test.
6.0 nmol/L (0.3–11.6) \( (P = 0.042) \). There were no significant differences in serum enterolactone concentration between intervention periods in subgroups by run-in serum enterolactone concentration below and above 20 nmol/L.

**Fecal pH and dry weight.** Fecal pH was not significantly changed during the study (Table 2). Fecal dry weight (percent) was significantly lower during the probiotics+GOS and probiotics+GOS+rye bread periods than at baseline (Table 2). Fecal dry weight was also lower during the probiotics+GOS period than during the probiotics period.

**Fecal enzyme activities.** Fecal activity of \( \beta \)-glucosidase was lower during the probiotics+GOS period than at baseline [mean difference (95% CI) \(-5.4 \text{ nmol-min}^{-1} \text{mg protein}^{-1}\) (\(-9.3 \text{ to } -1.5\)) \( (P = 0.045) \) and during the probiotics period \([\text{mean difference (95% CI) } -7.2 \text{nmol-min}^{-1} \text{mg protein}^{-1}\) (\(-11.3 \text{ to } -3.1\)) \( (P = 0.008) \) (Table 2). \( \beta \)-Glucosidase activity during the probiotics and probiotics+GOS+rye bread periods did not significantly differ from baseline. Activities of urease and \( \beta \)-glucuronidase were not significantly affected during the study (Table 2).

**Fecal bacterial counts.** Fecal counts of both *Lactobacillus* strains administered, LGG and LC705, as well as of total propionibacteria were significantly higher during each intervention period than at baseline (Table 3). Total bifidobacteria counts were significantly higher during the probiotics+GOS period than at baseline and during the probiotics period. There was no significant difference in total lactic acid bacteria during the probiotics, probiotics+GOS, or probiotics+GOS+rye bread periods from baseline.

**Fecal SCFA.** Total fecal SCFA content as well as fecal acetic acid, propionic acid, butyric acid, valeric acid, isobutyric acid, isovaleric acid, and caproic acid contents were not significantly different between the intervention periods (Supplemental Table 1).

**Associations between serum enterolactone and fecal markers.** We counted correlations between baseline values of serum enterolactone (nmol/L) and several fecal markers. Serum enterolactone concentration was not correlated with fecal pH or fecal dry weight. Serum enterolactone was positively correlated with \( \beta \)-glucosidase activity (nmol-min\(^{-1}\)mg protein\(^{-1}\)) \( (r = 0.57; P = 0.013) \) and tended to be positively correlated with urease \( (r = 0.46; P = 0.055) \) and \( \beta \)-glucuronidase \( (r = 0.45; P = 0.064) \) activity (nmol-min\(^{-1}\)mg protein\(^{-1}\)). \( \beta \)-Glucosidase activity was categorized according to the median (20.2 nmol-min\(^{-1}\)mg protein\(^{-1}\)). The median serum enterolactone was 22.2 and 29.5 nmol/L \( (P = 0.024) \) when the \( \beta \)-glucosidase activity was below or above the median, respectively (Fig 1A). Serum enterolactone tended to be positively correlated with 2 fecal bacterial counts (CFU/g dry weight): total bifidobacteria \( (r = 0.44; P = 0.067) \) and total lactic acid bacteria \( (r = 0.42; P = 0.086) \). Total bifidobacteria count was categorized according to the median \( (44.9\text{ CFU/g dry weight}) \). The median serum enterolactone was 22.2 and 29.5 nmol/L \( (P < 0.01) \) when the count of total bifidobacteria was below or above the median, respectively (Fig 1B). Correlations between serum enterolactone and other fecal bacteria were not significant. Serum enterolactone was positively correlated with 3 SCFA (\( \mu \text{mol/g dry weight} \) ): isobutyric \( (r = 0.56; P = 0.021) \), isovaleric \( (r = 0.56; P = 0.020) \), and caproic acids \( (r = 0.57; P = 0.019) \).

**Discussion**

Our aim in this study was to investigate the effects of a probiotic mixture alone and together with GOS on the serum enterolactone concentration and fecal metabolic activity. We hypothesized that GOS would enhance the colonic metabolism of probiotics and resident microbiota and thus lead to a larger change in the serum enterolactone concentration than probiotics alone. However, consumption of probiotics alone or with GOS did not have a significant effect on the serum enterolactone concentration. This is in line with the results of a previous in vitro study where LGG as well as 3 other *Lactobacillus* strains, *L. casei* Shirota, *L. johnsonii* La1, and *L. bulgaricus*, and *Bifidobacterium lactis* Bb12 did not convert a plant lignan 7-hydroxymatairesinol to enterolactone or to other metabolites (36). However, rye bread consumption increased the serum enterolactone concentration, consistent with a previous study (27), probably due mainly to rye bread’s high lignan content (26).

Lignans occur in plants as glucosides (10,37). In the present study, the activity of fecal \( \beta \)-glucosidase was positively correlated with the serum enterolactone concentration at baseline, consistent with a previous in vitro study where there was a trend toward positive correlation between \( \beta \)-glucosidase activity and enterolactone production by human fecal bacteria (38). Activity of fecal \( \beta \)-glucosidase was not significantly affected during the period of probiotics alone but decreased during the probiotics together with GOS treatment period compared both to baseline

**TABLE 2** Men’s fecal pH, dry weight and enzyme activities during a run-in period, sequential 2-wk interventions with probiotics, probiotics+GOS, and probiotics+GOS+rye bread, and a follow-up period

<table>
<thead>
<tr>
<th>Variable(^1)</th>
<th>Run-in</th>
<th>Probiotics</th>
<th>Probiotics+GOS</th>
<th>Probiotics+GOS+rye bread</th>
<th>Follow-up</th>
<th>( P^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecal pH</td>
<td>6.90 ( \pm ) 0.40</td>
<td>6.83 ( \pm ) 0.23</td>
<td>6.67 ( \pm ) 0.35</td>
<td>6.65 ( \pm ) 0.40</td>
<td>6.85 ( \pm ) 0.40</td>
<td>0.12</td>
</tr>
<tr>
<td>Fecal dry weight, %</td>
<td>19.6 ( \pm ) 0.77</td>
<td>19.4 ( \pm ) 1.12</td>
<td>18.4(^a) ( \pm ) 1.31</td>
<td>18.4(^b) ( \pm ) 1.20</td>
<td>18.9 ( \pm ) 1.36</td>
<td>0.007</td>
</tr>
<tr>
<td>Urease, nmol-min(^{-1})mg protein(^{-1})</td>
<td>32.5 ( \pm ) 26.6</td>
<td>32.6 ( \pm ) 23.1</td>
<td>23.0 ( \pm ) 12.5</td>
<td>30.9 ( \pm ) 21.9</td>
<td>35.9 ( \pm ) 18.3</td>
<td>0.20</td>
</tr>
<tr>
<td>( \beta )-glucuronidase, nmol-min(^{-1})mg protein(^{-1})</td>
<td>8.94 ( \pm ) 3.12</td>
<td>8.47 ( \pm ) 4.08</td>
<td>8.33 ( \pm ) 5.03</td>
<td>8.68 ( \pm ) 3.23</td>
<td>9.53 ( \pm ) 4.90</td>
<td>0.26</td>
</tr>
<tr>
<td>( \beta )-glucosidase, nmol-min(^{-1})mg protein(^{-1})</td>
<td>22.0 ( \pm ) 8.58</td>
<td>23.9 ( \pm ) 10.1</td>
<td>16.6(^c) ( \pm ) 5.32</td>
<td>18.2 ( \pm ) 4.72</td>
<td>23.3 ( \pm ) 7.86</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^1\) Values are means \( \pm \) SD, \( n = 18 \) except pH, \( n = 16 \). \(^a\)Different from run-in, \( P < 0.05 \); \(^b\)different from probiotics, \( P < 0.05 \); \(^c\)different from run-in, \( P < 0.01 \); \(^d\)different from probiotics, \( P < 0.01 \).

\(^2\) Friedman’s 1-way ANOVA for fecal dry weight and ANOVA for repeated measures for other variables. \( P\)-value refers to the global test. Bonferroni corrected comparisons.
and to the period of probiotics alone, suggesting that GOS either directly inhibited β-glucosidase activity or reduced the number or metabolism of bacterial species with high β-glucosidase activity. GOS addition led to very large and rapid reductions in the activity of this enzyme in a previous study using an in vitro human gut bacterial ecosystem, suggesting a direct inhibition (39), and GOS reduced fecal β-glucosidase activity in healthy men (40). However, in the present study, the decreased activity of fecal β-glucosidase during the consumption of probiotics together with GOS did not lead to decreased serum enterolactone concentrations, suggesting that despite a significant correlation with serum enterolactone, within the range of activities measured in the present study, fecal β-glucosidase does not have a central role in modifying serum enterolactone concentration.

Fecal counts of total propionibacteria, LC705, LGG, and total bifidobacteria were increased by the interventions, whereas counts of total lactic acid bacteria were not. The result that total bifidobacteria counts were increased during probiotics with GOS, compared with both baseline and probiotics alone, is in line with a previous finding that GOS alone increased the bifidobacterial population in healthy adults (41). In addition, trials using the same probiotic mixture as the present study indicated that fecal bifidobacteria counts significantly increased only when administered with GOS in infants (24,42) and slightly decreased when administered without GOS in IBS in adults (43). Total lactic acid bacteria counts in these studies, on the other hand, increased in infants given with or without GOS (24,42), although lactobacilli counts have not changed in adults with IBS (43).

Certain bifidobacteria species have been shown to synthesize high levels of β-glucosidase (44) and the administration of B. breve increases fecal β-glucosidase activity in healthy adults (45). In the present study, the increase in total bifidobacteria counts during probiotics with GOS did not increase β-glucosidase activity or serum enterolactone concentration. This suggests that the positive correlation between fecal bifidobacteria counts and serum enterolactone at baseline is explained by some other variable than fecal β-glucosidase activity, such as the amount of rye bread consumed, which has the potential to increase both (27,46,47). It is also possible that those bifidobacteria strains that increased during probiotics with GOS (Bb99 in the probiotic mixture or resident strains) do not synthesize high levels of β-glucosidase or that β-glucosidase activity is reduced by a direct inhibition by GOS (39).

Fecal pH or SCFA concentrations did not significantly change during the study. These findings are supported by previous intervention studies. The same probiotic mixture (without GOS) as that used in the present study had no significant effect on fecal SCFA concentrations in adults with IBS (43). GOS reduced pH and increased lactic acid and acetic acid concentrations in the proximal, but not distal, colon of pigs, without a significant effect on propionic acid and butyric acid concentrations in ei-

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**TABLE 3** Men’s fecal bacterial counts during a run-in period, sequential 2-wk interventions with probiotics, probiotics+GOS, and probiotics+GOS+rye bread, and a follow-up period

<table>
<thead>
<tr>
<th>Fecal bacteria</th>
<th>Run-in</th>
<th>Probiotics</th>
<th>Probiotics+GOS</th>
<th>Probiotics+GOS+rye bread</th>
<th>Follow-up</th>
<th>P&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>log&lt;sub&gt;10&lt;/sub&gt; CFU/g dry weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total lactic acid bacteria</strong></td>
<td>31.4 (27.1–35.2)</td>
<td>31.8 (28.5–33.8)</td>
<td>32.3 (30.1–38.3)</td>
<td>30.6 (24.4–32.9)</td>
<td>25.0 (16.1–30.7)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Total bifidobacteria</strong></td>
<td>44.9 (40.7–46.6)</td>
<td>46.6 (43.5–51.1)</td>
<td>51.0&lt;sup&gt;4&lt;/sup&gt; (48.6–55.1)</td>
<td>50.9 (44.5–54.3)</td>
<td>47.1 (34.7–51.4)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Total propionibacteria</strong></td>
<td>10.4 (10.1–12.1)</td>
<td>30.5&lt;sup&gt;5&lt;/sup&gt; (25.9–35.4)</td>
<td>32.6&lt;sup&gt;5&lt;/sup&gt; (27.9–35.9)</td>
<td>33.6&lt;sup&gt;5&lt;/sup&gt; (27.2–37.0)</td>
<td>10.7 (10.2–11.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LC705</strong></td>
<td>10.2 (9.9–10.6)</td>
<td>26.7&lt;sup&gt;6&lt;/sup&gt; (23.6–30.3)</td>
<td>30.4&lt;sup&gt;6&lt;/sup&gt; (24.8–34.0)</td>
<td>29.4&lt;sup&gt;6&lt;/sup&gt; (23.9–30.2)</td>
<td>11.1 (10.4–20.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>LGG</strong></td>
<td>10.2 (9.9–10.5)</td>
<td>24.5&lt;sup&gt;6&lt;/sup&gt; (20.1–29.1)</td>
<td>29.4&lt;sup&gt;6&lt;/sup&gt; (26.3–33.8)</td>
<td>24.1&lt;sup&gt;6&lt;/sup&gt; (16.9–28.6)</td>
<td>10.7 (10.2–21.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 Values are medians (IQR), n = 18 during each period. *Different from run-in, P < 0.05; different from probiotics, P < 0.01; different from run-in, P < 0.01.
2 Fecal counts of total lactic acid bacteria, total bifidobacteria, and total propionibacteria (expressed as log10 CFU/g wet weight) during run-in, Probiotics and Probiotics+GOS treatments have been published (35).
3 Friedman’s 1-way ANOVA. P-value refers to the global test.

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**FIGURE 1** Serum enterolactone concentrations at baseline in men with fecal β-glucosidase activity less than or greater than the median of 20.2 nmol min<sup>-1</sup> mg protein<sup>-1</sup> (A) and with fecal bifidobacteria counts less than or greater than the median of 44.9 log<sub>10</sub> CFU/g dry weight (B). Horizontal lines are medians (n = 9). *P < 0.03.
ther (48). In healthy men, fecal pH and SCFA concentrations remained unaltered during GOS administration, except for the acetic acid concentration, which increased (40). In the present study, rye bread together with probiotics and GOS appeared to increase concentrations of several SCFA compared with probiotics alone or with GOS. In our previous study, rye bread increased acetic acid, propionic acid, and butyric acid concentrations in feces and reduced fecal pH in constipated adults (49). SCFA are challenging to measure from feces, because they are efficiently absorbed from the colon, with only 10–20% being excreted in the feces (50).

A previous study showed that enterolactone concentrations in plasma reflected dietary change within 2 wk of the introduction of a new diet but they continued to increase for 4–6 wk (28). On the basis of this previous study, perhaps the intervention of 2 wk was too short to detect the maximal effect of interventions on serum enterolactone concentration. This is suggested by the fact that serum enterolactone concentrations were high during the follow-up.

In conclusion, the consumption of probiotics alone or with GOS in the present study did not have a significant effect on the serum enterolactone concentration, although probiotics with GOS, but not alone, decreased fecal β-glucosidase activity. This indicates that fecal β-glucosidase, at the activities measured, does not have a major role in modifying the serum enterolactone concentration.

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**Literature Cited**


