Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans

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De Preter V, Vanhoutte T, Huys G, Swings J, De Vuyst L, Rutgeerts P, Verbeke K. Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. *Am J Physiol Gastrointest Liver Physiol* 292: G358–G368, 2007. First published September 21, 2006; doi:10.1152/ajpgi.00052.2006.—Pre- and/or probiotics can cause changes in the ecological balance of intestinal microbiota and hence influence microbial metabolic activities. In the present study, the influence of oligofructose-enriched inulin (OF-IN), *Lactobacillus casei* Shirota, and *Bifidobacterium breve* Yakult on the colonic fate of NH₃ and *p*-cresol was investigated. A randomized, placebo-controlled, crossover study was performed in 20 healthy volunteers to evaluate the influence of short- and long-term administration of OF-IN, *L. casei* Shirota, *B. breve* Yakult, and the symbiotic *L. casei Shirota* + OF-IN. The lactose[^15]N[^15]Nureide biomarker was used to study the colonic fate of NH₃. Urine and fecal samples were analyzed for[^15]N content by combustion-isotope ratio mass spectrometry and for[^15]N content by gas chromatography-mass spectrometry. RT-PCR was applied to determine the levels of total bifidobacteria. Both short- and long-term administration of OF-IN resulted in significantly decreased urinary[^15]N excretion and[^15]N content. The reduction of urinary[^15]N excretion after short-term OF-IN intake was accompanied by a significant increase in[^15]N content of the fecal bacterial fraction. However, this effect was not observed after long-term OF-IN intake. In addition, RT-PCR results indicated a significant increase in total fecal bifidobacteria after long-term OF-IN intake. Long-term *L. casei Shirota* and *B. breve* Yakult intake showed a tendency to decrease urinary[^15]N excretion, whereas a significant decrease was noted in[^15]N excretion. In conclusion, dietary addition of OF-IN, *L. casei Shirota*, and *B. breve* Yakult results in a favorable effect on colonic NH₃ and[^15]N cecal metabolism, which, in the case of OF-IN, was accompanied by an increase in total fecal bifidobacteria.

**ammonia;[^15]N cecal; bifidobacteria; biomarker**

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IN RECENT YEARS, several human studies (1, 10, 28, 29, 31) have evaluated the possible health effects related to pre- and/or probiotic administration. The underlying mechanisms responsible for most of the effects of pre- and/or probiotic intervention have been extensively investigated and can be roughly classified as modifications of host immune reactivity (inhibition of NF-κB, modulation of apoptosis, and activation of macrophages) (14, 30) on the one hand and interference with colonic bacterial metabolism, which might result in a modulation of the colonic microbial ecosystem, on the other hand (7, 32). In general, pre- and probiotic administration aims at increasing the saccharolytic activity of colonic microbiota because of the beneficial effects attributed to end products of carbohydrate fermentation (i.e., short-chain fatty acids). Concomitantly, proteolytic fermentation resulting in the formation of potentially toxic metabolites is expected to be reduced.

In previous studies, we developed two biomarkers, i.e., lactose[^15][^15]Nureide and[^15]N cecal, used to monitor, in vivo, the degree of proteolytic fermentation by quantification of the formation of potentially toxic metabolites in the colon. As a result, these markers have been proposed as efficient tools to measure the effect of different pre- and probiotic substrates (8, 12). Because of its stable isotope label, lactose[^15][^15]Nureide is a safe biomarker that allows us to study the metabolic fate of ammonia in a noninvasive manner. Upon stimulation of bacterial growth and/or activity due to pre- or probiotic administration, it is generally assumed that more ammonia will be assimilated by bacteria, leading to reduced excretion in urine. The use of the[^15]N cecal marker is based on the fact that it is a unique bacterial metabolite of tyrosine that is not formed by human enzymes. It is absorbed from the colon and excreted in urine after sulfate or glucuronide conjugation in the mucosa or liver. As a consequence, a decrease in urinary[^15]N excretion reflects decreased proteolytic activity in the colon. Using these two biomarkers, we (9) have previously evaluated the efficacy of lactulose and the probiotic yeast *Saccharomyces boulardii* to reduce proteolytic colonic activity!

In the present study, the two biomarkers were used to study the effect of three commercially available pre- and probiotic substrates [i.e., bacterial strains *Lactobacillus casei* Shirota and *Bifidobacterium breve* Yakult as well as oligofructose-enriched inulin (OF-IN)] on colonic protein metabolism in healthy volunteers. For this purpose, we distinguished the immediate metabolic effects of the substrates in the colon (short-term effects) from the effects induced by a long-term dietary intervention, which are presumably caused by a change in the metabolic fate of ammonia. The two biomarkers were used to study the metabolic fate of ammonia in healthy human subjects, and the use of the[^15]N cecal marker is based on the fact that it is a unique bacterial metabolite of tyrosine that is not formed by human enzymes. It is absorbed from the colon and excreted in urine after sulfate or glucuronide conjugation in the mucosa or liver. As a consequence, a decrease in urinary[^15]N excretion reflects decreased proteolytic activity in the colon. Using these two biomarkers, we (9) have previously evaluated the efficacy of lactulose and the probiotic yeast *Saccharomyces boulardii* to reduce proteolytic colonic activity.

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relative composition of microbiota. Hence, immediate effects were evaluated when pre- and probiotics were first administered, whereas long-term effects were evaluated after a 4-wk intervention period and in the absence of substrates. In addition, we investigated whether the changes in metabolic activity could be correlated to quantitative changes in the total fecal Bifidobacterium population.

MATERIALS AND METHODS

Subjects

Twenty healthy volunteers (10 women and 10 men; age: 21 ± 1 yr) participated in the study. None of the subjects had a history of gastrointestinal or metabolic disease or previous surgery (apart from an appendectomy). Subjects did not receive antibiotic treatment or had received any other medical treatment influencing gut transit or intestinal microbiota for at least 3 mo before the start of the study. The Ethics Committee of the University of Leuven (Leuven, Belgium) approved the study, and all subjects gave informed consent.

Experimental Design

The healthy volunteers were randomly assigned to two different treatment groups of a placebo-controlled crossover trial. The study was conducted over a 16-wk period consisting of three ingestion periods of 4 wk, each separated by a 2-wk washout period: a probiotic period (days 1–28), a prebiotic period (days 42–70), and a placebo period (days 84–112). In group 2, an additional symbiotic period (days 126–154) was added to the setup of the study. Each subject received two different substrates twice a day during the ingestion periods (Table 1). Throughout the study, the volunteers consumed their usual diet, taking care that the diet remained as stable as possible over the four periods. In addition, they were advised to avoid intake of fermented milk products and food components containing high quantities of fermentable carbohydrates.

Immediately before the start of the study, at the start of each treatment period (short-term influence) and at the end of each treatment (long-term influence) and washout periods, volunteers consumed a test meal containing lactose-[15N,15N]ureide as a biomarker, as shown in Fig. 1. At the start of each dietary intervention period, the pre- and/or probiotic substrate was administered together with the labeled test meal as well as twice per day during the urine and fecal collection to evaluate the influence of the actual presence of the pre- and/or probiotic substrate in the colonic lumen. After each intervention period, no substrate was administered with the labeled test meal or during the urine and fecal collection to exclude effects caused by the actual presence of the substrate.

Pre- and Probiotic Substrates

OF-IN (Synergy 1, Orafti, Tienen, Belgium) was chosen as the probiotic substrate (17, 26). IN is a mixture of β-(2-1) linear fructans obtained from chicory root with a degree of polymerization (DP) ranging between 2 and 60 (average DP: 12). OF (DP: 2–8, average DP: 4) is obtained by partial enzymatic hydrolysis of IN, whereas a long-chain IN known as HP (DP: 10–60, average DP: 25) can be obtained by combining IN HP and OF. In the present study, a 1:1 (wt/wt) mixture was used.

As probiotic substrates, L. casei Shirota (incorporated in a single-strain fermented milk product) and lyophilized B. breve Yakult (both provided by Yakult Honsha, Tokyo, Japan) were chosen (37). The placebo for the probiotic milk was an identical milk product without L. casei Shirota strain (provided by Yakult Honsha); otherwise, maltodextrin (AVEBE Food, Foxhol, The Netherlands) was used as the placebo.

Test Meal

The test meal consisted of a pancake [8.4 g proteins, 11.2 g fat, and 26.7 g carbohydrates (243.5 kcal)] that contained 75 mg lactose,[15N,15N]ureide. The latter substrate was synthesized according to the method of Schoorl (33) as modified by Hofmann (16) with [15N,15N]urea (obtained from Eurisopo-top, St. Aubin Cédex, France). The absence of remaining [15N,15N]urea or lactose was confirmed using thin-layer chromatography (23). Whenever a correction for transit time was required, 185 kBq of [3H]polyethylene glycol ([3H]PEG, NEN Life Science Products, Boston, MA) was added to the test meal as an inert radiolabeled transit marker.

Sample Collection

Urine was always collected in dedicated receptacles to which neomycin was added for the prevention of bacterial growth. A basal urine sample was collected before volunteers consumed the test meal. After volunteers had consumed the test meal, a 48-h urine collection was performed in three fractions: 0–6, 6–24, and 24–48 h. After volumes were measured, samples were stored at −20°C until analysis.

After volunteers had consumed the test meal, all stools were collected for 72 h by the volunteers. Upon delivery of the fecal samples to the laboratory a 5-g (wt wt) aliquot was taken for DNA extraction and immediately frozen at −20°C. All stools collected on the same day were combined, weighed, and homogenized before further analysis. Samples of known weight were removed and freeze dried. Aliquots of the dried material were used for the subsequent analysis of total nitrogen, [15N], and [3H]PEG content or for separation into bacterial, fiber, and soluble fractions.

Analytical Procedures

Determination of urinary p-cresol content. The p-cresol content of all urine fractions was measured by gas chromatography (GC)-mass spectrometry (MS) technology. Urine samples with a volume of 950 µl were taken, and the pH of the samples was adjusted to pH 1 with concentrated H2SO4 (Merck, Darmstadt, Germany). This solution was heated for 30 min at 90°C to deproteinize and hydrolyze the conjugated phenols. After samples had been cooled down to room temperature, 50 µl of 2.6-dimethylphenol (20 mg/100 ml, Sigma-Aldrich Chemie, Steinheim, Germany) were added as an internal standard. p-Cresol was extracted with 1 ml of ethyl acetate (Merck). The ethyl acetate layer was dried, and 0.5 µl of this solution were analyzed by GC-MS (Trace GC-MS, Thermofinnigan, San José, CA). The analytical column was a 30-m × 0.32-mm inner diameter, 1-µm AT5-MS (Alltech). Helium GC grade was used as a carrier gas with a constant flow of 1.3 ml/min. The oven was programmed from 75°C (isothermal

Table 1. Ingestion periods of the different groups

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<tr>
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Ingestion periods were each for 4 wk; n = 10 volunteers/group. OF-IN, oligofructose-enriched inulin.
for 5 min) with 10°C/min to 160°C and to 280°C with 20°C/min. MS detection (single quadrupole) was performed in El full-scan mode from 20 to 590 at 2 scans/s. Results were expressed as total p-cresol content (in mg) excreted in urine.

**Determination of total nitrogen content and 15N enrichment in urine, feces, and bacterial pellets.** Total nitrogen content and 15N enrichment were determined by a continuous-flow elemental analyzer isotope ratio mass spectrometer (ANCA-2020, Europa Scientific, Crewe, UK). Therefore, known amounts of urine (15 μL, adsorbed on chromosorb (Elemental Microanalysis, Devon, UK)) feces (freeze-dried, 5–7 mg), or bacterial pellets (freeze-dried, 5–7 mg) were introduced in the oxidation-reduction module coupled to the isotope ratio mass spectrometer. In this module, the samples were oxidized using copper oxide, oxygen, and chromium oxide to nitrous oxides introduced in the oxidation-reduction module coupled to the isotope ratio mass spectrometer. The nitrogen content and 15N enrichment were measured. The 15N-to-14N isotope ratio of N2 was measured with reference to a calibrated laboratory standard (i.e., a standard ammonium sulphate solution). Results for urinary and fecal samples were expressed as total nitrogen content and as percentages of the administered dose of 15N (8). Total nitrogen and 15N excretion in the bacterial fraction were expressed, respectively, as milligrams per gram of bacteria and as nanograms per milligram of bacteria.

**Determination of fecal 1H/PKG.** The [1H]PEG content in fecal samples was measured with liquid scintillation counting (model 3375, Packard Tricarb Liquid Scintillation Spectrometer, Packard Instruments, Downers Grove, IL) after oxidation to [1H]H2O (model 306, Packard Sample Oxidizer, Packard Instruments). 1H contents in fecal samples were expressed as percentages of the administered dose recovered over 72 h and were used to correct for gastrointestinal transit by dividing the cumulative percentage of the administered dose of 15N recovered over 72 h by the cumulative percentage of the administered dose of 1H recovered over 72 h.

**Separation of dried fecal samples into bacteria, fiber, and soluble fractions.** The separation of freeze-dried fecal samples was performed according to the method described by Stephen and Cummings (38). An aliquot of 500 mg freeze-dried fecal material was taken and thoroughly mixed for 10 min with 30 ml formylsaline (1% formol in 0.9% saline) and 0.30 ml of 10% sodium lauryl sulfate. The mixture was filtered through a 20-μm filter (Varian, Palo Alto, CA) under vacuum, and the residue was washed with 30 ml of formylsaline, shaken, and filtered again. This procedure was repeated three times. The fraction on top of the filter was the fiber fraction. The filtrate was ultracentrifuged at 25,000 g during 36 min. The supernatant, containing water-soluble compounds, was discarded, whereas the sediment, containing bacteria, was dissolved in 2 ml sterile pyrogen-free water. After being weighed, the sediment was freeze-dried, and the dried material was weighed again.

**DNA extraction from fecal specimens.** Total bacterial DNA was extracted from fecal samples using a modified version of the method of Pitcher and co-workers (25) as previously described (40).

**Real-time PCR analysis.** Quantification of the fecal Bifidobacterium population was performed with the LightCycler system I (Roche, Mannheim, Germany) using a FastStart DNA Master SYBR Green I kit and a Bifidobacterium genus-specific PCR primer set (22). Data were expressed as log10 bifidobacteria per gram wet weight.

**Statistical Analysis**

Results are expressed as medians plus interquartile ranges (IQR). Statistical analysis was performed with SPSS software (SPSS 12.0 for Windows, SPSS, Chicago, IL). Given the low numbers of subjects in the treatment groups, nonparametric statistical analysis was used regardless of the distribution of results (Friedman ANOVA, Kruskal-Wallis test, Wilcoxon test, and Mann-Whitney test with the Bonferroni correction). The level for statistical significance was set at *P* < 0.05.

**RESULTS**

One female subject of group 2 withdrew from the study during the first ingestion period due to antibiotic intake. Data from this subject were excluded from further analysis. All other subjects completed the study.

**Total Nitrogen and 15N excretion**

The results of the urinary and fecal excretion of total nitrogen and 15N as well as different fecal parameters in the different test situations are shown in Figs. 2–9.

**Urinary excretion.** The cumulative nitrogen excretion was similar throughout the study in each group and varied from 15 to 20 g/48 h. In group 1, short-term intake of B. breve Yakult did not influence the cumulative percentages of 15N recovered in the 0- to 48-h urine collection, whereas a tendency to decreased 15N recovery was noted upon long-term administration of B. breve Yakult cells. On the other hand, the urinary 15N excretion was significantly lower after both short-term (*P* = 0.005) and long-term (*P* = 0.017) administration of OF-IN compared with baseline values and compared with placebo (*P* = 0.005 for short-term OF-IN intake and *P* = 0.022 for long-term intake). Also, in group 2, OF-IN administration significantly decreased urinary 15N content (*P* = 0.008 for short-term intake of OF-IN and *P* = 0.011 for long-term administration of OF-IN) compared with the baseline value and compared with placebo (*P* = 0.008 for both short and long term). For L. casei Shirota cells, only short-term administration resulted in a significant effect compared with the baseline condition (*P* = 0.038), although the urinary 15N excretion after the administration of L. casei Shirota cells was not significantly different from that after placebo intake. The symbiotic combination of L. casei Shirota with OF-IN resulted in a significant reduction of the cumulative excretion of the label compared with baseline (*P* = 0.008 for short- and long-term symbiotic administration). Also, compared with placebo intake, the urinary 15N excretion was significantly lower after symbiotic intake (*P* = 0.011 for short-term symbiotic administration and *P* = 0.008 for long-term symbiotic administration, respectively). No changes in urinary 15N content were observed after placebo treatment, and,

![Fig. 1. Schematic representation of the study design. The study was conducted over a 16-wk period consisting of 3 ingestion periods of 4 wk each separated by a 2-wk washout (WO) period. Immediately before the start of the study (baseline [Bas]), at the start of each treatment period [short-term (ST) effect], and at the end of each treatment [long-term (LT) effect] and WO period, a test was performed, and, each time, urine (48 h) and feces (72 h) were collected. In group 2, an additional symbiotic period was added to the study.](http://ajpgi.physiology.org/)

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after each 2-wk washout period, urinary $^{15}$N values had returned to baseline values. No significant differences in effect between the symbiotic and prebiotic were found.

**Fecal excretion.** Stool weight, fecal dry weight, pH, and total $^{15}$N excretion. The results obtained in the different test situations are shown in Table 2. After probiotic or placebo treatment, no significant effect was found on the total (combined urinary and fecal) excretion of $^{15}$N. After the short-term administration of OF-IN, the decrease in urinary $^{15}$N excretion was compensated by an increase in the fecal excretion of the marker, resulting in a similar total excretion of the label. However, the long-term administration of OF-IN and short- and long-term administration of the symbiotic combination resulted in significantly decreased total $^{15}$N excretion, suggesting that a higher fraction of $^{15}$N was retained within the human body.

**Total nitrogen and $^{15}$N enrichment in bacterial pellets.** The results of total nitrogen content and labeled nitrogen of bacterial pellets after pre- and/or probiotic intake in both groups are shown in Fig. 10. No influence of the different substrates on total nitrogen in the bacterial fraction was found in either of the groups. The mean nitrogen content varied from 20 to 30 mg/g bacterial solids. In both groups, a significant increase in $^{15}$N content in the bacterial fraction was found after short-term OF-IN intake compared with baseline [from 2.58 (IQR: 1.33–7.44) to 6.10 ng/mg bacterial solids (IQR: 1.89–9.53), $P = 0.047$, in group 1 and from 2.79 (IQR: 1.32–6.32) at baseline to 5.60 (IQR: 1.65–8.74) ng/mg bacterial solids (IQR: 1.98–9.53), $P < 0.05$ in group 2].
to 8.90 ng/mg (IQR: 7.50–16.28), $P = 0.008$, in group 2]. Also, short-term intake of the synbiotic combination of OF-IN and \textit{L. casei} Shirota cells resulted in a significant increase [2.79 (IQR: 1.32–6.32) vs. 12.14 ng/mg (IQR: 5.34–18.86), $P = 0.008$]. On the contrary, no significant effect on $^{15}$N content in the bacterial fraction was seen after long-term intake of OF-IN or synbiotic, correlating with the fact that no rise in the cumulative fecal excretion of $^{15}$N was observed. Neither probiotic nor placebo intake resulted in a significant change of $^{15}$N content in the bacterial fraction.

\textit{Urinary p-Cresol Excretion}

The results of the effects of pre- and/or probiotic administration on urinary excretion of \textit{p}-cresol are shown in Table 3. In group 1, short- and long-term intake of \textit{B. breve} Yakult resulted in a significant decrease of \textit{p}-cresol excretion compared with baseline ($P = 0.028$ and $P = 0.005$, respectively). In group 2, the long-term administration of \textit{L. casei} Shirota decreased \textit{p}-cresol excretion significantly ($P = 0.038$), whereas a tendency toward a lower \textit{p}-cresol excretion was observed after the short-term administration of the substrate. After the short-term OF-IN administration, the urinary excretion of \textit{p}-cresol was significantly reduced compared with baseline in both groups ($P = 0.013$ and $P = 0.025$ for groups 1 and 2, respectively). The long-term OF-IN administration resulted in significantly reduced \textit{p}-cresol excretion in group 2 ($P = 0.025$), whereas in group 1 no significant effect was observed. However, the combination of the results of both groups after
OF-IN administration resulted in a statistically significant reduction of p-cresol excretion compared with the combined baseline results ($P = 0.001$ and $P = 0.005$ for short- and long-term intake, respectively). The long-term synbiotic administration in group 2 resulted in a significant reduction of p-cresol excretion compared with baseline ($P = 0.021$). In both groups, placebo intake did not change p-cresol levels in urine.

Quantification of Fecal Bifidobacteria Levels Using Real-Time PCR.

Total fecal bifidobacteria were significantly increased after long-term OF-IN intake [from 7.54 (IQR: 6.70–8.03) log$_{10}$ bifidobacteria/g wet wt under baseline conditions to 8.19 (IQR: 7.97–9.08) log$_{10}$ bifidobacteria/g wet wt, $P = 0.006$]. In group 2, the long-term administration of the synbiotic resulted in a tendency to increased Bifidobacterium levels [7.88 (IQR: 6.81–9.10) log$_{10}$ bifidobacteria/g wet wt under baseline conditions vs. 8.76 (IQR: 7.92–10.04) log$_{10}$ bifidobacteria/g wet wt, $P =$ not significant].

DISCUSSION

The extent of urinary and/or fecal excretion of ammonia and p-cresol may give an indication of the degree of proteolytic colonic fermentation, which is considered to be relevant to colonic health (32). Both ammonia and p-cresol are considered important in the disease course of uremic syndrome (5).
have been proposed as putative markers relevant to colon cancer risk (4, 24). Several studies have investigated the ability of different types of dietary intervention to decrease the proteolytic activity of colonic microbiota, thereby reducing the generation and accumulation of these potentially toxic metabolites and to simultaneously increase saccharolytic activity (26).

The results of the present study demonstrated that the extent of the production of the biomarkers of proteolytic activity in the colon was reduced after the administration of the selected prebiotic (i.e., OF-IN) and, to a lesser extent, after the administration of bacterial probiotic substrates (i.e., **B. breve** Yakult or **L. casei** Shirota), whereas placebo intake did not result in any significant effects. Similar effects have previously been observed after lactulose administration (9). Remarkably, the decrease in urinary $^{15}$N excretion observed after the long-term administration of OF-IN or the symbiotic combination of OF-IN and **L. casei** Shirota was not correlated with a corresponding increase in fecal $^{15}$N excretion, resulting in an increased body retention of $^{15}$N. Previously, it has been shown that after the administration of lactulose, the decrease in urinary $^{15}$N excretion was compensated by an increased fecal
15N excretion, resulting in a similar body retention of 15N (9). Possibly, the observed retention in the colon may be linked to the fact that OF-IN does not only have a stimulating effect on the microbiota in the lumen of the colon but also promotes mucosa-associated microbiota. Recently, Langlands et al. (20) demonstrated both in vivo and in vitro that supplementation of a mixture of OF and IN significantly increased mucosal bifidobacteria. Also, in the study by Kleessen et al. (19), a significant increase in mucosal bifidobacteria was observed after rats harboring human fecal flora had been fed an OF-IN-supplemented diet.

Evaluation of the rate of urinary and/or fecal excretion of 15N and p-cresol after a 2-wk washout period demonstrated that the modification in the metabolic activity of colonic microbiota induced by dietary intervention was only temporary and readily disappeared once administration of substrate had ceased. These observations are in line with previous studies (6, 13, 37) in which 1–2 wk after the end of the intervention period, the induced changes of the composition of microbiota (i.e., an increase of bifidobacteria), accompanied with higher saccharolytic activity, disappeared and baseline values were restored. As a consequence, it was suggested that a continuous stimulation of microbiota through pre- and/or probiotic administration might be necessary to maintain the beneficial effects.

Further evaluation of the results demonstrated that the effects of prebiotic intake on colonic ammonia metabolism were more pronounced than those caused by probiotic intake both in short and long term. The clear effects caused by the short-term administration of the prebiotic can be explained by the rapid fermentation of the carbohydrate in the colon, resulting in a lower colonic pH (33). The short-term administration of probiotics does not immediately influence the colonic environment and metabolic activity. In addition, a moderate but significant correlation was found between the effect of short-term OF-IN intake on urinary p-cresol and 15N content (Fig. 11; Pearson correlation coefficient = 0.574 and P = 0.010). Although the colonic processes measured with both markers are different (fate of ammonia and protein degradation, respectively) and not related to each other, these results suggest that the influence of short-term OF-IN administration is similar on both processes. After long-term administration, this correlation was less pronounced (Fig. 12; Pearson correlation coefficient = 0.432 and P = 0.071).

Conversely, the impact of both probiotics was clearly more pronounced on p-cresol excretion than on the fate of ammonia. Contrary to prebiotics, probiotic intervention does not provide energy to colonic microbiota. As a consequence, it was not surprising that less-pronounced effects on 15N excretion were observed since this biomarker mainly reflects the stimulation of bacterial activity and/or growth. It is assumed that the decreased excretion of p-cresol is caused by an increased uptake of the amino acid tyrosine or metabolic products of protein putrefaction by a higher colonic availability of probiotic cells or by reduced protein fermentation due to inhibition of proteolytic bacteria by probiotic cells.

It was anticipated that the effects caused by symbiotic administration would exceed those of the separate compounds.
However, in our study, we could not observe a significant additive value of the synbiotic compared with the prebiotic, although a clear tendency was observed.

In the present study, the effects of the substrates were evaluated under “normal” conditions (i.e., in healthy people without imposing standard diets) since pre- and probiotics are often recommended as food supplements for healthy individuals in normal circumstances. A relatively homogeneous group of young volunteers was selected to minimize interindividual variability. Since it is known that the diversity of colonic microbiota decreases with aging, the impact of pre- and probiotics on colonic metabolism in middle-aged and elderly people might be different and should be investigated in future studies.

Furthermore, a reduction of proteolytic activity in the colon may not only benefit healthy individuals but could also play an important role in several pathological conditions. For instance, it has been demonstrated that as many as 50% of patients with irritable bowel syndrome (IBS) have abnormal colonic fermentation (18). The developed biomarkers could be used to determine whether $^{15}$N and $p$-cresol excretion in these patients differ from those of healthy volunteers and, second, to investigate whether pre- or probiotic administration could induce comparable effects as in healthy individuals.

There are indications that colonic microbiota can play a role in the pathogenesis of inflammatory bowel disease (IBD). Several studies have shown that inflammation is more abundant in those parts of the gastrointestinal tract where the highest numbers of bacteria are present (36). Furthermore, differences in the composition of colonic microbiota of patients with IBD compared with healthy volunteers have been demonstrated, which might lead to differences in the overall fermentation pattern (15, 21, 35). A higher degree of protein fermentation has the potential to cause damage to epithelial cells, resulting in higher inflammation levels. As a consequence, a modification of intestinal microbiota and changes in metabolic activity may have favorable effects on this syndrome (11).

Besides gastrointestinal conditions, a role for colonic microbiota was also described in the pathogenesis of chronic renal failure. Chronic renal failure is characterized by a progressive retention of a number of microbial metabolic end products (i.e., phenols, indols, and polyamines) (39), which cannot be eliminated any more due to kidney failure and can hardly be removed using dialysis techniques because of their high protein binding. In addition, Bammens et al. (2) demonstrated that the small intestinal assimilation (digestion and/or absorption) of
proteins is impaired in the case of renal failure, resulting in an increased availability of proteins for fermentation in the colon. As a consequence, besides reduced renal excretion, renal failure is also characterized by increased production of proteolytic fermentation metabolites and, thus, an accumulation of p-cresol. Furthermore, it has recently been demonstrated that lower p-cresol levels are correlated with lower mortality in uremic syndrome (3). For this reason, strategies that contribute to a lower generation of protein fermentation metabolites might constitute a significant improvement in the management of those patients.

In the present study, the use of two biomarkers demonstrated that OF-IN, L. casei Shirota, and B. breve Yakult exert favorable effects on colonic protein and ammonia metabolism in healthy volunteers. In the case of OF-IN, this effect was accompanied by an increase in total bifidobacteria levels in feces. Further exploitation of $^{15}$N excretion and p-cresol as biomarkers would particularly be useful during clinical trials involving specific patient groups, e.g., patients diagnosed with IBS and IBD.

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