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Impact of a Resistant Dextrin on Intestinal Ecology: How Altering the Digestive Ecosystem with NUTRIOSE®, a Soluble Fibre with Prebiotic Properties, May Be Beneficial for Health

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OBJECTIVES: The prebiotic potential of NUTRIOSE® – a sugar-free, digestion-resistant dextrin – was evaluated in two randomized, placebo-controlled trials that included 48 and 40 healthy volunteers, respectively. METHODS: In study 1, the effect on colonic bacteria of NUTRIOSE® 10, 15 or 20 g/day administered for 14 days was examined; in study 2, gut microbial changes in response to NUTRIOSE® 8 g/day for 14 days were monitored using real-time polymerase chain reaction analysis. RESULTS: NUTRIOSE® increased proliferation of *Bacteroides* and inhibited *Clostridum perfringens* in both studies, increased β-glucosidase activity (at 10 and 15 g/day) and decreased colonic pH (at 20 g/day). The increase in short-chain fatty acid production with NUTRIOSE® consumption was not statistically significant. There were no indications of gastrointestinal intolerance at any dose. CONCLUSIONS: According to commonly accepted definitions, NUTRIOSE® is a prebiotic soluble fibre that provides a beneficial effect on colonic ecology while preserving digestive comfort.

KEY WORDS: *Bacteroides*; Fermentation; Glucosidase; Intestinal microbiota; Low-digestible carbohydrate; Oligosaccharides

Introduction

NUTRIOSE® (Roquette, Lestrem, France) is a glucose polymer derived from wheat (NUTRIOSE® FB range) or maize starch (NUTRIOSE® FM range) via a highly controlled dextrinization process of partial hydrolysis and subsequent repolymerization.[1] In addition to typical α-1,4 and α-1,6 glucosidic linkages, repolymerization results in other linkages not found in starch, including linear and branched linkages β-1,6, α-1,2 and/or β-1,2, α-1,3 and/or β-1,3, and β-1,4. NUTRIOSE® is, therefore, resistant to hydrolysis by endogenous glucidolytic enzymes and can be classified as a soluble dietary fibre with a total fibre content of

almost 85% (NUTRIOSE® 06), according to the Association of Analytical Communities method.[1,2] Chromatography is used to tailor the molecular weight distribution and to increase fibre content further. Additional refining steps – including the removal of simple sugars and spray drying – result in a product with mono- and disaccharide content of < 0.5% dry weight.

NUTRIOSE® is a sugar-free dextrin that is not absorbed in the ileum and remains available for bacterial fermentation in the colon.[3] This bacterial fermentation has been shown to decrease colonic pH, alter the microflora and induce the production of short-chain fatty acids.[3] Wheat dextrin has been shown to have similar fermentability to inulin and partially hydrolysed guar gum (PHGG)[4] or psyllium[5] in *in vitro* batch-fermentation systems. Both wheat dextrin and inulin decreased pH, but inulin resulted in the production of significantly more hydrogen and total gas.[4] In addition, wheat dextrin and inulin produced significantly more total short-chain fatty acids than PHGG after 24 h.[4] Wheat dextrin, psyllium and inulin produced similar short-chain fatty acid concentrations at 24 h, but differences in fermentation rates and gas production may affect gastrointestinal tolerance.[5] NUTRIOSE® has been shown to have beneficial effects in humans, including increased lactobacilli numbers, decreased faecal pH (from 6.6 to 6.1) and increased faecal α- and β-glucosidase activities after consumption of 45 g/day for 35 days.[6] These results demonstrate the prebiotic potential of NUTRIOSE®.

Prebiotics were originally defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one (or a limited number) of the bacterial species already resident in the colon and, thus, attempt to improve host health.[7] This definition has been revised over time to include an increase in beneficial bacteria and/or a decrease in harmful bacteria, a reduction in intestinal pH, the production of short-chain fatty acids and changes in bacterial enzyme concentrations.[8] Several *in vitro* and *in vivo* studies (in rats and in humans) have been undertaken to explore the potential prebiotic effects of NUTRIOSE®,[6,9,10] and supplementation with NUTRIOSE® was found to increase the numbers of faecal lactobacilli.[6]

The aim of the present research was two-fold: first, to determine the effect of three different dosages of NUTRIOSE® on *Bacteroides* and *Clostridium perfringens* numbers; *Bacteroides* is the predominant saccharolytic genus of a normal gut flora;[11] secondly, to determine more precisely the gut microbial changes that occur, using real-time polymerase chain reaction (PCR) analysis.

Subjects and methods

STUDY 1

Subjects and study design

This prospective, single-centre, randomized, double-blind, placebo-controlled pilot study evaluated the impact of three different doses of NUTRIOSE® on the faecal flora of healthy volunteers over a 15-day period. Healthy male and female volunteers were locally recruited by advertisement by the Clinical Investigation Centre (CIC) at the Regional Teaching Hospital, Lille, France, between November 2002 and August 2003.

During a preinclusion visit, subjects provided a medical history and underwent physical examination. Inclusion criteria were: age 18 – 45 years; regular intestinal transit (meaning one or two stools per day, of normal consistency, excreted without any difficulty, for ≥ 3 months); no history of medical or psychiatric disorders; no history

of chronic gastrointestinal disorders; affiliation to the French public welfare system or similar. Exclusion criteria included: infection or antibiotic therapy in the previous 3 months; participation in any other trial in the previous month; use of drugs that may interfere with normal transit (antidiarrhoeals, laxatives, intestinal antiseptics, antibiotics); gluten or aspartame intolerance; wheat flour allergy; constipation; vegetarianism or veganism; consumption of more than three glasses of beer or wine per week; current pregnancy; subject under legal guardianship; partial or complete legal incapacity.

The Advisory Committee of People's Welfare in Biomedical Research in Lille, Regional Teaching Hospital, Lille, France, approved the study protocol (09/09/2002-CP 02/67), and subjects provided written informed consent.

Diet and supplementation

Subjects were required to follow a low-residue diet, containing a maximum of 20 g/day of total dietary fibre, and abstain from fermented dairy products containing bifidus, yoghurt, 'light' food products (i.e. sugar-free confectionery or beverages and artificial sweeteners), dietary supplements (pre- and probiotics) and meal substitutes. This diet was followed for a 7-day preinclusion period, or dietary lead-in period. On day 8, an inclusion visit allowed investigators to perform a clinical examination and record potentially interfering events before including and randomizing the subjects. Fresh stools were sampled and analysed, and the experimental products – either NUTRIOSE® or placebo – were then administered for ingestion, starting from day 8, for 14 days. On day 15 of the treatment period (i.e. day 23 of the study), the final visit took place.

Randomization of the subjects for assignment into one of four groups was done in blocks of 12 (blocks of two males and two females) by the CIC, after drawing lots using sealed envelopes pre-established by people independent from the study. The placebo group received 20 g/day glucose by mouth in two equal doses for 14 days. The remaining three groups received 10, 15 or 20 g/day NUTRIOSE® (Roquette, Lestrem, France) by mouth in two equal daily doses for 14 days. Experimental products used in all groups (including the placebo group) were rendered identical in taste and sweetness by the addition of aspartame. Both products were provided in powder form and were dissolved in 200 ml orange juice (given to all subjects by the investigators) prior to consumption with the afternoon and evening meals.

Faecal parameters

Subjects provided faecal samples at two time points: day 8 (inclusion visit), and day 23 (final visit on the day following the 14-day period of placebo/NUTRIOSE® consumption). Fresh faeces was collected and transported at ambient temperature in a sterile plastic container containing a moistened Anaerocult® A (Merck, Lyon, France), in order to generate an anaerobic environment. Bacteriological analysis was carried out within 4 h of sample collection.

Samples were diluted and spread on media specific for the culture of the different types of bacteria studied, then incubated in aerobic or anaerobic conditions as appropriate. Bacterial cultures were counted 48 h after inoculation for aerobic bacteria and 7 days after inoculation for anaerobic bacteria. Total anaerobic species were counted (Columbia agar medium [bioMérieux, Craponne, France] with 0.03% cysteine hydrochloride and 0.5% glucose, and supplemented with 5% horse blood);

separate counts were done for the anaerobic species: *Bacteroides* (*Bacteroides* bile esculin agar medium [Oxoid, Dardilly, France]); *Bifidobacterium* spp (Columbia agar medium with 0.03% cysteine hydrochloride and 0.5% glucose, and adjusted to pH 5.0 by adding propionic acid); *Chlostridium innocuum* (Columbia–mannitol agar medium with 0.03% cysteine hydrochloride and 1% mannitol [bioMérieux]); *C. perfringens* (brain–heart infusion broth with added 0.05% cysteine hydrochloride, then lactose–sulphite broth if positive); and *Lactobacillus* spp (de Man–Rogosa–Sharpe agar medium [Oxoid]). Separate counts were also performed for aerobic (facultative anaerobic) species; *Staphylococcus* spp (mannitol salt agar medium [bioMérieux]) and *Enterococcus* spp (MacConkey agar medium [bioMérieux]).

In addition, pH, β-glucosidase enzyme activity and short-chain fatty acid levels were assessed at both time points. The pH and enzyme activity were measured according to the method of van den Heuvel *et al.*[9] Short-chain fatty acid levels were assessed using the method described in Guérin-Deremaux *et al.*[12]

Statistical analyses

Statistical analyses were carried out with SPSS® statistical software, version 9.0 (SPSS Inc., Chicago, IL, USA) for Windows®. The Kolmogorov–Smirnov and Shapiro–Wilk tests were used to assess the normality of distribution of clinical and biological parameters. Homogeneity of the groups was analysed before the subjects received NUTRIOSE®/placebo treatment. Values were expressed as mean ± SD. Intragroup comparison of clinical parameters was carried out using the Friedman test for paired samples. Between-group tests were performed using Student's independent-sample *t*-test or the Kruskal–Wallis test, depending on the assumptions of normality. A P-value < 0.05 was considered to be statistically significant.

STUDY 2

Subjects and study design

This single-centre, randomized, double-blind, placebo-controlled, parallel-group study with aged-matched subjects evaluated the effect of NUTRIOSE® supplementation on faecal *Bacteroides* (total population and *B. fragilis* specifically) and *C. perfringens* numbers.

Female volunteers were recruited from the Food Manufacturer in YiWu, Zhejiang Province, China, between December 2007 and March 2008. Advertisements were placed on site by the CIC of Xinhua Hospital, affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China.

Inclusion criteria were: age 25 – 59 years; ability to understand the study procedures and provide informed consent; moderate intestinal disorders (including one or more of the following symptoms: diverticulosis or nonspecific symptoms; stool types 1 or 2 on the Bristol Stool Form Scale;[13] clinical constipation; difficult, painful, or extended faecal exoneration); body mass index (BMI) 24 – 27.9 kg/m^2. Exclusion criteria included: participation in any other trial in the previous 2 months; known chronic constipation; currently treated gastro-intestinal symptoms; analgesic treatment (except aspirin or paracetamol); regular intake of laxatives or other remedies to promote digestion in the 2 weeks prior to the study (at least once per week); consumption of dairy products or supplements containing probiotics in the 10 days prior to the study; current diarrhoea, or frequent urgent evacuation; current dietary fibre supplementation (except from food sources); previous

contraindication to fibre supplements (Crohn's disease); wheat allergy; dietary constraints limiting the consumption of pre- and probiotics; antibiotic therapy in the previous 3 months; diseases that contra-indicate dietary fibre supplementation.

The ethical committee 'Institutional Review Board of Shanghai', China (07/12/2007-EC [2007] No. 003) approved the study and all subjects provided written informed consent.

An urn randomization scheme stratified by age (29 – 39, 40 – 49 and 50 – 59 years) and BMI (using two distinct ranges: 24.0 – 25.9 kg/m^2 and 26.0 – 27.9 kg/m^2) was employed to assign participants to the groups. A fully detailed protocol to preserve blinding was communicated to the CIC on the logistics of assigning participants to one of the two groups according to this specified urn design. Subjects received either glucose 8 g/day (in dextrose liquid form) or NUTRIOSE® 8 g/day, diluted in 248 ml of apple juice, once a day for 14 days. Supplements were provided to subjects, already diluted in a total of 14 separate bottles of apple juice, for daily consumption before breakfast (at 08.00 h). Participants were asked to maintain their regular diet while avoiding the following in order to limit nonspecific fermentations and nonspecific digestive microflora selection: dairy products with added cultures, asparagus, salsify, rye bread, artichokes, leeks, garlic, onions and bananas.

Faecal parameters

Subjects provided faecal samples at baseline (between day –3 and day 0) and between day 11 and day 14. Real-time PCR analyses were performed using group-specific primers for *C. perfringens* and *Bacteroides* (developed by Matsuki *et al.*).[14] DNA was extracted from 20 mg faecal samples and real-time PCR amplification of 16S rDNA was performed in order to identify and quantify *Bacteroides fragilis* and *C. perfringens* populations. Real-time PCR was performed with an external control using a commercial kit (SYBR® Green I; Takara Bio Inc., Tokyo, Japan) according to the manufacturer's instructions. Each reaction mixture (25 µl) was composed of 10 × buffer (Mg^{2+} plus), 2.5 µl deoxyribonucleotide triphosphate 200 µM, 1:75 000 dilution of SYBR® Green I, 11 ng/µl of TaqStart™ antibody (Clontech Laboratories Inc., Palo Alto, CA, USA), 1 U of Taq DNA polymerase (Takara Bio Inc.), and each of the specific primers at a concentration of 0.25 µM. Primers were obtained[14] from the appropriate gene database (GenBank®; http://www.ncbi.nlm.nih.gov/genbank/). About 100 ng DNA from each sample was used for real-time PCR using an ABI 7300HT thermal cycler (Applied Biosystems, Foster City, CA, USA). The thermal cycling programme consisted of one cycle of 94 °C for 5 min, then 40 cycles of denaturation at 94 °C for 20 s, annealing at 55 or 50 °C for 20 s, and elongation at 72 °C for 50 s, followed by a final cycle of 94 °C for 15 s. The bacterial PCR products were quantified using real-time PCR, by measuring the gene-specific amplification curves and melting curves.

Bacteroides (total and *B. fragilis*) and *C. perfringens* populations were assessed at the clinical centre laboratory of Xinhua Hospital affiliated with Shanghai Jiao Tong University School of Medicine, Shanghai, China. Spot faecal samples were collected in a sterile gas-tight bag, in a plastic container containing an Anaerocult® A strip to create anaerobic conditions. Subjects recorded the time of defaecation. Samples were freeze-dried, stored at –18 °C, bulked as an individual's 24-h sample, weighed, ground into powder and homogenized, and portions of approximately 10 g each were stored at ambient temperature (protected from oxygen, humidity and light)

in airtight plastic bottles until the time of analysis. Agar plates were inoculated using general anaerobic medium broth supplemented with 1% glucose, and cultured anaerobically at 37 °C for 12 – 48 h. The resulting colonies were evaluated by microscopic examination and 2-(4-amidinophenyl)-6-indolecarbamidine dihydrochloride staining,[15] or by assessment of resistance to the antibiotics erythromycin, rifampicin, kanamycin and vancomycin.

Information regarding undesirable gastrointestinal events was collected daily during the 15 days of the study. Volunteers completed a notebook indicating whether they had experienced any of the following: abdominal pain, bloating, flatulence or other symptoms. The intensity of the event was graded by the subject himself as being minor (grade 1), moderate (grade 2) or strong (grade 3); absence of any symptom was also recorded (grade 0).

Statistical analyses

The number of subjects was calculated to provide a minimum of 85 – 90% statistical power in detecting a significant dose relationship, allowing for a maximum overall drop-out rate of 5% in each group and taking into account the short duration of the study.

Data were expressed as mean ± SD. One endpoint of the study was improvement in digestive discomfort and the assessment of frequency and severity of constipation, based on the Bristol Stool Form Scale.[13] Multiple regression analysis and generalized linear modelling were used to characterize the dose–response relationship.[16] Paired Student's *t*-tests were utilized to assess pre- to post-treatment changes in faecal parameters. The analyses were carried out using SAS and S-plus (SAS/STAT software version 9.2, SAS Institute Inc., Cary, NC, USA). A *P*-value < 0.05 was considered to be statistically significant.

Results

STUDY 1

Study 1 originally recruited 49 healthy volunteers (24 females and 25 males); all were randomized, but one subject dropped out after randomization due to an undesirable event (precise information is not available), leaving 48 subjects in total. Six female subjects were included in each group, while seven male subjects were included in the NUTRIOSE® 10 g group, six in the placebo and NUTRIOSE® 15 g groups, and five in the NUTRIOSE® 20 g group. The study population were healthy subjects of European origin, with a mean age of 28 years, mean weights (62.2 kg for females and 75.5 kg for males) within normal ranges, and a mean BMI of 24 ± 3 kg/m^2 for males and 22 ± 3 kg/m^2 for females. There were no statistically significant differences between the groups in terms of baseline demographic parameters.

After 14 days, consumption of 10 g/day NUTRIOSE® resulted in a significant increase in saccharolytic flora numbers (*Bacteroides*) compared with baseline ($P < 0.05$; Fig. 1). There were no intragroup significant differences in the numbers of other potentially beneficial bacteria (*Bifidobacterium* spp, *Lactobacillus* spp) whatever the dosage ingested. No other statistical differences were observed between any of the different treatment groups and placebo in terms of potentially beneficial bacteria.

Consumption of 15 g/day NUTRIOSE® led to a significant decrease in *C. perfringens* numbers after 14 days compared with baseline ($P < 0.05$; Fig. 2). There were no intragroup significant changes in the numbers of other potentially harmful bacteria

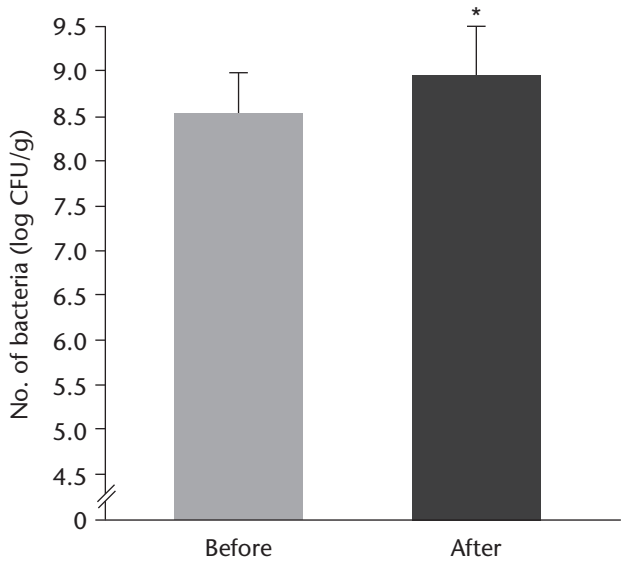

FIGURE 1: *Bacteroides* numbers (mean ± SD) in faecal cultures from healthy subjects before and after oral administration of NUTRIOSE® 10 g/day for 14 days. Results are expressed in log colony forming units (CFU)/g. *$P < 0.05$ compared with baseline, Kruskal–Wallis test

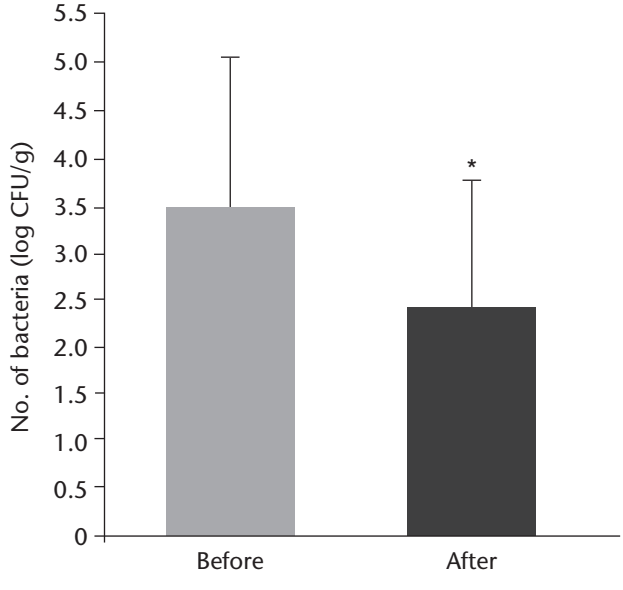

FIGURE 2: *Clostridium perfringens* numbers (mean ± SD) in faecal cultures from healthy subjects before and after oral administration of NUTRIOSE® 15 g/day for 14 days. Results are expressed in log colony forming units (CFU)/g. *$P < 0.05$ compared with baseline, Kruskal–Wallis test

(*C. innocuum*, *Staphylococcus* spp, *Enterococcus* spp) whatever the dosage ingested. In terms of these bacteria, no statistically significant differences were observed in the different groups compared with placebo.

There was a significant intragroup decrease in faecal pH with the consumption of 20 g/day NUTRIOSE® (from 6.67 ± 0.4 to 5.99 ± 0.5 after 14 days; $P < 0.05$; Fig. 3). Faecal pH decreases showed statistically significant between-group differences ($P < 0.05$) at the end of the study, with a more pronounced decrease being observed in the 20 g/day group compared with variations observed in the other groups.

There were no statistically significant differences in short-chain fatty acid levels before and after supplementation with NUTRIOSE® at any dose. Levels of acetate, propionate and butyrate were higher after NUTRIOSE® supplementation, but these increases were not statistically significant.

Oral supplementation with NUTRIOSE® significantly increased intragroup faecal β-glucosidase activity in the 10 g/day and 15 g/day groups compared with the placebo group (24.4 ± 14.0 and 22.6 ± 13.0 versus 12.9 ± 8.2 U/g, respectively; $P < 0.05$; Fig. 4).

Study subjects reported very good digestive tolerance for NUTRIOSE®: 10 and 20 g/day dosages resulted in significantly more frequent ($P < 0.05$) but milder flatulence than in the placebo group. The incidence of abdominal pain was significantly higher ($P < 0.05$) in the placebo group compared with the other groups, but with minor intensity, and the incidence of bloating was similar in all groups, remaining unchanged by NUTRIOSE® consumption. There were no cases of treatment-related drop-out, with a mean observance of 99%.

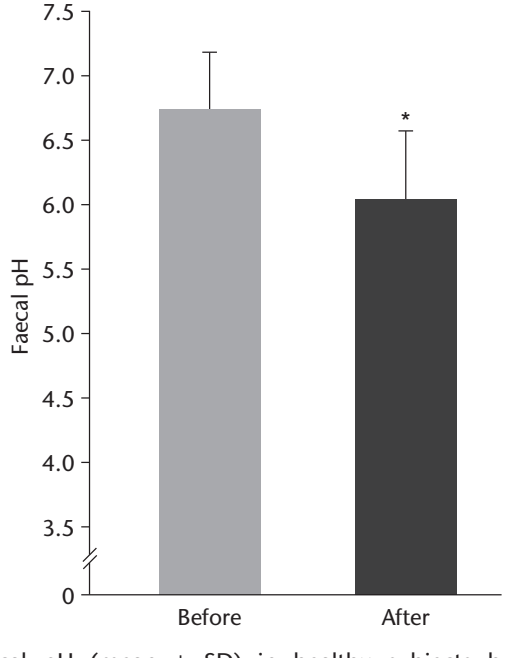

FIGURE 3: Faecal pH (mean ± SD) in healthy subjects before and after oral administration of NUTRIOSE® 20 g/day for 14 days. *$P < 0.05$ compared with baseline, Shapiro–Wilk test

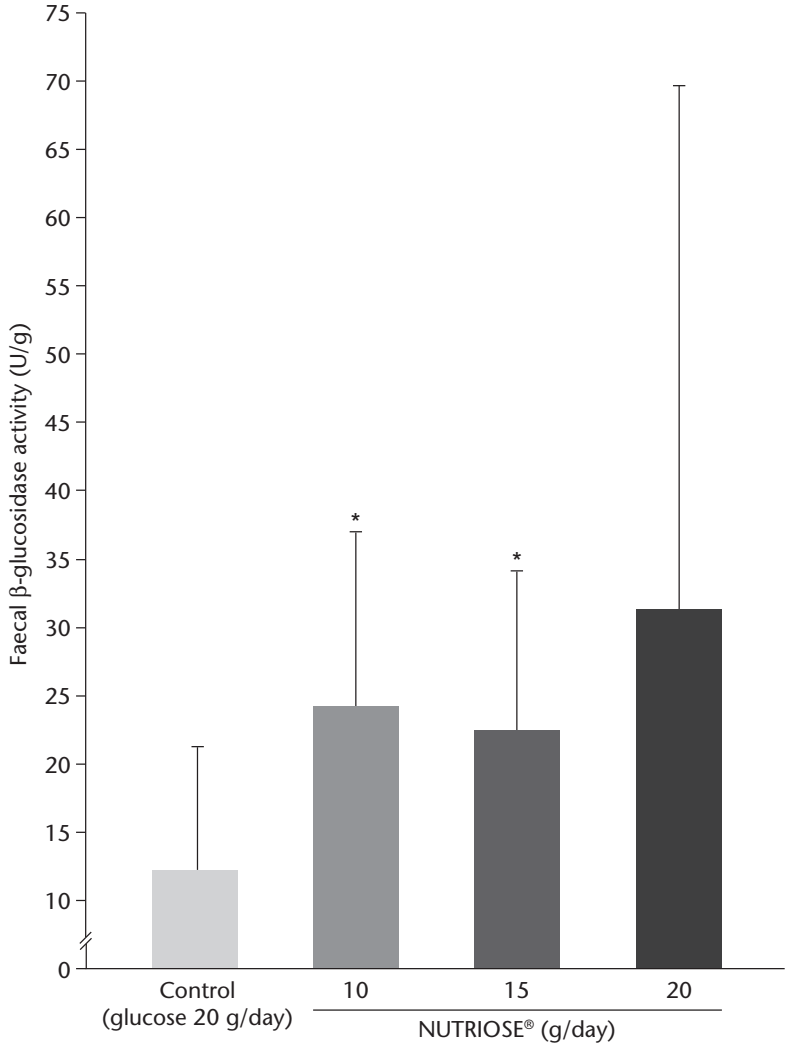

FIGURE 4: Faecal β-glucosidase activity (mean ± SD) in healthy subjects after oral administration of NUTRIOSE® 10, 15 or 20 g/day or glucose 20 g/day (control) for 14 days. *$P < 0.05$ compared with baseline, Shapiro–Wilk test

STUDY 2

Study 2 recruited 40 females, who were stratified by age and BMI before being randomly assigned to one of the two treatment groups. There were no statistical differences in the demographic data between subjects in the placebo and NUTRIOSE® groups: the mean ages of the groups were 48.7 and 43.9 years, and the mean BMI values were 24.6 and 24.6 kg/m^2, respectively.

Consumption of NUTRIOSE® 8 g/day for 14 days resulted in a significant intragroup increase in faecal *Bacteroides* numbers ($P < 0.05$; Fig. 5) and a significant intragroup decrease in faecal *C. perfringens* numbers ($P < 0.05$; Fig. 6) compared with baseline, as determined by real-time PCR. There was also a significant increase in the *Bacteroides* count

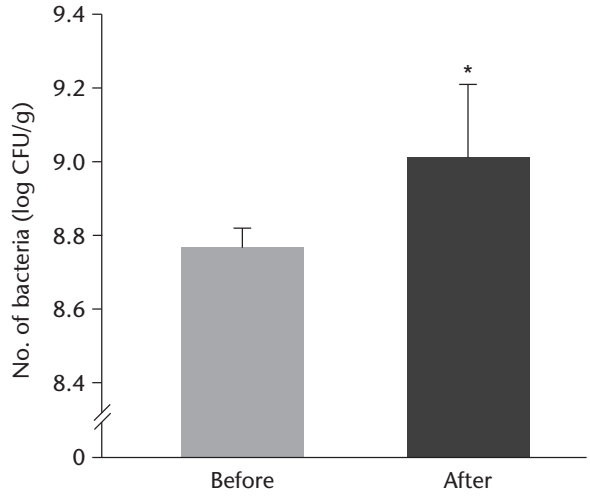

FIGURE 5: Faecal *Bacteroides* numbers (mean ± SD) assessed by real-time polymerase chain reaction in healthy subjects before and after oral administration of NUTRIOSE® 8 g/day for 14 days. Results are expressed in log colony forming units (CFU)/g. *$P <$ 0.05 compared with baseline, paired Student's *t*-test

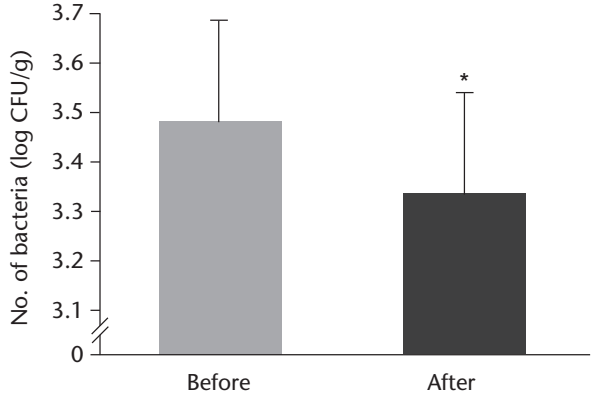

FIGURE 6: Faecal *Clostridium perfringens* numbers (mean ± SD) assessed by real-time polymerase chain reaction in healthy subjects before and after oral administration of NUTRIOSE® 8 g/day for 14 days. Results are expressed in log colony forming units (CFU)/g. *$P < 0.05$ compared with baseline, paired Student's *t*-test

($P < 0.00001$) and a significant decrease in the *C. perfringens* count ($P < 0.0016$) within the NUTRIOSE® group, between the beginning and the end of the study, compared with the variation observed in the placebo group. There were no statistically significant between-group differences in faecal classification according to the Bristol Stool Form Scale categories.[13]

Discussion

The present studies demonstrated that NUTRIOSE® has a specific colonic fermentation pattern in humans and induces beneficial effects on the colonic environment, in accordance with the findings of other research.[6] The specific colonic fermentation pattern is likely to be related to the molecular

structure of the dietary fibre and its physicochemical characteristics. NUTRIOSE®, a glucose polymer, may stimulate the proliferation of colonic bacteria that are able to adapt to nondigestible carbohydrates,[17] including the genus *Bacteroides*. *Bacteroides* are commonly found in the human intestine where they assist in the breakdown of food and produce valuable nutrients and energy: these bacteria are known to contribute to a healthy colonic ecology.[18] The present studies found that consumption of 8 or 10 g/day of NUTRIOSE® for 14 days increased the numbers of *Bacteroides*. In addition, numbers of *C. perfringens*, a potentially harmful bacterium, were decreased by the consumption of NUTRIOSE® 8 or 15 g/day for 14 days.

The growth of beneficial bacteria induces an increase in digestive enzymes including β-glucosidase, an inducible enzyme produced in particular by *Bacteroides*.[19] The increase in β-glucosidase activity after consumption of NUTRIOSE® 10 or 15 g/day for 14 days in the present study 1 indicates significant changes in the metabolic activity of the colonic flora;[17] this indicates a possible adaptation of the bacterial microflora to dietary substrates, leading to an optimization of energy harvesting.[20] This is consistent with another study of NUTRIOSE® consumption, which found an increase in both α- and β-glucosidase and in the amount of branched glucose (assumed to be a measure of enzymatic activity) present in faeces.[4] The action of β-glucosidase on residual undigested polysaccharides, for example vegetable residues, results in increased bioavailability of minerals and other micronutrients.[21]

Increased fermentation leads to a reduction in colonic (and therefore faecal) pH. A weak decrease in gut pH, coupled with propionic acid production, is known to be associated with a decrease in potentially harmful Gram-negative bacteria such as *C. perfringens*,[22] as observed during the present studies. This acidic environment may also help to solubilize minerals and further increase their absorption.[23]

Colonic short-chain fatty acids – indicators of the fermentation processes occurring after fibre consumption – are difficult to monitor in human clinical studies.[24] Study 1 described here examined faecal short-chain fatty acids but found no significant changes after consumption of NUTRIOSE®. This conflicts with findings from animal research, where short-chain fatty acid levels were increased after NUTRIOSE® consumption.[12]

The present research reinforced the classification of NUTRIOSE® as a prebiotic, in line with the definition: 'a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confers benefits upon host well being and health'.[25] A prebiotic has been further defined as, 'a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota'.[26] According to accepted definitions, a prebiotic fibre must alter the balance of gut microflora in a positive manner; it must induce an increase in beneficial bacteria and a decrease in deleterious bacteria. *In vitro*, animal and human studies have demonstrated that the resistant dextrin in NUTRIOSE® is fermented, lowers faecal pH and generates short-chain fatty acids.[4,6,27] The present studies found that dietary supplementation with dextrin not only increased *Bacteroides* numbers, but also decreased the numbers of pathogenic bacteria such as *C. perfringens*, in accordance with other studies.[6]

Well-known prebiotics include oligosaccharides such as inulin, fructo-oligosaccharides and galacto-oligosaccharides; all have a long history of good safety profiles.[28] The use of prebiotics in infant nutrition

reduces the risk of gastroenteritis and infection, improves general well-being and reduces the incidence of allergic symptoms such as atopic eczema.[28] Products that cause a selective modification in the composition of the gut microbiota could induce beneficial physiological effects in the colon and contribute towards reducing the risk of dysbiosis,[28] but there is some concern about excess production of digestive gas when they are consumed in large amounts.[29]

As a soluble dietary fibre, NUTRIOSE® is mostly resistant to digestion in the small intestine, being largely fermented in the colon, and shows an outstanding digestive tolerance.[6,30] This allows consumption in the amount best suited to achieving the desired beneficial changes to the gut ecosystem, as demonstrated in both short- and long-term use. The digestive tolerance threshold for NUTRIOSE® has been set at 45 g/day for healthy adults in short-term use:[30] short-term use is defined using studies in which digestive complaints of the tested volunteers are measured to determine a digestive threshold following 1 week's maximum consumption of the tested compound. The laxative threshold dose of NUTRIOSE® has been set at 100 g/per day.[6] Several factors contribute to these findings. First, NUTRIOSE® is only partially digested in the upper part of the intestinal tract, and the high degree of polymerization induces a lower osmotic pressure and a slower fermentation rate.[30] Secondly, the dextrin is slowly fermented throughout the colon, allowing the short-chain fatty acids produced to be progressively absorbed, inducing little osmotic effect[3] (compared with dietary fibres such as fructans that are rapidly fermented in the proximal colon).[31] Finally, the type of food matrix in which NUTRIOSE® is included, and the daily fibre consumption of the subject, may also influence digestive tolerance.[3]

Prebiotics may improve colon health via the reduction of inflammation and the stimulation of intestinal immunity.[28] Animal studies have found that the consumption of NUTRIOSE® significantly increases the levels of intestinal mediators involved in the regulation of pain, inflammation and immunity,[32,33] suggesting a potential influence of NUTRIOSE® on the regulation of local immunity.

NUTRIOSE® has a positive impact on diverse anthropometric and metabolic parameters,[27,34,35] possibly due to the modulation of microbial ratios in the gut flora.[36,37] In addition, the slow and prolonged production of short-chain fatty acids along the length of the colon may provide long-lasting energy and delay or reduce feelings of hunger.[38] Studies have described the dose-dependent increase in peptide-tyrosine tyrosine and proglucagon mRNA expression by butyrate *in vivo*, which may play an important role in the control of energy homeostasis[39,40] and promote satiety.[41] Further research is required to elucidate the underlying mechanism.

The present research had several limitations. First, the data were derived from two separate studies. Secondly, faecal sampling is not necessarily representative of total excretion. Thirdly, the method used for counting bacteria in study 1 may have led to viable cells being undercounted, since plate counting assumes that every colony is founded by a single cell, and requires lengthy incubation for colonies to become visible. Despite these limitations, this research supported and confirmed the efficacy of NUTRIOSE® as a prebiotic fibre ingredient or supplement, at doses fully compatible with both a beneficial biological effect on colonic ecology and a preserved digestive comfort. Additional studies, using

more precise and modern techniques, are required to investigate these findings further.

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Conflicts of interest

C Lefranc-Millot, L Guérin-Deremaux, D Wils and MH Saniez-Degrave are employed by Roquette, the manufacturers of NUTRIOSE®. C Neut and LE Miller had no conflicts of interest to declare in relation to this article.

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References

1 Fu B, Wang J, Roturier JM, *et al*: Determination of total dietary fiber in selected foods containing resistant maltodextrin by a simplified enzymatic-gravimetric method and liquid chromatography: interlaboratory study in China. *J AOAC Int* 2008; **91:** 614 – 621.
2 Gordon DT, Okuma K: Determination of total dietary fiber in selected foods containing resistant maltodextrin by enzymatic-gravimetric method and liquid chromatography: collaborative study. *J AOAC Int* 2002; **85:** 435 – 444.
3 Lefranc-Millot C, Wils D, Roturier JM *et al*: NUTRIOSE® Soluble Fiber. In: *Fiber Ingredients – Food Applications and Health Benefits* (Cho SS, Samuel P, eds). New York: CRC Press, 2009; pp 19 – 40.
4 Slavin JL, Savarino V, Paredes-Diaz A, *et al*: A review of the role of soluble fiber in health with specific reference to wheat dextrin. *J Int Med Res* 2009; **37:** 1 – 17.
5 Timm DA, Stewart ML, Hospattankar A, *et al*: Wheat dextrin, psyllium, and inulin produce distinct fermentation patterns, gas volumes, and short-chain fatty acid profiles *in vitro*. *J Med Food* 2010; **13:** 961 – 966.
6 Pasman W, Wils D, Saniez MH, *et al*: Long-term gastrointestinal tolerance of NUTRIOSE FB in healthy men. *Eur J Clin Nutr* 2006; **60:** 1024 – 1034.
7 Gibson GR, Roberfroid MB: Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995; **125:** 1401 – 1412.
8 Woods MN, Gorbach SL: Influences of fibres on the ecology of the intestinal flora. In: *Handbook of Dietary Fibre in Human Nutrition* (Spiller GA, ed). New York: CRC Press, 2001; pp 257 – 269.
9 van den Heuvel EG, Wils D, Pasman WJ, *et al*: Dietary supplementation of different doses of NUTRIOSE FB, a fermentable dextrin, alters the activity of faecal enzymes in healthy men. *Eur J Nutr* 2005; **44:** 445 – 451.
10 Lefranc-Millot C: NUTRIOSE® 06: a useful soluble dietary fibre for added nutritional value. *Nutrition Bulletin* 2008, **33:** 234 – 239.
11 Gibson GR, Willems A, Reading S, *et al*: Fermentation of non-digestible oligosaccharides by human colonic bacteria. *Proc Nutr Soc* 1996; **55:** 899 – 912.
12 Guérin-Deremaux L, Ringard F, Desailly F, *et al*: Effects of a soluble dietary fibre, NUTRIOSE®, on colonic fermentation and excretion rate in rats. *Nutr Res Pract* 2010; **4:** 470 – 476.
13 Lewis SJ, Heaton KW: Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997; **32:** 920 – 924.
14 Matsuki T, Watanabe K, Fujimoto J, *et al*: Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 2002; **68:** 5445 – 5451.
15 Jansen GJ, Wildeboer-Veloo AC, Tonk RH, *et al*: Development and validation of an automated, microscopy-based method for enumeration of groups of intestinal bacteria. *J Microbiol Methods* 1999; **37:** 215 – 221.
16 McCullagh P, Nelder JA: *Monographs on Statistics & Applied Probability 37: Generalized Linear Models*, 2nd edn. London: Chapman and Hall/CRC, 1989; pp 21 – 47.
17 Marteau P, Pochart P, Flourié B, *et al*: Effect of

chronic ingestion of a fermented dairy product containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* on metabolic activities of the colonic flora in humans. *Am J Clin Nutr* 1990; **52:** 685 – 688.
18 Xu J, Gordon JI: Honor thy symbionts. *Proc Natl Acad Sci USA* 2003; **100:** 10452 – 10459.
19 Dabek M, McCrae SI, Stevens VJ, *et al*: Distribution of β-glucosidase and β-glucuronidase activity and of β-glucuronidase gene *gus* in human colonic bacteria. *FEMS Microbiol Ecol* 2008; **66:** 487 – 495.
20 De Filippo C, Cavalieri D, Di Paola M, *et al*: Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010; **107:** 14691 – 14696.
21 Vermorel M, Coudray C, Wils D, *et al*: Energy value of a low-digestible carbohydrate, NUTRIOSE FB, and its impact on magnesium, calcium and zinc apparent absorption and retention in healthy young men. *Eur J Nutr* 2004; **43:** 344 – 352.
22 Duncan SH, Louis P, Thomson JM, *et al*: The role of pH in determining the species composition of the human colonic microbiota. *Environ Microbiol* 2009; **11**: 2112 – 2122.
23 Weaver CM, Martin BR, Nakatsu CH, *et al*: Galactooligosaccharides improve mineral absorption and bone properties in growing rats through gut fermentation. *J Agric Food Chem* 2011; **59:** 6501 – 6510.
24 Cummings JH: Short chain fatty acids in the human colon. *Gut* 1981; **22**: 763 – 779.
25 Gibson GR, Probert HM, Loo JV, *et al*: Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* 2004; **17:** 259 – 275.
26 Pineiro M, Asp NG, Reid G, *et al*: FAO technical meeting on prebiotics. *J Clin Gastroenterol* 2008; **42(suppl 3):** S156 – S159.
27 Li S, Guérin-Deremaux L, Pochat M, *et al*: NUTRIOSE dietary fiber supplementation improves insulin resistance and determinants of metabolic syndrome in overweight men: a double-blind, randomized, placebo-controlled study. *Appl Physiol Nutr Metab* 2010; **35:** 773 – 782.
28 Roberfroid M, Gibson GR, Hoyles L, *et al*: Prebiotic effects: metabolic and health benefits. *Br J Nutr* 2010; **104(suppl 2):** S1 – S63.
29 Marteau P, Flourié B: Tolerance to low-digestible carbohydrates: symptomatology and methods. *Br J Nutr* 2001; **85(suppl 1):** S17 – S21.
30 van den Heuvel EG, Wils D, Pasman WJ, *et al*: Short-term digestive tolerance of different doses of NUTRIOSE FB, a food dextrin, in adult men. *Eur J Clin Nutr* 2004; **58:** 1046 – 1055.
31 Cani PD, Dewever C, Delzenne NM: Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. *Br J Nutr* 2004; **92:** 521 – 526.
32 Rossi F, Morlacchini M, Gatti P, *et al*: Effects of a glucooligosaccharide supplement on the morphological characteristics of the gastro-intestinal tract and growth performance in weaned piglets. *Ital J Anim Sci* 2008; **7:** 185 – 198.
33 Pouillart PR, Dépeint F, Abdelnour A, *et al*: NUTRIOSE, a prebiotic low-digestible carbohydrate, stimulates gut mucosal immunity and prevents TNBS-induced colitis in piglets. *Inflamm Bowel Dis* 2010; **16:** 783 – 794.
34 Guérin-Deremaux L, Li S, Pochat M, *et al*: Effects of NUTRIOSE® dietary fiber supplementation on body weight, body composition, energy intake, and hunger in overweight men. *Int J Food Sci Nutr* 2011; **62:** 628 – 635.
35 Guérin-Deremaux L, Pochat M, Reifer C, *et al*: The soluble fiber NUTRIOSE® induces a dose-dependent beneficial impact on satiety over time in humans. *Nutr Res* 2011; **31:** 665 – 672.
36 Ley RE, Bäckhed F, Turnbaugh P, *et al*: Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102:** 11070 – 11075.
37 Ley RE, Turnbaugh PJ, Klein S, *et al*: Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444:** 1022 – 1023.
38 Stewart ML, Rushton A, Paredes-Diaz A, *et al*: Wheat dextrin (WD), inulin, and partially hydrolyzed guar gum (PHGG) produce unique short-chain fatty acid concentration in model colonic fermentation. *Gastroenterology* 2007; **132:** A-585.
39 Zhou J, Hegsted M, McCutcheon KL, *et al*: Peptide YY and proglucagon mRNA expression patterns and regulation in the gut. *Obesity (Silver Spring)* 2006; **14:** 683 – 689.
40 Zhou J, Martin RJ, Tulley RT, *et al*: Dietary resistant starch upregulates total GLP-1 and PYY in a sustained day-long manner through fermentation in rodents. *Am J Physiol Endocrinol Metab* 2008; **295:** E1160 – E1166.
41 Hamer HM, Jonkers D, Venema K, *et al*: Review article: the role of butyrate on colonic function. *Aliment Pharmacol Ther* 2008; **27:** 104 – 119.

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