Fn-type Chicory Inulin Hydrolysate Has a Prebiotic Effect in Humans

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ABSTRACT The partial enzymatic hydrolysis of chicory inulin (GFn; $2 \leq n \leq 60$) yields an oligofructose preparation that is composed of both GFn-type and Fn-type oligosaccharides ($2 \leq n \leq 7$; $2 \leq m \leq 7$), where G is glucose, F is fructose, and n is the number of $\beta(2\rightarrow1)$ bound fructose moieties. Human studies have shown that feeding GFn-type oligomers significantly modifies the composition of the fecal microflora especially by increasing the number of bifidobacteria. The experiments reported here were used to test the hypothesis that the Fn-type molecules have the same property. During a controlled feeding study, 8 volunteers (5 females and 3 males) consumed 8 g/d of an Fn-rich product for up to 5 wk. Fecal samples were collected and analyzed for total anaerobes, bifidobacteria, lactobacilli, bacteroides, coliforms and *Clostridium perfringens*. Both 2 and 5 wk of oligofructose feeding resulted in a selective increase in bifidobacteria ($P < 0.01$). In addition, a daily intake of 8 g of the Fn-type oligofructose preparation reduced fecal pH and caused little intestinal discomfort. J. Nutr. 130: 1197–1199, 2000.

KEY WORDS: • prebiotics • inulin • oligofructose • humans

The colon, along with its bacterial microflora, is an important organ that provides a great variety of functions, such as digestion, fermentation, metabolic, immunological and protective functions, as well as detoxifying functions, that are essential to the whole organism (Cummings 1997). Proliferation of bifidobacteria in fecal microflora, a surrogate marker for the colonic microbiota, has been associated with several beneficial effects. A dietary approach aimed at improving the composition of the fecal microflora by supplying substrates that allow selective proliferation of such indigenous bacteria, the prebiotic approach, has been proposed (Gibson and Roberfroid 1995) and validated in different human studies using different nondigestible oligosaccharides (Gibson et al. 1999). In particular, it has been shown that the consumption of chicory inulin or its partial hydrolysate (oligofructose), a mixture of $\beta(2\rightarrow1)$ bound GFn-type (glucosyl-[fructosyl]n-1-fructose) and $\beta(2\rightarrow1)$ bound Fn-type ([fructosyl]n-1-fructose) species (De Leenheer and Hoebregs 1994), significantly modifies the composition of the human fecal flora in such a way that bifidobacteria become numerically predominant (Roberfroid et al. 1998, Van Loo et al. 1999). Native chicory inulin is composed of >99% of the GFn-type species ($2 \leq n \leq 60$), but the oligofructose preparation, which is produced from inulin by partial enzymatic hydrolysis, is a mixture of both GFn[2] ($2 \leq n \leq 7$) and Fn ($2 \leq n \leq 7$)-type molecules [where G is glucose, F is fructose and n is the number of $\beta(2\rightarrow1)$ bound fructose moieties] which also occur naturally in plant foods such as banana, garlic, onion, salsify, asparagus, leek, wheat, chicory, etc. (Van Loo et al. 1995).

The objective of the present study was to test the hypothesis that, like the GFn-type, the Fn-type chicory oligofructose preparation selectively stimulates the growth of fecal bifidobacteria in humans. The protocol for the human study was very similar to recently published studies in terms of number of volunteers (8–12), protocol and bacteriological methodologies employed (Buddington et al. 1996, Gibson et al. 1995, Kleessen et al. 1997, Williams et al. 1994).

MATERIALS AND METHODS

Chemicals. All chemicals used in this study were of the purest grade available and were purchased from Merck (Darmstadt, Germany), Oxoid (Basingstoke, United Kingdom) or Sigma (St. Louis, MO).

Study food. The Fn-type-rich chicory oligofructose preparation was provided by ORAFTI (Tienen, Belgium) as Raftilose® L60, which is produced by partial enzymatic hydrolysis of a refined hot-water extract of chicory roots (i.e., inulin). It is available as an aqueous syrup containing 750 g/kg dry matter composed of 75 g (10%) glucose + fructose, 225 g (30%) sucrose and 450 g (60%) oligofructose [with 45 g (10%) GFn-type and 405 g (90%) Fn-type]. The product used in the experiments was of food-grade quality.

Volunteers. The study protocol was approved by the ad hoc ethical committee of the University (UCL-Brussels, Belgium) and complies with the Helsinki declaration of 1975 as revised in 1983. No history of gastrointestinal disease and no use of gastrointestinal or antibiotic medications for at least 3 mo prior to and during the trial were the inclusion criteria. Human subjects who participated in the trial were five women and three men aged between 20 and 50 y, having a body mass index between 19 and 25 kg/m^2, and between 18 and 24 kg/m^2, respectively. Subjects gave written consent to participate in the study.

Protocol for the human study. The eight volunteers participated in the experiment, which lasted for 7 wk divided into three successive periods: i) control, a period of 2 wk, during which the volunteers were all given a controlled diet without any addition of oligofructose; ii) treatment 1, a first treatment period of 2 wk, during which the controlled diet was supplemented with 8 g/d of chicory oligofructose; iii) treatment 2, a second treatment period of 3 wk, during which the volunteers consumed their usual home-cooked diet to which they added 8 g/d of chicory oligofructose. The chicory oligofructose (Raftilose® L60) composed of 90% Fn-type and 10% GFn-type molecules was incorporated into orange juice, various desserts (puddings, creams and fruit mousses), cakes and biscuits that were part of the

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[2] Abbreviations used: cfu, colony-forming units; GFn, G is glucose, F is fructose, and n is number of $\beta(2\rightarrow1)$ fructose moieties; Fn, F is fructose, and n is number of $\beta(2\rightarrow1)$ fructose moieties.

food consumed by the volunteers during the day, in such quantities as to provide a total daily intake of 8 g of chicory oligofructose, of which 90% (7.2 g) was pure Fn-type.

Feeding a controlled diet during periods 1 and 2 was intended to minimize the interindividual variations in food intakes that could have influenced the composition of the fecal microflora independent of oligofructose intake.

During these two periods, the volunteers were required to visit a central restaurant, where they had access to a buffet (breakfast and lunch) and were given a vacuum-sealed dinner to consume at home. These meals were prepared so as to minimize the consumption of naturally oligofructose/inulin-rich products (Van Loo et al. 1995) like onions, leeks, bananas, artichokes and wheat, as well as yogurts and fermented milk products. During these two periods, the foods given to the volunteers were very similar, except for the intake of chicory oligofructose (8 g/d) during period 2. During period 3, the volunteers were asked to consume their usual home-cooked meals but still excluding oligofructose/inulin-rich food products and fermented dairy products.

As in other studies on the bifidogenic effect of fructans (Buddington et al. 1996, Gibson et al. 1995, Kleessen et al. 1997, Williams et al. 1994), each volunteer acted as his/her own control and no separate placebo group was included. Using such a protocol avoids a cross-over design in which the length of the wash-out interval is often difficult to evaluate precisely.

Sample collection. Fresh stools were collected: sample 1 (last day of wk 2) at the end of the control period; sample 2 (last day of wk 4) at the end of the treatment 1 period; and sample 3 (last day of wk 7) at the end of the treatment 2 period.

During both the control and treatment 1 periods, the volunteers were requested to complete a daily well-being questionnaire, providing information about possible digestive discomfort (cramps, bloating, flatulence, soft stools or diarrhea) as well as frequency and appearance of stools.

Protocol for bacteriological analyses (Beerens 1991, Gibson et al. 1995). All stool samples (minimum weight 20 g) were processed anaerobically (desk-type home-made anaerobic glove-box containing an atmosphere of H_2, CO_2 and N_2, 10:10:80) within 60 min after defecation. Samples were weighed and then homogenized in 0.1 mol/L (pH 7) phosphate buffer to obtain a 100 g/L fecal suspension. Serial dilutions were prepared using half-strength Peptone water (Oxoid), the samples (0.1 mL) were inoculated onto agar medium specific for the growth of total anaerobes (Wilkins-Chalgren anaerobic agar), bifidobacteria (*Clostridia* agar supplemented with 0.0125 g/L iodoacetic acid, 0.02 g/L nalidixic acid, 0.05 g/L kanamycin, 0.009 g/L polymyxin, 0.025 g/L triphenyltetrazolium chloride), lactobacilli (Rogosa), coliforms (MacConkey #3), bacteroides (BMS supplemented with 5 g/L glucose, 0.5 g/L ammonium sulfate, 0.01g/L nalidixic acid and 0.003 g/L vancomycin) and *Clostridium perfringens* (Tryptose Sulfite Cycloserine Agar Base or TSC supplemented with fluorcult).

Anaerobic incubations (in duplicate) for colony development took place in anaerobic jars containing Anaerocult A (Merck). For each fecal sample, a count was made of viable colony-forming units (cfu) of total anaerobes after incubation at 37°C for 4 d, bifidobacteria (4 d), bacteroides (4 d), lactobacilli (3 d), coliforms (1 d) and clostridia (1 d). After incubation, individual colonies were removed from the plates and subcultured into peptone/yeast/glucose broth. Bacteria were characterized to genus level on the basis of colony appearance, Gram's reaction and cell morphology. Presumptive culture identities were confirmed through colony morphotype, microscopic characteristics and limited biochemical tests (Gibson et al. 1995).

Statistical analysis. The nonparametric Friedman test was used after logarithmic transformation of the data. This test, made by order of rank (rank averages) was chosen because it permits comparison of several mean values of nonindependent observations, which is the case in this study, where comparable samples were all taken from the same volunteers but during different feeding periods. Results were statistically analyzed on the basis of: i) a global comparison of mean values to identify differences between the three feeding periods and ii) paired comparisons to search for differences between periods. The significance threshold was set at 5% ($P < 0.05$).

TABLE 1

Effect of feeding 8 g/day chicory oligofructose (of which 7.2 g was Fn-type molecules) on the logarithm (log_{10}) of the number of the colony-forming units (cfu) of major bacteria in fresh fecal samples of male and female volunteers fed either a controlled (treatment 1) or a home cooked-diet (treatment 2)[1]

	Timing of microbiological analyses		
	Before treatment	After 2 weeks	After 5 weeks
Bacteria	Control value	Treatment 1	Treatment 2
Total anaerobes	10.3 ± 0.6	10.1 ± 0.5	10.4 ± 0.4
Bifidobacteria	8.6 ± 0.5	9.6 ± 0.3*	9.4 ± 0.6*
Lactobacilli	5.7 ± 1.0	6.0 ± 1.5	6.4 ± 0.7
Bacteroides	8.9 ± 0.2	8.8 ± 0.2	9.2 ± 0.7
Coliforms	7.0 ± 1.3	6.6 ± 1.6	6.5 ± 1.2
C. perfringens	3.5 ± 1.2	3.2 ± 1.0	3.2 ± 0.8

[1] Values are means ± SD, $n = 8$.
* Significantly different from before treatment.

RESULTS AND DISCUSSION

The key criterion for a prebiotic effect is the demonstration of the selective stimulation of growth of one particular, or a limited number of, potentially beneficial bacteria in the complex fecal microbiota following the consumption of a particular food. Data should demonstrate that the number (e.g., expressed as log_{10} cfu/g of feces) of bacteria in that particular population increased significantly, while the others did not change or even decreased (Gibson and Roberfroid 1995, Gibson et al. 1999, Roberfroid et al. 1998).

Table 1 reports the values of the total numbers of cfu (expressed as log_{10} cfu/g of feces) for the various bacteria analyzed in the feces of the eight volunteers fed a diet with and without chicory oligofructose. A global analysis of the different values reveals that the daily intake of 8 g of oligosaccharides did not significantly ($P > 0.05$) modify the counts of total anaerobes, lactobacilli, bacteroides, coliforms or C. *perfringens*, but it did significantly ($P < 0.01$) increase the counts of bifidobacteria.

The paired comparisons reveal that: i) at the end of the treatment 1 period, after eating a control diet supplemented with 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) for 2 wk, the number of bifidobacteria in feces had increased significantly ($P < 0.01$) compared to the end of the control period; ii) at the end of the treatment 2 period, after eating the usual home-cooked diet supplemented with 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) for an additional period of 3 wk, the number of bifidobacteria in feces were still significantly ($P < 0.01$) higher than at the end of the control period but not significantly different from the counts at the end of the treatment 1 period.

These data thus demonstrate that, as is the case with GFn-type oligofructose (Gibson et al. 1995, Roberfroid et al. 1998, Van Loo et al. 1999), a preparation of chicory oligofructose containing 90% of Fn-type molecules selectively stimulates the growth of colonic bifidobacteria in human volunteers, as evidenced by the increase in fecal number. Furthermore, the data demonstrate the selectivity of that

stimulation of growth, thus confirming the prebiotic nature of chicory Fn-type oligofructose.

At the end of the treatment 1 and treatment 2 periods, the fecal pH in all the volunteers had dropped by ~1 pH unit compared to the end of the control period. Such an effect is best explained by a change in colonic fermentation and confirms previous observations both in vitro (Wang and Gibson 1993) and in vivo (Gibson et al. 1995, Kleessen et al. 1997). The present study was not specifically designed to quantify changes in gut function parameters. However, when analyzing answers to the well-being questionnaires recorded during the control period vs. the treatment 1 period, changes in stool frequency (+ 12%) as well as in the appearance (softer) and the amount (evaluated qualitatively as "more than usual") of stools showed a tendency to confirm the bulking effect reported by Gibson et al. (1995) and by Den Hond et al. (1997). Moreover, an analysis of the intestinal side-effects associated with the meals during the periods of chicory oligofructose intake, as reported on the acceptability forms, revealed that from a total of 224 meals (8 volunteers receiving 2 meals/day for 2 wk), only six "mild" complaints were reported. These included one case of increased flatulence, three cases of intestinal distension and two cases of cramps in the intestine. It can be stated that the consumption of 8 g/d chicory oligofructose (of which 7.2 g was Fn-type molecules) is therefore not likely to cause significant intestinal discomfort.

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