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 ≤ n ≤ 60) yields an oligofructose preparation that is composed of both GFn-type and Fn-type oligosaccharides (2 ≤ n ≤ 7; 2 ≤ m ≤ 7), where G is glucose, F is fructose, and n is the number of β(2→1) 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, clostridia 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 β(2→1) bound GFn-type (glucosyl-fructosyl)n-1-fructose) and β(2→4) 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 ≤ n ≤ 60), but the oligofructose preparation, which is produced from inulin by partial enzymatic hydrolysis, is a mixture of both GFn2 (2 ≤ n ≤ 7) and Fn (2 ≤ n ≤ 7) type molecules where G is glucose, F is fructose and n is the number of β(2→1) 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, Klee- sen 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 Rafraflose® 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 (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 years, having a body mass index between 19 and 25 kg/m2, and between 18 and 24 kg/m2, 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 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 (Rafraflose® 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 diet.

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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 (Budding-
al. 1994), each volunteer acted as his/her own control and no sepa-
rate placebo group was included. Using such a protocol avoids a
cross-over design in which the length of the wash-out interval is often
tough to evaluate precisely.

Sample collection. Fresh stools were collected: sample 1 (last day
of wk 1) 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, provid-
ing 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 H2, CO2 and N2, 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
containing 90% (7.2 g) was pure Fn-type molecules selectively stim-
ulated the growth of colonic bifidobacteria in human volun-
tees, as evidenced by the increase in fecal number. Furthermore, the data demonstrate the selectivity of that

| Table 1 |

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

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 com-
plex fecal microbiota following the consumption of a particu-
lar food. Data should demonstrate that the number (e.g.,
expressed as log10 cfu/g of feces) of bacteria in that particu-
lar population increased significantly, while the others did not
change or even decreased (Gibson and Roberfroid 1995, Gib-
son et al. 1999, Roberfroid et al. 1998).

Table 1 reports the values of the total numbers of cfu (ex-
pressed as log10 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 differ-
ent values reveals that the daily intake of 8 g of oligosaccha-
rides did not significantly (P > 0.05) modify the counts of
total anaerobes, lactobacilli, bacteroides, coliforms or C. per-
fringens, 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/day 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 oligo-
fructose containing 90% of Fn-type molecules selectively stim-
ulates the growth of colonic bifidobacteria in human volun-
teers, 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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LITERATURE CITED