Digestion, excretion, and energy value of fructooligosaccharides in healthy humans[1–3]

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ABSTRACT   The fate of fructooligosaccharides (FOS) in the human gastrointestinal tract was evaluated in six healthy volunteers over an 11-d period. After an equilibration phase, 20.1 g FOS/d was given in three identical postprandial doses. Distal ileal output of FOS and their constituent components were determined by intestinal aspiration after a single meal, and the amounts of FOS excreted in stools and urine were also measured. Most of ingested FOS, 89 ± 8.3% ($\bar{x}$ ± SEM), was not absorbed in the small intestine, and none was excreted in stools, indicating that the portion reaching the colon was completely fermented by colonic flora. A small fraction of ingested FOS was recovered in urine. The mean estimated energy value of FOS was 9.5 kJ/g. We conclude that in healthy humans, FOS are only slightly digested in the small intestine and then fermented in the colon, resulting in reduced energy production.   *Am J Clin Nutr* 1996;64:324–8.

KEY WORDS   Digestion, fructooligosaccharides, indigestible carbohydrates, intestinal aspiration

INTRODUCTION

Fructooligosaccharides (FOS) are a mixture of oligosaccharides consisting of glucose linked to two, three, or four fructose units (1, 2). Links between fructose units are $\beta(1\rightarrow2)$. FOS are found in a variety of food products, including onion, asparagus root, Jerusalem artichoke tubers, garlic, salsify, and leek. They are produced on a commercial scale either from sucrose through the transfructosylating action of fungal fructofuranosidase or from chicory inulin by partial hydrolysis with endoglycosidases (2–5). Because of their physicochemical properties and sweetening power, FOS are consumed mainly in pastry, confectionery, and dairy products. Their energy value is theoretically lower than that of sucrose. Indeed, the energy value of sugars depends on the extent to which they are absorbed in the small intestine and fermented by the colonic flora (6–9). In the human small intestine there is no enzyme able to split the $\beta(1\rightarrow2)$ glycosidic linkages. Inulin, a $\beta(1\rightarrow2)$ fructan, which has a degree of polymerization within the range of 3 to 60, has been shown to be poorly digested in the small intestine of ileostomized patients (10) and in normal subjects by using the breath-hydrogen technique (11). Studies in rats showed that FOS are not hydrolyzed by salivary and pancreatic amylases (12, 13), and that few or none are hydrolyzed by intestinal brush border enzymes (13–15). Moreover, FOS recovery from rat small intestine was approximately the same as that of an unabsorbable marker (15). In humans, no change in blood glucose was noted after oral ingestion of FOS (12). On the other hand, FOS were fermented in the large intestine. In vitro incubation of [U-^{14}C]FOS with the cecal contents of rats showed that most of the label appeared in short-chain fatty acids (SCFAs) (16). In the same study, FOS fed to normal rats showed rapid fermentation, whereas germ-free animals delayed excretion of the label for many hours with substantial amounts appearing in feces. Breath-hydrogen studies in humans have also shown that FOS are fermentable, resulting in an amount of hydrogen in breath roughly similar to that excreted after ingestion of an identical load of lactulose (17). This also suggests that FOS are not digested in the human small intestine.

The present study assessed the extent of FOS absorption in the small intestine of healthy humans and their digestion in the colon as well as the in vitro hydrolysis of FOS by homogenates of human duodenal mucosa.

SUBJECTS AND METHODS

In vivo study

Subjects

Six healthy volunteers (three women, three men, 20–27 y of age) with no history of gastrointestinal disease (except appendectomy), no recent treatment with antibiotics or laxatives, and presenting no lactose malabsorption (negative lactose breath-hydrogen test) gave their written informed consent to participate in the study protocol. The study was approved by the Nantes University Ethics Committee.

Experimental design

Each subject was studied in random order during a placebo and a FOS period. These two periods were separated by ≥ 1 wk of washout. The placebo period was designed to provide baseline measurements for clinical tolerance and fecal analysis

with the same diet as that used in the FOS period. We used a previously described controlled diet (6) intended to maintain constant and roughly similar amounts of carbohydrates, proteins, and fats in three daily meals. All meals were prepared and eaten in the Clinical Research Center. The controlled diet did not contain foods rich in $\beta(1\rightarrow2)$ fructans (**Table 1**). Throughout both periods subjects were asked to record any significant symptoms (abdominal pain, borborygmus, bloating, excess flatus, and diarrhea). During the placebo period, subjects were given the controlled diet supplemented with placebo for 6 d. Ten grams of placebo (containing mainly sucrose, **Table 2**) were given three times daily at the end of each meal. After a 4-d equilibration phase, subjects stayed at the Clinical Research Center and stools were collected on days 5 and 6 and stored immediately at -20 °C. During the 11-d FOS period, FOS were initially ingested at one-fourth of the final daily dose and gradually increased to reduce possible digestive symptoms. From the 4th to 11th days, the full daily dose of FOS (20.1 g) was given in three equal portions at the end of each meal. We used FOS from Actilight (Eridania-Beghin-Say, Paris), which consist of 44% 1-kestose (GF2), 46% nystose (GF3), and 10% 1^F-β-fructofuranosyl nystose (GF4) (Table 2). From the 8th day, subjects stayed at the Clinical Research Center. Stools were collected on days 8 and 9 and stored immediately at -20 °C.

On day 10, urine was collected and stored at -20 °C and subjects were intubated with a triple-lumen polyvinyl tube led by a mercury bag that could be inflated with air to hasten its progression. When the bag reached the cecum (confirmed fluoroscopically), the progression of the tube was stopped by deflating the bag. Subjects then remained in a semirecumbent position. One lumen was connected to the bag and another

TABLE 1
Composition of daily test meals[1]

Breakfast	
Coffee or tea (mL)	250
Water (mL)	250
Sucrose (g)	10
White bread (g)	70
Jelly (g)	30
Eggs (*n*)	2
Butter (g)	10
Gruyère cheese (g)	30
Carbohydrate (g)	73
Fat (g)	27
Protein (g)	26
Total energy (kJ)	2713
Lunch or dinner (amounts per meal)	
Grilled meat or fish (g)	100
Or eggs (*n*)	2
Rice, potato, or potato purée (g)	200
Butter (g)	15
Gruyère or camembert cheese (g)	30
White bread (g)	40
Water (mL)	500
Orange juice (mL)	150
Carbohydrate (g)	85
Fat (g)	29
Protein (g)	30
Total energy (kJ)	3014

[1] Calculated from food tables (18, 19).

TABLE 2
Composition of placebo and products containing fructooligosaccharides (FOS) used in the in vivo and in vitro studies[1]

		Product containing FOS	
	Placebo	In vivo[2]	In vitro[3]
		% of dry matter	
Fructooligosaccharides (GF2:GF3:GF4)[4]	0	67 (44:46:10)	94.9 (41:49:10)
Maltodextrin	30	30	0
Sucrose	70	2.4	3.3
Glucose	0	0.3	1.0
Fructose	0	0.1	0.8
Aspartame	0.03	0.12	0
Acesulfame K	0.07	0.23	0

[1] Determined by HPLC (*see* Methods).
[2] Actilight; Eridania-Beghin-Say, Paris.
[3] Actilight P; Eridania-Beghin-Say.
[4] GF2, 1-kestose; GF3, nystose; GF4, 1^F-β-fructofuranosyl nystose. Purified GF2, GF3, and GF4 were provided by Meiji Seika, Tokyo.

ending 5 cm above were used to collect distal ileum fluid samples; a third lumen, positioned 25 cm proximal to the aspiration port, was used for perfusion. The perfusate contained 154 mmol NaCl/L and 10 g PEG 4000/L as a recovery marker to estimate water flow through the distal ileum. On day 11, ileal perfusion started at 0700 at a rate of 2 mL/min. The solution was maintained at 37 °C and stirred until the end of perfusion. After 1 h of equilibration, the intestinal contents were aspirated and subjects were given a breakfast similar to that served on previous days (Table 1) containing 6.7 g FOS. No more food was allowed before the end of the sampling period. Intestinal contents were continuously collected for 10 h after the meal began by manual aspiration to obtain as much fluid as possible. Samples were divided into 30-min aliquots. The pH of ileal fluid was measured immediately (pH meter; Synectics, Stockholm) and always found to be in the alkaline range (pH 7.5–8.5). Samples were then frozen in liquid nitrogen and stored at -20 °C. This treatment was used to prevent bacterial degradation of carbohydrates. Maintenance of probe position was confirmed fluoroscopically at the end of all studies.

Analytical methods

In ileal samples PEG was measured by turbidimetry (20). FOS were determined in ileal, urine, and fecal contents. An aliquot of each ileal sample was centrifuged (2000 × *g* for 25 min at 4 °C) and then ultrafiltrated on 10-kD porous membranes (Microsep; Filtron Technology Corporation, Northborough, MA). Urine was first incubated in the presence of a mixture of ion-exchange resins to eliminate salts and some of the organic acids, and then ultrafiltrated. Each stool was weighed, partially thawed, diluted in distilled water, and homogenized. An aliquot of each stool was then centrifuged and ultrafiltrated in the same way as ileal samples.

FOS were determined on an HPLC apparatus (Dionex, Sunnyvale, CA) equipped with a gradient pump, a degas module, and a pulse amperometric detector (F Ouarne, A Guibert, D Brown, F Bornet, unpublished observations, 1995). The column used was a Carbopac TM PA1 (4 × 250 mm) (Dionex) with a Carbopac PA (3 × 25 mm) precolumn (Dionex). The injected volume was 25 μL. Standard solutions were prepared

from purified standards (Meiji Seika, Tokyo). A linear response was obtained with concentration values ranging from 0.15 to 28 mg/L. The lower detection limit, based on a peak height at least three times that of the background noise, was 0.15 mg/L. Repeatability of the method was determined by injecting solutions of GF3 with known concentrations of 7.12 and 14.15 mg/L, 10 times each. The results obtained in the 20 samples showed that the repeatability of the method varied by a maximum of 0.5% around the theoretical values. Recovery experiments were also performed by adding various amounts (1 and 5 mg/L) of FOS in fecal samples. Of FOS added to fecal samples, 98 ± 1% were recovered. To verify that measured oligosaccharides were truly FOS, ileal, fecal, and urine samples were further incubated (6 h at 40 °C) with invertase (Max invert, with a final activity of 2316 U/L; Gist Brocades, Delft, Netherlands), which is able to hydrolyze the $\beta(1{\rightarrow}2)$ links between fructose units in FOS. The samples obtained were then analyzed again by HPLC to confirm peak disappearance.

Data analysis

Water flow rate. The volume flowing through the terminal ileum for each 30-min period of sampling was calculated on the basis of PEG dilution (21). In each ileal sample, the amounts of FOS and their constituent components were determined from the calculated ileal volume and the corresponding concentrations of sugars. The total ileal content for 10 h was obtained by summation of the results for individual periods. Urine and fecal data were expressed as mean 24-h outputs.

FOS energy value. The energy value of FOS was estimated by using the following equation (6, 8):

$$\text{Energy value} = [(1 - A - B) \times 16.7 \text{ kJ/g}]$$
$$+ [(A - C) \times 8.4 \text{ kJ/g}]$$

where A is the fraction not absorbed in the small intestine, B is the fraction excreted in urine, C is the fraction excreted in stools, 16.7 kJ/g is the energy content of FOS absorbed from the small intestine, and 8.4 kJ/g is the estimated energy available from products of FOS digestion in the large intestine (*see* Discussion). Data are expressed as means ± SEMs.

In vitro study

To investigate hydrolysis by intestinal brush border enzymes, apparently normal duodenal mucosa were sampled during gastroduodenal endoscopy in 15 patients referred for dyspeptic symptoms. Biopsies were immediately frozen in liquid nitrogen and stored at −20 °C. Duodenal biopsies were pooled, placed in 300 μL 154 mmol NaCl/L and then ground in a Potter Thomas mill (Prolabo, Paris). The solution thus obtained was centrifuged 20 min at 5000 × g at 4 °C. Treatments applied to mucosa were carried out at 4 °C to avoid the denaturing of enzymes. The supernate and pellet were stored at −20 °C.

Enzyme kinetics were studied separately for the supernate and pellet. The supernate was incubated at 37 °C with five volumes of Actilight (50 g/L in 50 mmol/L phosphate buffer, pH 6), which contains a higher amount of FOS and is devoid of maltodextrin (Table 2). Aliquots were collected at 0, 30, 60, 90, and 150 min, and the enzymes were denatured (immersed in water at 100 °C for 5 min). The solutions obtained were centrifuged 15 min at 5000 × g at 4 °C, and the supernates filtered (15 min at 2000 × g at 4 °C) on 10-kD porous membranes

(Microsep; Filtron) to eliminate proteins. FOS were assayed by the method used in the in vivo study. The pellet was dissolved in 0.125 g 154 mmol NaCl/L and incubated at 37 °C with five volumes of Actilight P (50 g/L in 50 mmol/L phosphate buffer, pH 6). Aliquots collected at 0, 30, 60, 90, and 150 min underwent the same steps as the supernate.

RESULTS

In vivo study

None of the volunteers experienced symptoms during the placebo and FOS periods. The mean flow of water through the distal ileum during the 10 h was 1445 ± 144 mL/10 h (range: 1020–1875 mL/10 h, **Table 3**). The ileal flow rate increased for 2 h after the meal and then remained roughly stable until the end of the sampling period. FOS appeared in the distal ileum within 0.5–1 h after its ingestion (**Figure 1**) and were recovered for 8 h. The mean amount of FOS not absorbed in the small intestine was 5988 ± 558 mg (range: 4050–7293 mg, Table 3). When data were expressed in relation to the amount of FOS ingested, the mean percentage of unabsorbed FOS was 89 ± 8% (range: 60–109%, Table 3). In ileal samples, fructose was not detected, and the percentages of constituent oligosaccharides (GF2, GF3, and GF4) were close to those of ingested FOS (**Figure 2**). However, variations in GF2 and GF3 percentages were noted from the sixth hour after the meal, when the recovery of FOS was very low.

The mean 24-h amount of FOS recovered in urine was 24 ± 12 mg (range: 11–60 mg, Table 3), which corresponded to 0.12 ± 0.04% of the amount of FOS ingested (range: 0.05–0.30%). The mean percentages of GF2, GF3, and GF4 in urine were 41%, 52%, and 8%, respectively.

The chromatograms showed peaks with retention times identical or close to those of FOS. However, the same peaks were found for stools from the placebo period and were not modified after incubation of stool with invertase. It was thus concluded that feces contained no FOS (Table 3). In our experimental

TABLE 3
Amounts of fructooligosaccharides (FOS) recovered in the distal ileum and in 24-h urine and stool samples

	$\bar{x} \pm$ SEM
Ileal flow rate (mL/10 h)	1445 ± 144
FOS in ileal contents (g/10 h)	5.988 ± 0.558
Percentage FOS recovered in ileal contents (%)[1]	89.4 ± 8.3
Urine volume (mL/24 h)	1015 ± 124
FOS in urine (mg/24 h)	24 ± 10
Percentage FOS in urine (%)[2]	0.12 ± 0.04
Stool weight, placebo period (g/24 h)	210 ± 12.6
Stool weight, FOS period (g/24 h)	222 ± 29.7
Percentage FOS in stool (%)[3]	0 ± 0
Energy value (kJ/g)[4]	9.5 ± 0.6

[1] Ratio of FOS in ileal contents to ingested FOS (6.7 g).
[2] Percentage of ingested FOS (6.7 g × 3) recovered in 24-h urine sample.
[3] Percentage of ingested FOS (6.7 g × 3) recovered in 24-h stool sample.
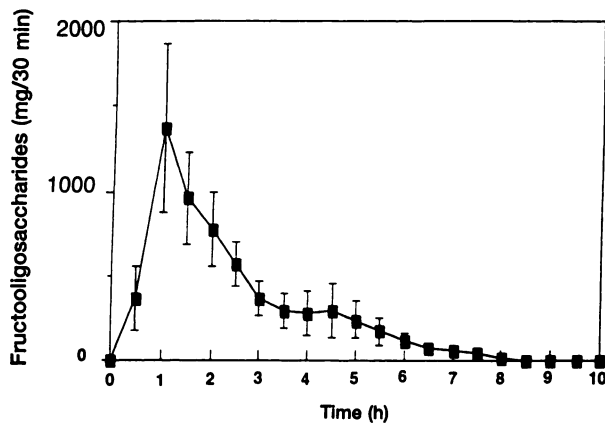[4] Energy value = [(1 − ileal FOS − urine FOS) × 16.7 kJ/g] + [(ileal FOS − stool FOS) × 8.4 kJ/g].

FIGURE 1. Recovery of total fructoologisaccharides from the ileal contents of six healthy subjects. $\bar{x} \pm$ SEM.

FIGURE 2. Percentages of the different fractions of fructooligosaccharides (FOS) in the ingested FOS and in the ileal contents recovered in six healthy subjects. $\bar{x} \pm$ SEM. GF2, 1-kestose; GF3, nystose; GF4, 1^F-β-fructofuranosyl nystose

conditions, the mean estimated energy value of FOS was 9.5 ± 0.6 kJ/g (range: 8.3–11.7 kJ/g) (Table 3).

In vitro study

Only sucrose was hydrolyzed during incubations with homogenates from duodenal mucosa (1.8 and 0.8 U/g mucosa for supernate and pellet, respectively), whereas the constituent oligosaccharides of FOS were not hydrolyzed at all.

DISCUSSION

The nondigestible properties of FOS can cause gastrointestinal symptoms. However, subjects in this study reported no side effects for a total postprandial FOS dose of 20 g/d, reached by a gradually increased dose. This agrees with previous reports showing no or few gastrointestinal side effects for the dose and consumption conditions used here (17, 22).

To quantify the passage of FOS into the colon of normal subjects, we used the direct method of measuring the flow rate in the distal ileum by an intubation technique validated previously in ileostomized dogs and humans (21). This method also indicated the fate of FOS in the colon, thus providing an estimate of their energy value. A mean 89% of ingested FOS was delivered to the colon, and analysis of the constituent oligosaccharides in ileal contents showed that most unabsorbed FOS were in an intact unhydrolyzed form. Because FOS are not metabolized by rats when infused intravenously (13), it may be assumed that their excretion in urine resulted from absorption of the intact form in the small intestine. Approximately 1% of the FOS that disappeared from the small intestine was recovered in urine. The small amount of FOS absorbed in intact form thus appears close to that previously shown with other low-molecular-weight and unabsorbable sugars, such as cellobiose and lactulose (23, 24). A fraction of ingested FOS was hydrolyzed during passage through the gastrointestinal tract and then absorbed as glucose and fructose. The in vitro study showed that enzymes from human duodenal mucosa were not able to split FOS. Thus, it may be postulated that slight hydrolysis of ingested FOS occurred either in the terminal ileum, where a low level of fermentation may physiologically take place, or in the stomach (25). Indeed, a low rate of FOS hydrolysis occurs in vitro, though at a very low pH (26).
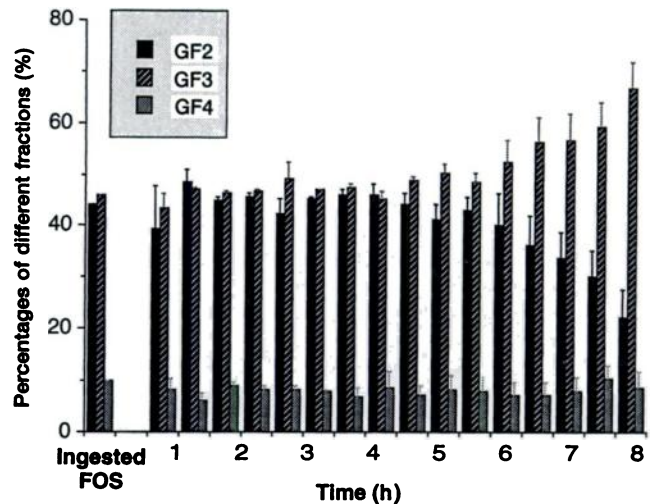
FOS not hydrolyzed in the upper gastrointestinal tract were completely fermented in the colon because none were found in stools. The colonic fermentation of carbohydrates is responsible for SCFAs, lactic acid and gas production, bacterial maintenance and growth, and heat dissipation (27), which results in a loss of energy for the host estimated to be equal to 50% of the energy content of carbohydrate (6–8). We found that the energy value of FOS was 9.5 kJ/g, a value somewhat higher than the 6.3 kJ/g reported by Hosoya et al (28). Those authors used a radiorespirometric method in healthy humans ingesting [U-^{14}C]FOS and also measured gas and SCFA production from labeled FOS in anaerobic incubation with feces. They postulated that SCFAs were completely absorbed in the large intestine but they used a very low mean energy content for SCFAs (10.0 kJ/g) to calculate the energy value of FOS. The heat produced by combustion of acetic, propionic, and butyric acids is 14.6, 20.7, and 25.0 kJ/g, respectively (29), whereas absorbed SCFAs and lactic acid produce 15–25% less energy for maintenance requirements than does glucose (8, 30, 31). Accordingly, the metabolizable energy from acetic, propionic, and butyric acids is 10.9–12.6, 15.5–17.6, and 18.8–21.3 kJ/g, respectively. On the basis of these figures, the energy value of FOS would have ranged between 8.4 and 9.2 kJ/g, a value close to that found in the present study. We conclude that FOS in healthy humans are only slightly digested in the small intestine and then fermented in the colon, resulting in reduced energy production (about one-half that of sucrose).

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