Prebiotic digestion and fermentation[1–3]

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ABSTRACT Prebiotics, as currently conceived of, are all carbohydrates of relatively short chain length. To be effective they must reach the cecum. Present evidence concerning the 2 most studied prebiotics, fructooligosaccharides and inulin, is consistent with their resisting digestion by gastric acid and pancreatic enzymes in vivo. However, the wide variety of new candidate prebiotics becoming available for human use requires that a manageable set of in vitro tests be agreed on so that their nondigestibility and fermentability can be established without recourse to human studies in every case. In the large intestine, prebiotics, in addition to their selective effects on bifidobacteria and lactobacilli, influence many aspects of bowel function through fermentation. Short-chain fatty acids are a major product of prebiotic breakdown, but as yet, no characteristic pattern of fermentation acids has been identified. Through stimulation of bacterial growth and fermentation, prebiotics affect bowel habit and are mildly laxative. Perhaps more importantly, some are a potent source of hydrogen in the gut. Mild flatulence is frequently observed by subjects being fed prebiotics; in a significant number of subjects it is severe enough to be unacceptable and to discourage consumption. Prebiotics are like other carbohydrates that reach the cecum, such as nonstarch polysaccharides, sugar alcohols, and resistant starch, in being substrates for fermentation. They are, however, distinctive in their selective effect on the microflora and their propensity to produce flatulence. *Am J Clin Nutr* 2001;73(suppl):415S–20S.

KEY WORDS Prebiotic, oligosaccharide, bacteria, large intestine, fermentation, short-chain fatty acids, carbohydrate

INTRODUCTION

Prebiotics are food ingredients that stimulate selectively the growth and activity of specific species of bacteria in the gut, usually bifidobacteria and lactobacilli, with benefits to health. In practice, they are short-chain carbohydrates (SCCs) that are nondigestible by human enzymes and that have been called resistant SCCs (1). They are sometimes referred to as nondigestible oligosaccharides (NDOs). However, NDOs are not strictly oligosaccharides and their nondigestibility is largely assumed and not always proven.

Some of the SCCs currently available for human consumption that are candidate prebiotics are shown in **Table 1**. They are probably best defined as "carbohydrates with a degree of polymerization (DP) of two or more, which are soluble in 80% ethanol and are not susceptible to digestion by pancreatic and brush-border enzymes" (1). The accepted definition of an oligosaccharide (2) is "…a molecule containing a small number (2 to about 10) of monosaccharide residues connected by glycosidic linkages." Some of the carbohydrates that are currently potential prebiotics clearly fall outside this definition in that several have a DP >10. What does distinguish them chemically, however, is their solubility in 80% ethanol, together with their in vitro resistance to pancreatic and brush border enzymes.

Analysis of these preparations indicates that although some are very pure, containing 86–87% oligosaccharides, eg, inulin and oligofructose, in others the oligosaccharide fraction is minor, ≈20–30%, the rest being free monosaccharides, starch, and nonstarch polysaccharides. For example, xylooligosaccharide (Suntory, Japan) contains 29.4% oligosaccharide, 41% starch, and 15% monosaccharide. It is important to bear purity in mind when interpreting human or animal feeding studies of prebiotics.

DIGESTIBILITY

To be effective, prebiotics need to reach the cecum in some form. Although it is likely that, because of their chemical structure, a fraction of the substances listed in Table 1 escapes digestion by pancreatic and small-bowel enzymes in the human gut and therefore arrives in the large bowel, the experimental proof of this is difficult and time consuming to collect. Studies showed that when either inulin or oligofructose is fed to ileostomy subjects, average recovery at the terminal ileum lies between 86% and 89% of the material fed (**Table 2**) (3, 4). Similarly, when gut contents are aspirated from the terminal ileum after test meals containing oligofructose are consumed, 89% is recovered (5).

Other evidence of nondigestibility is more circumstantial. Several studies showed that after intake of prebiotics, breath-hydrogen excretion increases. Although this is evidence of the fermentability of prebiotics, it does not provide information on the true extent of their nondigestion. Sucrose 1F-fructosyltransferase (EC 2.4.1.99) catalyzes the transfer of fructose remnants onto sucrose, which leads to fructooligosaccharides (FOS) of the type $(\beta\text{-fructosyl})_n$-

TABLE 1
Compositions of some candidate prebiotics available for human consumption[1]

Product name	DM	Ara	Xyl	Man	Gal	Glc	Total
				% by wt			
Fructooligosaccharide[2]	94.0	—	—	34.0	0.2	53.3	87.4 ± 1.3
Isomaltooligosaccharide[3]	77.8	—	—	—	—	29.8	29.8 ± 0.5
Oligomate[4]	74.9	0.1	—	0.8	18.6	22.7	42.2 ± 2.5
Palatinose[5]	92.6	0.1	—	10.5	—	35.7	46.3 ± 2.8
Polydextrose	89.8	—	—	0.3	1.9	36.5	38.7 ± 2.6
Pyrodextrin[6]	94.3	—	—	—	0.2	18.8	20.0 ± 0.5
Raftiline[2]	93.3	—	0.1	34.7	0.8	50.2	85.8 ± 3.4
Soybean oligosaccharide[7]	76.5	—	0.1	7.5	8.4	15.6	32.3 ± 3.9
Xylooligosaccharide[8]	94.9	0.8	25.9	0.6	—	1.6	29.4 ± 1.3

[1] Recovery of fructose was measured as mannose (Man) and glucose (Glc). Total values are the mean ± SD of 3 samples. DM, dry matter; Ara, arabinose; Xyl, xylose; Gal, galactose. Data from reference 1.
[2] Orafti, Belgium.
[3] Showa Sangyo, Japan.
[4] Yakult, Japan.
[5] Sudzucker, Germany.
[6] Matsutani, Japan.
[7] Calpis, Japan.
[8] Suntory, Japan.

sucrose or GF(n + 1); where n = 1 to ≤10. GF2 and GF3 have been incubated in vitro with either human saliva or rat pancreatic homogenate and reported to be "hardly digested" (6). No change in blood glucose or insulin was seen when 25 g neosugar (a fructooligosaccharide mixture of GF2, GF3, and GF4) was given to healthy subjects (6), nor when fructans extracted from Jerusalem artichokes (30% GF7 or greater) were consumed in doses of 5, 10, or 20 g either alone or with other carbohydrates (7). Nilsson et al (8) incubated various cereal fructan fractions in fresh human gastric juice for 1 h and showed that at pH 1.05, 10–15% was hydrolyzed, but at a pH > 1.8, <1% was degraded. When incubated with homogenized rat intestinal mucosa, the rate of hydrolysis of fructan was <1% of that of sucrose. In the same study, there was virtually no disappearance of fructan from the intubated rat small bowel in vivo.

All of this work relates to fructans of various molecular size. No convincing evidence for their digestion in the stomach and small bowel has been obtained, and this would agree with the known specificity of mammalian digestive enzymes. However, many other SCCs are present in the diet and more are being produced every year by various enzyme-based industrial processes. A relatively simple set of criteria is needed to assess the likely digestibility of these SCCs in humans. Human studies are time consuming and it

would be unreasonable to expect that every new prebiotic, either discovered or synthesized, undergo this process of testing. In practical terms, the in vitro properties of new prebiotics will probably relate reasonably well to their physiologic function and analytic results, and these can be used to screen potential prebiotics. These analytic results should include *1*) a detailed description of the chemical structure, *2*) measurement of resistance to gastric juice, *3*) measurement of resistance to pancreatic enzymes, and possibly *4*) measurement of resistance to brush border enzymes. The fermentability of the prebiotic should also be assessed.

FERMENTABILITY

Any carbohydrate that reaches the cecum is a potential substrate for fermentation by the microbiota, and much evidence supports the belief that the currently identified prebiotics are fermented. In a small number of human feeding studies, fecal recovery of inulin or FOS was measured and found to be zero (5, 9, 10). Indeed, there are abundant data from both in vitro and in vivo studies of fermentation of these carbohydrates by the bacteria of the large intestine.

In vitro, many different SCCs support bacterial growth and produce various fermentation-derived end products (6, 11–13).

TABLE 2
Digestibility of prebiotics in human upper intestine

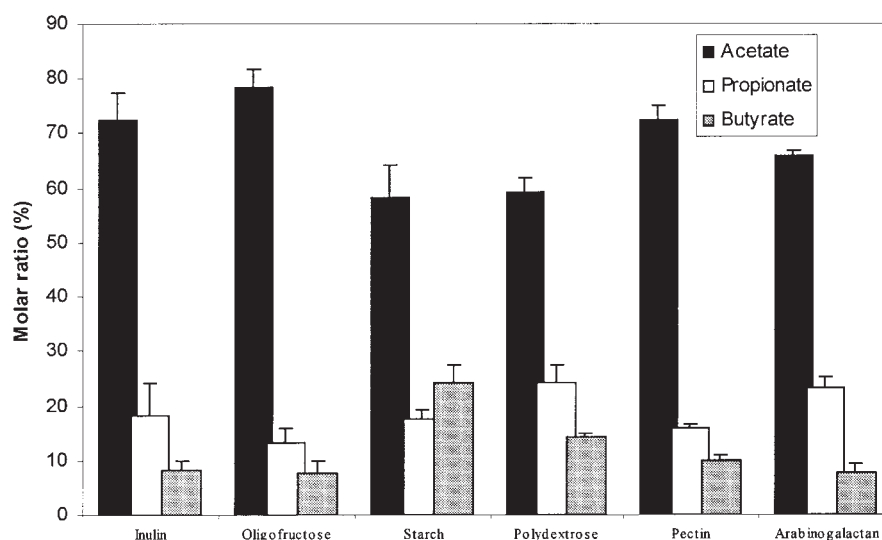| Reference, year, and prebiotic source	Model system	Intake	Recovery	Percentage recovery[1]
		g	*g*	*%*
Bach Knudsen and Hessov (3), 1995				
Inulin	Ileostomy	7.07	6.1	86
		21.2	18.4	87
Ellegard et al (4), 1997				
Inulin	Ileostomy	17.0	15.0	88
Oligofructose	Ileostomy	15.5	13.8	89
Molis et al (5), 1996				
Oligofructose	Aspiration from ileum	20.1	6.0	89

[1] Average recovery 88%.

FIGURE 1. Molar ratios of acetate, propionate, and butyrate produced by carbohydrate fermentation of slurries of mixed human fecal bacteria. $\bar{x} \pm$ SEMs of triplicate determinations from 6 volunteers (12).

Two early studies (6, 11) both showed that a range of bifidobacteria could utilize low-DP fructans (GF2–4), although *Bifidobacterium bifidum* appeared to be less effective than the other strains tested. Bifidobacteria also utilized lactulose and glucose. However, other enteric bacteria were able to grow on a range of prebiotics, especially *Bacteroides* species. Utilization of fructans by lactobacilli, *Escherichia coli*, and *Clostridium perfringens* was poor. Using gas and short-chain fatty acid (SCFA) production as endpoints, Wang and Gibson (12) showed that fecal slurries fermented oligofructose, along with a wide range of other carbohydrates, but that oligofructose and inulin selectively stimulated growth of bifidobacteria. In pure culture experiments, 8 different strains of bifidobacteria, including *B. bifidum,* grew well on oligofructose, as did *E. coli* and *C. perfringens*—in direct contrast with the earlier results of Hidaka et al (6) and Mitsuoka et al (11). However, these latter 2 organisms showed somewhat better growth rates on glucose, whereas of the bifidobacteria, only *Bifidobacterium longum* did. In competition experiments, Gibson and Wang (14) showed that in pH-controlled coculture of *Bifidobacterium infantis, E. coli,* and *C. perfringens* with oligofructose as the sole carbohydrate substrate, the bifidobacteria grew well and exerted an inhibitory effect on the growth of the other 2 species. These findings were subsequently confirmed in vivo in human feeding studies (15).

Whether the nature of the carbohydrate determines its fermentability is a question that has barely been addressed. Van Laere et al (13) produced a range of different SCCs with widely different sugar compositions and molecular sizes and tested their breakdown by several strains of *Bifidobacterium*, *Clostridium*, *Bacteroides*, and *Lactobacillus*. Fermentability differed with oligosaccharide structure. The fructans were extensively fermented, except by clostridia, whereas few species were able to break down arabinoxylan under the conditions of the experiment. Xylooligosaccharides were well fermented. Linear oligosaccharides were catabolized to a greater degree than were those with branched structures. Bifidobacteria utilized low-DP carbohydrates first and bacteroides utilized those with a high DP. Metabolic collaboration among species was evident in carbohydrate breakdown. Both the structure of the carbohydrate and the bacterial species present in the ecosystem are probably important factors in controlling the fermentation of SCCs.

SHORT-CHAIN FATTY ACIDS

The major products of prebiotic metabolism are SCFAs, the gases hydrogen and carbon dioxide, and bacterial cell mass. Much has been written about SCFA production in the hindgut and about the differing metabolic significance of the individual acids (16). Prebiotics have been shown to be a source of SCFAs both in vitro and in vivo, although the relative yield per gram of substrate fermented has not been investigated. No particular distinguishing feature of the pattern of SCFA production has emerged as yet. Using fecal innocula from 6 healthy volunteers, Wang and Gibson (12) compared in vitro production and molar ratios of SCFAs from 17 different carbohydrate sources. The molar ratios from 6 of these, of which 2 are established prebiotics (FOS and inulin), are shown in **Figure 1**. As was shown in other studies (17), starch consistently produces relatively more butyrate whereas oligofructose and inulin are the lowest producers. Arabinogalactan and polydextrose yield relatively more propionate, and oligofructose yields predominantly acetate. In an in vitro study similar to that presented in Figure 1 that used feces from 2 subjects who had been eating 20 g oligofructose daily for 4 wk, the molar ratio of acetate:propionate:butyrate at 12 h was 63:12:25 (18).

In vivo, the study of SCFAs is more difficult and relies mostly on determination of the concentrations in feces. Because of the limitations of this approach, it is not surprising that little has been learned from it about the fermentation of prebiotics. In the 3 comprehensive human studies that have been published, neither inulin nor oligofructose at doses of between 4 and 40 g/d produced any significant change in the concentration or molar ratios of fecal SCFAs (9, 15, 19).

GAS

The gases carbon dioxide and hydrogen are inevitable products of fermentation but are also the major clinical disincentive to

consumption of prebiotics. Unwanted symptoms relating to gas production in the gut are widely reported in human prebiotic feeding studies (7, 14, 20–23). In Stone-Dorshow and Levitt's study (20), 112 subjects took 15 g FOS daily for 12 d. When compared with a group of 5 subjects taking sucrose, symptoms of abdominal pain, eructation, flatulence, and bloating were all significantly more severe in the FOS group. There was no adaptation over the 12-d period but symptoms were all reported as no worse than mild. Breath hydrogen after a 10-g challenge of FOS did not differ significantly between the groups at 12 d and was not significantly different from breath hydrogen after a similar dose of lactulose. Other studies of FOS at doses of 5 and 20 g/d showed dose-related increases in breath hydrogen and mild flatulence and borborygmi. In general, only isolated individuals experienced somewhat more discomfort (15, 18, 24). Paradoxically, 8 healthy subjects taking 10 g transgalactooligosaccharides/d reported a decrease in breath-hydrogen excretion and no digestive symptoms (25).

Inulin leads similarly to increased breath-hydrogen excretion (15, 26). At a dose of 14 g/d, highly significant increases in flatulence, rumbling, stomach and gut cramps, and bloating were seen in a group of 64 women taking inulin in a double-blind crossover study over 4-wk periods. Twelve percent of the volunteers considered the flatulence severe and unacceptable. No adaptation in symptoms occurred over time (21).

An explanation of these various and idiosyncratic effects of prebiotics on symptomatology and hydrogen metabolism is difficult to find. Wide individual variation is known to occur in response to fermentation of prebiotics (22) and it is likely that the stoichiometry of fermentation differs for carbohydrates of differing chain length and monosaccharide composition (27). With use of breath-hydrogen excretion alone, it was shown that lactitol, isomalt, and polydextrose each increase breath hydrogen by 112%, 73%, and 11%, respectively, when given as equal doses to healthy subjects (28). These findings were broadly reflected by in vitro fermentation studies and suggest that molecules with longer chain lengths are fermented more slowly and with less net hydrogen excretion. A similar result was obtained by Brighenti et al (26) when comparing lactulose, inulin, and resistant starch in healthy subjects. Breath hydrogen was only 4.7 $ppm \cdot h^{-1} \cdot g$ resistant starch^{-1} compared with 19.1 for inulin and 26.6 for lactulose at similar doses. When Christl et al (27) studied absolute hydrogen-excretion rates using a human calorimeter, total hydrogen excretion for starch was only 40% of that from an equivalent dose of lactulose.

Interpreting hydrogen metabolism by using studies with breath measurement alone is complicated not only by the differing stoichiometries for individual carbohydrates, but also by the changing distribution of hydrogen excretion between flatus and breath and the alternative pathways for hydrogen disposal via methane, sulfide, and acetate. Nevertheless, prebiotics are clearly a major source of hydrogen generation in the gut, and for some people, the rapid generation of gas and its volume is a major hindrance to their consumption. Experiments to produce different chain lengths, degree of branching, and DPs might lead to less flatulent prebiotics and might alter their properties to benefit health through selectively affecting the microflora.

BOWEL HABIT

Carbohydrates that reach the large intestine, such as non-starchy polysaccharides and resistant starch, have a laxative effect on bowel habit. The mechanism works via stimulation of microbial growth, increase in bacterial cell mass, and thus, stimulation of peristalsis by the increased bowel content (29). It can be predicted, therefore, that prebiotics will be laxative. However, current evidence is patchy, largely because of study design and the type of volunteers used.

The clearest demonstration of a laxative effect was in the controlled diet study of Gibson et al (15), which showed that 15 g FOS increased stool output significantly from 136 to 154 g/d ($n = 8$). In a smaller group of subjects, 15 g inulin was also laxative; mean daily stool output was 92 g/d for the control, 123 g/d for inulin ($n = 4$). Three other human experiments have not shown an increase (9, 23, 25) but in none of these was diet controlled, which would tend to mask a small effect. In the study of Alles et al (9), subjects started with unusually high fecal weights with the control diet, 272 ± 26 g/d. In that of Bouhnik et al (25), volunteers were given 10 g transgalactooligosaccharides/d for 21 d without an effect on bowel habit. Ito et al (23), who fed 4.8–19.2 g oligomate (52% galactooligosaccharides)/d to 12 healthy subjects, also did not show a change in bowel habit, despite showing bifidogenicity, and the subjects did report an increase in abdominal symptoms. In 3 studies reporting only qualitative data, either FOS or inulin "improved" constipation in small groups of hospitalized subjects (19, 30, 31).

FOS and inulin are probably laxative, but because the effect is small, it is difficult to detect except in carefully controlled studies. In the study of Gibson et al (15), there were 1.3- and 2.0-g increases in stool wet weight per gram of prebiotic fed. This is less than that seen with nonstarchy polysaccharide sources such as wheat bran (5.4 g) or fruit and vegetables (4.7 g), but similar to that produced by more rapidly fermented polysaccharides such as pectin (1.2 g) (32).

The increase in fecal output is likely to be due to an increase in biomass. Along with the increase in excretion of dry matter, there is a significant increase in fecal nitrogen (**Figure 2**). In the study by Gibson et al (15), the additional excretion of 0.32 g N/d when FOS was added to the diet was equivalent to 5 g bacterial solids (33), which at the moisture content of stool is equivalent to 20–25 g wet stool. This is exactly the change in stool output seen in this study (15).

FECAL MICROBIAL ENZYMES

Apart from their selective effect on microbial growth, prebiotics change microbial activity in other ways. When studied in a 3-stage, compound continuous culture model of the colon, FOS caused profound and rapid change in microbial enzyme activities. Azoreductase [NAD(P)H dehydrogenase (quinone); EC 1.6.99.2] and nitroreductase expression were increased. A small increase in arylsulfatase was seen (34). These changes have been described as dysbiotic because increased activities of these enzymes are thought to favor carcinogen formation in the colon (35). In vivo, however, 4 g FOS (neosugar)/d decreased β-glucuronidase and glycocholic acid hydroxylaseactivities in 12 subjects but did not affect nitroreductase activity (36). Similarly, in the study of Bouhnik et al (37), 12.5 g FOS/d had no effect on the activities of nitroreductase, azoreductase, or β-glucuronidase in feces. Likewise, Kleessen et al (19) were unable to show changes in β-glucuronidase and β-glucosidase activities with inulin consumption in constipated elderly subjects.
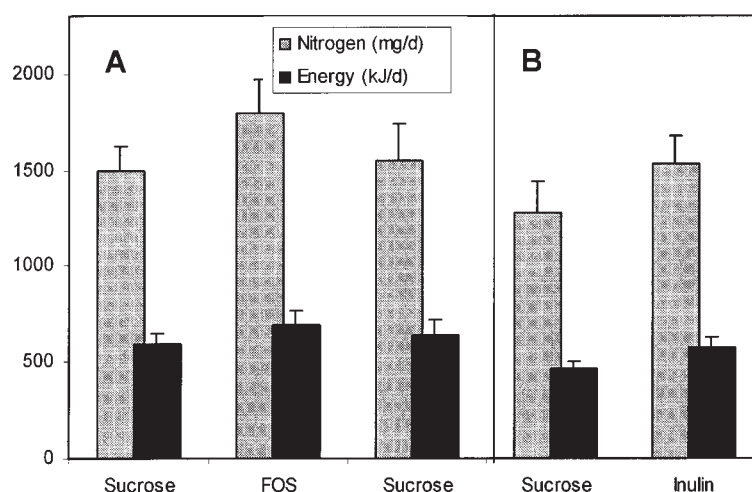
FIGURE 2. Fecal nitrogen and energy excretion from (A) 8 healthy volunteers fed 15 g fructooligosaccharides (FOS)/d for 15 d compared with 2 sucrose control periods and from (B) 4 healthy volunteers fed 15 g inulin/d for 15 d compared with a single sucrose control period (15).

Studies of fecal enzyme activities are notoriously difficult to interpret and the in vitro system may well be a better model of what is going on in the more proximal gut. Furthermore, whether changes in enzyme activity translate into increased product formation depends on substrate availability, pH, and a host of other factors.

CONCLUSIONS

Prebiotics, so called because of their selective stimulation of the activity of certain groups of colonic bacteria, have many other effects in the large intestine. The evidence that they are fermented is convincing, although not many candidate prebiotics have been tested in humans as yet. Through fermentation, prebiotics affect bowel habit and microbial enzyme activity and lead to the production of SCFAs and gas. This latter property is the cause of some discomfort in people and a barrier to the wider acceptance of prebiotics as a functional food that may benefit health.

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