Selective Stimulation of Bifidobacteria in the Human Colon by Oligofructose and Inulin

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Background/Aims: **Oligofructose and inulin are naturally occurring indigestible carbohydrates. In vitro they selectively stimulate the growth of species of *Bifidobacterium*, a genus of bacteria considered beneficial to health. This study was designed to determine their effects on the large bowel microflora and colonic function in vivo.** ***Methods:*** **Eight subjects participated in a 45-day study during which they ate controlled diets. For the middle 15 days, 15 g · day^{-1} oligofructose was substituted for 15 g · day^{-1} sucrose. Four of these subjects went on to a further period with 15 g · day^{-1} inulin. Bowel habit, transit time, stool composition, breath H_2 and CH_4, and the predominant genera of colonic bacteria were measured.** ***Results:*** **Both oligofructose and inulin significantly increased bifidobacteria from 8.8 to 9.5 $\log_{10}$ g stool^{-1} and 9.2 to 10.1 $\log_{10}$ g stool^{-1}, respectively, whereas bacteroides, clostridia, and fusobacteria decreased when subjects were fed oligofructose, and gram-positive cocci decreased when subjects were fed inulin. Total bacterial counts were unchanged. Fecal wet and dry matter, nitrogen, and energy excretion increased with both substrates, as did breath H_2. Little change in fecal short-chain fatty acids and breath CH_4 was observed.** ***Conclusions:*** **A 15-g · day^{-1} dietary addition of oligofructose or inulin led to *Bifidobacterium* becoming the numerically predominant genus in feces. Thus, small changes in diet can alter the balance of colonic bacteria towards a potentially healthier microflora.**

The human large intestine contains a substantial and diverse population of bacteria that is important to human health. This predominantly anaerobic microflora is able to salvage energy for the host through the bacterial fermentation of undigested carbohydrates and protein to short-chain fatty acids,[1] which are then absorbed. The gut microbiota may also synthesize vitamins,[2] protect against invasive species that are often pathogenic,[3,4] and possibly contribute to the economy of essential amino acids in humans.[5,6] However, not all intestinal bacteria are beneficial to health, and a long-established concept is that of beneficial and harmful species.[7] Beneficial genera include *Bifidobacterium* and *Lactobacillus,* both of which are saccharolytic, whereas species such as *Clostridium perfringens* and *Escherichia coli* are considered detrimental.[8,9]

Bifidobacteria are the numerically predominant bacterial genus in the feces of breast-fed infants. It is believed that this may contribute to the protection that breast feeding provides against gut infections.[10–12] Most adults also carry bifidobacteria in their colons[12,13] but in lower numbers than in breast-fed infants. Because of their potentially beneficial properties, there have been attempts to increase their relative proportion in the adult colon. One method has been the feeding of probiotic microorganisms to introduce more bifidobacteria into the bowel. Bifidobacteria administered in this way are able to pass through the terminal ileum[14] and are detected in feces at about $10^{8.8}$ g^{-1}.[15] However, they rapidly disappear from feces when oral dosing ceases.

When administered by mouth, bifidobacteria can alter fecal bacterial enzyme activities,[16] reduce antibiotic-induced side effects,[17] inhibit 2-amino-3-methylimidazo[4,5-*f*] quinoline–induced mammary and liver tumors in rats,[18] and reduce 1,2-dimethylhydrazine induced colonic carcinogenesis in mice in conjunction with oligofructose.[19] They may be partly responsible for colonization resistance, which the resident microflora offers against invading pathogens,[20,21] and bifidobacteria stimulate the immune system towards certain tumors[22,23] and bacterial invasion.[21,24]

What controls the growth of bifidobacteria in the gut? In breast milk, the "bifidus factor" is a glycoprotein containing glucose, galactose, fucose, and *N*-acetyl glucosamine.[12] In uncontrolled studies of elderly people administered 8 g · day^{-1} of a fructooligosaccharide (Neosugar; Meija Seika, Tokyo, Japan), bifidobacterial numbers increased in feces, as did total anaerobes.[25,26] Using in vitro cultures of human fecal bacteria, we have shown that two structurally similar carbohydrates, oligofructose (OF) and inulin, selectively stimulate bifidobacterial growth while maintaining potential pathogens such as

Abbreviations used in this paper: OF, oligofructose; PYG, peptone yeast glucose.

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E. coli and clostridia at low levels.[27] Moreover, in defined coculture experiments, various species of bifidobacteria inhibited the growth of *E. coli* and *C. perfringens*. This effect was caused by the secretion of an inhibitory substance that was independent of changes in the culture pH. Plating experiments showed that this antimicrobial substance variably suppressed species belonging to the genera *Salmonella, Listeria, Campylobacter,* and *Shigella,* as well as *Vibrio cholerae.*[28]

To determine whether the addition of OF or inulin to a normal diet can lead to changes in the gut microflora, we fed these carbohydrates for 15 days to 8 healthy volunteers under controlled dietary conditions, substituting them for 15 $g \cdot day^{-1}$ sucrose in the basal diet. The effect of this dietary change on the major genera of fecal bacteria and the colonic function, including stool output and composition and breath H_2 and CH_4, was measured.

Materials and Methods

Subjects

Eight healthy volunteers (7 men and 1 woman) with a mean age of 33.6 years (range, 21–48 years) and a mean body mass index of 22.4 (range, 18.7–25.4) were used in the study. All subjects underwent a medical examination, were healthy, and had not taken antibiotics for at least 3 months before the commencement of the study. Written consent was obtained from each person, and the protocol was approved by the Medical Research Council Dunn Nutrition Ethical Committee. As part of the initial assessment, a stool sample was collected from the volunteers for bacteriological analyses. All had an initial viable count of bifidobacteria in the range of 10^8–10^9 g wet wt $feces^{-1}$.

Oligofructose and Inulin

Oligofructose was the oligosaccharide fraction of Raftilose (Orafti, Tienen, Belgium). It is composed of molecules of the GFn and Fn type [G, glucose; F, fructose; n, number of fructose moieties linked by β (2,1) linkages in a ratio of about 2:1], with n being between 2 and 6, with an average degree of polymerization of 4. Inulin was the oligosaccharide fraction of Raftiline (Orafti), which was obtained by the extraction of chicory roots. It is composed of molecules of the GFn type, with n ranging from 2 to 60 and an average degree of polymerization of 10.

Diet and Experimental Design

The study was conducted using controlled diets with each subject fed for 15 days. The protocol included an initial few days to settle into the Unit's routine on a free diet and then 45 days that were divided into 15 days of initial control, 15 days when 15 $g \cdot day^{-1}$ sucrose was replaced with 15 $g \cdot day^{-1}$ OF, and finally 15 days of a second control period (15 $g \cdot day^{-1}$ sucrose). This was then followed by 5 days on a free diet for stool collections to complete fecal marker recoveries. Four of the subjects went on to participate in another 25-day study, comprising the same control sucrose diet for 10 days, with 15 $g \cdot day^{-1}$ sucrose being substituted by 15 $g \cdot day^{-1}$ inulin during a further 15 days, and again followed by 5 days of stool collections to recover all markers. Subjects lived in the metabolic suite of the Medical Research Council Dunn Clinical Nutrition Centre. No food other than the diets provided or any alcohol were allowed. Subjects' normal energy requirements were assessed before the study from height, weight, and food records. The diets were weight maintaining.

Three 1-day menus comprising normal foods were designed and fed in rotation to the subjects throughout the study. The basal diet provided 9 MJ energy and had the following composition[29]: protein, 63 g; fat, 93 g (polyunsaturated/saturated fat ratio, 0.19); starch, 142 g (resistant starch, 4.7 g[30]); sugars, 124 g; and nonstarch polysaccharides, 16.4 g.[31] Energy intakes were adjusted to meet individual needs by adding 1-MJ increments of the same composition as the basal diet to that diet. Energy intakes ranged from 9 to 15 $MJ \cdot day^{-1}$. Five grams of sucrose, OF, and inulin were administered with breakfast in a free form, and the other 10 g was incorporated into biscuits. Volunteers kept a daily diary in which to record their weight, times of radiopaque markers taken, stools passed, and any unusual events such as diarrhea and flatulence.

Collection and Analysis of Breath

End expiratory breath samples were collected in duplicate using a dedicated sampling device[32] together with a room air sample at 7 PM and 10 PM on days 10, 13, and 15 of each experimental period, except for during the control sucrose period before inulin, when breath samples were collected on days 8 and 10. Hydrogen was measured using an exhaled hydrogen monitor (GMI, Renfrew, Scotland) that was adjusted to detect to 0.1 ppm and was calibrated daily using a 52-ppm standard. Methane was measured using a Pye 104 gas chromatograph[32] (Pye Unicam; Cambridge, Cambridgeshire, England) that was calibrated with 4.8- and 48-ppm CH_4 standards. Breath CH_4 was reported as the concentration in the sample less that in room air. All samples were analyzed within 24 hours of collection and corrected for H_2 losses during storage using previously obtained correction factors for the syringes.

Stool Collection and Estimation of Intestinal Transit Time and Balance Markers

All stools passed were collected and, apart from those required for bacteriologic analyses, were immediately weighed and frozen at −20°C. During the last 6 days of each dietary period, three small subsamples of well-mixed stool were obtained within 1 hour of defecation for immediate bacteriologic analyses, and another sample was frozen for short chain fatty acid measurements. Mean transit time was measured using the continuous marker method.[33] Volunteers were given 10 radiopaque shapes with each meal (30 per day), and marker type was changed every 15 days with diet. All stools were x-

rayed before any sampling, and the markers in each stool were counted. The mean transit time was calculated, and marker excretion was used to correct stool weights, nitrogen, energy, and ash outputs so that results represented a complete 5-day collection period.[34]

Diet and Fecal Analysis

All stools from the last 5 days of each dietary period were pooled, weighed, and lyophilized to constant weight. The resultant dry sample was milled in a centrifugal mill and used for fecal analyses. Total nitrogen was measured using an automated Kjeldahl procedure, energy was determined by an adiabatic bomb calorimeter, and ash was quantified at 500°C after an initial burning off of organic residues over a flame. Fecal subsamples for short-chain fatty acid analysis were defrosted at 4°C, mixed, and 1 g was diluted to 10 mL and centrifuged. One milliliter of the supernatant was then acidified and extracted into diethyl ether over ice,[35] along with three calibration standards. 2-Methyl-valerate was used as an internal standard. The ether was dried with fused calcium chloride, and 0.5 μL ether was injected onto a Pye 204 gas liquid chromatograph fitted with a flame ionization detector and a 25-m BP21-fused silica capillary column (inside diameter, 0.53 mm) (S.G.E. Australia, Ringwood, Victoria, Australia). The column was held at 100°C with helium as the carrier gas at 11 psi of inlet pressure. There was linearity of extraction and recovery for all short-chain fatty acid analysis. All samples were analyzed in duplicate.

Bacterial Enumeration and Identification

All bacteriologic analyses of feces were performed within 1 hour of defecation. Five grams of fresh stool was homogenized using 50 mL of anaerobic 0.1 mol/L sodium phosphate buffer (pH 7.0) to provide a 10% (wt/vol) fecal slurry. The slurry was sieved (aperture 250 μm), and 1 mL was diluted serially in half-strength Wilkins Chalgren anaerobic broth (Oxoid, Basingstoke, Hampshire, England) in an anaerobic cabinet ($H_2/CO_2/N_2$, 10:10:80). Plates were inoculated in triplicate using selective media for the enumeration of total anaerobes, total aerobes, coliforms, gram-positive cocci, bifidobacteria, bacteroides, fusobacteria, lactobacilli, and clostridia, and plates were incubated aerobically or anaerobically as appropriate.[35–39]

After incubation, individual colonies were removed from the media plates and subcultured into peptone yeast glucose (PYG) broth.[35] Bacteria were then characterized to genus level on the basis of colonial appearance, gram reaction, spore production, cell morphology, and fermentation end-product formation. Bifidobacteria were further identified by the production of acetate and lactate in PYG broth and a positive detection of fructose-6-phosphate phosphoketolase activity in crude cell extracts.[39]

Statistical Analyses

Systat version 5.2.1 was used for all statistical analyses. Diet and fecal results were analyzed using paired Student's *t* tests and presented as mean (SEM), and breath and short chain fatty acids results were analyzed by analysis of variance (ANOVA) and presented as SEM. Before statistical analysis of bacteriologic results, the normality of a representative set of data was checked by means of the Kolmogorov–Smirnov test. Bacterial counts were logarithmized to fit a normal distribution, and the normality of the data was confirmed by Box and Whisker plots of the logged counts. ANOVA was used to show the effect of the diet on bacteriologic analyses. To confirm unequivocally statistical results of the bacterial count data, the analysis was repeated by means of the nonparametric Kruskal–Wallis test, which does not assume normality of the distribution. Bacteriologic results are presented as mean values (SD).

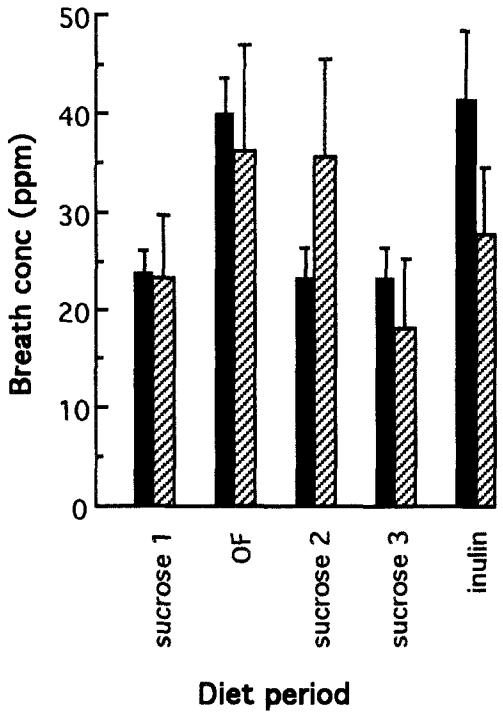

Figure 1. Mean breath H_2 (■) and CH_4 (▨) concentrations in 8 subjects with 15 $g \cdot day^{-1}$ of sucrose, OF, or inulin added.

Results

All 8 subjects completed the controlled diet study without any periods of ill health or abnormal bowel habit and maintained their weight. One subject complained of flatulence intermittently throughout, and 1 complained of flatulence and abdominal pain during the OF feeding period.

Breath H_2 and CH_4

The inclusion of OF in the diet resulted in a significant increase ($P = 0.001$) in breath H_2 concentration to reach 40.1 ± 3.6 ppm compared with the first and second control sucrose periods of 23.8 ± 2.0 ppm and 23.5 ± 2.9 ppm (Figure 1). This increase occurred for all the subjects. Only 3 of the 8 subjects had detect-

Table 1. Bowel Habit, Fecal Composition, and Apparent Digestibility of Nitrogen, Energy, and Ash

	Sucrose 1	OF	Sucrose 2
Stools (*per 5 days*)	4.1 ± 0.52	5.3 ± 0.41[a]	5.3 ± 0.53[a]
Mean transit time (*h*)	51.5 ± 8.65	53.8 ± 9.74	56.6 ± 12.2
Dry matter (%)	22.4 ± 1.97	22.2 ± 1.84	23.5 ± 1.76
Corrected wet wt ($g \cdot day^{-1}$)	135.8 ± 22.8	154.1 ± 22.9	131.3 ± 19.5[b]
Corrected dry wt ($g \cdot day^{-1}$)	27.8 ± 2.79	32.2 ± 3.39	29.0 ± 3.43
Nitrogen density (*% dry matter*)	5.49 ± 0.22	5.76 ± 0.18	5.38 ± 0.21[b]
Nitrogen excreted ($g \cdot day^{-1}$)	1.51 ± 0.12	1.83 ± 0.17[a]	1.55 ± 0.19[b]
Energy density ($kJ \cdot g$ *dry matter*$^{-1}$)	21.5 ± 0.50	21.5 ± 0.53	21.9 ± 0.57[a]
Energy excreted ($kJ \cdot day^{-1}$)	596.6 ± 56.4	695.6 ± 78.3	639.9 ± 83.3
Ash density (*% dry matter*)	14.1 ± 0.78	12.9 ± 0.73[a]	14.1 ± 0.74[b]
Ash excreted ($g \cdot day^{-1}$)	3.87 ± 0.30	4.02 ± 0.17	3.94 ± 0.22
Dry matter digestibility (%)	96.2 ± 0.34	95.7 ± 0.37	96.1 ± 0.44
Nitrogen digestibility (%)	91.9 ± 0.75	90.1 ± 0.92[a]	91.6 ± 1.06[b]
Energy digestibility (%)	96.2 ± 0.40	95.6 ± 0.49	95.9 ± 0.59
Ash digestibility (%)	80.3 ± 1.73	79.2 ± 2.04	79.7 ± 2.20

NOTE. Values are expressed as mean ± SEM.
[a]Significantly different from sucrose 1.
[b]Significantly different from OF ($P < 0.05$).

able CH_4 in breath during the study period, and these subjects showed no obvious relationship between OF intake and breath CH_4 concentration (Figure 1) (23.5 ± 6.2 ppm and 35.4 ± 10.1 ppm for sucrose 1 and 2, respectively; 36.2 ± 10.7 ppm for OF).

When subjects were fed inulin, a similar significant increase in breath H_2 was observed (from 23.4 ± 3.11 ppm to 41.5 ± 6.94 ppm; $P = 0.031$). Of the 4 subjects who received inulin, 3 were methanogenic, and breath CH_4 increased in 2 of these 3 but decreased in the third, resulting in an increase, which was not significant in mean breath CH_4, from 18.0 ± 7.22 ppm to 27.7 ± 6.72 ppm.

Bowel Habit, Stool Composition, and Digestibility

Both OF and inulin increased stool frequency, the excretion of wet and dry matter, nitrogen, and energy (Tables 1 and 2), with increases in wet matter and nitrogen excretion being significant when subjects were fed OF. Moisture content was unaffected, and there was an increase in transit time that was not significant throughout all diet periods, suggesting a slight underlying constipating effect of the basal diet. Ash excretion also increased when subjects were fed inulin, although changes were not significant. When considering only the results from the 4 subjects who participated in both OF and inulin diet periods (Table 2), the inclusion of inulin resulted in a larger increase in fecal wet and dry weight than did OF (34% and 23% vs. 14% and 12%); however, it also slowed mean transit time more than OF did.

All measured components of the diet were highly digestible (Table 1), and the inclusion of OF had very little effect on apparent digestibility, except a very small but significant decrease in apparent nitrogen digestibility compared with both the sucrose diet periods ($P < 0.05$).

Total short-chain fatty acids in feces (Table 3) averaged between 111 and 131 $mmol \cdot kg^{-1}$ for each dietary pe-

Table 2. OF and Inulin Fecal Results for the 4 Subjects Who Participated in Both Studies

	OF			Inulin	
	Sucrose 1	OF	Sucrose 2	Sucrose 3	Inulin
Stools (*per 5 days*)	3.3 ± 0.85	4.5 ± 0.65[a]	4.5 ± 0.87[a]	3.8 ± 0.63	4.3 ± 0.63
Mean transit time (*h*)	55.9 ± 14.7	56.4 ± 17.3	60.7 ± 22.0	51.1 ± 7.73	57.1 ± 18.3
Dry matter (%)	25.0 ± 3.4	24.1 ± 3.6	24.1 ± 3.5[a]	24.6 ± 1.9	23.5 ± 2.6
Corrected wet wt ($g \cdot day^{-1}$)	107.1 ± 22.4	121.9 ± 18.0	104.8 ± 24.4	92.4 ± 12.6	123.4 ± 24.0
Corrected dry wt ($g \cdot day^{-1}$)	24.5 ± 2.7	27.4 ± 1.3	22.8 ± 2.7	22.2 ± 1.9	27.3 ± 3.3
Nitrogen density (*% dry matter*)	5.71 ± 0.10	6.11 ± 0.09[a]	5.59 ± 0.17[b]	5.88 ± 0.25	5.81 ± 0.26
Nitrogen excretion ($g \cdot day^{-1}$)	1.41 ± 0.17	1.68 ± 0.10	1.29 ± 0.19	1.31 ± 0.16	1.56 ± 0.14
Energy density ($kJ \cdot g$ *dry matter*$^{-1}$)	21.3 ± 0.13	21.4 ± 0.36	21.9 ± 0.03[a]	21.0 ± 0.17	20.8 ± 0.28
Energy excretion ($kJ \cdot day^{-1}$)	523 ± 56	585 ± 19	497 ± 60[a,b]	466 ± 42	565 ± 64
Ash density (*% dry matter*)	14.4 ± 1.16	13.0 ± 0.83[a]	14.9 ± 0.90[b]	14.9 ± 0.39	14.0 ± 0.89
Ash excretion ($g \cdot day^{-1}$)	3.44 ± 0.15	3.55 ± 0.11	3.33 ± 0.25	3.28 ± 0.21	3.74 ± 0.33

NOTE. Values are expressed as mean ± SEM; n = 4.
[a]Significantly different from sucrose 1.
[b]Significantly different from OF ($P < 0.05$).

Table 3. Fecal Short-Chain Fatty Acid Concentrations and Molar Ratios During Three Diet Periods

	Sucrose 1 (n = 15)	OF (n = 16)	Sucrose 2 (n = 16)
Acetate	80.2 ± 9.22	76.8 ± 6.77	64.3 ± 5.64
Propionate	23.5 ± 3.04	23.0 ± 2.68	21.8 ± 2.44
Isobutyrate	2.09 ± 0.198	2.10 ± 0.234	2.46 ± 0.299
Butyrate	19.3 ± 2.30	18.5 ± 2.20	16.0 ± 2.00
Isovalerate	2.92 ± 0.331	2.98 ± 0.426	3.67 ± 0.501
Valerate	2.33 ± 0.304	2.67 ± 0.390	2.46 ± 0.264
Caproate	0.81 ± 0.247	0.92 ± 0.285	0.52 ± 0.170[a]
Acetate	60.4 ± 1.59	60.4 ± 1.36	58.1 ± 1.13
Propionate	17.9 ± 1.38	17.8 ± 1.26	19.0 ± 1.26
Isobutyrate	1.92 ± 0.27	1.90 ± 0.27	2.43 ± 0.30
Butyrate	14.5 ± 0.66	14.1 ± 0.93	13.9 ± 0.80
Isovalerate	2.72 ± 0.43	2.75 ± 0.46	3.66 ± 0.51
Valerate	1.92 ± 0.23	2.17 ± 0.26	2.27 ± 0.26
Caproate	0.72 ± 0.21	0.80 ± 0.23	0.58 ± 0.17[a,b]

NOTE. Two fecal samples were collected on separate days from each of 8 subjects (1 sample was unobtainable). Values are in millimoles per kilogram of feces and are expressed as mean ± SEM.
[a]Significantly different from OF ($P < 0.008$).
[b]Significantly different from sucrose 1 ($P = 0.034$).

riod. No changes were observed in fecal short-chain fatty acid concentrations during any of the dietary periods, except for a minor decrease in caproate during the second sucrose period ($P = 0.008$). Similarly, molar ratios (Table 3) were unaltered, again with the exception of caproate ($P = 0.002$).

Stool Bacteriology

The addition of both OF and inulin had little effect on total viable counts of aerobes or anaerobes. However, for both carbohydrates, bifidobacterial counts (Table 4) were significantly higher than during the sucrose periods ($P < 0.01$). This increase was observed in 7 of the 8 volunteers when they were fed OF and all 4 subjects who were fed inulin. Numbers of bifidobacteria declined significantly ($P < 0.01$) when OF was withdrawn, showing that the increase was directly attributable to the addition of OF to the diet and not to some underlying change in the microflora from becoming a resident at the Dunn Clinical Nutrition Centre on a controlled diet.

A significant decrease in counts of bacteroides ($P < 0.01$) was observed during the OF diet period with the result that bifidobacteria became the numerically predominant species in 7 of the 8 volunteers. Although bacteroides numbers did not change when subjects were fed inulin, bifidobacteria still became the predominant species in 3 of the 4 volunteers.

Clostridia and fusobacteria both declined significantly when subjects were fed OF ($P < 0.05$ and $P < 0.01$). In most of the volunteers, subsequent ingestion of sucrose was required for a relatively long period (>13 days) for counts of fusobacteria to recover to levels detected during the initial control period. Thus, viable counts in the second sucrose period were significantly lower ($P < 0.05$) than those recorded in the first sucrose period (Table 4). Counts of fusobacteria and clostridia were unchanged by the addition of inulin, although counts of gram-positive cocci decreased significantly ($P < 0.001$). In all the volunteers, numbers of lactobacilli were not affected by OF, but inulin increased lactobacilli counts, although not significantly ($P = 0.075$). Counts of coliforms were unaffected by any dietary addition (Table 4).

Discussion

Although the resident bacteria of the colon depend on exogenous (dietary) substrates to a large extent for energy and growth, particularly nondigested carbohy-

Table 4. Mean Viable Bacterial Counts From Three Fecal Samples With Either 15 g·day^{-1} of Sucrose, OF, or Inulin Added to a Controlled Diet

Bacteria	Sucrose 1 (n = 8)	OF (n = 8)	Sucrose 2 (n = 8)	Sucrose 3 (n = 4)	Inulin (n = 4)
Total aerobes	6.4 ± 1.3	6.2 ± 1.0	6.3 ± 0.9	6.7 ± 0.88	6.7 ± 1.0
Coliforms	6.0 ± 1.2	5.9 ± 0.7	5.8 ± 1.0	6.3 ± 1.2	6.2 ± 1.4
Gram-positive cocci	5.8 ± 1.0	5.8 ± 0.9	5.5 ± 0.8	6.0 ± 0.32	5.5 ± 0.27[d]
Total anaerobes	9.9 ± 1.4	10.2 ± 0.9	10.3 ± 0.8	10.6 ± 0.22	10.7 ± 0.25
Bifidobacteria	8.8 ± 0.5	9.5 ± 0.7[a]	8.9 ± 0.9[b]	9.2 ± 0.46	10.1 ± 0.44[d]
Bacteroides	9.4 ± 0.8	8.8 ± 1.1[a]	8.9 ± 0.9[c]	9.7 ± 0.47	9.8 ± 0.50
Fusobacteria	8.5 ± 0.7	7.7 ± 0.9[a]	8.1 ± 0.8[c]	8.8 ± 0.44	8.9 ± 0.62
Clostridia	8.0 ± 1.2	7.5 ± 0.9[c]	7.7 ± 0.7	8.3 ± 0.54	8.1 ± 0.72
Lactobacilli	6.8 ± 1.2	7.0 ± 1.4	7.1 ± 1.0	6.0 ± 1.1	6.3 ± 0.76

NOTE. Values are in $\log_{10}$ grams wet weight of feces^{-1} and are expressed as mean ± SD.
[a]Significantly different from sucrose 1 ($P < 0.01$).
[b]Significantly different from OF ($P < 0.01$).
[c]Significantly different from sucrose 1 ($P < 0.05$).
[d]Significantly different from sucrose 3 ($P = 0.0002$).

drates, it has proved experimentally difficult to produce consistent changes by dietary manipulation. The colon seems to be a self-regulating environment. The advent of "dietary fiber" approximately 20 years ago led to a number of studies that aimed to show beneficial effects on the microflora, but most failed to show any significant change.[40,41] Some alterations in fecal bacterial enzyme activity have been noted[42,43] but no consistent changes in bacterial numbers. Similar findings emerge from studies of meat[44] and fat[45] on the microflora. An increase in overall numbers of bacteria in the colon with additional fermentable carbohydrate in the diet is apparent from studies of fecal biomass excretion,[46] but the balance of groups seems difficult to change by dietary means alone. Although specific effects of diet on the microflora are rarely observed, we have recently shown that the growth of sulfate-reducing bacteria could be stimulated by adding sulfate to the diet.[47]

The present study has shown that a small alteration in diet, namely, the substitution of 15 $g \cdot day^{-1}$ sucrose by 15 $g \cdot day^{-1}$ OF or inulin, can lead to significant changes in the balance of the constituent microflora in the large intestine. Despite the relatively insensitive nature of bacteriologic techniques for enumerating microorganisms in feces and wide individual variation, bifidobacteria increased significantly when subjects were fed OF and inulin, whereas numbers of potential pathogens decreased with OF. These observations confirm results from in vitro studies[27,48,49] that showed that the stimulation of bifidobacterial growth was relatively specific to OF and the related carbohydrate inulin. In vivo studies with other fermentable carbohydrates have failed to show a bifidogenic effect. It is believed that bifidobacteria have relatively high amounts of β-fructosidase that is selective for β1-2 glycosidic bonds present in fructooligosaccharides.[50] Subsequent transport mechanisms and rates of hydrolysis may also be faster. After oligosaccharide hydrolysis, monomers then serve as an efficient growth substrate for the bifidus pathway of hexose fermentation.[51] In addition, the inhibitory effects of bifidobacterial growth on other colonic organisms[28] are likely to assist in the competitive influence that occurred with OF and inulin.

OF is a lower-molecular-weight version of inulin, and the two forms exist in plants such as artichokes, chicory, onions, leeks, garlic, asparagus, with smaller amounts in many cereals. Inulins are mostly linear polymers of fructose with glucose as the terminal sugar. OF, usually DP 2–5, is produced commercially in one of two ways: either by partial hydrolysis using endoglycosidases, e.g., Raftilose, which is made from chicory inulin, or by synthesis from sucrose using fungal fructofuronosidase, e.g., Neosugar.[25] OF is almost certainly not digested in the human small intestine, although final proof of this is still lacking.[52] Its recovery from the small intestine of rats is approximately the same as that of an unabsorbed marker, polyethylene glycol.[53] OF is not hydrolyzed when incubated with human salivary enzymes or rat pancreatic homogenate,[25] and oral dosing of OF does not affect blood glucose in humans.[25,54,55] Fermentation in the large bowel is most probable. In vitro incubation of ^{14}C-labeled OF with the cecal contents from rats showed 66% of the label appearing as short-chain fatty acids, with some being metabolized to CO_2 or incorporated into the biomass.[56] In the same study, feeding OF to conventional rats showed rapid fermentation of OF, whereas germ-free animals delayed excretion of label for many hours, and substantial amounts appeared in feces. Breath H_2 studies in humans have indicated that the majority of OF is fermented.[54] These oligosaccharides cannot be detected in feces even after inulin feeding.[57] In the present studies, a clear increase in breath H_2 was observed during OF and inulin feeding, although changes in breath CH_4 were more erratic in the 3 methanogenic subjects.

Previous studies in humans have shown that OF had a modifying effect on stool frequency, reducing functional constipation[58] and relieving both constipation and loose stools.[55] In our study, stool frequency increased when OF was included in the diet. However, the increase persisted after OF was replaced by sucrose, suggesting that this effect was not mediated via the presence of a fermentable substrate in the colon but more likely some other factor, such as a change in the composition of the colonic microflora. Increases (14% and 34%) in daily stool output were observed when OF and inulin were introduced into the diet. In this respect, they are acting like any other indigestible carbohydrate, such as nonstarch polysaccharides[59] and resistant starch,[60] which produce a laxative effect through fermentation. In terms of the magnitude of the effect determined in the present study (1.3 g and 2.0 g increase in stool weight per gram in subjects fed OF and inulin, respectively), this is less than that observed with sources of nonstarch polysaccharides, such as bran (5.4 g) and fruit and vegetables (4.7 g), but similar to that produced by rapidly fermented nonstarch polysaccharides, such as pectin (1.2 g).[59] The increase in fecal output is likely to be attributable to an increase in biomass that is confirmed by the significant increase in nitrogen excretion observed for both substrates. The additional 0.32 g of N excreted is equivalent to approximately 5 g of bacterial solids,[61] and at the moisture content of the stools in the present study, this amount is the equivalent of 20–25 g of wet stool. This is almost exactly the increase observed (Tables 1 and 2) in stool wet weight output.

Fifteen grams of OF has a gross energy of about 240 kJ. During the OF dietary period, about 77 $kJ \cdot day^{-1}$ additional fecal energy was excreted, which suggested that although fermentation of OF resulted in some increased excretion of energy, much of the energy provided became available through the absorption of short-chain fatty acids. Although there was a larger increase in fecal energy excreted when subjects were fed inulin (about 99 $kJ \cdot day^{-1}$), energy from this increase was still available through fermentation. The fact that all subjects maintained their weight when 15 g of sucrose was substituted by OF or inulin confirms that, at this level of inclusion, they had little effect on total metabolizable energy. Previous studies have shown that feeding subjects OF increases fecal mineral losses[62]; however, in this study, OF had no effect on total minerals (ash) excreted, although inulin feeding did result in an increase in excreted minerals that was not significant. In general, OF and inulin had very little effect on bowel habit and fecal composition other than increasing output. The most important effects were those on the colonic microflora.

Both OF and inulin acted as selective bifidogenic factors by increasing their numbers in absolute terms and as a proportion of total anaerobes, thus resulting in *Bifidobacterium* becoming the predominant bacterial genus. Are the changes which these oligosaccharides induce in the colonic microflora of any benefit to health? Bifidobacteria can represent up to 95% of the total gut microflora of breast-fed infants compared with only 25% in adults.[12,13] This is believed to be a principal reason why such infants suffer relatively less gastrointestinal infection than bottle-fed infants. Although bifidobacteria have been implicated in some opportunistic infections,[63] they are generally regarded as benign and even beneficial. They stimulate immune function, particularly against malignant cells, and produce vitamins of the B group, and perhaps most importantly, we have shown in vitro that they suppress the growth of pathogenic species.[28] In this present study, numbers of bacteroides, clostridia, and fusobacteria were significantly decreased when subjects were fed OF. All of these may be pathogenic, e.g., through their proteolytic capabilities and toxin production.

In conclusion, this study has shown that a relatively straightforward dietary manipulation may alter the composition of the gut bacteria to produce a potentially healthier community.

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