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Influence of Dietary Neosugar on Selected Bacterial Groups of the Human Faecal Microbiota

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Despite an increasing awareness of the relationships between the intestinal microbiota and health, there are few definitive guidelines about dietary interventions to adventitiously influence species composition of the microbiota. Therefore, standard microbiological methods were used to determine changes in the abundance of selected bacteria in anaerobic faecal samples from ten adult human volunteers who consumed 4 g of neosugar, a mixture of short-chain fructooligosaccharides, daily for 2 wks. The diet was not otherwise controlled. Total anaerobic counts increased or remained relatively stable in nine subjects. The percentage of total bacteria counts represented by aerobes increased over ten-fold, but enterics declined by over 90 per cent (from 2·3 per cent to <0·2 per cent). Although bifidobacteria increased from 1·3 per cent to 6·8 per cent of the total bacteria, there was wide individual variation in responses; bifidobacteria were not detected in two subjects at either date ($<10^4$ colony forming units/g faeces). Lactobacilli increased in six of the subjects, but were not a significant component of the microbiota at either date (<0·0001 per cent). Individual variation in responses to supplemental neosugar are probably caused by differences in diet, initial microbiota, and environmental conditions. The results demonstrate that supplementing the diet with neosugar influences the relative abundances of selected bacteria with some of the changes consistent with those considered advantageous.

KEY WORDS—Fructooligosaccharide; Bifidobacteria; Lactobacilli; Enterics; Anaerobes; Aerobes.

INTRODUCTION

An increasing awareness of the relationships between diet, the intestinal microbiota, and health has encouraged development of dietary strategies that promote the proliferation of bacterial groups perceived to be beneficial.[4] The majority of research has been directed at increasing the densities of bifidobacteria and lactobacilli in the gastrointestinal tract, which are thought to be important in human health.[10] Reported benefits associated with higher densities of bifidobacteria and lactobacilli include immunopotentiation, competition with putrefactive and pathogenic bacteria for nutrients and attachment sites, and production of volatile fatty acids that provide metabolic energy for the host, acidify the bowel, and inhibit growth of many potential pathogens.[12] Higher densities of bifidobacteria have also been reported to reduce biogenic production of carcinogenic substances.[8]

The most commonly pursued dietary strategies for increasing numbers of bifidobacteria and lactobacilli involve supplementing food products with either stable cultures of bifidobacteria and lactobacilli,[7] or, more recently, substances that promote the growth of beneficial bacteria already present in the gastrointestinal tract.[12,13] A number of naturally occurring substances have bifidogenic properties. For example, 'neosugar', a mixture of short-chain fructooligosaccharides, is known to increase faecal concentrations of bifidobacteria and lactobacilli when fed to elderly Japanese at levels of 8 and 15 g per day[5,11] and has shown benefits when included in animal diets.[3] Neosugar is not metabolised by many of the putrefactive and pathogenic bacteria which may be present in the gastrointestinal tract.[11] Even though neosugar is not hydrolysed by digestive enzymes produced by vertebrates,[14,18] volatile fatty acids produced by its fermentation can be absorbed and metabolised by the host. Recovery of about 50 per cent of ^{14}C-labelled neosugar in expired CO_2 indicates

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that neosugar provides 1·5 calories of metabolisable energy per g of neosugar.[6]

Population sizes of 10^9–10^{10} colony forming units (c.f.u.) of bifidobacteria per gram of faeces are not uncommon for adults, with wide individual variation probably correlated with differences in diet composition.[1,19] Therefore, the benefits of supplementing diets with neosugar to increase faecal populations of bifidobacteria and lactobacilli may be more pronounced for North American adults with a diet higher in fat, protein, and simple carbohydrates, and lower in complex carbohydrates, such as oligosaccharides, when compared to Japanese eating their traditional diet. The present study examines the responses of selected bacterial groups in the faeces of ten North American adult human subjects before and after consuming 4 g of supplemental neosugar daily for 14 days. To reduce variability in bacterial populations due to dietary and cultural differences, all subjects were at least second generation US citizens. All were of good health and assessments of feeding habits showed their diets to be typical of North American adults. Thus they would be expected to consume 2–8 g of inulin and oligosaccharides each day,[15] of which neosugar would represent about 0·1–1·0 g.[2]

MATERIALS AND METHODS

All phases of the project were approved by the MSU IRB (protocol approval no. 92-144). Each of ten adult volunteers (20–40 yrs of age; five females and five males) provided an initial faecal sample prior to and a second sample after consuming 4 g of neosugar daily for 14 d. Neosugar was provided by Nutrilite Products Inc. (Buena Park, CA, USA) and was presented in the morning as chewable tablets (1 g) and as a drink in the evening (3 g). The diets of the subjects were not controlled. None of the subjects received antibiotics 2 wks prior to or during the study.

Faecal samples (10–50 g) were collected in sterile containers, capped, and placed immediately (within 1 min after defaecation) in an anaerobic chamber (Forma, Model 1028) with a gas mixture of 80 per cent N_2; 10 per cent H_2; 10 per cent CO_2. The samples were processed in the anaerobic chamber according to established methods of anaerobic microbiology.[17] To reduce potential influences of exposure to atmospheric conditions and contaminants, after the faecal sample was in the chamber it was broken open, 1 g was obtained from the centre, and serially diluted in a ten-fold manner in prereduced, anaerobic diluent. Appropriate dilutions were plated in duplicate.

Total anaerobes were enumerated using Brucella blood agar, and bifidobacteria and lactobacilli were enumerated using bifidobacterium agar and lactobacillus agar, respectively, with a 3–5 d anaerobic incubation (35°C). Aerobic plate counts were performed using tryptic soy agar (TSA) with 5 per cent sheep blood for total aerobes and MacConkey agar for enterics; both were incubated for 48 h at 35°C in ambient atmospheric conditions.

Anaerobic and aerobic colony counts were performed after appropriate incubations. Colonies suggestive of bifidobacteria and lactobacilli were Gram-stained, evaluated for aerotolerance, subcultured, and identified using the Anident System (Analytical Products, Plainview, NY, USA). Counts were expressed as c.f.u. per g faecal matter.

Statistical analyses

Values in the table and figures represent means and standard errors. Analysis of variance and paired *t*-tests were used to test differences between initial and final bacterial populations, with $P<0·05$ accepted as the critical level of significance.

RESULTS

Table 1 presents population sizes for bacteria in faecal samples collected from the subjects before and after 14 d of supplementing their normal diets daily with 4 g of neosugar. Total bacterial population sizes increased in nine of the ten. The increases were caused by higher populations of anaerobes in six subjects and aerobes in nine subjects, with the magnitude of increases greater for the aerobes.

Bifidobacteria increased by an average of over 40-fold (Figure 1), but with a wide range of response (from 3·7- to over 300-fold increases). Bifidobacteria were not detected in faecal samples of two subjects at either date (below 10^4 c.f.u./g). Lactobacilli were detected in the faecal sample of only one subject before supplementing the diet with neosugar; the other nine subjects had $<10^3$ c.f.u./g. Because of low initial levels for lactobacilli, samples collected at day 14 were plated at lower dilutions. Although lactobacilli were detected in faecal samples of eight subjects, due to the low sensitivity of the assay at day 1, it is not

Table 1. Bacterial levels for faecal samples collected from the ten human subjects before and after consuming 4 g of neosugar per day for 14 days. Values represent colony forming units (c.f.u.) per gram faeces

	Total anaerobes		Total aerobes		Bifidobacteria		Lactobacilli		Enterics	
Subject	1	14	1	14	1	14	1	14	1	14
1	$1·32 \times 10^{10}$	$2·25 \times 10^{10}$	$2·93 \times 10^{6}$	$1·67 \times 10^{9}$	$1·03 \times 10^{8}$	$4·80 \times 10^{9}$	$<1·00 \times 10^{3}$	$5·20 \times 10^{3}$	$2·39 \times 10^{6}$	$1·04 \times 10^{8}$
2	$1·38 \times 10^{10}$	$4·30 \times 10^{10}$	$1·97 \times 10^{7}$	$2·82 \times 10^{9}$	$1·04 \times 10^{9}$	$9·10 \times 10^{9}$	$<1·00 \times 10^{3}$	$1·00 \times 10^{2}$	$1·10 \times 10^{7}$	$1·98 \times 10^{7}$
3	$1·88 \times 10^{10}$	$4·10 \times 10^{10}$	$3·90 \times 10^{7}$	$8·00 \times 10^{7}$	$1·79 \times 10^{8}$	$2·78 \times 10^{9}$	$<1·00 \times 10^{3}$	$8·30 \times 10^{4}$	$2·31 \times 10^{7}$	$4·00 \times 10^{7}$
4	$2·29 \times 10^{10}$	$2·05 \times 10^{10}$	$1·04 \times 10^{7}$	$2·28 \times 10^{8}$	$4·40 \times 10^{7}$	$5·90 \times 10^{8}$	$<1·00 \times 10^{3}$	$1·00 \times 10^{2}$	$2·70 \times 10^{5}$	$2·21 \times 10^{8}$
5	$1·91 \times 10^{10}$	$1·97 \times 10^{10}$	$1·45 \times 10^{6}$	$2·52 \times 10^{9}$	$<1·00 \times 10^{4}$	$<1·00 \times 10^{4}$	$<1·00 \times 10^{3}$	$8·00 \times 10^{2}$	$1·16 \times 10^{6}$	$8·50 \times 10^{4}$
6	$4·50 \times 10^{9}$	$2·52 \times 10^{10}$	$1·03 \times 10^{7}$	$3·10 \times 10^{10}$	$<1·00 \times 10^{4}$	$<1·00 \times 10^{4}$	$<1·00 \times 10^{3}$	$4·00 \times 10^{2}$	$5·80 \times 10^{6}$	$1·50 \times 10^{4}$
7	$1·63 \times 10^{10}$	$1·71 \times 10^{10}$	$2·45 \times 10^{9}$	$3·05 \times 10^{10}$	$7·00 \times 10^{6}$	$2·27 \times 10^{9}$	$<1·00 \times 10^{3}$	$2·00 \times 10^{3}$	$2·44 \times 10^{9}$	$1·44 \times 10^{7}$
8	$2·09 \times 10^{10}$	$6·20 \times 10^{10}$	$3·50 \times 10^{6}$	$2·35 \times 10^{9}$	$5·06 \times 10^{8}$	$6·90 \times 10^{9}$	$<1·00 \times 10^{3}$	$9·10 \times 10^{3}$	$8·40 \times 10^{5}$	$5·30 \times 10^{4}$
9	$2·83 \times 10^{10}$	$1·99 \times 10^{10}$	$1·80 \times 10^{6}$	$3·41 \times 10^{10}$	$1·89 \times 10^{8}$	$7·03 \times 10^{8}$	$9·20 \times 10^{5}$	$1·80 \times 10^{3}$	$1·46 \times 10^{6}$	$3·60 \times 10^{4}$
10	$9·50 \times 10^{9}$	$2·98 \times 10^{13}$	$1·60 \times 10^{9}$	$1·89 \times 10^{9}$	$5·60 \times 10^{7}$	$3·40 \times 10^{8}$	$<1·00 \times 10^{3}$	$8·14 \times 10^{5}$	$1·12 \times 10^{9}$	$1·75 \times 10^{9}$

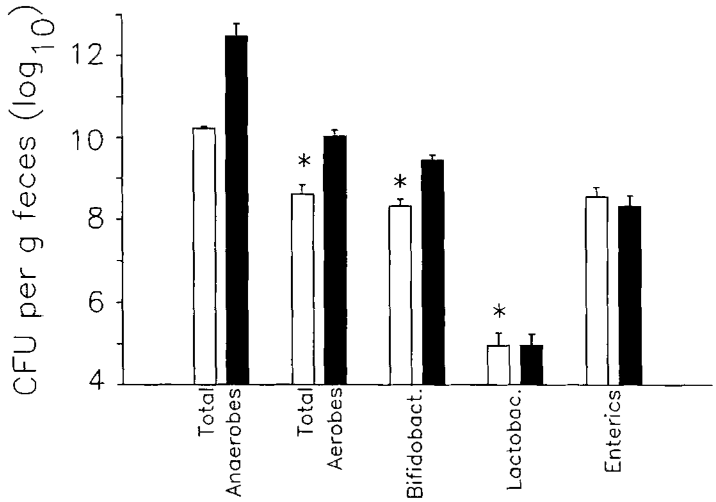

Figure 1. Population sizes for the different bacterial groups (c.f.u./g) in the faeces of the ten subjects before (open bars) and after 14 d (solid bars) of 4 g/d supplemental neosugar. Bars and lines represent means and standard errors. Differences ($P<0{\cdot}05$) between days 1 and 14 are indicated by *

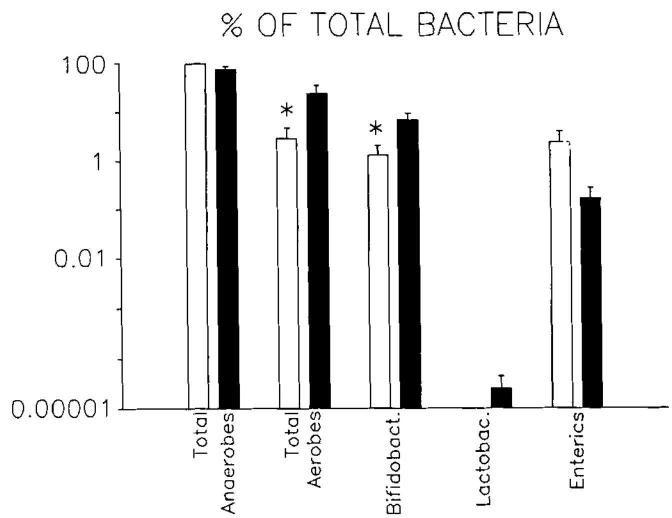

Figure 2. Percentages of the total bacterial counts represented by the different bacterial groups before (open bars) and after (solid bars) the ten subjects consumed 4 g of neosugar per day for 14 d. Bars and lines represent means and standard errors with significant differences ($P<0{\cdot}05$) between days 1 and 14 indicated by *

clear for four subjects (nos 2, 4, 5, 6) if lactobacilli actually increased. Interestingly, the single subject with lactobacilli at day 1 showed a decline (no. 9). Although this subject showed the largest increase in total aerobes, the number of enterics declined by almost 98 per cent. The remaining five subjects showed increases in lactobacilli.

Figure 2 illustrates the changes in numbers of bacteria expressed as percentages of the estimated total bacterial numbers. Total anaerobes dominated the microbiota at both dates. Although the numbers of anaerobes were higher at day 14 relative to day 1 (Figure 3), because of the dramatic increase in total aerobes there was a slight,

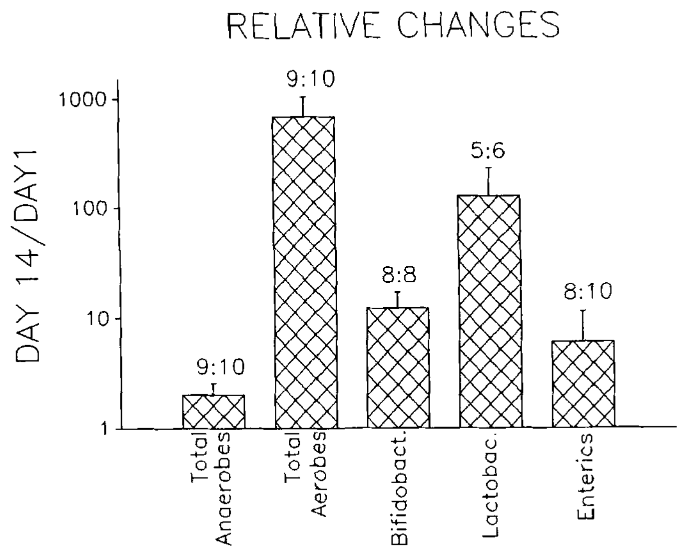

Figure 3. Relative changes in the population sizes for the different bacterial groups calculated as the ratio of counts at day 14 relative to day 1 when the ten subjects started to consume 4 g of neosugar each day. Ratios at the bottom of the figure indicate the number of subjects showing the specific trend (e.g. eight of the ten subjects showed increases in total anaerobes; only eight and six subjects could be used to calculate ratios for bifidobacteria and lactobacilli, respectively). Bars and lines represent means and standard errors

non-significant decline by day 14 in the percentage of total bacteria represented by anaerobes. Whereas bifidobacteria increased from 1·3 per cent to 6·8 per cent of the total microbiota, lactobacilli were never a significant fraction (<0·0001 per cent of total counts). Responses of the enterics were variable. Although enterics increased in five subjects, when all subjects are averaged there was a decline (Figure 1). When expressed as a percentage of the total microbiota, enterics decreased in eight of the ten subjects from an average of 2·3 per cent to 0·2 per cent (Figure 2).

DISCUSSION

The addition of 4 g of neosugar each day for 14 days to the diet of ten adult human subjects resulted in increased population sizes of bifidobacteria and lactobacilli. However, the increases in bifidobacteria and lactobacilli were not sufficient to account for the higher total bacterial counts at day 14. Therefore, the daily supplement of 4 g neosugar encouraged proliferation of other bacterial groups. Although positive identifications were not made, based on colony morphology and growth characteristics, *Streptococcus* spp. responded positively to the neosugar. These findings demonstrate that supplementing the typical diet of adult North Americans with neosugar effectively increases the population sizes and relative proportions of at least some bacterial groups perceived as being beneficial and causes a decline in the proportion of the microbacteria represented by enterics.

The variation in responses between subjects is likely to have been caused by a combination of at least two factors. First, there were individual differences at day 1 in the concentrations and relative proportions of the different bacterial populations. Exemplary are the two subjects without detectable bifidobacteria. Second, the subjects were exposed to different environmental conditions during the 14 day period, including diets.

Bifidobacteria are known to exhibit a dose-dependent response to levels of neosugar with up to 1000-fold increases at high levels of dietary neosugar.[5] The present findings with 4 g of neosugar are comparable to those of elderly, hospitalised Japanese patients fed 8 g neosugar per day for two weeks (about 10-fold increases in bifidobacteria).[5,11] There is also wide individual variation in responses of the bifidobacteria. Of the eight subjects with detectable bifidobacteria, seven showed modest, but significant increases (average of 2·5-fold ± 0·6; $P<0{\cdot}05$), with a large increase in one subject (no. 7, 313-fold). It is therefore possible, but not proven, that supplementing the diet with neosugar may provide greater benefits to some North American adults eating a diet higher in fat, protein and simple carbohydrates when compared to Japanese eating their traditional diet. However, even after consuming neosugar for 2 weeks, relative densities of bifidobacteria (up to 6·3 per cent of total bacterial counts) remained lower than those of Japanese eating a traditional diet (up to 20 per cent).[9]

The low densities of lactobacilli were not unexpected because population sizes of this group are known to be highest in the lower small intestine and proximal colon.[1] Because of low assay sensitivity and lactobacilli levels at both dates we were unable to determine if lactobacilli changed in four of the ten subjects. Of the remaining six, five showed increases in lactobacilli, although actual increases can not be computed. It is possible the increases would be of greater magnitude in the proximal bowel. If so, this would result in higher production of organic acids and other metabolites that acidify the bowel, liberation of free bile acids, and competition with pathogenic species for attachment sites and nutrients. It is interesting that increases in lactobacilli of the five subjects were greater than those reported for Japanese subjects fed 8 g of neosugar.[11]

Responses of the selected bacterial groups correspond with respective abilities to metabolise neosugar. Enterobacteriaceae, *Clostridium perfringens*, *Salmonella* spp. and many pathogenic groups are unable to metabolise neosugar, corresponding with lower absolute and relative populations sizes at day 14, though not all subjects showed this trend. In contrast, the fermentation of neosugar by most bifidobacteria and lactobacilli[11] coincides with increased population sizes and would provide potentially more than half of the energy content to the host as volatile fatty acids.[6,16] Furthermore, because diet is known to alter the metabolic activity of the microbiota,[4,16] supplemental neosugar may elicit shifts in the metabolic activities of bifidobacteria, lactobacilli, and possibly other bacteria. Therefore, the potential health benefits of the observed changes may be of greater magnitude than suggested by the increased population sizes of bifidobacteria and lactobacilli. This possibility has yet to be demonstrated.

In summary, the present results and those obtained from Japanese adults demonstrate supplementing the diet with neosugar selectively encourages the proliferation of bacterial groups perceived as being beneficial (e.g. bifidobacteria and lactobacilli). The benefits may be greater for subjects who consume the typical North American diet which is low in fibre, and high in fat and animal protein and have lower levels of bifidobacteria and lactobacilli than Japanese eating their traditional diet. By acting like a 'fertiliser', neosugar encourages the growth of the host's existing microbiota, and thereby avoids perceptual and procedural problems associated with adding live bacterial cultures to food products. Future studies using controlled diets are needed to evaluate the responses of the microbiota to varying doses and periods of supplemental neosugar, and to better define health benefits.

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