Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects1–3

Randal K Buddington, Carol H Williams, Sui-Chi Chen, and Steven A Witherly

ABSTRACT The influence of dietary fructooligosaccharide (neosugar) on the fecal flora and activities of reductive enzymes was studied in 12 healthy, adult human subjects fed a controlled diet for 42 d and given 4 g neosugar/d between days 7 and 32. Fecal samples were collected before, during, and after supplementation with neosugar to enumerate total anaerobes, aerobes, bifidobacteria, and enterobacteria, and to assay for β-glucuronidase, nitroreductase, and glycocholic acid hydroxylase. Although the controlled diet caused an increase in total anaerobes and bifidobacteria, the highest densities occurred during supplementation with neosugar. Total aerobes and enterobacteria were less affected by diet and neosugar. Neosugar caused β-glucuronidase and glycocholic acid hydroxylase activities to decrease 75% and 90%, respectively; both increased after supplementation with neosugar was stopped. Nitroreductase activity declined 80% after the control diet was started, but was not affected by neosugar. These findings indicate that 4 g neosugar/d alters the fecal flora in a manner perceived as beneficial by decreasing activities of some reductive enzymes. Am J Clin Nutr 1996:63:709–16.

KEY WORDS Fructooligosaccharide, bifidobacteria, microflora, reductive enzymes, humans, fecal flora

INTRODUCTION The complex interactions between microflora resident in the gastrointestinal tract and health are now recognized from clinical studies, but remain poorly understood. Of particular interest is the relation between the species composition and metabolic characteristics of the microflora and the risk of colon cancer. Although direct evidence for this relation is lacking, certain groups of the microflora are known to produce toxins and carcinogens from endogenous and exogenous substrates (1, 2). Diet is known to influence the species composition and metabolic characteristics of the intestinal microflora, and therefore the conversion of procarcinogens to carcinogens (3–5). Notable is the association among the high-fat, high-protein diet of Western societies, higher densities of putrefactive bacteria, elevated activities of reductive enzymes, and an increased incidence of colorectal cancer (1). Further evidence is provided by the inverse relation between risk of colon cancer and dietary fiber (6). These and other findings have highlighted the need to better understand the role of diet in moderating the interactions between the microflora and health.

The most likely influence of diet is the increased proliferation of bacterial groups that have different abilities to convert procarcinogens to active forms. For example, activities of reductive enzymes are lower in bifidobacteria and lactobacilli relative to Escherichia coli and clostridia (2). Corresponding with this, dietary interventions that promote bifidobacteria and lactobacilli and reduce E. coli and clostridia are perceived as beneficial. Previous studies showed that this can be accomplished by supplementing the diet with fructooligosaccharides and other forms of oligosaccharide (7–11). However, the specific health benefits of such dietary interventions in vivo are still not well established.

In light of the recent interest in “prebiotics,” which selectively encourage the growth of species that are perceived as beneficial (12), the present study examined the effect of supplementing a controlled diet with a short-chain fructooligosaccharide (neosugar, or Nutraflora; Golden Technologies, Westminister, CO). Classical microbiology techniques were used to describe quantitative and qualitative responses of the microflora and involved enumerating total anaerobes and aerobes, enterobacteria, and bifidobacteria. Metabolic characteristics were evaluated by assaying for three reductive enzymes. Two of the enzymes hydrolyze exogenous substrates of dietary origin. β-Glucuronidase, which has been implicated in carcinogenesis, releases aglycones from glycosides. Nitroreductase produces aromatic amines, often from nitro groups associated with pollutants (1). The third enzyme, glycocholic acid hydroxylase, is involved with metabolism of bile acids and may be linked to the increased risk of cancer associated with high-fat diets (2).

SUBJECTS AND METHODS

Recruitment of subjects All phases of the research were approved by the Mississippi State University Institutional Review Board Committee. We

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recruited 12 subjects (6 males and 6 females) enrolled in undergraduate and graduate research programs at Mississippi State University. This decision was based on three considerations. First, we sought participants that would adhere to protocols. Second, we wanted a population of similar age and exposed to comparable environmental conditions. Third, to promote adherence and retention in the program, we sought individuals who could eat most of the meals as a group. Informed consent was obtained from all the subjects. The subjects ranged in age from 20 to 34 y, were in good health, and had not taken antibiotics for ≥ 1 mo before starting the study. Ten of the subjects were at least second-generation US citizens. The remaining two students were from China and Indonesia.

Assessment of subjects’ normal diets
Before the study, each subject provided a 3-d record of food and fluid consumption, which was used to estimate normal intake of macronutrients and energy. Intake of energy and fiber, and percentage of energy from fat, protein, and carbohydrate, were assessed by using published values (NUTRITIONIST III, version 7.0; N-Squared Computing, Salem, OR).

Composition and feeding of the controlled diet
A 7-d cycle menu was used during the course of the 42-d controlled feeding period. A detailed description of the daily menus and the amount for a single portion is provided in Appendix A. During the week (Monday breakfast to Friday lunch), meals were provided by the Mississippi State University dining services. Friday night’s meal was provided by a local restaurant. All of the week’s meals were prepared to exact specifications. Weekend meals (Saturday morning to Sunday night) were selected such that the students could leave the campus yet still adhere to the controlled diet. To minimize nutrient variability, foods were purchased in case lots. Baked goods, milk, and fresh produce were purchased from the same vendor weekly.

All meals were provided to the subjects in controlled portions. Energy intake was adjusted during the first d of the study by altering the size of the portions to prevent weight changes of > 10% of original body weight and to ensure satiety of the subjects. Subjects were requested to eat the entire meal and were allowed to do so at their leisure. Some subjects preferred to consume meals throughout the period preceding the next meal. When a subject was unable to consume the entire meal, he or she was asked to eat the various components in equal proportions. Soft drinks (diet and regular) were provided for lunch and dinner and intake was not restricted; additionally, coffee and tea intakes were not limited. Although graham crackers were provided as a snack for subjects who became hungry between meals, none of the subjects reported consuming the crackers during the feeding period. The subjects kept a daily log to record changes in stool volume, consistency, and frequency; flatulence; and any other aspects of general health.

An extra portion was always prepared for each meal served from Monday to Friday. At random intervals the extra portion was collected and frozen (− 20 °C), and this continued until the entire menu for each of the weekdays had been obtained. Representative menu items consumed on Saturday and Sunday were collected during a single weekend. All three meals for each day were pooled, homogenized in a blender, lyophilized, and sent to Amway Corporation (Hazelton, WI) for proximate analysis. Energy content was determined by bomb calorimetry. Fat and protein contents were assayed by Soxhlet extraction and Kjeldahl analysis (percentage nitrogen × 6.25), respectively. Ash amounts were determined by exposing the pooled samples to 625 °C for 48 h. Total carbohydrate was estimated by difference.

The neosugar supplement
On each day between days 7 and 32 of the controlled diet period, the subjects consumed 4 g neosugar. A portion of the neosugar (1 g) was taken in the morning in the form of chewable tablets. The remaining 3 g was provided in the evening as a flavored drink mix that was added to water. In addition to the neosugar, the tablets and drink mix provided an additional 20 g carbohydrate, mostly fructose as a sweetener.

Collection of samples
Each subject provided nine stool samples. The first sample was collected at the start of the controlled diet period. The second sample was obtained 7 d later, and samples 3–7 were collected after 5, 10, 15, 20, and 25 d of supplemental neosugar, respectively (corresponding with days 12, 17, 22, 27, and 32 of the controlled diet period). The final two samples were obtained 5 and 10 d after supplementation with neosugar was stopped (days 37 and 42 of the controlled diet period). Subjects were required to collect fecal samples in sterile cups with caps in a bathroom near the microbiology laboratory. As a result, all samples were in the anaerobic chamber within 2 min of defecation, and had minimal exposure to atmospheric conditions and contaminants.

Microbiology
Fresh fecal samples were obtained from the center of each stool and diluted 1:10 with reduced 0.05% yeast extract. The samples were homogenized and filtered (0.2-mm filter) to remove large particulates and serial dilutions were prepared from 10−2 to 10−10. At the time the samples were diluted, the consistency was evaluated on a scale of 1 to 4, with the same person evaluating all the samples. Firm, hard stools were assigned a value of 1 whereas diarrhea was assigned a value of 4.

Duplicate spread plates were made with 0.1-mL aliquots of appropriate dilutions. Total anaerobes were counted through use of CDC anaerobic blood agar (BBL, Cockeysville, MD); total aerobes and enterobacteria were counted through use of trypticase soy agar II with 5% sheep blood and MacConkey II agar (BBL). A selective medium was used to count Bifidobacterium spp. (13). No attempts were made to distinguish the species of enterobacteria and bifidobacteria.

Anaerobic plates remained in the chamber for incubation (3–5 d at 35 °C) and counting of colonies. Colony types growing on the different media were numbered and their characteristics described and recorded. Total anaerobe counts were corrected for facultative anaerobes by evaluating aerotolerance of the different colony types. Therefore, reported values represent obligate anaerobes. Bifidobacterium spp. form white mucoid colonies on the selective medium used, but strepto-
cocc, lactobacilli, and actinomycetes form similar colonies. Therefore, suspicious colonies were gram stained, examined microscopically, and tested for aerotolerance. *Bifidobacterium* sp. are gram-positive, obligate anaerobic, non-spore-forming bacilli that are typically branching and bifurcated. Colonies believed to be *Bifidobacterium* spp. were verified (API An-IDENT, Hazelwood, MO).

After the anaerobic plates were prepared, the dilutions were removed from the chamber and used to prepare aerobic plates. The aerobic plates were incubated at 35 °C in atmospheric conditions for 24–48 h before colonies were counted. Total aerotolerant colonies were counted on the basis of growth on trypticase soy agar II with 5% sheep blood. Aerotolerant colonies growing on MacConkey II agar were considered representative of enterobacteria, without distinction regarding lactose fermentation. Duplicate plate values were averaged and bacterial densities were expressed as number of colony forming units (CFU) per gram wet stool.

**Enzyme assays**

A sample of fresh stool from each subject was placed in an airtight vial while in the anaerobic chamber and stored at −75 °C until analyzed (within 2 wk after collection of the final sample). The sample vials were placed in the anaerobic chamber and after partial thawing, a portion of each stool was homogenized in cold (2–4 °C) buffer containing 0.1 mol potassium phosphate/L (pH 7.0) with 0.15 mol NaCl/L, which had been placed in the anaerobic chamber 24 h before the assay to remove dissolved oxygen. The homogenates were transferred to capped microfuge tubes, removed from the chamber, and centrifuged for 3 min at 13 800 × g at room temperature.

Two of the tubes were returned to the chamber to be assayed for nitroreductase and the other two were kept out of the chamber for β-glucuronidase and glycocolic acid hydroxylase assays. Except when in the microfuge, the homogenates were kept on ice.

Nitroreductase activity was assayed at 35 °C in the anaerobic chamber by adding aliquots of supernate to equal volumes of deoxygenated potassium phosphate buffer (0.1 mol/L with 0.9% NaCl, pH 7.4). The reaction was initiated by adding oxygen-free, 10 mmol *p*-nitrobenzoic acid/L in 70% ethanol, and stopped after 10 and 60 min by adding aliquots of the reaction mixture to 20% trichloroacetic acid. After the samples were centrifuged (3000 × g for 5 min at room temperature), the concentration of *p*-aminobenzoic acid in the supernate was determined by sequentially adding sodium nitrite, ammonium sulfamate, and *N*-(1-naphthyl)ethylenediamine dihydrochloride; recording absorbance at 545 nm; and comparing this measurement with that for a *p*-aminobenzoic acid standard.

β-Glucuronidase was assayed at 37 °C in atmospheric conditions with *p*-nitrophenyl-β-glucuronide as the substrate (3 mmol/L in buffer containing 0.1 mol potassium phosphate/L, pH 7.0). The reaction was stopped after 5 and 20 min by adding cold Na2CO3 (1 mol/L). After the samples were centrifuged (3000 × g for 5 min at room temperature), concentrations of *p*-nitrophenol were determined by comparing absorbance at 405 nm with a standard.

Glycogallic acid hydroxylase was also assayed at 37 °C in atmospheric conditions by adding aliquots to 1 mmol glycolgallic acid/L in potassium phosphate buffer (0.02 mol/L, pH 5.8). Aliquots were removed at 5 and 20 min and added to 20% trichloroacetic acid. After the samples were centrifuged (3000 × g for 5 min), glycine concentrations were determined with ninhydrin reagent and a glycine standard.

All assays were replicated, and repeated when CVs exceeded 10%. Concentrations of reaction products present in the stool and substrates were accounted for by simultaneously adding stop and substrate solutions to fecal supernate. Enzyme activities (micromoles of substrate hydrolyzed per minute) were normalized to wet stool weight and total densities of anaerobic bacteria.

**Statistics**

Values in tables and figures represent means and SEs. The influences and interactions of diet and the neosugar supplement on bacterial densities and enzyme activities were assessed by analysis of variance (ANOVA) with the SAS statistical software package (SAS Institute Inc., Cary, NC). When a significant effect was detected (*P* < 0.05), Student-Newman-Keul’s test was applied to search for differences between specific sampling intervals. Potential sex effects were assessed by ANOVA.

**RESULTS**

**Diet composition**

The 3-d diet history showed that US citizens ate a diet with a higher percentage of energy from fat and protein and less fiber than did the diets of the two subjects from China and Indonesia (Table 1). When averaged over the entire week, the control diet provided 27% of energy as fat and 22% as protein (Table 2).

**General observations**

Reviews of the personal logbooks and interviews with the subjects revealed that no subjects had digestive or health problems requiring antibiotics during the course of the feeding trial. Interestingly, two subjects reported that some meals apparently interacted with neosugar to cause looser and more frequent stools. Both subjects thought the problems were associated with sweet potatoes. They did not report similar symptoms before and after receiving supplemental neosugar, or from past experiences with the same food items. Weight loss was reported by seven of the subjects, all of whom considered themselves to be overweight. No subjects reported weight gains of

<p>| TABLE 1 |
| Daily intakes of the subjects before the study&lt;sup&gt;1&lt;/sup&gt; |</p>
<table>
<thead>
<tr>
<th>US citizens (n = 10)</th>
<th>Non-US citizens (n = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>9560 ± 741</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>10 ± 2</td>
</tr>
<tr>
<td>Crude fiber (g)</td>
<td>3 ± 1</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>18 ± 1</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>39 ± 2</td>
</tr>
</tbody>
</table>

<sup>1</sup> Because of distinct differences in feeding habits on the basis of 3-d diet histories, the 12 subjects were separated into two groups consisting of US and non-US citizens. ± ± SE.

<sup>2</sup> Significantly different from US citizens, *P* < 0.05.
TABLE 2
Pooled energy content and composition of the three meals consumed each day by the subjects during the 40-d controlled feeding period

<table>
<thead>
<tr>
<th></th>
<th>Monday</th>
<th>Tuesday</th>
<th>Wednesday</th>
<th>Thursday</th>
<th>Friday</th>
<th>Saturday</th>
<th>Sunday</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/100 g dry wt)</td>
<td>1824</td>
<td>1903</td>
<td>1887</td>
<td>1845</td>
<td>1866</td>
<td>1899</td>
<td>1937</td>
</tr>
<tr>
<td>Fat (% by wt)</td>
<td>11.1</td>
<td>13.6</td>
<td>14.3</td>
<td>11.3</td>
<td>13.0</td>
<td>13.8</td>
<td>15.3</td>
</tr>
<tr>
<td>Protein (% by wt)</td>
<td>20.3</td>
<td>25.6</td>
<td>19.1</td>
<td>22.4</td>
<td>24.8</td>
<td>19.8</td>
<td>19.8</td>
</tr>
<tr>
<td>Carbohydrate (% by wt)</td>
<td>63.6</td>
<td>57.6</td>
<td>61.4</td>
<td>62.2</td>
<td>57.5</td>
<td>62.6</td>
<td>61.5</td>
</tr>
<tr>
<td>Ash (% by wt)</td>
<td>4.9</td>
<td>3.2</td>
<td>5.1</td>
<td>4.1</td>
<td>4.7</td>
<td>3.8</td>
<td>3.4</td>
</tr>
</tbody>
</table>

> 2% of original body weight, nor did any subject complain of excessive hunger or a need to eat graham crackers between meals.

Although all 12 subjects remained enrolled in the study, one subject was removed from the analysis because of persistent constipation that either prevented, or delayed by ≥ 1 d, collection of several stool samples. Unlike the other participants, this subject reported that the addition of neosugar to the diet reduced stool frequency and volume. All other subjects were able to provide stool samples on the designated collection dates. Although total volume and water content of the stools were not measured, observations of stool consistency at the time of sample collection indicated that stools of the remaining 11 subjects became softer after the start of the neosugar supplementation period. Stools of all subjects became even less formed after supplementation with neosugar was stopped. Flatulence was reported by some of the subjects, but in most cases this began after the start of the control diet without an increase after the neosugar was added.

Microbiology

In addition to measurements presented as CFU/g wet stool wt, densities of bifidobacteria, aerobes, and enterobacteria are expressed as percentages of total anaerobes to examine shifts in the relative proportions of the different groups. The controlled diet alone resulted in higher densities of total anaerobes (Figure 1), as evident by comparing values for day 1 with those for days 7, 37, and 42 (P < 0.05). Anaerobe densities were highest when the diet was supplemented with neosugar (P < 0.0003 and < 0.05 for comparisons with densities before and after the supplementation period).

Densities of bifidobacteria were also higher after the first week of the control diet alone (Figure 2). Densities increased about one logarithm more with supplementation with neosugar (P < 0.03). The decline in bifidobacteria after neosugar was removed from the diet was not significant. Bifidobacteria represented ~4% of total anaerobes before the diet was supplemented with neosugar. Relative densities were unchanged after 5 d of neosugar, then increased and remained higher even after the neosugar was discontinued.

Aerobes never exceeded 0.3% of total anaerobes (Figure 2). Except for the transient elevation after 15 d of supplemental neosugar, densities of aerobes did not differ between sampling dates and were comparable before, during, and after supplementation with neosugar. Enterobacteria represented 33%, 41%, and 40% of the aerobes before, during, and after supplementation with neosugar, respectively (coinciding with 0.07%, 0.09%, and 0.10% of total anaerobes). The patterns of responses of enterobacteria to the controlled diet and the neosugar supplement (Figure 2) were similar to those described for aerobes.

Activities of reductive enzymes

Enzyme activities were normalized to wet stool weight and, as an additional indicator of metabolic activity, to densities of total anaerobes. Activity of β-glucuronidase per gram stool differed between sample dates (P < 0.02); the highest values were detected 5 d after the start of supplementation with neosugar [29 ± 8 (μmol/L) · g⁻¹ · h⁻¹; which was significant for all comparisons except with sample 9]. Activity per gram stool was comparable at all other sample dates. However, β-glucuronidase activity normalized to 10¹⁰ anaerobes was lowest in the last four samples taken during the period of supplemental neosugar (Figure 3). Activity per anaerobe remained low for ≥ 5 d after supplementation with neosugar was stopped, but by the end of the study was significantly higher (P < 0.05).

Activity of glycocholic acid hydroxylase per gram stool and per 10¹⁰ anaerobes also differed between sample dates (P < 0.0001 and 0.005, respectively). Activity increased significantly after neosugar was withdrawn: the highest activity was measured at the conclusion of the study [37 (μmol/L) · g⁻¹ · h⁻¹; Figure 3]. There was a transient but nonsignificant increase in activity per gram stool after neosugar supplementation was started (values for samples 4 and 5 were on average more than threefold higher than those of other samples collected during supplementation with neosugar). However, because of higher anaerobe densities, activity per 10¹⁰ anaerobes was not significantly higher. Continued consumption of

![Figure 1](https://example.com/figure1.png)

**FIGURE 1.** Densities of total anaerobes present in the nine stool samples collected from the human subjects before, while, and after the control diet was supplemented with 4 g neosugar. Values for before and after the subjects received neosugar are presented as open circles; values for during the period of supplemental neosugar are presented as filled circles. x ± SE; CFU, colony forming units.
neosugar led to a decline in activity; samples 6 and 7 had the lowest activities per gram stool [2.6 and 8.7 (μmol/L)·g⁻¹·h⁻¹, respectively] and per 10¹⁰ anaerobes. Activity of nitroreductase was significantly higher at the start of the study, whether expressed per gram stool [6.3 ± 2.5 (μmol/L)·g⁻¹·h⁻¹] or per 10¹⁰ anaerobes (Figure 3). There was no noticeable influence of neosugar, as evident from the lack of change during and after the period of supplementation.

DISCUSSION

The present study shows that dietary interventions can increase densities of anaerobic microbiota, including bifidobacteria, by several logarithms. Furthermore, the increases corresponded to lower activities of two fecal reductive enzymes (β-glucuronidase and glycocholic acid hydroxylase) associated with conversion of procarcinogens to carcinogens.

Bacterial responses

The influence of diet on the fecal flora occurs over two time scales. First, the diet consumed during an individual’s life influences both total fecal bacterial densities and the relative abundance of the constituent species, as evident from comparisons of stool samples from citizens of Japan (9) and the United States (7, and the present study). Coinciding with this observation, the fecal flora of the two subjects from China and Indonesia, who were eating their traditional diets before the start of the controlled diet, differed from that of the US citizens. The differences between US and non-US citizens persisted, but were less evident at the conclusion of the study. Second, the fecal flora exhibit short-term responses to diet composition, although until recently many investigators considered these to be inconsistent or minor (3, reviewed in reference 8). The present study shows that a switch to a diet lower in fat and protein and with a higher fiber content is sufficient to increase the densities of anaerobes. Responses were rapid and were evident after only 5 d of the controlled diet. The fecal flora of the two subjects from China and Indonesia showed
little change, reflecting the less dramatic changes in diet composition. The higher densities of bifidobacteria during the period of supplemental neosugar (on average, $7.2 \times 10^9$ CFU/g) compared with before and after the supplemental period (average of before and after, $2.5 \times 10^9$ CFU/g; $P < 0.02$) correspond to an ability to use neosugar as a metabolic substrate (9, 14). As a result, the proportion of total anaerobes represented by bifidobacteria increased from 3.5% before to 9.5% during the supplemental neosugar period ($P < 0.01$). However, this represented only $\approx 14\%$ of the increase in total anaerobes. Similarly, the decline in bifidobacteria after supplementation with neosugar was stopped represented only 13% of the decrease in total anaerobes. These findings are comparable with those of Gibson et al (8), who reported that bifidobacteria increased from 8% of total anaerobes before to 20% after supplementation of a control diet with 15 g oligofructose/d, and represented 32% of the increase in total anaerobes. These data provide three insights about bacterial responses to neosugar. First, neosugar elicits responses that augment those associated with a healthy diet that is lower in fat and higher in complex carbohydrates, particularly fermentable fiber. Second, bacterial groups other than bifidobacteria respond to the presence, or absence, of neosugar in the diet. Although we did not attempt to identify the other groups, likely candidates include the lactobacilli and streptococci and a limited number of other groups (7, 14). Third, the significant declines in densities of bifidobacteria, but not of aerobes and enterobacteria, after supplementation with neosugar stopped show that the effects are transient. These observations suggest that the potential health benefits of neosugar persist for only as long as it is present in the diet. However, the declines in total anaerobes and bifidobacteria after neosugar supplementation was stopped were not as rapid as those reported after supplementation with probiotics was stopped (15).

Except for the transient, and as yet unexplained, increase in densities after 10 d of neosugar, aerobes and enterobacteria were not responsive to diet and neosugar. These findings agree with previous reports of the apparent inability of many aerobes to use neosugar as a substrate (8, 9). Of particular importance were the reductions of clostridia and fusobacteria in subjects fed oligofructose (8). Similar to our results, coliforms were not affected by dietary treatment.

**Enzyme activities**

There are known differences between bacterial species in the activities of reductive enzymes (2). The present study shows that a diet that increases densities of anaerobic bacteria, including bifidobacteria, results in lower activities of some reductive enzymes implicated in carcinogenesis. However, increasing dietary fiber alone is not sufficient to alter enzyme activities (15). Our findings corroborate results from studies with probiotics, which showed that increasing the proportion of the fecal flora represented by bifidobacteria and lactobacilli is associated with lower activities of reductive enzymes (15–18). Although less well understood, species composition of the microflora is also thought to influence absorption of mutagens and carcinogens (19). From a public health perspective, one factor that has been implicated in the lower incidence of colon cancer in Asian populations is the traditional diet, which is associated with higher densities of bifidobacteria and other bacteria considered to be beneficial.

The reciprocal shifts in activities of glycocholic acid hydrolase and $\beta$-glucuronidase and densities of bifidobacteria during and after supplementation with neosugar can be caused by a combination of diet-induced shifts in the species composition of the fecal flora and in the metabolic characteristics of individual species or strains (3, 4). Although our data are not adequate to determine the relative role of each, the different patterns of responses after supplementation with neosugar were stopped for $\beta$-glucuronidase (delayed), glycocholic acid hydrolase (immediate), and nitroreductase (no response) suggest that fecal activities of these three enzymes 1) are regulated independently by different factors and processes and 2) respond differently to diet composition.

Even with optimal dietary intervention, there will be residual activity. The actual health risk may be related to a combination of total enzyme activity and duration of exposure. A review of the subjects’ daily dietary and health logs and interviews showed that the controlled diet increased stool frequency, probably as the result of a higher fiber content relative to the subjects’ normal diets. A further increase was noted by most of the subjects after neosugar was added to the diet. Although stool volumes were not determined, on the basis of the higher stool water content and information related to us by the subjects, there was an apparent increase. This would be consistent with more rapid transit times. If so, the combination of lower enzyme activity and more rapid transit would effectively reduce exposure to potential carcinogens produced by the reductive enzymes. Although an interesting difference between sexes in $\beta$-glucuronidase activity has been reported (17), sex-related differences were not detected for any of the three hydrolases.

**Additional relevance to health**

A population of microflora with higher densities and proportions of bifidobacteria and lactobacilli is thought to confer other benefits. Fermentation of undigested dietary components provides valuable metabolites (eg, volatile fatty acids and vitamins). Lactic acid bacteria, such as the bifidobacteria, may also increase resistance to disease by reducing the growth of pathogenic and putrefactive bacteria by lowering pH, competing directly for substrates and mucosal attachment sites, producing inhibitory molecules, and stimulating the enteric immune system (14, 20, 21).

**Perspectives**

The present study and recent reports of the influence of neosugar and other related carbohydrates highlight the need for further studies. We draw attention to five. First, the dose-response relation between neosugar and the microflora has not been adequately established. Related to this, and even less well understood, is the degree of change in the fecal flora necessary to cause benefits. Second, there are two basic approaches that can be used to alter the microflora: probiotics and dietary changes or supplements (prebiotics) that selectively encourage the growth of species that are perceived as beneficial (12). Probiotics have been shown to be effective in lowering the
activities of reductive enzymes, but the influence persists for only as long as the microbes are fed (16). When an ecosystem-based perspective is considered, the use of dietary supplements may prove to be a more effective approach for modifying the fecal flora. Specifically, even though communities within established ecosystems, such as the human adult colon, are often resistant to invasion by new species, they are usually responsive to the addition of nutrients. Neosugar appears to represent a nutrient that selectively encourages the growth of specific bacterial groups that are already adapted to and present in the gut. The stability of the colonic ecosystem is apparent from the return of the microflora to a state similar to that at the start after supplementation with neosugar was stopped. Third, the present study did not attempt to identify bacterial species or strains. However, characterizing the responses of a group, such as the bifidobacteria, is probably not adequate. Different species and strains of bifidobacteria are known to vary in how they respond to oligofructose (14). Fourth, fecal microbiology does not provide insights about the responses of bacterial populations in the small bowel or adhering to the intestine. However, these bacteria are likely to have the greatest effect on health of the host. Finally, the present and previous studies represent a relatively simplistic view of the dietary responses of the microflora and the activities of associated enzymes. Future studies are needed to elucidate the interactions between diet and other variables known to influence the composition and metabolic activities of the microflora. These include stress, physiologic state, age, host species or strain, and a variety of environmental factors (5).

REFERENCES


APPENDIX A

Components and quantities for a single portion for each of the meals making up the 7-d rotating diet that was fed to the subjects during the 6-wk study period.

<table>
<thead>
<tr>
<th>Monday</th>
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<tbody>
<tr>
<td><strong>Breakfast</strong></td>
<td><strong>Lunch</strong></td>
</tr>
<tr>
<td>Corn flakes (21 g)</td>
<td>Ham sandwich (57 g ham on 2 slices bread with 15 g mayonnaise)</td>
</tr>
<tr>
<td>Whole-wheat toast (2 slices with 28 g grape jelly)</td>
<td>Tossed salad (228 mL)</td>
</tr>
<tr>
<td>Orange juice (118 mL)</td>
<td>Potato chips (28 g)</td>
</tr>
<tr>
<td>1%–Fat milk (122 mL)</td>
<td>Canned peach halves (2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Dinner</strong></th>
<th><strong>Tuesday</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked boneless chicken breast (57 g) with mushrooms and herbs</td>
<td>Corn flakes (21 g)</td>
</tr>
<tr>
<td>Mashed potatoes (114 mL)</td>
<td>Plain sugar-glazed doughnut (1)</td>
</tr>
<tr>
<td>Green beans (114 mL)</td>
<td>Orange juice (118 mL)</td>
</tr>
<tr>
<td>Whole-wheat bread (1 slice)</td>
<td>1%–Fat milk (122 mL)</td>
</tr>
<tr>
<td>Fresh apple (1)</td>
<td>Plain tuna (57 g)</td>
</tr>
<tr>
<td>Whole-wheat bread (2 slices)</td>
<td>Mayonnaise (15 g)</td>
</tr>
<tr>
<td>Mayonnaise (28 g)</td>
<td>Sweet relish (28 g)</td>
</tr>
<tr>
<td>Fresh orange (1)</td>
<td></td>
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</tbody>
</table>
APPENDIX A
Continued

<table>
<thead>
<tr>
<th></th>
<th>Menu</th>
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</thead>
</table>
| Dinner | Hamburger patty (57 g) with beef gravy (30 mL)  
                              White rice (114 mL)  
                              Steamed broccoli (114 mL) with fresh lemon juice  
                              Whole-wheat bread (1 slice)  
                              Pineapple packed in juice (114 mL) |
|       | Breakfast | Blueberry muffin (1)  
                              Corn flakes (21 g)²  
                              Banana (1/2)  
                              1%-Fat milk (122 mL) |
|       | Lunch | Tossed salad (228 mL) with thousand island dressing (28 g)  
                              Fresh orange (1) |
|       | Dinner | Roast turkey (57 g)  
                              Baked sweet potato (114 mL)  
                              Steamed spinach (114 mL) with fresh lemon juice  
                              Tossed salad (228 mL) with Italian dressing (28 g)  
                              Corn bread (58 g)  
                              Fruit cocktail in water (114 mL)  
                              Margarine (5 g) |
|       | Thursday | Raisin bran muffin (1)  
                              Cheerios (18 g)³  
                              1%-Fat milk (122 mL)  
                              Banana (1/2) |
|       | Lunch | Roast pork (57 g) with mushroom gravy (30 mL)  
                              Rice pilaf (114 mL)  
                              Steamed mixed vegetables (114 mL)  
                              Whole-wheat bread (1 slice)  
                              Canned pear halves (2) |
|       | Dinner | Baked boneless chicken breast (57 g)  
                              Scalloped potatoes (114 mL)  
                              Parsley carrots (114 mL)  
                              Tossed salad (228 mL) with thousand island dressing (28 g)  
                              Whole-wheat bread (1 slice)  
                              Fresh apple (1) |
|       | Friday | Corn flakes (21 g)²  
                              Whole-wheat bread (1 slice) with strawberry jam (14 g)  
                              Banana (1/2)  
                              1%-Fat milk (122 mL) |
|       | Lunch | Baked fish (58 g) with lemon and herbs  
                              Pimento whole-kernel corn (115 mL)  
                              Coleslaw (114 mL)  
                              Whole-wheat bread (1 slice)  
                              Fresh orange (1) |
|       | Dinner | Grilled sirloin steak (85 g)  
                              Baked potato (1) with light sour cream (15 g)  
                              Tossed salad (228 mL) with Italian dressing (28 g)  
                              Hard white roll (1)  
                              Beer (244 mL) |
|       | Saturday | Sausage egg McMuffin (1)⁴  
                              McDonald’s chef salad⁴  
                              Crackers (28 g)  
                              McDonald’s vanilla frozen yogurt (90 g)⁴ |
|       | Lunch | Frozen mesquite chicken dinner⁴  
                              Whole-wheat bread (1 slice)  
                              Fresh apple (1)  
                              Graham crackers (4) |
|       | Dinner | Breakfast fajita⁴  
                              Orange juice (118 mL)  
                              Frozen beef dinner⁵  
                              Whole-wheat bread (1 slice)  
                              Fresh apple (1)  
                              Small French fries with ketchup (18 g)  
                              Fresh pear (1) |

¹ Coffee, tea, and soft drinks (sugar and sugar-free) were not restricted and were available for all meals.  
² Kelloggs, Battle Creek, MI.  
³ General Mills, Minneapolis, MN.  
⁴ Purchased from McDonalds’ restaurants the day of consumption.  
⁵ Healthy Choice frozen dinners (Con Agra Frozen Foods, Omaha, NE) were purchased frozen and heated by the subjects before consumption.