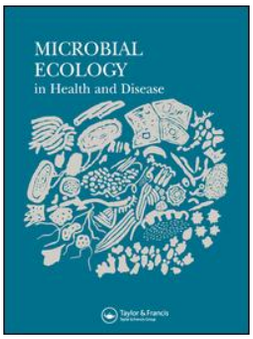

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The Colonic Bacteria and Rates of Small Intestinal Nutrient Transport of Mice Fed Diets with Inulin and Oligofructose

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The densities and metabolic characteristics of the gastrointestinal tract bacteria are responsive to dietary supplements of nondigestible oligosaccharides (NDO), but little is known about the influences on the small intestine. Therefore, the colonic bacteria and the dimensions and transport functions of the small intestine were compared among mice (B6C 3F1 strain) fed diets with 10% cellulose (control) or with the cellulose replaced entirely with the NDO inulin or oligofructose or partially (2.5% oligofructose and 7.5% cellulose). Mice fed diets with 10% inulin or oligofructose had higher densities of anaerobes, aerobes, bacteroides, and lactobacilli, and lower proportions of enterics than mice fed a diet with 10% cellulose. Inulin, but not oligofructose, resulted in higher densities of streptococci. The small intestine was longer and weighed more when mice were fed 10% inulin with intermediate values for 10% oligofructose. Relative to control mice, rates of glucose transport and absorption of leucine, proline and glycyl-sarcosine (nmol/mg-min) were lower when mice were fed diets with 10% oligofructose and inulin, whereas only leucine was lower when mice were fed the diet with 2.5% oligofructose. Our findings indicate supplementing diets with the NDO oligofructose and inulin 1) change the assemblages of colonic bacteria, 2) influence the dimensions and absorptive functions of the small intestine, and 3) may be useful for managing the gastrointestinal ecosystem, but 4) the specific responses vary among the types and amounts of NDO and among animal models. *Key words*: nondigestible oligosaccharides, fermentable fiber, prebiotics, glucose, proline.

INTRODUCTION

Supplementing diets with soluble nondigestible oligosaccharides (NDO) increases the densities of lactic acid producing bacteria (LAB), such as lactobacilli and bifidobacteria (1). This response has been associated with several purported health benefits, including lower densities of pathogenic and putrefactive bacteria in the gastrointestinal tract (GIT) (2), lower activities of reductive enzymes that are implicated in carcinogenesis (3, 4), direct observations of reduced tumor formation in animal models (5, 6), and indirect evidence of reduced risk of cancer from epidemiology studies (7). A summary of additional benefits includes improved bowel habits, enhanced immune system functions, increased absorption of calcium and other minerals, and lower serum lipids (reviewed in 8).

The addition of NDO to the diet also causes an increase in the production of short chain fatty acids (SCFA), which reduce the pH of the gastrointestinal tract (GIT) environment and provide energy to the host. Recent findings demonstrate that the higher concentrations of SCFA increase the mass of intestinal mucosa by stimulating the proliferation of enterocytes and colonocytes (9–11). In different animal models SCFA also stimulate the expression of several genes, including the one coding for the basolateral sugar transporter of the small intestine (GLUT-2) (12, 13). These findings are corroborated by the larger small intestines and higher rates of carrier-mediated glucose transport when dogs are fed a diet supplemented with NDO (14).

In light of the demonstrated and purported health benefits, there is increasing interest in supplementing the diet with NDO. Among the several different types that are being considered, the fructooligosaccharides (FOS), which include oligofructose and inulin, have received the majority of attention. Oligofructose and inulin are not digestible by vertebrates, selectively encourage the growth of LAB, particularly bifidobacteria (1, 7), and are considered to provide health benefits (15). The present study used a strain of mouse commonly used for studies of immunotoxicology (B6C3F1) to determine if the influences of oligofructose and inulin on the colonic bacteria assemblages would be associated with changes in the dimensions and nutrient absorption functions of the small intestine.

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MATERIALS AND METHODS

Mice and their care

All aspects of the research that involved animals were approved by the Mississippi State University Institutional Animal Care and Use Committee. A total of 60 female B6C3F1 mice were obtained from a single supplier at 32–35 days of age and were distributed to cages (5 per cage) located in facilities accredited by the American Association for Accreditation of Laboratory Animal Care. The room was maintained at 21–22°C and with a 12:12 light-dark cycle. Food was provided as pellets and water by nipples, with both available continuously.

Diets

The mice were fed diets based on the AIN 76A rodent diet, with 10% of the final weight as fiber (Table I). The control diet contained 10% of the insoluble and poorly fermented fiber cellulose. Two experimental diets had the cellulose replaced entirely by oligofructose (Raftilose® P95; Orafti, Belgium) or inulin (Raftiline® HP; Orafti, Belgium), which are fermented by the GIT bacteria and differ with respect to the average degree of polymerization (DP = 4 and 25, respectively). A third experimental diet had 2.5% of the cellulose replaced by oligofructose. The control and experimental diets were fed for 6 weeks to allow the GIT ecosystem (structure, functions and resident bacteria) of the mice to adapt to the respective diets (n = 15 for each diet).

Table I

Composition of the control and experimental diets[1] fed to the mice for 6 weeks before isolating and enumerating bacteria present in the contents of the colon and measuring intestinal dimensions and rates of nutrient transport

Ingredient	gm
Casein, 30 mesh	200
DL Methionine	3
Corn Starch	150
Sucrose	450
Corn Oil	50
Salt Mix S10001[2]	35
Vitamin Mix V10001[3]	10
Choline Bitartrate	2
Fiber[4]	100

[1] Diets were formulated and prepared by Research Diets, Inc (New Brunswick, NJ) and were based on the AIN 76A rodent diet.

[2] Composition of the salt mixture (amount in 35 g): calcium phosphate dibasic (Ca = 5.2 g; *P* = 4.0 g), magnesium oxide (Mg = 0.5 g), potassium citrate (K = 3.6 g); potassium sulfate (S = 0.33 g), chromium potassium sulfate (Cr = 2.0 mg), sodium chloride (Na = 1.0 g; Cl = 1.6 g), cupric carbonate (Cu = 6.0 mg), potassium iodate (I = 0.2 mg), ferric citrate (Fe = 45 mg), manganous carbonate (Mn = 59 mg), sodium selenite (Se = 0.16 mg), zinc carbonate (Zn = 29 mg), with sucrose as the remainder.

[3] Composition of the vitamin mixture (amount in 10 g): vitamin A palmitate (4000 IU), vitamin D_3 (1000 IU), vitamin E acetate (50 IU), menadione sodium bisulfite (0.5 mg menadione), biotin (0.2 mg), cyanocobalamin (10 ug), folic acid (2 mg), nicotinic acid (30 mg), calcium pantothenate (16 mg), pyridoxine-HCl (7 mg), riboflavin (6 mg), thiamin HCl (6 mg), with sucrose as the remainder.

[4] The control diet contained cellulose as the only fiber source whereas the experimental diets had either 100 g of inulin or oligofructose or a combination of 25 g oligofructose and 75 g cellulose.

Sampling

Mice were killed by carbon dioxide asphyxiation after which the alimentary canal, extending from the stomach to the rectum was removed. The associated mesentery was cut allowing the small and large intestine to be isolated and straightened. The length of the small intestine was recorded before it was placed in cold (2–4°C), aerated (95% O_2 with 5% CO_2) mammalian Ringers. The entire colon was transferred within 3–5 min after death to an anaerobic chamber filled with a 80:10:10 mixture of N_2, H_2, and CO_2.

Bacteriology

The contents of the colon were homogenized and serially diluted using reduced yeast broth. A spiral autoplater (Model 4000, Spiral Biotech, Bethesda, MD) was used to plate appropriate dilutions for enumerating the following bacterial groups (medium): anaerobes (CDC anaerobe blood agar, BBL; Becton-Dickinson Co., Cockeysville, MD), aerobes (TSA with sheep blood; BBL), enterics (MacConkey agar, BBL), bacteroides (CDC laking blood agar with kanamycin and vancomycin, BBL), lactobacilli (LBS agar (16)), bifidobacteria (BIM-25 agar (17)), and streptococci (Columbia CNA agar with 5% sheep blood; BBL). Anaerobe plates were reduced before use by placing in the anaerobic chamber for at least 12 h, and after plating were maintained in the chamber at 35°C for 3–5 d before identifying and counting colonies. Aerobic plates were incubated for 2 d at 35°C under atmospheric conditions before enumerating colonies.

Representative colonies were identified by colony morphology, Gram reactivity, aerotolerance, and biochemical characteristics (Crystal system; BBL). Colonies suspected as bifidobacteria were further examined using the fructose-6-phosphate phosphoketolase assay (18). Counts for each group were expressed as the number of colony forming units per gram of wet colon contents.

Measurements of nutrient transport

The everted sleeve method (19) was used to measure rates of transport for glucose and absorption for the peptide glycyl-sarcosine and five amino acids that are substrates for four amino acid carriers [aspartate (acidic), lysine (basic), leucine and methionine (neutral), proline (imino)]. Briefly, the small intestine was everted, cut into three

regions of equal length (proximal, mid, and distal), and 1 cm long sleeves were cut from the middle of each region. The sleeves were tied onto stainless steel rods that approximated the diameter and yielded a snug fit (3 and 4 mm).

After the tissues were mounted onto the rods they were held in cold, aerated Ringers until 45 min after death at which time the sleeves were transferred to 37°C, aerated Ringers for 5 min. The sleeves were then suspended for 2 min in tubes containing 37°C Ringers with added nutrient that was aerated and stirred (1200 rpm) to maintain tissue viability and reduce unstirred layer influences. Accumulation of nutrients by the tissue sleeves was quantified by adding tracer levels of radiolabeled nutrient (^{14}C D-glucose and ^{3}H peptide and amino acids). Tracer levels of ^{3}H L-glucose were added to glucose solutions for simultaneous correction of labeled D-glucose that was absorbed passively and associated with the adherent fluid. Therefore, transport values for glucose represent uptake by carrier-mediated pathways. ^{14}C polyethylene glycol (PEG; MW = 4000) was added to tubes containing the peptide and amino acids and allowed for correction of nutrient in the adherent fluid. Because PEG does not allow for the correction of carrier-independent absorption, the resulting values are for total absorption, which is the sum of carrier-mediated uptake and apparent diffusion (the component of absorption that is not saturated at 50 mmol/L).

After exposure to the glucose solutions tissues were rinsed for 20 s in cold Ringers to reduce the activity in the adherent fluid before they were removed from the rods and placed in tared vials. Tissues exposed to the peptide and amino acids were not rinsed. After wet mass was recorded, the tissues were solubilized (TS-1, Research Products International, Mount Prospect, IL), scintillant was added (4A20; Research Products International, Mount Prospect, IL), and radioactivity was measured using a dual channel liquid scintillation counter. Rates of glucose transport and amino acid and peptide absorption were calculated and normalized to tissue mass (19).

Rates of glucose transport were measured in all three regions using 50 mmol/L. The relationship between glucose concentration [tracer (0.04), 0.25, 2.5, 25, and 50 mmol/L] and rates of transport was defined in the proximal region. Similarly, rates of proline absorption were measured at 50 mmol/L in all three regions and as a function of concentration in the mid region, which is where rates of amino acid absorption are usually highest. The rates of glucose and proline absorption in the three regions were integrated with small intestinal weight to estimate the absorptive capacities with values expressed as the mmol of glucose and proline that could be absorbed per day at a concentration of 50 mmol/L. Rates of absorption for aspartate, leucine, lysine, methionine, and the peptide were measured in the mid intestine only and at tracer and 50 mmol/L concentrations. Each measurement used a minimum of five sleeves prepared from different animals.

The accumulation of tracer by the tissues was compared when the tracer was present alone divided by accumulation in the presence of 50 mmol/L unlabeled nutrient. These accumulation ratios were used as an indicator of whether a component of absorption was saturable. Specifically, if there are limited numbers of transporters, then accumulation of tracer will be lower in the presence of 50 mmol/L unlabeled nutrient because of competition for transporter sites. Therefore, accumulation ratios that exceeded a value of 1.0 were considered to indicate that a portion of absorption was by a saturable component.

Statistics

Means and standard errors are presented in tables and figures. Values for body weight and intestinal dimensions are based on sample sizes of 15. Means for rates of absorption are calculated from a minimum of five animals. The main effects of diet were examined using the PROC GLM procedure of SAS (Statistical Analysis System, V 6.11, Cary, NC). When a significant effect was detected, Duncan's test was used to identify specific differences between diets. Densities of bacteria were log transformed for the analyses. The Univariate procedure of SAS was used to determine if the accumulation ratios differed from a value of 1.0. For all tests, $P < 0.05$ was accepted as the critical level of significance.

The relationships between nutrient concentration and rates of glucose transport and proline absorption were defined using nonlinear regression analysis (Enzfitter, Biosoft, Elsevier, 1987). Glucose data were fit to an equation for a single carrier, whereas data for proline were fit to an equation that included a single carrier and a passive component to account for the apparent diffusion pathway.

RESULTS

Body weights and intestinal dimensions

Body weights did not differ between the four groups of mice, but the small intestine was longer and heavier when mice were fed the diet with 10% inulin (Table II). The intestines of mice fed the diets with 2.5 and 10% oligofructose were not significantly longer or heavier than those of mice fed the diet with cellulose.

Bacteriology

Densities enumerated from the colon contents of mice fed the control and 2.5% oligofructose diets did not differ for any of the groups of bacteria studied (Fig. 1). The only possible exception is for the lactobacilli. Specifically, densities of lactobacilli were below the level of detection for mice fed the control diet (< 100 cfu), but densities for mice fed the diet with 2.5% oligofructose (2.86×10^3) were significantly greater than the limit of detection. Bifidobacteria were not detected in the colon contents of any of the mice.

Table II

Body weights and intestinal lengths and weights of mice fed the control diet with 10% cellulose and the three experimental diets containing 10% inulin or oligofructose, or 2.5% oligofructose with 7.5% cellulose. Values with different letter superscripts are significantly different ($P < 0.05$; Duncan's test).

	Cellulose	10% Inulin	10% Oligofructose	2.5% Oligofructose	Mean Error
Body Weight (g)	24.0	23.4	23.6	23.5	1.6
Intestine Length (cm)	36.0[a]	40.0[b]	37.4[a]	35.7[a]	2.4
Intestine Weight (g)	0.82[a]	1.00[b]	0.88[a b]	0.79[a]	0.13

Compared to mice fed the control diet, those fed the diets with 10% oligofructose and inulin had higher densities of anaerobes, aerobes, and lactobacilli, and lower densities of enterics. Densities of streptococci and bacteroides were highest in mice fed the diet with inulin; the 10% oligofructose resulted in intermediate values for bacteroides, but did not affect the streptococci.

Nutrient transport

Rates of glucose transport at 50 mmol/L and tracer concentrations averaged for the three regions were lower for mice fed the 10% oligofructose (1.84 ± 0.33) and inulin (1.85 ± 0.19) compared to those fed the control diet (3.4 ± 0.49; $P < 0.05$) due to lower rates of uptake in the proximal and mid small intestine (Fig. 2). Mice fed the diet with 2.5% oligofructose (2.82 ± 0.33) were intermediate due to lower rates of glucose uptake in the proximal, but not mid, intestine. Diet effects were not detected in the distal segment. Rates of proximal small intestine glucose transport were also lower when measured at tracer concentrations in mice fed the 10% oligofructose and inulin diets ($P < 0.05$) with intermediate values for mice fed 2.5% oligofructose.

Rates of proline absorption at 50 mmol/L averaged for the three small intestinal regions did not differ among mice fed the diets with 10% cellulose (7.86 ± 0.97), 2.5% and 10% oligofructose (8.21 ± 0.52 and 7.68 ± 0.67), and 10% inulin (7.51 ± 0.44). Proline absorption was more evenly distributed along the entire length of the small intestine than glucose transport, with lower rates of absorption in the distal small intestine of mice fed the diet with 2.5% oligofructose providing the only exception (Fig. 2).

Rates of absorption by the mid intestine measured at 50 mmol/L were lower for leucine when mice were fed the diets with 10% oligofructose and inulin compared to those fed 10% cellulose with intermediate values for mice fed the diet with 2.5% oligofructose (Fig. 3). Rates of glycyl sarcosine absorption at 50 mmol/L were lower when mice were fed 10% oligofructose with intermediate values from mice fed 10% inulin. At tracer concentrations, rates of mid intestine absorption were depressed by 10% inulin for aspartate and proline and at 10% oligofructose for proline and glycyl sarcosine. Although tracer leucine absorption was not lower when the 10% oligofructose and inulin diets were considered separately, pooled data for both groups were lower compared to mice fed the control and 2.5% oligofructose diets.

Tracer accumulation ratios exceeded a value of 1.0 for all of the solutes (Fig. 4) indicating a saturable, carrier-mediated pathway was present for each of the nutrients studied. Tracer accumulation ratios for mice fed the diets with 10% oligofructose and inulin were comparable, but tended to be lower compared to those fed the control diet, significantly so for glucose, leucine, proline, and the peptide.

Estimated maximum rates of carrier-mediated glucose transport (V_{max}) were higher for mice fed the control (1.86 nmol/mg-min ± 0.19) and 2.5% oligofructose (2.1 ± 0.26) compared to those fed 10% oligofructose (0.52 ± 0.02) and inulin (0.70 ± 0.01) diets. Apparent affinity constants (K_m^*) did not differ indicating differences for V_{max} are due to differences in densities of transporters, not a shift to another transporter type. Although accumulation ratios exceeded 1.0 for proline, a large proportion of absorption appear to be via an apparent diffusion pathway that is not saturable at 50 mmol/L. As a result, it was not possible to obtain accurate kinetic estimates for the carrier-mediated component of absorption.

DISCUSSION

The structure, functions, and resident biota of ecosystems are altered when energy is added, with the specific responses varying according to the amount and type of energy. The GIT can be considered as a small, but complex ecosystem (20), and a synthesis of the present and previously reported findings shows that the structural components (physical, chemical, and biotic features) and functional elements (transfer of energy and materials) are affected when the diet is supplemented with NDO. Similar to other ecosystems, the specific responses of the GIT are dependent on the types and amounts of fermentable fiber added to the diet.

Responses of the bacteria

Agricultural and ecological research has provided numerous examples about how the number and biomass of organisms in ecosystems are responsive to the amounts and types of energy inputs. Corresponding with this, adding energy in the form of oligofructose, inulin, and

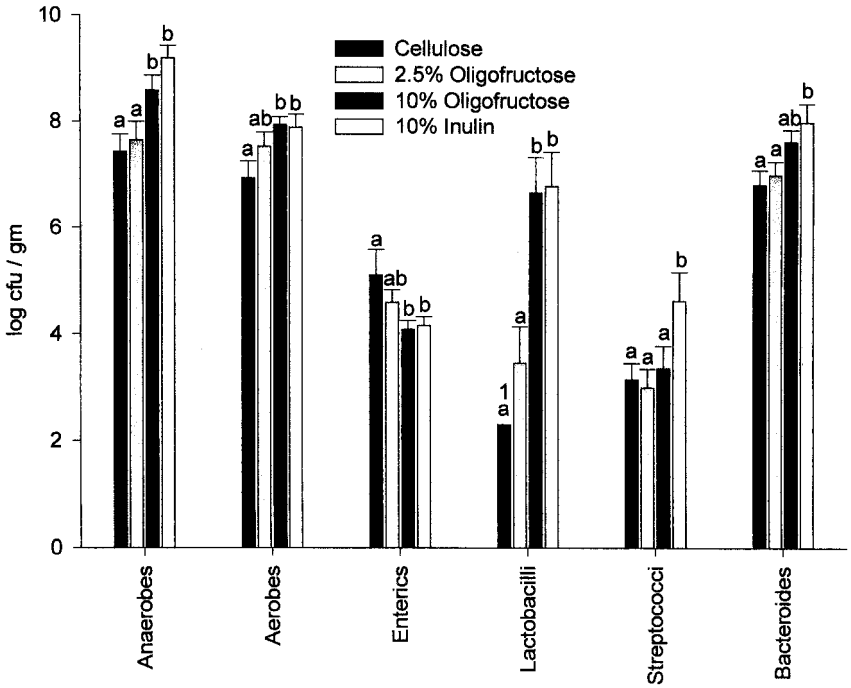

Fig. 1. Bacterial densities present in the colon contents (log cfu/g wet weight) of mice fed the control (10% cellulose) and experimental diets with 10% inulin or oligofructose, or 2.5% oligofructose with 7.5% cellulose. Values with different letter superscripts are significantly different ($P < 0.05$; Duncan's test).

other NDO increases the total densities of anaerobic and aerotolerant bacteria present in the colon (present study; 2122). However, not all bacteria are able to use NDO as energy substrates (8, 9). As a consequence, adding inulin and oligofructose to the diet result in some bacterial groups representing a larger proportion of the bacterial assemblage in the colon contents (e.g., LAB), whereas other groups declined in abundance (e.g., enterics), and still others were not affected (e.g., bacteroides). Although dose-response relations are known for NDO and the bacteria resident in the GIT (21), this is not universally accepted (23). The results presented in Figure 1 suggest that a dietary supplement of 2.5% oligofructose may not be sufficiently high to cause significant shifts in the densities for any bacterial group in this mouse model.

The present findings indicate the responses will vary among NDO. Despite differing only in the degree of polymerization, oligofructose did not elicit a change in the densities of streptococci at either dose, whereas densities were significantly higher when mice were fed the diet with inulin. The relevance of the inulin induced increase in streptococci on health of the host (mice or humans) is not known, but the genus *Streptococcus* includes several species that are considered to be beneficial and have been used in probiotic preparations (24). However, the present bacteriologic approach was not designed to identify species, and this will be necessary to fully understand the potential impact on the host. It can be expected that the wide diversity of NDO being considered as dietary supplements will elicit an even wider range of differences than what was detected for oligofructose and inulin.

The responses of ecosystems to energy inputs are dependent on the resident organisms. In light of the variation in bacterial assemblages present in the GIT of different spe-

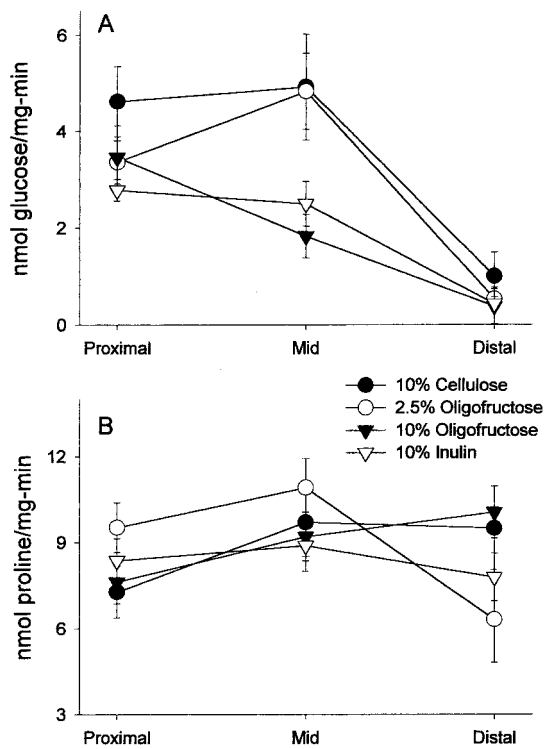

Fig. 2. Rates of glucose transport (A) and proline absorption (b) at 50 mmol/L in the three regions of the small intestine of mice fed the control (10% cellulose) and experimental diets with 10% inulin or oligofructose, or 2.5% oligofructose with 7.5% cellulose.

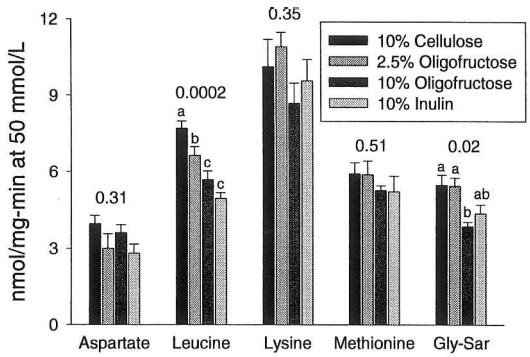

Fig. 3. Rates of absorption for four amino acids and the dipeptide glycyl sarcosine when measured at 50 mmol/L using the mid small intestine from mice fed the control (10% cellulose) and experimental diets with 10% inulin or oligofructose, or 2.5% oligofructose with 7.5% cellulose. Values above sets of bars are the *P* values from the ANOVA with different letters indicating specific differences ($P < 0.05$) among treatments as identified by Duncan's test.

cies and individuals, variation can be expected for the influences of different types and amounts of NDO. Many strains of commercially available rodents, including the B6C3F1 strain used for the present study, are derived from gnotobiotic animals that are inoculated with a probiotic consisting of only 8 species (the 'Altered Schaedler Flora' (25)). Although the bifidobacteria are a significant, and are sometimes the dominant, component of the bacterial assemblages present in the colons of humans, they are not included in the Altered Schaedler Flora. Coinciding with this, bifidobacteria were not detected in the B6C3F1 mice or other strains of mice we have studied (26). It is not surprising that the responses to NDO by the assemblages of bacteria resident in the GIT of the experimental mice

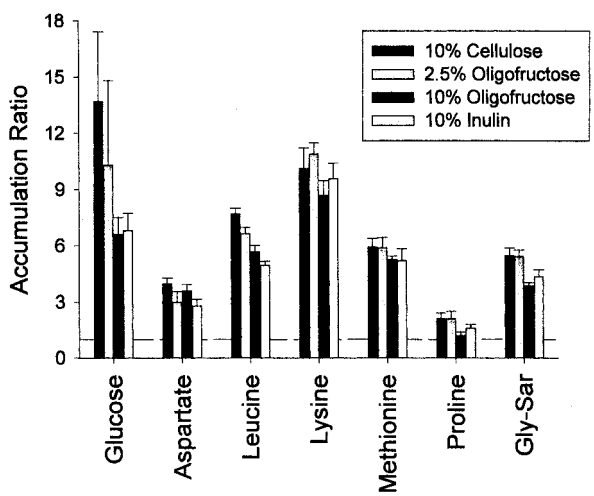

Fig. 4. Tracer accumulation ratios for glucose in the proximal and the five amino acids and the dipeptide in the mid small intestine of mice fed the control (10% cellulose) and experimental diets with 10% inulin or oligofructose, or 2.5% oligofructose with 7.5% cellulose. The dashed line is at 1.0.

differ from those reported for humans and other animals with more 'normal' assemblages of bacteria that include bifidobacteria as well as other species that are absent from the altered Schaedler flora.

Influences on small intestine length and absorptive functions

The intestines of omnivores have the capacity to modulate rates of nutrient absorption in response to changes in diet composition (27). Since oligofructose and inulin are not digested by vertebrates (7), the observed changes in intestinal length and transport functions must have been mediated by other signals. Likely candidates are the SCFA produced by the fermentation of oligofructose, inulin and other NDO. Lumenal SCFA increase rates of proliferation for enterocytes and colonocytes (9–11), and when administered systemically enhance adaptation of the remnant small intestine after small bowel resection (28). These findings are consistent with the longer, heavier intestines of mice fed the diet with 10% inulin. However, it is uncertain if SCFA exert trophic influences directly or indirectly by stimulating the production of glucagon-like peptide-2 and other mediators of intestinal growth and functions. It is also possible that NDO alter the movement of digesta along the length of the GIT and this may elicit adaptive changes in intestinal structure and functions.

It is puzzling why mice fed diets with 10% NDO had lower rates of transport for glucose and absorption of some amino acids and the peptide. SCFA are known to increase glucose uptake by intact tissues (14), possibly by stimulating expression of genes coding for glucagon-like peptide-2 and the intestinal apical and basolateral glucose transporters (12, 13).

Our data verify previous findings that rates of absorption by the mouse intestine vary among the different classes of amino acids (29). Although carrier-mediated uptake pathways have been detected previously in another strain of mice for the same classes of amino acids (29), the insignificant accumulation ratios (not different from 1.0) for aspartate and lysine are surprising in that they suggest an apparent lack of saturable pathways of absorption for acidic and basic amino acids. There are several possible explanations for the discrepancies between mouse models. The densities of transporter sites in the present model might be so low for these amino acids that saturation occurs at tracer concentrations. Alternatively, the carriers might exist in such high densities that they are not saturated at 50 mmol/L. Or the affinities of the carriers are sufficiently low and capacities high that they are not saturated by 50 mmol/L. The present study was not designed to address this question.

Perspectives

Despite the increasing interest in NDO as supplements to a healthy diet, it remains difficult to predict the responses

of the GIT ecosystem. Ecologists have recognized for many years how difficult it is to understand how various ecosystems will respond to nutrient enrichment. The principal reason is that no two ecosystems are identical, and this applies to the GIT ecosystems of various species, and even individuals, as evident from the differences in the GIT bacteria of human subjects. As a consequence, the influences of 'nutrient enrichment' on the GIT ecosystem will not be consistent among species, can elicit different responses in the structural and functional characteristics of the GIT (present study vs 14), and will be virtually impossible to predict. Furthermore, the various oligosaccharides do not elicit the same responses among the numerous species comprising the resident bacteria (30) or yield the same concentrations and relative proportions of SCFA (9).

There is a need to better understand how the bacteria in the several regions of the GIT ecosystem respond to different amounts and types of NDO. It is possible that the responses are of greater magnitude in more proximal regions of the GIT ecosystem than what is evident from the distal colon or stool samples (31). Similarly, when adding fertilizers to the headwaters of a river system, the responses are less evident and harder to detect where the river empties into the ocean. Coinciding with this, stool samples, which are generally the only sample available from clinical patients, may provide only limited insights about the actual responses of the resident bacteria to NDO and this may limit interpretations of possible relations among NDO, the resident bacteria, and influences on GIT functions.

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