Dietary Modulation of the Human Gut Microflora Using the Prebiotics Oligofructose and Inulin[1]

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ABSTRACT Although largely unproven in humans, better resistance to pathogens, reduction in blood lipids, antitumor properties, hormonal regulation and immune stimulation may all be possible through gut microflora manipulation. One approach advocates the oral intake of live microorganisms (probiotics). Although the probiotic approach has been extensively used and advocated, survivability/viability after ingestion is difficult to guarantee and almost impossible to prove. The prebiotic concept dictates that non viable dietary components fortify certain components of the intestinal flora (e.g., bifidobacteria, lactobacilli). This concept has the advantage that survival of the ingested ingredient through the upper gastrointestinal tract is not a prerequisite because it is indigenous bacterial genera that are targeted. The feeding of oligofructose and inulin to human volunteers alters the gut flora composition in favor of bifidobacteria, a purportedly beneficial genus. Future human studies that exploit the use of modern molecular-based detection methods for bacteria will determine the efficacy of prebiotics. It may be possible to address prophylactically certain gastrointestinal complaints through the selective targeting of gut bacteria. J. Nutr. 129: 1438S–1441S, 1999.

KEY WORDS: • *prebiotics* • *gut microbiota modulation* • *inulin* • *oligofructose*

Bacterial fermentation in the human colon

Functions of the human gut include absorption of water, certain minerals, and the storage and excretion of waste materials. However, because of the resident microbiota, it is clear that the colon has an important role in human nutrition and possibly health (Gibson and Macfarlane 1995). It is known that many disease states involve bacterial metabolism. However, the gut microflora may also be considered relevant to host welfare. Gut bacteria carry out a multidisciplinary process known as fermentation in which dietary and endogenously produced residues are metabolized in a process that involves a large amount of cross-feeding by the microflora. Large intestinal microorganisms have a strictly anaerobic metabolism; the numbers of obligate anaerobes are many orders of magnitude higher than those of facultative anaerobes. Numerically predominant anaerobes are gram-negative rods belonging to the genus *Bacteroides*. These bacteria can represent up to 30% of the total microbial flora. Other groups that have been identified to date as present in high numbers include bifidobacteria, clostridia, eubacteria, lactobacilli, gram-positive cocci, coliforms, methanogens and dissimilatory sulfate-reducing bacteria. It is thought that between 400 and 500 different bacterial species are present in the human large intestine.

The principal substrates for gut bacterial growth are dietary carbohydrates that have escaped digestion in the upper gastrointestinal tract. In addition, amino acids can also be effective as growth substrates for colonic bacteria; bacterial secretions, lysis products, sloughed epithelial cells and mucins may also make a contribution.

A number of different microbial metabolic niches, bacterial habitats and interrelationships occur in the large gut and respond mainly to substrate availability, the physicochemical environment of the gut and the metabolic capabilities of the microflora (Freter 1992). Gut bacteria are able to metabolize substrates for increased energy and growth. The major end-products of metabolism are short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate, but a variety of other metabolites are also produced, including electron sink products such as lactate, pyruvate, ethanol, hydrogen and succinate (**Table 1**). These substances are formed to maintain the redox balance during fermentation. Electron sink products act as fermentation intermediates because they are further metabolized to SCFA by other species. Although the products of gut proteolysis may be generally thought of as toxic toward host health, those of carbohydrate digestion may be considered benign and in some cases can contribute positively (Table 1).

Although the gut microflora contain certain bacteria that are pathogenic, there may also be a positive aspect to gut microbiology. In this context, the intestinal flora are considered to be key in influencing human well-being. Under normal homeostatic conditions, the intestinal microflora are of central importance in preventing coloniza-

[1] Presented at the conference Nutritional and Health Benefits of Inulin and Oligofructose held May 18–19, 1998 in Bethesda, MD. This symposium was supported in part by educational grants from the National Institutes of Health Office of Dietary Supplements, the U.S. Department of Agriculture and Orafti Technical Service. Published as a supplement to *The Journal of Nutrition*. Guest editors for the symposium publication were John A. Milner, The Pennsylvania State University, and Marcel Roberfroid, Louvain University, Brussels, Belgium.

TABLE 1

Predominant products of carbohydrate metabolism in the human colon

End product	Bacterial group involved	Metabolic fate
Acetate	bacteroides, bifidobacteria, eubacteria, lactobacilli, clostridia, ruminococci, peptococci, veillonella, peptostreptococci, propionibacteria, fusobacteria, butyrivibrio	Metabolized in muscle, kidney, heart and brain
Propionate	bacteroides, propionibacteria, veillonella	Cleared by the liver, possible gluceogenic precursor, suppresses cholesterol synthesis
Butyrate	clostridia, fusobacteria, butyrivibrio, eubacteria, peptostreptococci	Metabolised by the colonic epithelium, regulator of cell growth and differentiation
Ethanol, succinate, lactate, pyruvate	bacteroides, bifidobacteria, lactobacilli, eubacteria, peptostreptococci, clostridia, ruminococci, actinomycetes, enterococci, fusobacteria,	Absorbed, electron sink products, further fermented to short-chain fatty acids
Hydrogen	clostridia, ruminococci, fusobacteria	Partially excreted in breath, metabolized by hydrogenotrophic bacteria

tion by pathogens;they are also thought to have many beneficial local and systemic roles such as improved lactose tolerance, supply of SCFA as energy substrates for the host, antitumor properties, neutralization of certain toxins, stimulation of the intestinal immune system and possibly reduction of blood lipid levels (Fuller 1989,1992 and 1997, Gorbach et al. 1988, Isolauri et al. 1991, Kohwi et al. 1978, Lin et al. 1989, Sanders 1994).

For obvious reasons, there is much interest in increasing numbers and activities of beneficial bacteria in the large gut, preferably at the expense of more harmful species. A way in which this can be achieved is through dietary supplementation.

Probiotics

Fuller (1992) defined a probiotic as *A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance*. The probiotic organism(s) used for human consumption are usually lactic acid excretors.

The history of probiotics dates back as far as the first intake of fermented milks, over 2000 years ago. However, it is probably from the work of Metchnikoff (1907) in the early years of this century that the first scientific assessments of probiotics were made. Common probiotics include the following: *1*) Lactobacilli such as *Lactobacillus acidophilus*, *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, *L. reuteri*, *L. brevis*, *L. cellobiosus*, *L. curvatus*, *L. fermentum*, *L. plantarum*; *2*) Gram-positive cocci such as *Lactococcus lactis* subsp. *cremoris*, *Streptococcus salivarius* subsp. *thermophilus*, *Enterococcus faecium*, *S. diaacetylactis*, *S. intermedius*; and *3*) Bifidobacteria such as *Bifidobacterium bifidum*, *B. adolescentis*, *B. animalis*, *B. infantis*, *B. longum*, *B. thermophilum*.

Selection criteria for probiotics is an area of much debate and should be taken into account when defining appropriate strains (Huis In't Veld and Havennar 1991). One important characteristic is survival (and establishment) of the fed microorganism after ingestion. Some studies with feces rely on phenotypic traits of the probiotics, such as different morphologies or biochemical tests. However, these are probably unreliable because the bacteria may exhibit metabolic variation. Future developments in molecular techniques directed toward gut microbiology will more clearly define the survival characteristics of probiotics (McCartney and Gibson 1997).

Prebiotics

Because the viability of live bacteria in food products and during transit through the gastrointestinal tract may be variable, the prebiotic concept has been developed. Here, the selective growth of indigenous gut bacteria is required. *A prebiotic is a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health* (Gibson and Roberfroid 1995). Thus, the prebiotic approach advocates the administration of nonviable entities and therefore overcomes survival problems in the upper gastrointestinal tract. Certain oligosaccharides that cannot be digested, except through bacterial activity, are prebiotics. Those that contain fructose can alter the composition of the human gut flora, by a specific fermentation, towards a community predominated by bifidobacteria.

Oligofructose and inulin as prebiotics

Criteria that allow the classification of a food ingredient as a prebiotic, include the following: *1*) It must be neither hydrolyzed, nor absorbed in the upper part of the gastrointestinal tract. *2*) It must be selectively fermented by one or a limited number of potentially beneficial bacteria in the colon. *3*) It must alter the composition of the colonic microbiota towards a healthier composition. *4*) It must preferably induce effects that are beneficial to the host health.

Any food that reaches the colon such as nondigestible carbohydrates, some peptides and proteins, as well as certain lipids, is a prebiotic candidate. Nondigestible carbohydrates, in particular fructose oligosaccharides, are authentic prebiotics. Fructooligosaccharides are β-D-fructans with degrees of polymerization (DP) varying between 2 and 60 (inulin) and 2 and 20 (oligofructose).

In vitro studies have indicated that inulin and oligofructose have a specific fermentation (Hidaka et al. 1986, Wang and Gibson 1993). This has also been confirmed in human volunteer trials that assessed the bifidogenic effects of both

TABLE 2

Differences in fecal microbial counts of volunteers fed a controlled diet supplemented with 15 g/d sucrose, oligofructose or inulin[1,2]

Bacterial group	Sucrose	Oligofructose	Inulin
Total aerobes	6.5 ± 1.0	6.2 ± 1.0	6.7 ± 1.0
Coliforms	6.0 ± 1.2	5.9 ± 0.7	6.2 ± 1.4
Gram positive cocci	5.8 ± 0.7	5.8 ± 0.9	5.5 ± 0.27
Total anaerobes	10.3 ± 0.8	10.2 ± 0.9	10.7 ± 0.25
Bifidobacteria	8.9 ± 0.6	9.5 ± 0.7	10.1 ± 0.44
Bacteroides	9.3 ± 0.7	8.8 ± 1.1	9.8 ± 0.5
Fusobacteria	8.5 ± 0.6	7.7 ± 0.9	8.9 ± 0.62
Clostridia	8.0 ± 0.8	7.5 ± 0.9	8.1 ± 0.72
Lactobacilli	6.6 ± 1.1	7.0 ± 1.4	6.3 ± 0.76

[1] Counts are $\log_{10}$/g wet weight of feces and are given as mean values ± SD.
[2] See Gibson et al. (1995) for study details.

inulin and oligofructose in vivo. The influence of oligofructose (Raftilose, P95) on the fecal bacterial composition in healthy persons was evaluated during a 45-d feeding period in which the volunteers were given a strictly controlled diet (Gibson et al. 1995). Eight volunteers participated in the experiment. They had never suffered from any form of gastrointestinal disorder and had not taken antibiotics for at least 3 mo before the start of the study. During the first 5 d, subjects were given a noncontrolled diet; at that time, a stool sample was collected for bacteriological analysis. Subsequently, the volunteers were given the controlled diet supplemented with 15 g of sucrose for a 15-d period. This was then replaced by 15 g of oligofructose for a further 15 d, followed by another period with sucrose. Stool samples were taken periodically for bacterial enumeration. In summary, the use of oligofructose as a replacement for sucrose in diet caused a marked increase in bifidobacteria, whereas bacteroides, fusobacteria and clostridia all decreased. Other bacteria tested (total aerobes, total anaerobes, lactobacilli, coliforms and gram-positive cocci) remained more or less unchanged. Bacteroides was the numerically predominant genus with sucrose consumption, whereas bifidobaceria dominated with oligofructose. Similar results were detected during the feeding of inulin (Raftiline, ST) (**Table 2**). Other investigators have since confirmed the prebiotic effect of inulin and oligofructose in vivo (Buddington et al. 1996, Kleesen et al. 1997).

Health aspects of prebiotics

Although prebiotics offer one rational approach to the probiotic concept, the health consequences have not yet been defined. In theory, a number of potential benefits may arise. However, it may be that improved resistance to pathogens offers the most feasibility. The lactic microflora of the human gastrointestinal tract are thought to play a significant role in improved colonization resistance. Increased bifidobacterial numbers in the breast-fed infant may be one factor that contributes towards improved competitive exclusion of pathogens seen in this group compared with those who are formula fed (Gibson et al. 1997).

In terms of the mechanism of inhibition, metabolic end products, such as acids excreted by these microorganisms, may lower the gut pH, in a microniche, to levels below those at which pathogens are able to effectively compete. Another factor that could be considered is a competitive effect by occupation of normal colonization sites (by anti-infective prebiotics or probiotic microrganisms) and competition for available nutrients. Enhanced immune function would also be a further important factor.

Many lactobacilli and bifidobacterial species are able to excrete natural antibiotics, which can have a broad spectrum of activity (e.g., lactocins, helveticins, lactacins, curvacins, nisin or bifidocin). For the bifidobacteria, our studies have indicated that some species are able to exert antimicrobial effects on various gram-positive and gram-negative intestinal pathogens including salmonellae, campylobacters and *Escherichia coli* (Gibson and Wang 1994).

The outbreak of *E. coli* 0157 in Lanarkshire, Scotland at the end of 1996 resulted in 20 fatalities. The deaths have highlighted the continuing concern about bacterial gastroenteritis to consumers, the food industry, researchers and the medical profession. In recent laboratory tests, we have also shown that some bifidobacteria exert powerful antagonistic effects towards *E. coli* 0157. The inhibition was variable in species of bifidobacteria, with *Bifidobacterium infantis* and *B. longum* exerting the greatest effect on *E. coli* 0157. The possibility exists therefore that increased levels of bifidobacteria (and consideration of the species type) in the large gut, together with other factors such as immune status, may offer improved protection.

In humans older than ~55 y, fecal bifidobacterial counts are known to show a marked decrease in comparison to those of younger persons (Kleessen et al. 1997, Mitsuoka 1990). It may be of some relevance that the UK fatalities during the *E. coli* outbreak all involved the elderly, whereas hundreds of people in different age groups reported the infection. A potential analogy exists with reduced pathogen resistance, decreased numbers of bifidobacteria in the elderly and the production of natural resistance factors. In essence, the natural gut flora may have been compromised through reduced bifidobacterial numbers and possibly a diminished ability to deal with the pathogen. The design of prebiotic-based health foods for selected populations such as the elderly may therefore have much virtue.

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