Critical Review

Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics

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ABSTRACT Because the human gut microbiota can play a major role in host health, there is currently some interest in the manipulation of the composition of the gut flora towards a potentially more remedial community. Attempts have been made to increase bacterial groups such as *Bifidobacterium* and *Lactobacillus* that are perceived as exerting health-promoting properties. Probiotics, defined as microbial food supplements that beneficially affect the host by improving its intestinal microbial balance, have been used to change the composition of colonic microbiota. However, such changes may be transient, and the implantation of exogenous bacteria therefore becomes limited. In contrast, prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health. Intake of prebiotics can significantly modulate the colonic microbiota by increasing the number of specific bacteria and thus changing the composition of the microbiota. Nondigestible oligosaccharides in general, and fructooligosaccharides in particular, are prebiotics. They have been shown to stimulate the growth of endogenous bifidobacteria, which, after a short feeding period, become predominant in human feces. Moreover, these prebiotics modulate lipid metabolism, most likely via fermentation products. By combining the rationale of pro- and prebiotics, the concept of synbiotics is proposed to characterize some colonic foods with interesting nutritional properties that make these compounds candidates for classification as health-enhancing functional food ingredients. J. Nutr. 125: 1401–1412, 1995.

INDEXING KEY WORDS:

• *colon* • *humans* • *microbiota*
• *prebiotics* • *probiotics*

The human gastrointestinal tract consists of the mouth, oral cavity, esophagus, stomach, small intestine and colon. The large intestine starts at the ileocecal junction, with anatomically distinct regions of this organ being the cecum, ascending colon, transverse colon, descending colon and sigmoid colon (Macfarlane and Cummings 1991). Biologically important functions of the large gut include the absorption and secretion of certain electrolytes and water, as well as the storage and excretion of waste materials (Drasar and Hill 1974, Eastwood 1982). In the past decade, however, much attention has been diverted towards those colonic functions that affect host health and nutrition. Of overriding importance in this respect is the gut microbiota.

The large intestine is by far the most heavily colonized region of the digestive tract, with up to 10^{12} bacteria for every gram of gut contents. Through the process of fermentation, colonic bacteria are able to produce a wide range of compounds that have both positive and negative effects on gut physiology as well as other systemic influences. For instance, colonic bacteria produce short-chain fatty acids (SCFA) from the metabolism of complex carbohydrates and proteins (Cummings 1981, Cummings and Macfarlane 1991, Rerat et al. 1986). The host may then salvage energy and regulate metabolism from SCFA absorption (see later). There is, therefore, some interest in the manipulation of the composition of the gut flora towards a more salutary regimen. That is, an increase in numbers and activities of bacterial groups (such as *Bifidobacterium* and *Lactobacillus*) that may have health-promoting properties is desirable. In this review we introduce the concept of prebiotic foodstuffs, which can be added to the diet in order to increase the health-promoting attributes of certain aspects of the resident gut microbiota. Prior to this, however, a brief description of this microbiota and its activities is given.

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COMPOSITION AND ACTIVITIES OF THE COLONIC MICROBIOTA

Colonic bacteria. The human large intestine can be described as a complex microbial ecosystem. It is thought that at least 50 genera of bacteria reside in the colon, comprising several hundred species (Finegold et al. 1974, 1975 and 1983, Moore and Holdeman 1972 and 1974). The activities of colonic bacteria are affected by the physiology and architecture of the hindgut. The bacteria present have fluctuating activities in response to substrate availability, redox potential, pH, O_2 tension and distribution in the colon (Cummings and Macfarlane 1991). For example, those microorganisms resident in the proximal colon (right side) have a plentiful supply of dietary nutrients and thus grow at a fast rate, causing a decrease in pH as a result of intense SCFA production. In the distal (left) colon, however, substrate availability is lower; bacteria grow more slowly and the pH frequently approaches neutrality (Cummings et al. 1987, Macfarlane and Gibson 1994). Thus, a high degree of heterogenity exists within this ecosystem.

The gastrointestinal tract of the newborn is inoculated by the mother's vaginal and fecal flora during birth (Mevissen-Verhage et al. 1987). Initially, a predominance of facultatively anaerobic strains such as *Escherichia coli* or enterococci exists (Rotimi and Duerden 1981, Yoshiota et al. 1991). These bacteria may therefore create a highly reduced environment that then allows the growth of strict anaerobes. It has been recognized that gross differences exist with respect to the composition of the gut microbiota in response to the infant's feeding (Drasar and Roberts 1989). The fecal flora of bread-fed infants is dominated by populations of bifidobacteria, with only ~1% enterobacteria. In contrast, formula-fed infants have more complex microbiota, with bifidobacteria, bacteroides, clostridia and streptococci all prevalent (Yoshiota et al. 1991). After weaning (>2 y of age), a pattern that resembles the adult flora becomes established.

Most human large intestinal microorganisms have a strictly anaerobic metabolism, and numbers of facultative anaerobes are many orders of magnitude lower than those of the obligate anaerobes (Finegold et al. 1975, Moore and Holdeman 1972, Reddy et al. 1975). Information regarding the composition of the gut microbiota has largely arisen as a result of studies with feces. Data reported by Finegold and colleagues (Finegold et al. 1983) give the distribution of various bacterial genera in a random selection of 141 individuals with various dietary and disease states. The numerically predominant anaerobes were Gram-negative rods of the genus *Bacteroides*. These microorganisms can constitute to 30% of the total fecal flora and as such can have an important impact on colonic microbiological processes. Other numerically predominant groups are bifidobacteria (Gram-positive rods), eubacteria (Gram-positive rods), clostridia (Gram-positive rods), lactobacilli (Gram-positive rods) and Gram-positive cocci (**Fig. 1**). A number of other groups exist in lower proportions, including enterococci, coliforms, methanogens and dissimilatory sulfate-reducing bacteria.

Considering the wide range of bacterial species residing in the large gut, as well as the different growth substrates available, it is not surprising that the colonic microbial ecosystem harbors a multiplicity of nutritional patterns (Macfarlane and Cummings 1991). A variety of different metabolic niches, bacterial habitats and interrelationships have arisen. Different nutritional types of bacteria include saccharolytic species (Macfarlane and Gibson 1994), nitrogen utilizers (Pittman et al. 1976) and bacteria that metabolize such gases as hydrogen (Allison and Macfarlane 1988, Gibson et al. 1988, Miller and Wolin 1986).

In general, intestinal bacteria may be divided into species that exert either harmful or beneficial effects on the host (Fig. 1). Pathogenic effects include diarrhea, infections, liver damage, carcinogenesis and intestinal putrefaction; health-promoting effects may be caused by the inhibition of growth of harmful bacteria, stimulation of immune functions, lowering of gas distention problems, improved digestion and absorption of essential nutrients, and synthesis of vitamins.

Characteristics of fermentation in the human colon. The principal substrates for bacterial growth are dietary carbohydrates that have escaped digestion in the upper gastrointestinal tract (Cummings et al. 1989). Between 10 and 60 g/d of dietary carbohydrate reaches the colon (Cummings and Macfarlane 1991). It is estimated that 8–40 g/d of resistant starch contributes a major part of the available fermentable substrate, followed by about 8–18 g/d non-starch polysaccharides, 2–10 g/d unabsorbed sugars and 2–8 g/d oligosaccharides (Van Loo et al. 1995). There is also a contribution of about 2–3 g/d from endogenous carbohydrates. In addition, protein can be utilized for growth (about 3–9 g/d is dietary and 4–6 g/d is endogenous, such as pancreatic enzymes) (Macfarlane and Cummings 1991).

The majority of simple sugars and oligosaccharides ingested and digested by humans are absorbed in the small intestine (Bond et al. 1980). However, some such as lactose, raffinose, stachyose, fructooligosaccharides (such as oligofructose or inulin) are able to reach the colon intact (Hudson and Marsh 1995, Roberfroid et al. 1993, Salyers 1979, Salyers and Leedle 1983, Wolin and Miller 1983). In addition, many food additives and sugar alcohols, e.g., sorbitol and xylitol, are not absorbed (Calloway and Murphy 1968, Tadesse et al. 1980).

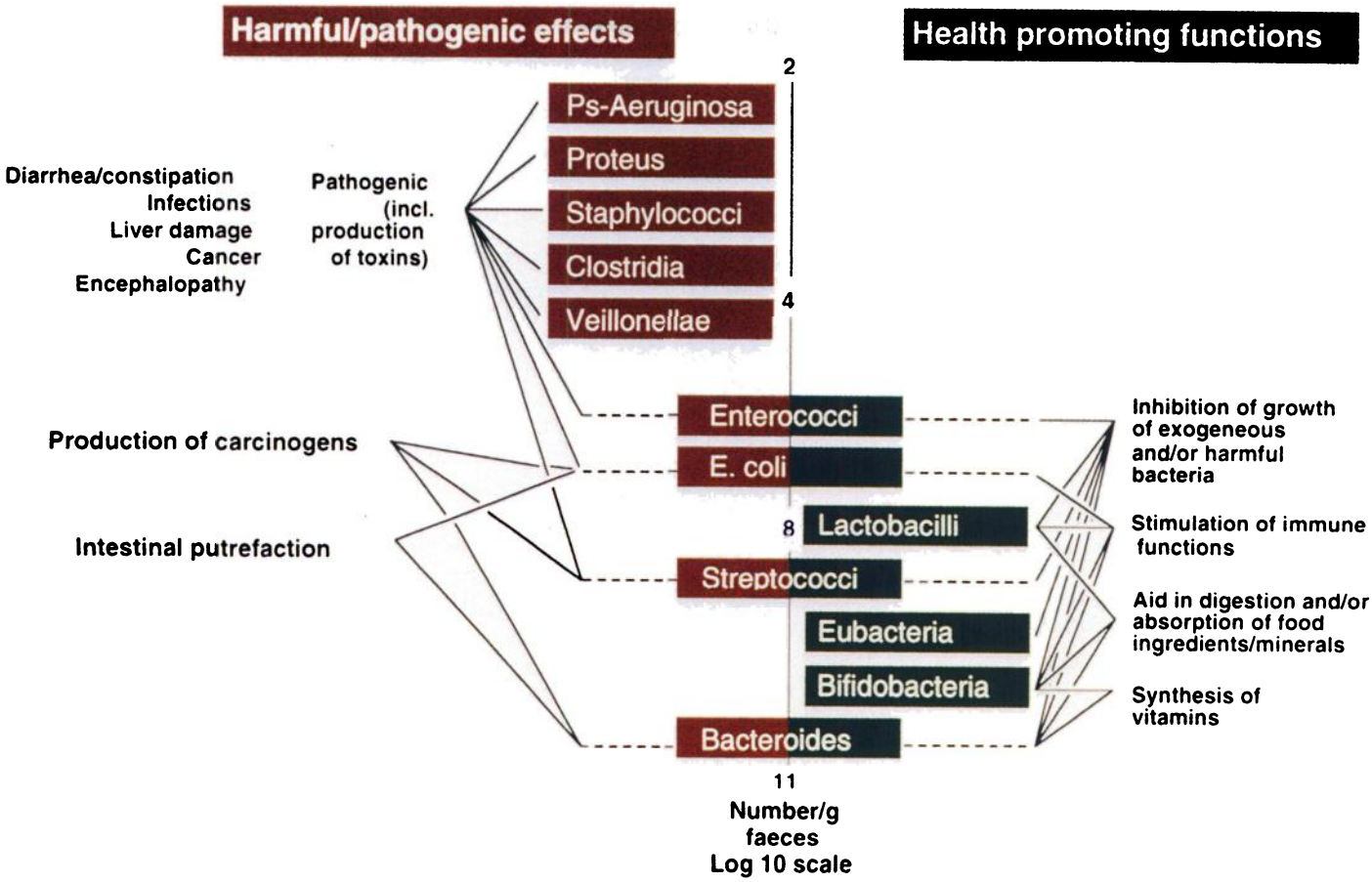

FIGURE 1 Generalized scheme of the composition and health effects of predominant human fecal bacteria. The figure shows approximate numbers of the different genera. The bacteria are generally split into those groups that have harmful or pathogenic influences on human health, those that have beneficial effects, and those that may have both. Potential reasons for the classification scheme are given.

In addition to growth substrates provided by the diet, the host is itself capable of producing fermentable carbohydrates. These include glycoproteins (e.g., mucins) and other derivatives of polysaccharides (e.g., chondroitin sulfate). Studies from in vitro incubations have indicated that the gut microbiota is able to rapidly metabolize endogenously produced substances. The important genera in this respect are thought to be bifidobacteria, ruminococci and some bacteroides (Hoskins and Boulding 1981, Hoskins et al. 1985, Macfarlane and Gibson 1991, Roberton and Stanley 1982, Salyers et al. 1977 and 1982).

Each of the vast range of bacterial species growing in the colon has a specialized ecological niche. Because of the diversity and metabolic capabilities of the microbiota, gut fermentation is a complicated process. In most cases, the metabolic end products excreted by one individual species can serve as a growth substrate for another. The most numerous, as well as the most versatile, polysaccharide utilizers in the colon belong to the genus *Bacteroides* (Macy and Probst 1979, Salyers 1979). Other bacteria able to grow on carbohydrates are saccharolytic species belonging to the genera *Bifidobacterium*, *Ruminococcus*, *Eubacterium*, *Lactobacillus* and *Clostridium*. Because of the extremely complex nature of the gut ecosystem, many groups of bacteria are unable to degrade polymerized carbohydrates directly. These species grow by cross-feeding on fragments produced by primary polysaccharide degraders. Saccharolytic bacteria are highly adapted for growth on complex carbohydrates by means of their ability to produce a variety of polyhydrolases and glycosidases (Salyers and Leedle 1983). Although some bacteria in the colon can synthesize many different types of saccharolytic enzymes, carbohydrate metabolism is likely to be dependent on the cooperation of different enzymes and various bacterial species taking part in the process.

A generalized scheme of fermentation is shown in **Figure 2**. The principal end products of this process are short-chain fatty acids (SCFA) (Cummings 1985,

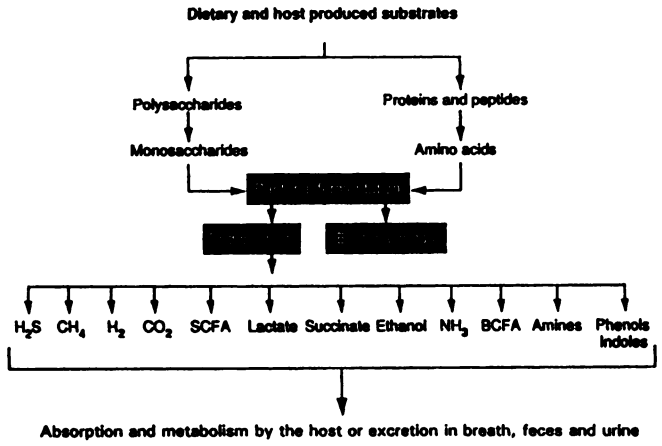

FIGURE 2 Generalized scheme of fermentation by human colonic microbiota. A number of different bacterial metabolic interactions cumulate in the process of fermentation, for which diet- or host-derived carbohydrates and proteins are the major substrates. Various intermediate and end products are formed, including gases, short-chain fatty acids (SCFA), organic acids, branched-chain fatty acids (BCFA), and other products of proteolysis.

Rechkemmer et al. 1988), because the majority of saccharolytic colonic bacteria metabolize carbohydrates by the Embden-Meyerhoff pathway (Wolin and Miller 1983). A number of gases including hydrogen, carbon dioxide and methane, are also produced (Levitt et al. 1994). Hydrogen is further removed by some bacteria, or it may simply be excreted in breath and flatus (Christl et al. 1992b, Cummings et al. 1989).

Some fermentation intermediates are also produced in the colon, including ethanol, lactate, succinate and pyruvate, and these may, in addition, be further fermented to SCFA (Turton et al. 1983). This process allows further energy gain for the host from fermentation. Proteolytic species may cause an accumulation of end products such as ammonia, phenolic compounds and amines (Cummings and Macfarlane 1991, Macfarlane and Cummings 1991). Amino acid metabolism yields branched-chain fatty acids such as isobutyrate and isovalerate (Macfarlane et al. 1992). In addition, from the products that arise from fermentation, bacteria are also able to obtain energy for growth and the maintenance of cellular function.

The composition of an individual's colonic microbiota is usually considered to be fairly stable over long periods (Holdeman et al. 1976, Simon and Gorbach 1982). However, a number of physicochemical factors can influence the pattern and extent of fermentation of particular substrates. These include competition for nutrients, the physicochemical environment of the large gut, various host conditions, metabolic interactions among bacteria, and individual dietary preferences (Berg 1981, Hentges and Freter 1962, Hill 1986, Rowland and Tanaka 1993).

It is likely that the composition of diet is able to determine the nature and activity of the gut microbiota. For example, many of the purported beneficial effects of a high fiber intake are directly related to bacterial metabolism (Finegold et al. 1983, Stephen et al. 1987). Also, highly sulfated foodstuffs can potentially influence hydrogen utilization pathways (Christl et al. 1992a).

BIFIDOBACTERIA AND THEIR BENEFICIAL EFFECTS ON HUMAN HEALTH

Among the major genera of colonic bacteria, *Bifidobacterium* and *Lactobacillus* are not usually thought to be pathogenic (Bezkorovainy and Miller-Catchpole 1989, Fuller 1992, Gilliland and Speck 1977). In the past, a number of studies have concentrated on the advantageous physiological functions of lactobacilli in the human colon with regard to a promotion of health (see Fuller 1992 and references therein). The positive biological activities of bifidobacteria are currently receiving some attention (Tamura 1983). *Bifidobacterium* is a major group of saccharolytic bacteria in the colon, and constitutes up to 25% of the total population in the gut of adult and 95% in newborns (Kawaze et al. 1981). The potential positive effects of bifidobacteria on human health are summarized in **Figure 3** and include the following aspects:

1) Bifidobacteria produce strong acids as metabolic end products (acetate, lactate). These lower the pH of the medium and may thus exert an antibacterial effect (Kawase 1982, Rasic 1983). Further work has indicated that bifidobacteria are able to excrete a metabolic end product that is directly inhibitory to a range of Gram-positive and Gram-negative pathogenic bacteria (Gibson and Wang 1994a and 1994b).

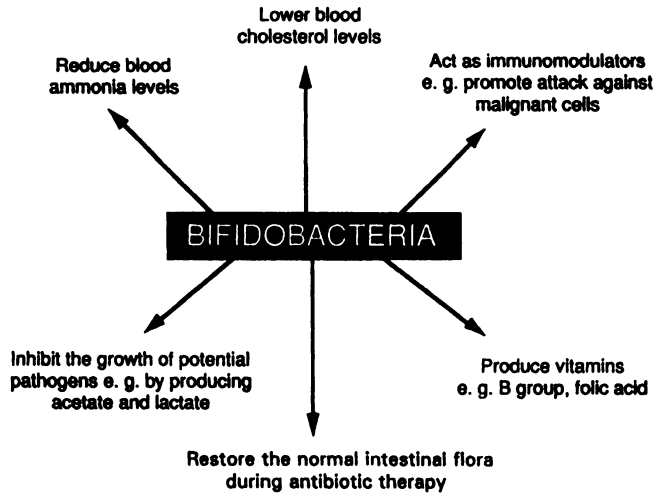

FIGURE 3 Properties of bifidobacteria that are purported to occur and be beneficial to human health.

2) An added effect of acid production is the protonation of potentially toxic ammonia (and amines) to produce NH_4^+, which is nondiffusable and thus lowers blood ammonia levels (Hansen 1985). Moreover, these bacteria do not form aliphatic amines, hydrogen sulfide or nitrites (Bezkorovainy and Miller-Catchpole 1989).

3) Bifidobacteria produce vitamins, largely of the B group (Nishizawa 1960, Liescher 1961), as well as digestive enzymes such as casein phosphatase and lysozyme (Kawase 1982, Minagawa 1970).

4) Certain cellular components of bifidobacteria act as "immunomodulators", i.e., they promote immunological attack against malignant cells (Mizutani and Mitsuoka 1980, Sekine et al. 1985). This activation of the immune system will also contribute towards improved host resistance to pathogens.

5) These bacteria have also been used to restore the normal intestinal flora during antibiotic therapy (Korshunov et al. 1985).

An increase in the number and activity of bifidobacteria (and lactobacilli) in the colon is therefore likely to be desirable for a range of reasons. One approach has been the addition of live cultures to foodstuffs such as fermented milk products (e.g., yogurts, Modler et al. 1990). The rationale is that a number of the exogenous bacterial additions remain in a viable form during transit through the gastrointestinal tract and become active on reaching the colon, where appropriate physicochemical conditions for their growth occur. This technology is often referred to as "probiotics."

PROBIOTICS

Probiotics can be described as *organisms and substances which contribute to intestinal microbial balance* (Sperti 1971). However, Fuller (1989) has redefined a probiotic as *a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.* In this form, the probiotic therefore needs to be viable and is as such more restricted. That is, antibiotics and related compounds are not included. The latter definition is now most widely accepted.

The following criteria must be met before a probiotic can be described as useful (Fuller 1991 and 1992):

1) The probiotic must be capable of being prepared in a viable manner and on a large scale (e.g., for industrial purposes).

2) During use, and under storage, the probiotic should remain viable and stable.

3) It should be able to survive in the intestinal ecosystem.

4) The host animal should gain beneficially from harboring the probiotic.

In humans, lactobacilli (e.g., *Lactobacillus acidophilus, L. casei, L. delbruekii*) are commonly used as probiotics, either as single species or in mixed culture with other bacteria. Other genera that have been used are bifidobacteria (e.g., *Bifidobacterium adolescentis, B. bifidum, B. longum, B. infantis*) and streptococci (e.g., *Streptococcus salivarius* ss. *thermophilus, S. lactis*). It has been hypothesized that probiotics administered to humans can have positive effects in a number of biomedical conditions. These include diarrhea, constipation, colitis, recolonization by pathogens, flatulence, gastroenteritis, gastric acidity, immunostimulation, hypercholesterolemia, hepatic encephalopathy and carcinogenesis. For a discussion of these claims, the review by Goldin and Gorbach (1992) should be consulted.

The classical and most well-used example of probiotic technology is the addition of lactobacilli and/or bifidobacteria to fermented milk products (Colombel et al. 1987, Modler et al. 1990). In this case, it is proposed that the exogenous bacteria reach the large intestine in an intact and viable form and thus help maintain the "balance" of the gut flora. In the process, the bacteria are confronted by a number of physical and chemical barriers in the gastrointestinal tract. These barriers include gastric acid and bile acids. There is some evidence that at least a proportion of the bifidobacteria added in this manner is able to reach the colon (Pochart et al. 1992). Intake of food as a bolus is undoubtedly of some importance here, as is the development of species that are relatively acid resistant.

A further problem exists however. The probiotic microorganism needs to establish in the colon and, preferably, become active. For increased persistence the probiotic may need to adhere to the intestinal epithelium. Here difficulties are likely to arise. The probiotic is likely to be in some sort of stressed state due to its encounters with the adverse conditions referred to above. This would probably compromise its chances of survival. Next, the bacteria would need to compete for nutrients and ecological sites of colonization with a previously established microbial flora comprising several hundred other bacterial species. Indeed, the data of Bouhnik et al. (1992) indicate that when the product containing the probiotic is not longer consumed, the added bacteria are rapidly washed out of the colon.

Another approach that, at least partly, overcomes the limitations of probiotics is to use prebiotics, which are not viable entities but rather are growth substrates specifically directed toward potentially beneficial bacteria already growing in the colon.

THE CONCEPT OF PREBIOTICS

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, and thus improves host health.

In order for a food ingredient to be classified as a prebiotic (**Fig. 4**), it must *1)* be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract; *2)* be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are stimulated to grow and/or are metabolically activated; *3)* consequently, be able to alter the colonic flora in favor of a healthier composition; and *4)* induce luminal or systemic effects that are beneficial to the host health.

Among the food ingredients, nondigestible carbohydrates (oligo- and polysaccharides), some peptides and proteins, and certain lipids (both ethers and esters) are candidate prebiotics. Because of their chemical structure, these compounds are not absorbed in the upper part of the gastrointestinal tract or hydrolyzed by human digestive enzymes. Such ingredients could be called "colonic foods," i.e., foods entering the colon and serving as substrates for the endogenous colonic bacteria, thus indirectly providing the host with energy, metabolic substrates and essential micronutrients.

Amongst the colonic foods, nondigestible carbohydrates are naturally occurring and are able to fulfill the criteria of prebiotics as defined above. Although some peptides and proteins, mostly from milk and plants, are known to be (partly) nondigestible and to have some beneficial effects both by facilitating the intestinal absorption of cations (mainly Ca and Fe) and by stimulating the immune system, the role of colonic fermentation in mediating such effects has not been demonstrated. On the contrary, anaerobic proteolysis is likely to produce potentially harmful compounds (e.g., ammonia, amines) (Macfarlane and Cummings 1991).

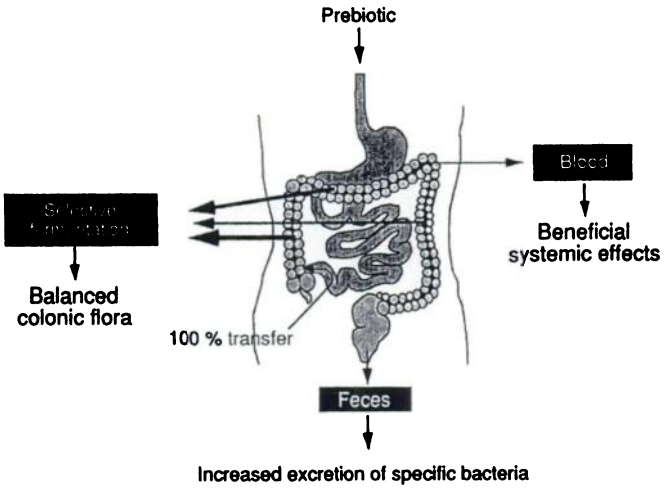

FIGURE 4 Schematic representation of the properties that allow classification of food ingredients as prebiotics. The prebiotics must directly target the colon, have a selective fermentation and help maintain a balanced microflora, preferably by being utilized by promoting species. Other systemic effects may also occur after absorption of fermentation products into the bloodstream.

TABLE 1

Classification of certain carbohydrates as colonic foods and prebiotics

Carbohydrate	Colonic food	Prebiotic
Resistant starch	Yes	No
Non-starch polysaccharides		
Plant cell wall polysaccharides	Yes	No
Hemicelluloses	Yes	No
Pectins	Yes	No
Gums	Yes	No
Nondigestible oligosaccharides		
Fructooligosaccharides	Yes	Yes
Galactooligosaccharides	Yes	?
Soybean oligosaccharides	Yes	?
Glucooligosaccharides	?	No

The presence in common food products of naturally occurring nondigestible lipids has not been extensively investigated, and hence the metabolism of lipids by the colonic microbiota is largely unknown.

Nondigestible carbohydrates include miscellaneous compounds such as resistant starch, non-starch polysaccharides (plant cell wall polysaccharides, hemicellulose, pectins, gums), and nondigestible oligosaccharides (Delzenne and Roberfroid 1994). However, even though they can all be classified as colonic foods, not all are prebiotics (**Table 1**). Indeed, for most of these substances, the process of colonic fermentation is rather nonspecific. When ingested they stimulate, in the colon, the growth and/or metabolic activity of different bacterial species, including species that are both potentially harmful and beneficial (Drasar et al. 1976, Maczulak et al. 1993, Salyers et al. 1982, Wang and Gibson 1993). Consequently, they lack the necessary metabolic selectivity for one or a limited number of beneficial bacteria such as lactobacilli and bifidobacteria, a critical criterion for classification as prebiotic.

FRUCTOOLIGOSACCHARIDES AS PREBIOTICS

Nature and origin. Among the natural nondigestible oligosaccharides that fulfill the criteria of a colonic food, fructooligosaccharides are the only products presently recognized and used as food ingredients that meet all the criteria allowing classification as prebiotics. Other compounds for which experimental evidence indicates such a classification are transgalactosylated disaccharides (Ito et al. 1990 and

1993, Rowland 1992, Tanaka et al. 1983) and soybean oligosaccharides (Hayakawa et al. 1990, Saito et al. 1992).

Chemically, fructooligosaccharides are short- and medium-length chains of β-D fructans in which fructosyl units are bound by a β 2-1 osidic linkage (**Fig. 5**). Because their synthesis in plant cells starts by the transfer of a fructosyl moiety between two sucrose molecules (Edelman and Dickerson 1966), some of these molecules have a glucose unit as the initial moiety. The β 2-1 osidic bond of fructooligosaccharides, including the first glucose-fructose bond, is not hydrolyzed to a great extent by any mammalian digestive enzymes (Rumessen et al. 1990, Stone-Dorshow and Levitt 1986). A recent study in ileostomy subjects has shown that after correction for partial fermentation due to bacterial contamination of the small intestine, ingested fructooligosaccharides are quantitatively recovered as nondigested material (Bach Knudsen and Hessov 1995). Depending on the chain length, as defined by the number of osyl units and called the degree of polymerization (DP), fructooligosaccharides are named oligofructose (DP <9, average DP = 4.8) or inulin (DP up to 60, average DP = 12) (Fig. 5). Inulin is prepared by hot water extraction of chicory roots, and oligofructose is obtained by partial enzymatic hydrolysis of inulin under strictly controlled conditions. A number of common foodstuffs such as garlic, onion, artichoke and asparagus have high oligofructose and inulin contents (Van Loo et al. 1995). The term fructooligosaccharides will be used here to encompass both oligofructose and inulin, which are commercially available as RAFTILOSE® and RAFTILINE®, respectively.

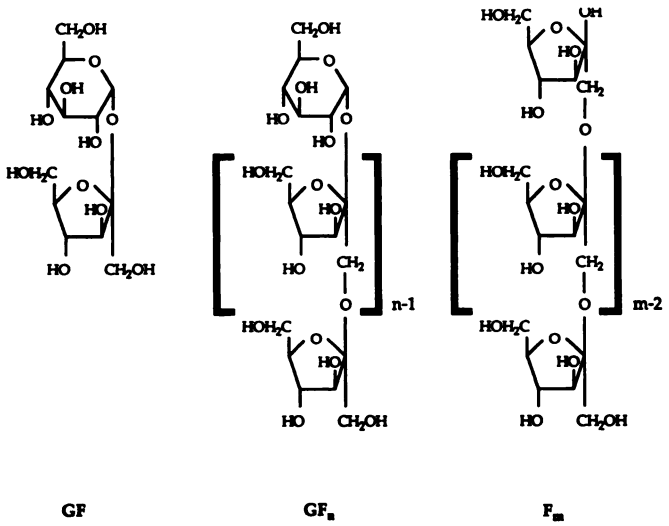

FIGURE 5 Chemical structures of sucrose (GF) and fructooligosaccharides (GFn and Fm). G = glucosyl; F = fructosyl. Short chains of fructooligosaccharides are known as oligofructose (n is 4.8 on average, maximum = 9), and medium chains as inulin (n is 12 on average, maximum = 60).

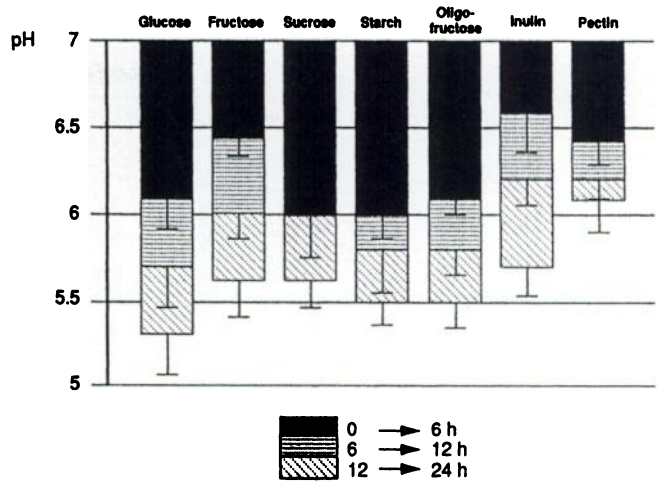

FIGURE 6 Comparative pH values, expressed during a time course, from in vitro fermentations of various carbon sources by anaerobic slurries of human fecal bacteria. Substrate concentration was 7 g/L, and the inoculum concentrations was 100 g/L. Adapted from Gibson and Wang (1994b) and Wang and Gibson (1993).

Selective fermentation. That fructooligosaccharides are fermented by colonic microbiota has been demonstrated in vitro using mixed human fecal bacteria. Like other carbohydrates such as glucose, fructose, sucrose, starch and pectin, fructooligosaccharides induce a decrease in pH of the culture medium during anaerobic fermentation (**Fig. 6**) (Gibson and Wang, 1994b, Wang and Gibson 1993). In a recent in vitro study using both pure strains of colonic bacteria and mixed human fecal cultures, Wang and Gibson (1993) demonstrated that, in comparison with other simple or complex carbohydrates, fructooligosaccharides are selectively fermented by most strains of bifidobacteria (**Fig. 7**). Moreover, when bifidobacteria grow on such substrates, they seemingly do so at the expense of bacteroides, clostrida or coliforms, which are maintained at low levels or may even be reduced (Wang and Gibson 1993). Such a specificity of bifidobacteria for these fructooligosaccharides is likely to be due to the production of β-fructosidases, as demonstrated in pure culture (Wang 1993). The accepted mechanism for the inhibition of growth of other bacteria by bifidobacteria is thought to involve a decrease in pH as a consequence of the production of large quantities of carboxylic acids, mainly acetate and lactate (see Fig. 2). However, this is by no means the only mechanism, as shown by fermentation experiments in which, even though the pH was maintained at a neutral level, an inhibition of growth of a *Clostridium* and *E. coli* still occurred in co-culture with *Bifidobacterium infantis*. The potentially important demonstration that bifidobacteria might secrete a bacteriocin-type substance that is active against clostridia, *E. coli*, and many other

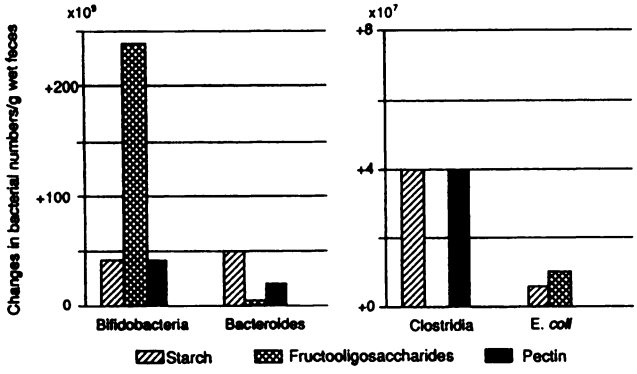

FIGURE 7 Growth of different genera of colonic bacteria during the in vitro fermentation of various carbon sources by anaerobic slurries of mixed human fecal bacteria. Substrate concentration was 7 g/L, while the inoculum concentration was 100 g/L. Results are averaged differences between counts taken at 0 h and 12 h from three separate volunteers. Adapted from Wang and Gibson (1993).

pathogenic bacteria such as listeria, shigellas, salmonellas and *Vibrio cholerae* is presently under investigation. The selective fermentation of fructooligosaccharides by bifidobacteria has also been confirmed in vivo in healthy human volunteers. Bacteriological analysis of fecal samples collected at the end of a 2-wk feeding period showed that ingestion of 3 × 5 g fructooligosaccharides/d significantly increased the proportion of bifidobacteria from 6 to 22%, whereas bacteroides, clostridia and fusobacteria were decreased from 25 to 4%, 1 to 0.2% and 4 to 0.4%, respectively (**Fig. 8**).

Systemic effects. Being selectively fermented by various strains of bifidobacteria, fructooligosaccharides are almost quantitatively utilized so as to give a mixture of SCFA, L-lactate and carbon dioxide according to the following stoichiometry (for review and discussion see Roberfroid et al. 1993):

$$1\ C_6H_{12}O_6 \quad >1.5\ CH_3COOH + 0.33\ CH_3CH_2COOH + 0.15\ CH_3(CH_2)_2COOH + 0.33\ CH_3CHOHCOOH + 0.33\ CO_2 + 0.33\ H_2O.$$

However, as discussed above, colonic fermentation is a symbiotic process in which many different bacteria participate. Moreover, bifidobacteria and others utilize energy released by such a metabolism to grow and to incorporate carbon atoms from the fermented carbohydrates into their own structural and functional molecules. In terms of carbon atoms, as discussed recently (Delzenne and Roberfroid 1994, Roberfroid et al. 1993), the balance of such a complex process is likely to be: 40% as SCFA, 15% as L-lactate, 5% as carbon dioxide, and 40% as bacterial biomass. On the basis of such calculations, it has been proposed that the caloric value of fructooligosaccharides must be on the order of 4.2 to 6.3 kJ/g (1.0 to 1.5 kcal/g), or 25 to 40% that of a digested fructose molecule (Delzenne and Roberfroid 1994, Roberfroid et al. 1993).

It has recently been hypothesized that highly fermentable carbohydrates could, possibly via the production of SCFA and lactate in the colon, improve the metabolic absorption of various ions, including Ca, Mg and Fe (Scharrer et al. 1992, Schulz et al. 1993). Using rats, Delzenne and Roberfroid (1994) demonstrated that consumption of a diet supplemented with oligofructose or inulin has a positive effect (+60–65%) on the intestinal uptake of these ions. Similar data have been obtained by Japanese researchers working with "Neosugar," a synthetic fructooligosaccharide (Ohta et al. 1994). Like other fermentable carbohydrates, fructooligosaccharides increase fecal excretion of nitrogen and decrease uremia (N. M. Delzenne, Université Catholique de Louvain, Brussels, Belgium, personal communication).

After being absorbed, SCFA are metabolically utilized by various tissues: butyrate by the colonic epithelium, propionate, L-lactate and acetate (partly) by the liver, and acetate (partly) by muscle and other peripheral tissues (Demigné et al. 1986, Rémézy and Demigné 1983, Schumann et al. 1991). As hypothesized by various authors, including ourselves, SCFA are also likely to play a regulatory role in modulating endogenous metabolism (Demigne et al. 1986). In particular, acetate and propionate, possibly in combination with L-lactate, might play a role in regulating lipid and cholesterol metabolism. But the physiological significance of these effects, which have been demonstrated in vitro, remains unclear.

Recent experiments (Delzenne et al. 1993, Fiordaliso et al. 1995) have demonstrated that consumption by rats of a diet supplemented with

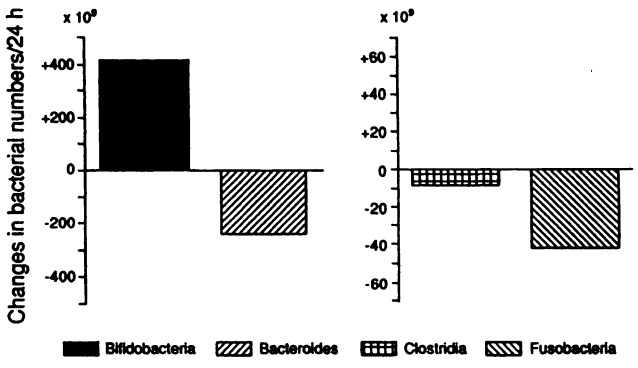

FIGURE 8 Mean changes in fecal bacteriology (plus or minus number of specific bacterial genera during a 24-h period) from eight healthy volunteers given a controlled diet supplemented with 15 g/d fructooligosaccharides for 15 d. As a control, sucrose was used at the same concentration. Bifidobacteria increased during the test period, whereas bacteroides, clostridia and fusobacteria all decreased. Adapted from Gibson et al. (1995).

TABLE 2

In vivo data showing the effect of dietary fructooligosaccharides on lipid metabolism in male rats[1,2,3]

Variable	Result
Total body fat	–35%
Triglyceridemia	–35%
Phospholipidemia	–25%
Lipoproteinemia	Decrease in the number of VLDL particles

[1]Male wistar rats were fed a standard diet supplemented with 10–15% (wt/v) fructooligosaccharides for 3-4 mo.
[2]Body weight gain and food intake of fructooligosaccharides-fed rats were not statistically different from values for controls.
[3]Adapted from Delzenne and Roberfroid (1994), Delzenne et al. (1993) and Fiordaliso et al. (1995).

10–15% fructooligosaccharides induces a significant reduction in total body carcass fat deposition, triglyceridemia (–35%) and phospholipidemia (–25%) (**Table 2**), most likely because of a reduction in the number of circulating VLDL particles. Because, when incubated in vitro, hepatocytes isolated from fructooligosaccharide-fed rats incorporate significantly less palmitate into triglycerides than do control liver cells, it can be hypothesized that the hepatic metabolism of lipids is modified.

CONCLUSIONS AND PERSPECTIVES

It is becoming increasingly accepted that the colonic microbiota may play an important role in the maintenance of host health. Keeping the colonic flora in a balanced state seems to be a contributory factor. A preferred microbiota is that in which the so-called beneficial strains predominate over the potentially harmful species (see Fig. 1). Among the generally recognized beneficial species are the bifidobacteria and lactobacilli, to which have been attributed various health-promoting functions such as the production of SCFA (which acidify gut contents), immunostimulation and inhibitory effects on the growth of harmful bacteria (Fig. 3). Diet can influence the microbiota in two ways: *1)* by including viable microorganisms that, because of their resistance to digestion (i.e., the effects of gastric acid, digestive enzymes, bile acids, etc.) reach the colon and (temporarily) implant, grow and become metabolically active; and *2)* by including nondigestible substrates that resist digestion and feed the colon so as to stimulate the growth and metabolism of resident bacteria. The former are probiotics; the latter are colonic foods, which, if they have the additional characteristic of being selective, may be known as prebiotics.

That a prebiotic is indeed an efficient way to significantly modify the composition of the gut microbiota has now been demonstrated, both in vitro and in vivo, for the nondigestible fructooligosaccharides. That consumption of these food ingredients may positively influence host health is supported by experimental data showing increased cation absorption, improved lipid profile, certain effects associated with dietary fiber, and a low energy value.

Compared with probiotics, prebiotics are likely to have distinct advantages such as the in situ stimulation of the growth of certain resident (endogenous/commensal) bacteria, activation of bacterial metabolism, and their own physiological effects (Delzenne and Roberfroid 1994, Roberfroid 1993, Roberfroid and Delzenne 1994) such as various dietary fiber–like properties. Moreover, prebiotics offer, for the first time, an opportunity to design human studies aimed at studying the health benefits of a colonic microbiota in which bifidobacteria are predominant.

One approach that may be encouraged for future research is the combination of both probiotics and prebiotics as synbiotics, which may be defined as *a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare.*

Such an approach (Tanaka et al. 1983) could ultimately produce the following nutritional benefits: improved survival of live bacteria in food products with, as a consequence, prolonged shelf life; an increased number of ingested bacteria reaching the colon in a viable form; stimulation in the colon of the growth and implantation of both exogenous and endogenous bacteria; and activation of the metabolism of these bacteria (it is important to emphasize that only the metabolically active bacteria can promote health). This approach will be particularly important if the bacteria that are targeted utilize specific substrates, as is the case for bifidobacteria.

Beyond nutritional benefits, probiotics, prebiotics and (perhaps most importantly) synbiotics have potential pharmaceutical applications. Indeed, increased levels of certain bacteria growing in the human intestine have been implicated as causative agents or maintenance factors involved not only in colonic disorders but also in systemic disorders. These intestinal pathologies include antibiotic-associated colitis, inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, colorectal cancer, necrotizing enterocolitis, and ileocecitis; the systemic disorders are gut origin septicemia, pancreatitis and multiple organ failure syndrome. Overgrowth of pathogenic bacteria such as clostridia and *E. coli*, as well as implantation of parasites, viral

infections, extensive burn injury, postoperative stress, and antibiotic therapy are major factors in the etiology of these disorders, which are often associated with bacterial translocation due to intestinal barrier failure (Gardiner et al. 1993, Gibson and Macfarlane 1994, Solomons 1993). Few studies have been published that support the hypothesis that probiotic- and prebiotic-type therapy could be effective in the treatment of these conditions: *Bifidobacterium longum* used as a probiotic has been reported to prevent bacterial translocation (Yamazaki et al. 1985), and a prebiotic-type colonic food (such as soybean fiber) has been shown to delay disease onset and prolong survival in experimental *Clostridium difficile* ileocecitis (Frankel et al. 1994).

Among the colonic foods, fructooligosaccharides such as oligofructose and inulin are naturally occurring ingredients for which convincing experimental evidence in favor of a health-promoting effect is available. These fructooligosaccharides thus belong to the class of prebiotics and, because of their strong bifidogenic activity, can be combined with bifidobacteria to produce a synbiotic. In addition to their nutritional properties, they may also have technological advantages, because they contribute to improve palatability of food products. Moreover, inulin can be treated to prepare a cream that can be used as a fat replacer in products such as spreads, ice creams and margarines.

Probiotics, prebiotics and synbiotics in general, and oligofructose and inulin in particular, thus have the properties of a health-enhancing functional food ingredient: *a food ingredient that affects the functions of the body in a targeted manner so as to exert positive effects that may, in due course, justify health claims.*

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