INULIN: A REVIEW OF NUTRITIONAL AND HEALTH IMPLICATIONS

LINDA S. BOECKNER

Panhandle Research and Extension Center
University of Nebraska
Scottsbluff, NE 69361
USA

MARILYNN I. SCHNEPF

Department of Nutritional Science and Dietetics
University of Nebraska at Lincoln
R Leverton Hall, 316C
Lincoln NE 68583-0806
USA

BRYAN C. TUNGLAND

Imperial Sensus
8106 Hwy. 90A, PO Box 9
Sugar Land, TX 77487-0009
USA

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I. INTRODUCTION

A growing awareness of the complex interactions between diet, the intestinal microflora resident in the gastrointestinal tract and health has encouraged the development of dietary strategies promoting the growth of specific bacterial groups perceived to be beneficial. Even though these complex relationships are now recognized from clinical studies, they remain poorly understood. Of particular interest is the use of a new class of dietary foodstuffs termed non-digestible oligosaccharides (NDO). Due to their unique chemical structure some of these carbohydrates resist digestion by the human alimentary tract. Consequently, they reach the ceco-colon essentially as intact molecules, not providing the body with digestible monosaccharides. Because these carbohydrates enter the colon as intact compounds they elicit systemic physiological functions and act as fermentable substrates for colonic microflora – influencing the species composition and metabolic characteristics of the intestinal microflora, and therefore providing important health attributes. Inulin is a natural NDO extracted in industrial quantities from chicory with potential use in producing physiological functional foods and promoting human health.

Evidence of the growing interest in inulin is the increased research in both short-chained fructooligosaccharides (FOS) and the longer-chained inulin. A number of review papers have been published discussing various aspects of potential health benefits of FOS and inulin. These include Fuchs, 1992; Farnworth, 1993; Roberfroid, 1993, 1996; Gibson and Roberfroid, 1995; Van Loo *et al.*, 1995; Gibson *et al.*, 1996; Yun, 1996; and Roberfroid *et al.*, 1998.

II. IDENTITY

Inulin falls under a general class of fructose-containing polymers known as fructans. Fructans serve as storage polymers in many members of the Compositæ family such as *Cichorium intybus* (chicory), *Inula helenium* (elecampane), *Taraxacum officinalis* (dandelion) and *Helianthus tuberosus* (Jerusalem artichoke). Inulin extracted from chicory is a natural polydisperse carbohydrate (Phelps, 1965). It is a fructan which consists predominantly of linear chains of 1,2-β-linked d-fructofuranose units bound by a (αl–β2) type linkage (as in sucrose) to a terminal glucose moiety. All fructans found in the dicotyledons, as well as some monocotyledons, are of this type. By comparison, fructans composed predominantly of linear fructose units bound by a β-(2→6) glycosidic bond are typically levans that are produced by many soil and oral bacteria, yeasts and fungi.

The gross molecular formula of inulin is GF_n with G being a terminal glucosyl unit, F representing the fructosyl units and n representing the number of fructosyl units. The basic GF_2 trimer in inulin and the shortest fructan of the inulin type is 1-kestose. The same bonds link the ensuing fructosyl units, i.e. β-(2→1) as that in 1-kestose (Fig. 1). Short chain fractions of fructooligosaccharides such as 1-kestose, the major GF_2 compound in chicory roots or Jerusalem artichoke, and neokestose in onion do not differ analytically (Van Loo *et al.*, 1995). Further, fructan chains linked to either of these naturally occurring trisaccharides have the β-(2→1) configuration, implying that with the exception of one glycosidic linkage within the basic trisaccharide there is no difference between a fructan molecule based on 1-kestose or neokestose. The chain length or degree of polymerization (DP) is $n + 1$.

Pollock *et al.* (1993) reviewed the enzymology of fructan synthesis *in vivo*. The natural biosynthesis of inulin within plant cells begins by the cells using a vacuolar enzyme, sucrose-sucrose fructosyltransferase (SST), to catalyze the reaction (Equation 1) that produces trisaccharides (isokestose, kestose and neokestose) and a glucose molecule from two sucrose molecules (Edelman and Dickerson, 1966).

$$\mathrm{GF} + \mathrm{GF} \overset{\mathrm{SST}}{\Rightarrow} \mathrm{GFF} + \mathrm{G} \qquad (1)$$

Sucrose-sucrose fructosyltransferase is only active at high concentrations of sucrose (6–15%) so fructan metabolism is an extension of sucrose metabolism. Following trisaccharide production, the polymeric chain is lengthened by the action of another vacuolar enzyme, fructan-fructan

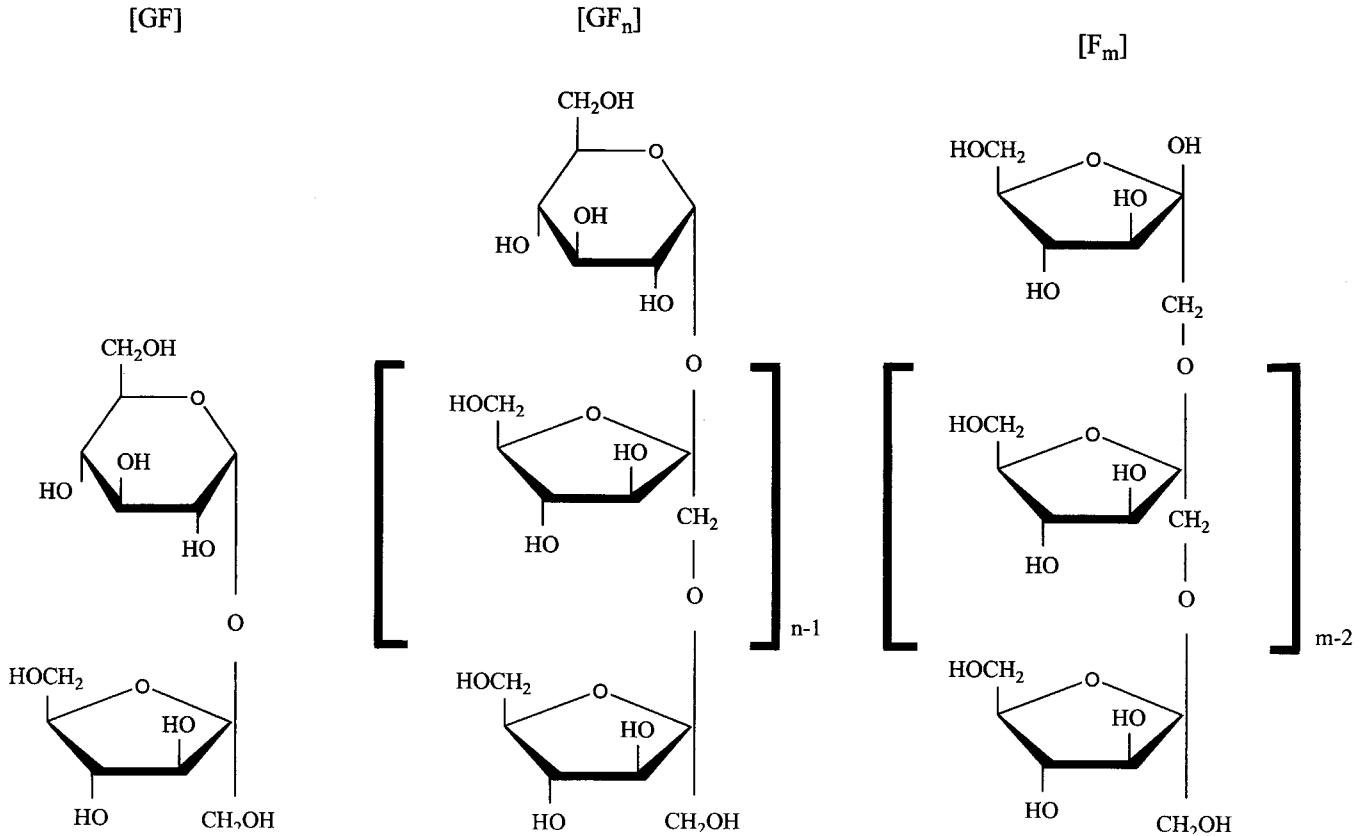

FIG. 1. Chemical structure of sucrose (left), inulin (center), oligofructose (right).

fructosyl transferase (FFT), catalyzing the transfer of a unit of fructose from one donor polymer to another acceptor. The enzyme uses the trisaccharide formed by SST for further chain elongation of the fructan polymer, releasing sucrose (Equation 2). Therefore, SST and FFT have some overlapping activities. During this transfer, sucrose molecules only act as acceptors and cannot function as donors. The balance mechanism between the various fructans with a different DP and in the absence of new syntheses appears to have a regulatory effect on the plant's physiology (Marchetti, 1993a).

$$G(F)_n + G(F)m \xrightarrow{FFT} G(F)_{n-1} + G(F)_{m+1} \quad (2)$$

Inulin from chicory is a mixture of oligomers and polymers of fructose having varying degrees of polymerization (DP), but typically having a DP range from three (corresponding to GF_2) to about 60 with a modal chain length of approximately nine. Native inulin from chicory often is made up of about 1–2% β-(2→6) branching with short side-chains (Van Loo *et al.*, 1995). In addition to having predominately linear chains of the GF_n-type, native inulin extracted from fresh chicory roots also has been shown to contain about 1–1.5% (F_m) compounds on a dry weight basis. These homopolymers of fructose are bound by a β-(2→1) linkage (Van Loo *et al.*, 1995). The *m* represents the number of fructosyl moieties in the homopolymers. Both GF_n and F_m have very similar physicochemical properties except that F_m-type products are reducing, due to the presence of a reducing fructose-end group, whereas GF_n compounds are not.

An understanding of different terms used to describe fructose containing polymers is important as more commercial products become available. Oligofructose was introduced as a synonym for fructooligosaccharides in 1989 (Coussement, 1999). The oligofructose product is a partial enzymatic hydrolyzate of native chicory inulin containing predominantly molecules of the F_m-type (homopolymers of fructose bound by a β-(2→1) glycosidic linkage having no terminal glucose). Oligofructose has been defined by the IUB-IUPAC Joint Commission on Biochemical Nomenclature and the AOAC as fructose oligosaccharides containing 2–10 monosaccharide residues connected by glycosidic linkages (Niness, 1999). As previously stated, inulin extracted from chicory root is a polydisperse fructan with chain lengths ranging from 2 to 60 units having a modal DP of approximately 10 monomeric units. Chicory inulin is composed of approximately 2% monosaccharides, 5% disaccharides and 93% inulin. Neosugar is a FOS mixture of ketose ($n = 2$), nystose ($n = 3$) and 1F-*B*-fructofuranosyl nystose ($n = 4$). Basically these are sucrose molecules to which one to three

additional fructose units have been added. The development and isolation of Neosugar was first reported in the Japanese literature in 1983 (Oku *et al.*, 1984).

Neosugar can be isolated from brans of triticale, wheat and rye or synthesized by the action of the fungal enzyme *B*-fructofuranisidase, naturally produced by *Aspergillus niger* (Fishbein *et al.*, 1988). Using the most widely available and accepted nomenclature, all FOS and inulins are fructans, all FOS are inulins, but not all inulins are FOS. Those inulin molecules having a degree of polymerization of < 10 fructose units generally are considered to represent FOS.

A. ORIGIN AND HISTORY

Inulin was discovered by Rose, a German scientist, who in 1804 found "a peculiar substance" from plant origin in a boiling water extract from the roots of *Inula helenium,* a genus of perennial herbs of the group *Compositæ,* natives of the temperate regions of Europe, Asia, and Africa (Goudberg, 1913). The substance was named inulin but was also identified by other names such as helenin, alantin, meniantin, dahlin, sinanternin and sinisterin. The biochemical production was elucidated around the middle of the 19th century.

Inulin belongs to the group of naturally occurring carbohydrates known as non-digestible oligosaccharides (NDO). It is produced naturally in over 36,000 plants worldwide, including 1200 native grasses belonging to 10 families. After starch, they are the most plentiful carbohydrates occurring in nature (Carpita *et al.*, 1989; Marchetti, 1993b). It has been estimated that as much as one third of the total vegetation on earth consists of plants that contain fructans. Inulin-type carbohydrates obtained from fungal fermentation have been reported in commercial use but the predominant commercial source for inulin/FOS is of chicory root origin. Inulin has extensive documented historical human use through the consumption of edible plants and fruits, a variety of which are common foodstuffs (Table I).

Inulin is an energy-reserve carbohydrate and may act as an osmoprotectant in plants. Because inulin is soluble in water, it is osmotically active. By changing the DP of the molecule in the plant's vacuole, the plant can readily change the osmotic potential of its cells without altering the total amount of carbohydrate. The internal hydrolysis of inulin by endoinulinase to lower DP F_m and GF_n molecules allows plants to osmoregulate, surviving winter periods in cold to moderately cold and drought-stricken regions (Edelman and Jefford, 1968).

Historically, several inulin-laden foods, especially chicory, dahlia, Jerusalem artichokes, murnong and yacon, have been used as staple food or as sustenance crops. Australian aborigines ate murnong, a tuberous plant,

TABLE I

INULIN AND OLIGOFRUCTOSE CONTENT OF FOODS EATEN BY AMERICANS (g/100g)

	Inulin		Oligofructose	
	Range[a]	Midpoint[b]	Range	Midpoint
Banana				
Raw	0.3–0.7	0.5	0.3–0.7	0.5
Raw – dried	0.9–2.0	1.4	0.9–2.0	1.4
Canned	0.1–0.3	0.2	0.1–0.3	0.2
Asparagus				
Raw	2.0–3.0	2.5	2.0–3.0	2.5
Boiled	1.4–2.0	1.7	1.4–2.0	1.7
Chicory root	35.7–47.6	41.6	19.6–26.2	22.9
Dandelion greens				
Raw	12.0–15.0	13.5	9.6–12.0	10.8
Cooked	8.1–10.1	9.1	6.5–8.1	7.3
Garlic				
Raw	9.0–16.0	12.5	3.6–6.4	5.0
Dried[c]	20.3–36.1	28.2	8.1–14.5	11.3
Globe artichoke	2.0–6.8	4.4	0.2–0.7	0.4
Jerusalem artichoke	16.0–20.0	18.0	12.0–15.0	13.5
Leeks				
Raw	3.0–10.0	6.5	2.4–8.0	5.2
Onions				
Raw	1.1–7.5	4.3	1.1–7.5	4.3
Raw – dried	4.7–31.9	18.3	4.7–31.9	18.3
Cooked	0.8–5.3	3.0	0.8–5.3	3.0
Wheat				
Bran – raw	1.0–4.0	2.5	1.0–4.0	2.5
Flour – baked	1.0–3.8	2.4	1.0–3.8	2.4
Flour – boiled	0.2–0.6	0.4	0.2–0.6	0.4
Barley				
Raw	0.5–1.0	0.8	0.5–1.0	0.8
Cooked	0.1–0.2	0.2	0.1–0.2	0.2
Rye				
Baked	0.5–0.9	0.7	0.5–0.9	0.7

[a] Source: van Loo *et al.* (1995) and personal communication with Dr Jan Van Loo, December 1997.

[b] Calculated as an average of the range.

[c] Calculated using a total solids approach.

in the 19th century as their main vegetable food with a reported daily intake of 200–300 grams (Gott, 1984).

Chicory is indigenous to Europe and has been cultivated since the 16th century with the roots and greens (known as Belgian endive) being used for human consumption. Post-World War II populations in England and

Germany roasted root of the chicory plant to use as an extender or substitute for coffee beans (Meijer *et al.*, 1993). This concept is still in use in the southern United States, particularly Louisiana. A cup of chicory coffee may contain three grams of inulin (Douglas and Poll, 1986; Van Loo *et al.*, 1995).

In the South Pacific a variety of yacon was introduced to Japan from New Zealand and grew in popularity (Asami *et al.*, 1989). Native Americans used the Jerusalem artichoke tuber, native to North America, for food. Jerusalem artichokes were found by Champlain at Cape Cod, Massachusetts and were introduced in France in 1605, becoming the main source of carbohydrate in Western Europe until it was superseded by the potato in the middle of the 18th century (Wyse and Wilfahrt, 1982). Jerusalem artichokes were again cultivated as a staple crop by the post-Second World War French and Germans due to scarcity of the potato at the time.

B. COMMON INTAKES

Historically, daily inulin intake was estimated to be approximately 25 to 32 grams. Today, the average daily intake of inulin and its hydrolysis products in Western Europe is estimated between 2–12 g/person/day (Roberfroid *et al.*, 1993). The US consumption is estimated at 2–8 g/person/day, based on data from the US Nationwide Food Consumption Survey 1987–88 (Roberfroid *et al.*, 1993).

A more recent USDA study by Moshfegh and others (1999) showed that American diets provide about 2.6 g of inulin and 2.5 g of oligofructose. Mean intakes varied by gender and age groups with a range from 1.3 g for young children to 3.5 g for teenage boys and adult males. Per 1000 calories, mean intake ranged from 0.9 to 1.5 g in American diets. Significant differences exist between variable sociodemographic categories. Whites, who make up 73% of the US population, consume significantly more of these inulin-containing components than Blacks or Hispanics (Moshfegh *et al.*, 1999). The primary sources of inulin in American diets are wheat and onions. Many other commonly consumed fruits and vegetables contain considerable amounts of inulin and contribute varying levels of inulin in daily human diets (Fig. 2).

C. PRODUCTION

Until recently, inulin as a pure compound was not produced economically on an industrial scale and was not available as a food ingredient for human consumption. Inulin was produced on a pilot scale in Deutsche Kulörfabrik

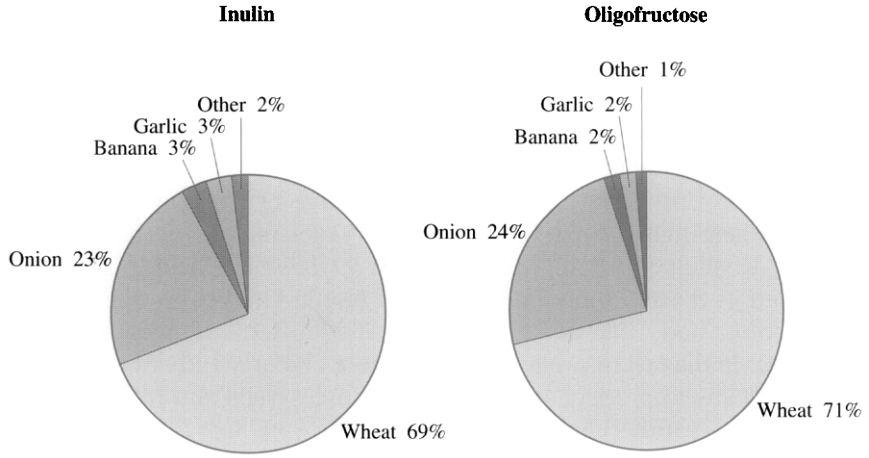

FIG. 2. Contribution of food sources to inulin and oligofructose in American diets. Data presented in this figure are from the 1994–96 Continuing Survey of Food Intakes by individuals using the specialized database for inulin and oligofructose (Moshfegh *et al.*, 1999).

in the early 1920s (Schöne, 1920), and later was extracted on an industrial scale. In 1927 Belval reported several German sugar factories extracted inulin from chicory, similar to sugar extraction from sugar beets. Like sugar from sugar beets, the extract was high in impurities that were removed by lime-carbon dioxide purification. Chilling precipitation further purified the inulin. However, the production process was deemed uneconomical due to the recovery process using precipitation by chilling (Gibson *et al.*, 1994). Today's process is significantly more efficient and economical.

The primary industrial source of pure inulin is the chicory root *(Cichorium intybus* L.). Chicory is a member of the Compositœ family. Other significant inulin-containing members of this family are dandelion, lettuce, globe and Jerusalem artichoke, dahlia and yacon. Other inulin-containing plants belong to the Liliales family, e.g. leeks, onion, garlic and asparagus. Although chicory has been cultivated for centuries, it still remains quite wild, having had only very minor influence in its genetic basis since its early cultivation occurred in England in 1548. The ancestral form of chicory is a perennial plant (var. silvestre) that probably originated in East India and grew widespread in the Mediterranean areas of Europe and Near Asia. Cultivated root chicory is a biennial plant that is grown as a long-season annual. The root is indigenous to Western Europe and was cultivated on a larger scale beginning in the 16th century before

being exported to the United States in 1806. Chicory is now a common purplish-blue-flowering weed growing wild in roadway ditches in the United States and Canada.

A distinction is made in cultivation between the three basic forms of chicory. The slightly bitter, curled dandelion-like greens (called Italian dandelion) are grown and used as potherbs. Witloof chicory, *Cichorium endivia* Linn Endive (also called French endive or Belgian endive) is a perennial herb that is forced as a blanched vegetable and used as a salad delicacy. Root chicory varieties, *Cichorium intybus* Linn var. sativum, have been used as roasted roots in Europe and North America to impart additional color, body and sedative action as a coffee extender or coffee substitute. In the last ten years, European root chicory cultivation has provided a means to produce high-purity fructose syrups and refined inulin products with various chain lengths.

Chicory root is a biennial plant requiring soil and climatic conditions resembling those for sugar beets: deep, fertile, permeable and cool soil with pH tending towards neutral; intolerance of droughts or poor drainage. The best conditions for high yields are mild maritime-like climates, rich light-clay to sandy-clay soils and long periods of daily illumination, similar to that in Western European countries like France, Holland and Belgium. The plant, being similar to the sugar beet root, shares similarities in agronomic practices and inulin production technologies. The inulin production process involves three general steps: extraction of raw inulin with hot water, purification of the raw inulin, and spray drying of the purified juice to a pure inulin powder. Although inulin is spray dried, the molecule is quite flexible, and crystallizes easily (French, 1989). Further outline of the process is given in Fig. 3.

III. BIFIDOGENIC PROPERTIES

The large intestine is the most heavily colonized region of the digestive tract, with up to 10^{11} bacteria for every gram of intestinal content (Gibson and Roberfroid, 1995). Gut bacteria comprise one hundred different species which include both beneficial and potentially deleterious bacteria in a balance that affects how food is digested and energy is obtained. When the main types of generally recognized beneficial bacteria, bifidobacteria and lactobacilli, are at optimum levels they constitute approximately one-third of the bacterial population in the adult gastrointestinal tract. In some cases the numbers of beneficial bacteria may be so low they are undetectable. The numbers of bifidobacteria are regarded as a marker of the stability of the human intestinal microflora (Mutai and Tanaka, 1987).

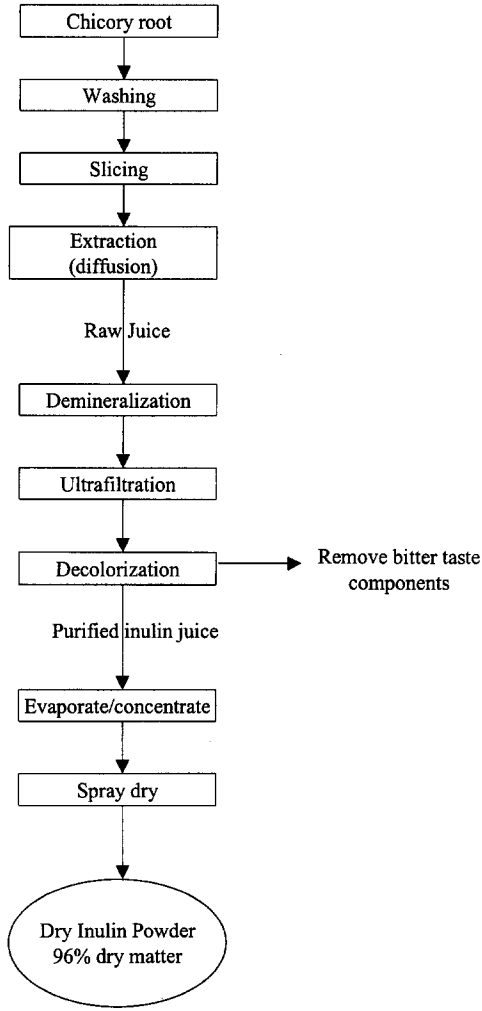

FIG. 3. Inulin production and process.

The importance of an indigenous microflora population as a natural means to fight potential pathogenic microorganisms was originally recognized by Metchnikoff during research on cholera in the 19th century (Bibel, 1988). Decades later, the importance of a well-balanced gut microbial ecosystem is becoming more widely recognized for host health. Research by Mutai and Tanaka (1987) stimulated further efforts to identify the role of bifidobacteria (Romond and Romond, 1987; Mitsuoka, 1990), and other

lactic acid bacteria, mainly lactobacilli (Tannock, 1990; Salminen *et al.*, 1993a, 1993b) with regard to host health. Miller-Catchpole (1996) noted that bifidobacteria, even though implicated in incidental opportunistic anaerobic infections (as in individuals with weakened immune systems), are generally regarded as safe. Stimulation of bifidobacterial numbers as well as the numbers of lactobacilli in the colon is beneficial to host health.

Populations of bifidobacteria can represent up to 95% of the total intestinal microflora in breast-fed infants, in comparison with about 25% in the adult (Gibson, 1995). The mother's delivery canal and fecal flora inoculate the gastrointestinal tract of the newborn during birth (Gibson and Roberfroid, 1995). Drasar and Roberts (1989) recognized that fecal flora of breast-fed infants is dominated by populations of bifidobacteria, with only 1% enterobacteria. By contrast, formula-fed infants have a more complex microflora, with bifidobacteria, bacteroides, clostridia and streptococci all prevalent (Gibson and Roberfroid, 1995). Thus, it is thought that presence of these healthy microorganisms in breast-fed infants contributes to their alleged health advantages compared to formula-fed infants. Following weaning, the microflora pattern in breast-fed infants begins to resemble that of adults.

As a consequence to findings by Mutai and Tanaka (1987), Romond and Romond (1987), Drasar and Roberts (1989) and Mitsuoka (1990) and a number of other researchers, scientists now generally ascribe to the beneficial health effects of bifidobacteria in the colon.

Predominant gut microflora, generally recognized as producing health promoting functions, are the *Bacteroides, Bifidobacterium, Lactobacillus,* and *Eubacterium* (Gibson and Roberfroid, 1995). These beneficial microflora, particularly the bifidobacteria and lactobacilli, may play critical roles in immunological responses, by either helping to resist infection, or by creating conditions which reduce the number of pathogenic bacteria. These beneficial bacteria may act as wards regulating the activity of the other bacteria in the colon. The other bacteria, such as *Salmonella, Shigella, Clostridia, Staphylococcus aureus, Candida albicans, Campylobacter jejuni, Escherichia coli, Veillonella* and *Klebsiella,* have varying potential to cause disease and are much less numerous. However, these pathogenic bacteria and several strains of yeasts, most notably *Candida albicans,* can produce harmful local and systemic effects if they overgrow as a consequence of a gut microflora imbalance (Elmer *et al.*, 1996). Research has shown beneficial bacteria, particularly bifidobacteria and lactobacilli, keep these potential disease-causing organisms under control, preventing several disease-related dysfunctions related to an imbalanced GI situation (Elmer *et al.*, 1996).

Bifidobacteria, while significantly influencing other colonic microflora,

are also affected by many exogenous and endogenous factors. Factors affecting intestinal flora observed by Rasic (1983) were: animal species, habits, age, sex, climate, diet, stress, exogenous organisms, and immune mechanisms of the host. Other modifiers of the gut microflora are surgery on the stomach or small intestine, kidney or liver disease, cancer, pernicious anemia, blind loop syndrome or a change in the acidity of gastric juices (Modler *et al.*, 1990). In addition, other pathogenic disorders, such as liver cirrhosis and impaired intestinal motility may modify colonic microflora. During stages of acute infection, antibiotic therapy used to combat the bacterial invasion can also induce digestive disorders due to alteration of normal gut flora. Bifidobacteria have been shown to be resistant to streptomycin, but have only moderate resistance to penicillin, tetracycline, neomycin and novobiocin (Kurmann and Rasic, 1991). Bifidobacteria can be completely eradicated from the colon when antibiotics such as erythromycin, spiramycin and chloramphenicol are used to combat other bacterial infections.

Reducing or eliminating more of the healthy gut microflora, like bifidobacteria, has its consequences. When the human diet influences the species composition and metabolic characteristics of the intestinal microflora, toxic metabolite production is affected, such as the conversion of procarcinogens to active carcinogens (Rowland, 1998; Perman, 1989; Roland *et al.*, 1993). *E. coli* and clostridia are known to produce ammonia, amines, carcinogens and cancer promoters such as nitrosamines, phenols, cresols, indole and skatole, estrogens, secondary bile acids and aglycones. Chadwick and coworkers (1992) reported reductive enzyme activities are lower in bifidobacteria and lactobacilli relative to *E. coli* and clostridia. Bacteroides are generally thought to be health promoting, while *Enterococcus faecalis* produce nitrosamines, aglycones and secondary bile acids, and proteus produces ammonia, amines and indole (Drasar and Hill, 1974; Koizumi *et al.*, 1980; Mitsuoka, 1982, 1990; Kanbe, 1988). In addition to producing toxic metabolites, several harmful bacteria such as salmonella, shigella, listeria, proteus, *E. coli, Clostridium perfringens* and *Vibrio cholerae* also have been associated with diarrhea, infections, liver damage, carcinogenesis and intestinal putrefaction. It is possible the health-promoting effects prompted by bifidobacteria and other healthful bacteria were due to the growth inhibition of harmful bacteria, stimulation of immune functions, lowering of gas distension problems, improved digestion/absorption of essential nutrients and synthesis of vitamins (Roberfroid and Delzenne, 1993; Gibson, 1995; Gibson and Roberfroid, 1995).

Pathogenic effects associated with harmful intestinal microflora such as *E. coli*, clostridia, proteus, salmonella, shigella, and *Vibrio cholerae* not only include colonic disorders but also have implication with possible

vaginal infections and systemic disorders. Intestinal pathologies include antibiotic-associated diarrhea (AAD), inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease, colorectal cancer, necrotizing entercolitis, and ileocecitis. Vaginal infections include candidal vaginitis while systemic disorders include gut origin septicemia, pancreatitis and multiple organ failure syndrome (Gibson and Roberfroid, 1995). Major factors in the biology of these disorders are the overgrowth of pathogenic bacteria such as clostridia, *E. coli*, as well as parasites, viral infections, extensive burn injury, post-operative stress and antibiotic therapy, which are often associated with bacterial translocation due to intestinal barrier failure (Gardiner *et al.*, 1993; Solomons, 1993; Gibson and MacFarlane, 1994).

Mechanisms related to microflora modification to a healthier environment vary for individual microflora groups. However, the antagonistic effects of lactic acid bacteria have been attributed to favorable competition for active sites on the colonic epithelial wall, the production of primary metabolites, such as acetic, lactic and butyric acids, and hydrogen peroxide and the secretion of specific bacteriocins, e.g. Lactacins B, F and Lactocin 27 (Modler *et al.*, 1990; Gibson and Wang, 1994c). Numerous other investigators have also reported the ability of lactic acid bacteria to produce antibacterial substances, which are active against certain pathogenic and putrefactive organisms (Mehta *et al.*, 1983). Some *Streptococcus* spp. have also been shown to produce bacteriocidal substances (Miller-Catchpole, 1989). *L. acidophilus* strains produce three substances, namely acidolin, acidophilin and lactocidin; *L. bulgaricus* produces one substance, bulgarican.

Although bifidobacteria do not produce hydrogen peroxide, observations by Gibson and Roberfroid (1995) hint that some bifidobacteria might secrete a bacteriocin-type substance that is active against clostridia, *E. coli*, and many other pathogenic bacteria, such as listeria, shigella, salmonella, and *Vibrio cholerae,* or antimicrobal substances. Anand and coworkers (1985) tested six strains of *B. bifidum* for their antibacterial activity and reported that antibacterial activity differed among the strains with maximum inhibitory action shown by one strain against *M. flavus* followed by *Staph. aureus, B. cereus, E. coli, Ps. flourescens, S. typhosa and Sh. dysenteriae.*

Like lactobacilli, bifidobacteria produce strong acids, i.e acetic and lactic acid (Scardovi, 1986). The production of these acids reduces intestinal pH. One effect of lowering the gastrointestinal pH might be the protonation of toxic ammonia (NH_3) to produce ammonium ion (NH_4^+), which is non-diffusable and could result in lower blood ammonia levels and a reduced hepatic load (Miller-Catchpole, 1989; Levrat *et al.*, 1993). An

additional, and potentially more important, effect is restriction or prohibition of the growth of many potential pathogens and putrefactive bacteria.

Acetic acid has been observed to exert a greater antimicrobial effect than lactic acid, most likely due to a greater amount of undissociated acid at intestinal pH values (5.8) common to bifidobacteria and lactobacilli (Modler *et al.*, 1990). Because bifidobacteria produce almost two-fold more acetate than lactate, the undissociated acetic acid would be approximately 11-fold greater than lactate. This is an important factor since the growth of many potential pathogens and putrefactive bacteria is very sensitive to concentrations of undissociated acid (Modler *et al.*, 1990).

Scardovi (1986) suggested the optimum pH for bifidobacteria is between 6.5 and 7.0 with little or no growth below the pH range of 4.5 to 5.0 or above 8.0 to 8.5. Wang and Gibson (1993) observed that specific growth rates of *B. infantis, E. coli* and *Cl. perfringens* were approximately equal at neutral pH. As the pH was lowered the bifidobacteria growth rate remained relatively unaffected, while the growth of *E. coli* and *Cl. perfringens* was completely inhibited at pH 5.0 and 4.5.

Bifidobacteria do not form aliphatic amines, hydrogen sulfide or nitrites (Bezkorovainy and Miller-Catchpole, 1989). They produce vitamins, largely of the B-group, such as biotin, thiamine, riboflavin, niacin, pyridoxine, cyanocobalamin and folic acid (Deguchi *et al.*, 1985; Hartemink *et al.*, 1994; Gibson *et al.*, 1995). These bacteria also produce digestive enzymes such as lactase (β-galactosidase) that may improve lactose tolerance and digestibility of dairy products (Hughes and Hoover, 1991).

A. PROBIOTICS AND PREBIOTICS

To minimize the potential for imbalances in gut microflora which could lead to intestinal, systemic and, possibly, vaginal infections, researchers have investigated various means of achieving a greater population of healthy gut microflora. There is growing evidence that live selected bacteria, such as bifidobacteria and lactobacilli, when added to food (such as fermented milk beverages or yogurt) or used as a dietary aid, are beneficial for these purposes. Probiotics are defined as living microbial feed supplements added to the diet which have beneficial effects on the host by improving its intestinal microflora balance (Fuller, 1989). In humans, lactobacilli, either as single species or in mixed culture with other bacteria such as bifidobacteria and streptococci, are common probiotics (Gibson, 1995).

When probiotics are added to the diet as a large bowel target to bring about microflora balance, these organisms must reach their intended destination intact and become viable. There is evidence that some probiotics

added in the diet are able to reach the colon and provide health benefits (Kageyama *et al.*, 1984; Pochart *et al.*, 1992; Elmer *et al.*, 1996). However, due to fluctuating activities in response to substrate availability, redox potential, pH, oxygen tension and colonic distribution, the survivability and effectiveness of ingesting living microorganisms for purposes of targeting colon microflora modulation is variable.

In addition, incorporation of healthy viable bacteria into processed foods is also somewhat difficult because of their high sensitivity to oxygen, shear, heat, and pH (Tomomatsu, 1994). Intake of food as a bolus and the development of species that are more oxygen- and acid-resistant are probably of importance. Further, due to competition for nutrient sources and colonization sites with previously well-established endogenous microflora, individual probiotic fixation and activities are strain-dependent. There is also evidence that probiotic effects are transient. When consumption of the probiotic product ceases, the added bacteria are excreted (Bouhnik *et al.*, 1992). However, in order for bifidobacteria to benefit host health, it is necessary that these organisms be metabolically active in the lower gastrointestinal tract (Tamura, 1983; Hashimoto, 1985).

As a prerequisite to their survivability, these bacteria require a carbohydrate source to use for fermentation that has not been metabolized by the human digestive system before reaching the colon. Selective non-digestible carbohydrate food sources that promote the proliferation of bifidobacteria and lactobacilli have been defined as prebiotics (Gibson and Roberfroid, 1995). Prebiotics that would have potential use as ingredients in foods should be non-digestible, very shelf stable, require no refrigeration, be easily and effectively incorporated into processed foods and nourish all endogenous beneficial bacteria. The combined form of a probiotic and a prebiotic, as described by Gibson and Roberfroid (1995), is termed a synbiotic. In a review, Fuller and Gibson (1997) discussed a variety of mechanisms which may be responsible for the beneficial effects of pro- and prebiotic supplements. A comparison of the terms probiotic, prebiotic and synbiotic is presented in Table II.

Various *in vitro* and *in vivo* studies have shown that a diet supplemented with β-(2$\rightarrow$1) fructans (inulin/FOS) provide an effective means to promote the growth of bifidobacteria and lactobacilli, while selectively reducing the growth of pathogenic microorganisms and potentially treating intestinal dysfunctions (Rowland and Tanaka, 1993; Terada *et al.*, 1993; Wang and Gibson, 1993; Gibson and Wang, 1994a, 1994b, 1994c; Kleessen *et al.*, 1994, 1997; Williams *et al.*, 1994; Gibson and Roberfroid, 1995; Gibson *et al.*, 1995; Cummings *et al.*, 1997; Hartemink *et al.*, 1997; Roberfroid *et al.*, 1998). As a consequence of a European Commission-funded project on non-digestible oligosaccharides, the

TABLE II

SUMMARY OF THE VARIOUS MECHANISMS USED TO MODULATE THE COMPOSITION OF THE HUMAN LARGE INTESTINAL MICROFLORA

Term	Definition	Examples	Advantages	Possible future developments
Probiotic	A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance	Lactobacilli, bifidobacteria enterococci streptococci	Strain may have proven health values Useful when gut flora may be compromised	New product developments based on synbiotics, that may improve probiotic survival
Prebiotic	A non-digestible 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	Fructooligosaccharides, galactooligosaccharides, inulin	Genus-level changes occur in gut flora Product survival not problematic Low dose required and can be incorporated into many different foods	Manufacture of novel multiple-function prebiotics, that may: stimulate the beneficial flora; exert anti-adhesive properties; attenuate pathogen virulence Prebiotics derived from dietary fiber-type polysaccharides
Synbiotic	A mixture of pro- and prebiotics which beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract	Fructooligosaccharides + bifidobacteria; lactitol + lactobacilli	Dual effect of both entities Probiotic survival should be improved	Design of new synbiotics through molecular engineering (based on specific prebiotic enzymes)

Reference: Reproduced with permission from Gibson and McCartney, 1998, *Biochem. Soc. Trans*, **26**: 222–228 © the Biochemical Society.

ENDO project has determined by consensus that there is now strong scientific evidence that β-(2→1)-type fructans are prebiotic (Van Loo *et al.*, 1999). In addition, inulin used as a general human gastrointestinal aid (Paul, 1996), as a method of treating ulcerative colitis (Garleb and Demichele, 1995) and to inhibit *C. difficile* infections (Garleb *et al.*, 1997) are defined in United States patents.

Several researchers have reported the selective fermentation of inulin from a variety of sources and production processes as well as other NDO using *in vitro* and *in vivo* studies and pure cultures of human colon microflora. Results from many of these researchers are summarized in Table III.

In review, Modler *et al.* (1990) and Roberfroid (1993) defined inulin as a good substrate for most *Bacteroides* and *Bifidobacterium* species, except *Bifidobacterium bifidum* and some strains of *B. longum*. Chain length of inulin seems to be an important determinant for some specific species. McBain and Macfarlane (1997) used a three-stage fermentation system to reproduce several nutritional and environmental characteristics of the proximal large intestine and the distal colon. Bifidobacterial populations were stimulated within three hours of FOS addition and were increased by approximately 10-fold within 12 hours. Total anaerobic populations and the *B. fragilis* group also increased as did peptostreptococci and enterococcal numbers. *Clostridium perfringens* increased transiently while fusobacterial populations were suppressed. These researchers also studied the effect of FOS on enzymes capable of metabolizing a wide range of compounds in the diet to form toxic, mutagenic and carcinogenic products. They found that the addition of FOS increased the production of arylsulphatase with *B*-glucosidase and *B*-glucuronidase showing a small decrease three hours following addition of FOS. These studies highlight the complexity of the human intestinal microbiota and the potential problems that could be encountered in changing its metabolism. They concluded that, even though there was some synthesis of putatively 'genotoxic enzymes', carbohydrate administration should not be viewed as potentially harmful.

Wang and Gibson (1993) and Gibson and Wang (1994a) while working *in vitro* with several bifidobacteria strains, *Cl. perfringens* and *E. coli*, demonstrated that native chicory inulin (modal DP 10 units) allowed more rapid development of *B. infantis, B. pseudolongum* and *B. angulatum* as compared to glucose. *B. longum* had a slower development in comparison to glucose, and the development of four other species was not significantly different. Inulin (modal 10 DP units) was demonstrated to significantly suppress the growth of both *E. coli* and *C. perfringens*. During the fermentation of inulin, mainly CO_2, medial H_2 and relatively no CH_4 were produced.

TABLE III

UTILIZATION OF VARIOUS NON-DIGESTIBLE OLIGOSACCHARIDES BY SELECTED HUMAN GUT BACTERIA[a]

Bacterial species	FOS[b]	INU	LOL	PHGG	LAC	LAT	TOS	RAF	GLL	IMO
Bifidobacterium spp[c]	+	+	V	–	+	+	+	+	+	+
Lactobacillus acidophilus-group	V	+	+	–	+	+	+	–	+	V
L. casei	V	+	+	–	–	+	+	–	–	–
Bacteroides fragillis	+	+	+	–	+	+	+	V	+	–
B.thetaiotamicron	+	+	+	–	+	+	+	V	+	+
B. vulgatus	+	+		–	+	+	+	V	+	+
B. ovatus	+	+		+	+	+	+	V	–	+
B. distasonis	+	+	+	–	+	+	+	V	+	+
Eubacterium lentum	–	–			+	+	+	–	–	–
E. limosum	–	–				–	–	–	–	–
Fusobacterium necrophorum	–	–		–		–	–	–	–	–
Enterococcus faecalis	+	+			+	+		V		
E. faecium	+	+			+	+		V		
Propionibacterium granulosumi	–	V								
Escherichia coli	V	–	–	–	+	V	+	–	–	–
Peptostreptococcus prevotil	+	–			–			+		
Clostridium perfringens	V	V	+	–	+	+	V	V	–	+
C. paraputrificum	–	–			+	+		–		
C. clostridiiforme	V	–	+	–	+	–		V	–	–
C. difficile	–	–			–	–		–		
C. romosum	+	+	+	–	+	+		V	–	+
C. butyricum		–	+	+		+	–	+	–	–
Megasphaera elsdenii	–	–	–	–	–	–	–	–	–	
Veillomella parvula	–	–	–		–	–	–	–	–	–

[a] Results from various studies involving oligosaccharides having comparable chemical composition were combined. When studies and/or a majority of the strains showed positive or negative results, the strain is displayed (+) or (–), respectively. Studies having no agreement are displayed as (V). Data obtained using different methods are combined.

[b] FOS = fructooligosaccharides (including ketose, nystose, fructosylnystose, neosugar, Profeed, Meioligo, oligofructose), INU = inulin (including average DP 9–10 units), LOL = lactitol, PHGG = partially hydrolyzed guar gum, LAC = lactose, LAT = lactulose, TOS = transgalactosyloligosaccharides, RAF = raffinose, GLL = 4′-galactosyllactose, IMO = isomalto-oligosaccharides.

[c] Bifidobacterium bifidum negative for most oligosaccharides.

References: Hayakawa *et al.*, 1990; Tanaka et al., 1983; Hartemink *et al.*, 1997; Yanahira *et al.*, 1995; Asano *et al.*, 1994; Kitler *et al.*, 1992.

Saito and coworkers (1992) performed an *in vitro* fermentation study with monocultures of 125 strains of human intestinal bacteria of 18 different genera, including 29 strains from five species of *Bifidobacterium* on media containing five different carbohydrate substrates: refined soybean oligosaccharides, stachyose, raffinose, fructooligosaccharide or glucose. The multiple unit carbohydrate sources elicited a slower growth rate than glucose for *Lactobacillus, Bacteroides,* and *Enterococcus* and did not support the growth of potential pathogenic *Clostridium* species, *Veillonella, E. coli,* and *Kiebsiella.* The growth rate of *Bjfidobacterium,* except *B. bifidus,* was similar on all carbohydrate sources. It should be noted that Wada (1990) identified raffinose as a fermentation substrate for *C. perfringens.*

In vitro study using *B. infantis* and *B. breve,* and various inulin fractions showed that inulin (DP ≥ 15, modal value of 22 units) induced a slower growth rate than glucose or lactose for *B. infantis* (Yazawa and Tamura, 1982). The reported data emphasized that the molecular weight of a carbohydrate should be relatively large and that its reducing end be occupied by fructose to selectively grow bifidobacteria. Bacterial generation times were equal for inulin fractions, irrespective of the molecular weight (from 1200 to 4500). It was further shown that *B. infantis* required about four hours for adaptation to these substrates. Consequently, when inulin is used as substrate with *B. infantis,* the bifidobacteria should be adapted to it and ingested at the same time (Yazawa and Tamura, 1982).

McKellar *et al.* (1993) characterized the growth and inulinase production by *Bifidobacteria* spp. on fructooligosaccharides and observed that these strains grew equally well on short-chain fructooligosaccharides (average DP 3.7) and inulin as a native extract of chicory root (modal DP 10 monomer units). Several strains of animal origin *(B. thermophilum, B. minimum,* and *B. cuniculi)* grew significantly better than strains from human origin on inulin (DP ≥ 15, modal value of 22 units).

Several references identified *Klebsiella pneumoniae* as an inefficient or non-inulin fermenter (Brenner, 1980; Yazawa and Tamura, 1982; McKellar *et al.*, 1993). The relative growth parameters of *K. pneumoniae* are chain dependent, as the organism does not possess highly active intracellular 2,1 β-D-fructan-fructanohydrolase (EC 3.2.1.7) enzyme. As analytical grade, long-chain inulin (DP ≥ 15, modal value of 22 units) has typically been used as control substrate in research and for identification of *K. pneumoniae.* The pathogen *K. pneumoniae* is indicated as growing well on short-chain fructooligosaccharides, 1-kestose, nystose, fructosylnystose and FOS synthesized from sucrose and composed of GF_n [$n\beta$-(2→1) linked fructose moieties bound to a glucose molecule; $2 < n < 4$] (Mitsuoka *et al.*, 1987).

In addition to bacteria, some yeasts also have active exo-inulinase

enzyme to break the β-(2→1) linkage of inulin. They can potentially grow in periods following antibiotic therapy or in individuals that are immune-compromised. However, because yeasts are primarily opportunistic pathogens their overgrowth is normally controlled and candidiasis is prevented by competition from healthy, lactic acid-producing organisms that are nourished selectively by inulin. Of approximately 590 species of yeast only 13 have clinical significance and only five of the 13 have positive or variable growth on inulin (Barnett *et al.*, 1990).

As for *in vivo* studies, Bornet and others (1997b) working with a short-chain inulin fraction (average DP 3.7) observed fecal bifidobacteria increases in healthy humans was dose-response related. They noted that doses of 5 and 10 g/day significantly increased colonic bifidobacteria ($p < 0.05$) while doses equal to or less than 2.5 g/day showed no statistically significant modification effects. In a related study, 12 elderly adults, aged 69 ± 2 years, ingested 8 g/day for four weeks and had bifidobacteria counts increase from 8.52 ± 0.26 to 9.17 ± 0.17 log CFU/g ($p < 0.05$) (Bornet *et al.*, 1997a). However, Roberfroid *et al.* (1998) stated that log increases in bifidobacteria counts do not necessarily correlate with daily doses administered, but rather depend more on the initial number of bifidobacteria. Lower initial numbers of bifidobacteria have been shown to produce greater increases, irrespective of dose, within a range of 4–20 g or more per day. An increase of bifidobacteria less than one log unit is difficult to assess, and the absolute increase in number of bifidobacteria is likely to be less important than the statistical significance of the increase (Roberfroid *et al.*, 1998).

Gibson *et al.* (1995) showed that humans consuming 15 g/day of inulin (DP 2 to 60; average 10 units) significantly increased the bifidobacteria population over a two-week period ($\log_{10}$ 9.2 to $\log_{10}$ 10.1 per gram, $p < 0.001$), rendering them the dominant population (Fig. 4). The numbers of gram-positive cocci decreased from $\log_{10}$ 6.0 to $\log_{10}$ 5.5 ($p < 0.001$); and the total aerobic and anaerobic counts and the numbers of other groups of bacteria stayed at the same level. Buddington and others (1996) found that adding Neosugar to the diet of healthy subjects increased anaerobes represented by bifidobacteria from 3.4% to 9.5%. Total aerobes and enterobacteria were less affected by Neosugar.

In a study to determine pre- and probiotic effects on intestinal microbial composition, Bouhnik and others (1996a) reported that prolonged ingestion of *Bifidobacterium* sp. as a probiotic increased the proportion of bifidobacteria in the colonic flora of healthy human subjects. However, the concurrent administration of inulin as a prebiotic did not enhance this effect. No changes in fecal total anaerobe counts, pH, nitrate reductase, nitroreductase and azoreductase activities were found in either group.

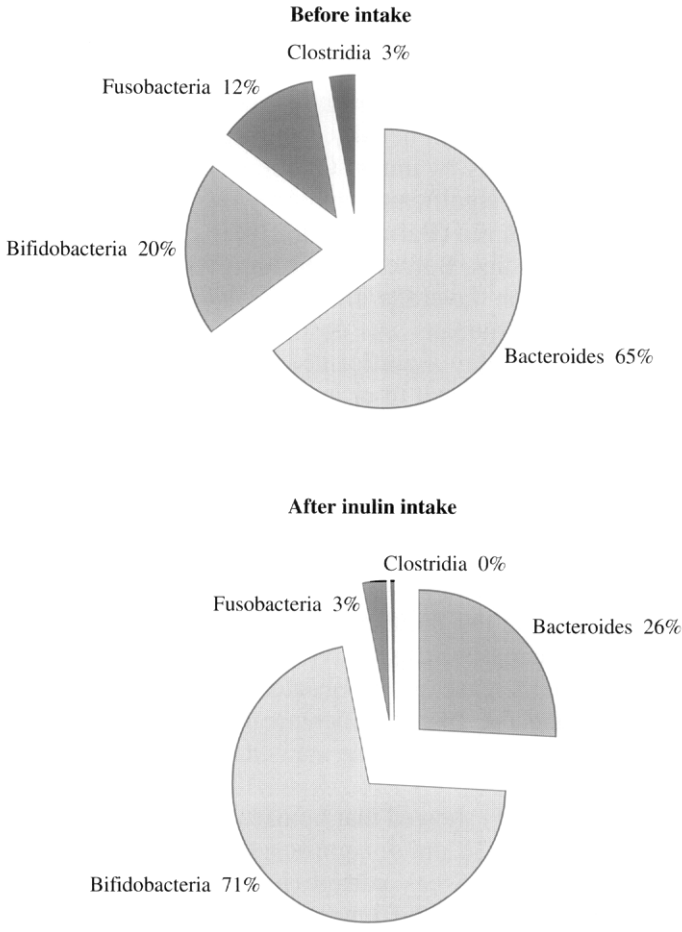

FIG. 4. Selective bifidus stimulation from inulin intake – human *in vivo* study. (Adapted from Gibson *et al.*, 1995.)

In another *in vivo* study involving 35 elderly female subjects, mean age of 76.4 years and suffering from constipation, inulin was compared to lactose to determine effects on fecal microflora, microbial activity and bowel habit. Results showed that progressive increases in inulin ingestion from 20 g/day to 40 g/day for 19 days increased bifidobacteria significantly from 7.9 to 9.2 $\log_{10}$/g dry feces, and decreased enterococci in number and frequency, while not changing the total bacterial counts (Kleessen *et al.*, 1997).

Inulin may also affect intestinal and hepatic enzymes important in the

elimination of toxic compounds from the body. Roland and others (1994) found that the specific activity of glutathione-S-transferase (GSH-T) in rats fed inulin was significantly higher than in rats fed other dietary fibers. The effects on the zenobiotic-metabolizing enzymes (XEM) could not be predicted based on the solubilities of the fibers. Buddington and others (1996) supplemented a controlled diet of 12 healthy human subjects with four grams of Neosugar. Neosugar caused β-glucuronidase and glycocholic acid hydroxylase activities to decrease by 75% and 90%, respectively. Nitroreductase activity declined by 80% after the control diet was started but was not affected by Neosugar. The responses by these three enzymes suggest that they are regulated independently by different factors and processes, and respond differently to diet composition.

Causey and others (2000a) used a double-blind cross-over study involving 12 healthy male volunteers who ate 20 g/day of inulin (DP 2 to 60; average 9 units). When inulin was consumed there were significant increases in total anaerobes (1.98 E10 CFU ± 1.65 E10 vs. 2.82 E10 ± 1.79 E10, $p = 0.03$) and lactobacillus species (1.55 E09 ± 2.44 E09 vs. 2.85 E09 ± 4.30 E09, $p = 0.05$) and a significant decrease in fecal ammonia levels (87.50 ± 42.90 ppm vs. 51.50 ± 28.68 ppm, $p = 0.001$) and β-glucuronidase activity (15.49 ± 3.94 μmol/L·g·h vs. 10.51 ± 4.34 μmol/L·g·h; $p = 0.02$). The study further showed a trend toward decreased β-glucosidase, fecal pH, clostridia, and enterobacteriaceae species. Glycocholic acid hydroxylase activity was unchanged by inulin consumption.

B. PRODUCTS OF FERMENTATION

Upon reaching the large intestine inulin is preferentially utilized by a group of healthy bacteria, bifidobacteria and lactobacilli, that are present in the ceco-colon. During the fermentation process, energy is provided for bacterial proliferation and increased cell mass. A few species of lactobacilli produce carbon dioxide gas during their fermentation (Hartemink and Rombouts, 1997). Bifidobacteria have not been found to produce hydrogen or carbon dioxide (Holdeman and Moore, 1977). The bacterial mass and gas production are metabolically of no benefit to the host. Gas production from inulin is likely a result of its fermentation by strict anaerobic species, such as bacteroides, some non-pathogenic species of clostridia, anaerobic cocci, and some species of lactobacilli. The hydrogen and carbon dioxide produced from these bacteria may be further metabolized to methane by methanogenic bacteria (Wolin and Miller, 1983). However, Roland and others (1995) found that rats colonized with human fecal microflora and fed inulin at 116 g/kg/feed produced almost no methane but significantly more hydrogen gas.

In addition to these fermentation products, short-chain fatty acids (SCFA), acetate, propionate and butyrate are also formed along with L-(+)-lactate. Rats consuming inulin had significantly higher production of short-chain fatty acids (SCFA) in the cecum ($p < 0.05$) in comparison to other fibers tested: wheat bran, pea hull, oat husk, cocoa seed and carrot fiber (Roland *et al.*, 1995). SCFAs are important anions in the colonic lumen, affecting both colonocyte morphology and function. By stimulating sodium and water absorption, SFCAs act to minimize effects due to diarrhea. SCFAs may enhance ileal motility and increase intestinal cell proliferation by local action and by increasing mucosal blood flow (Scheppach, 1994). In addition to their effects on gut morphology and function, the SCFAs are absorbed through the colonic epithelial cells into the portal blood, thus becoming a source for host energy and regulators of several metabolic processes.

Butyrate, remaining from colonic metabolism, propionate and L(+)-lactate enter the liver from the portal blood and are completely metabolized. Propionate is transformed into methylmalonyl-SCoA and then succinyl-CoA. L(+)-lactate is the precursor in gluconeogenesis. The small amount of butyrate reaching the liver is a precursor in lipogenesis (Roberfroid and Delzenne, 1993). About 50–75% of acetate is metabolized in the liver to produce energy and, like butyrate, serves as a lipogenic substrate. The remaining acetate fraction passes into peripheral muscle tissue where it is metabolized (Roberfroid and Delzenne, 1993).

The ratios of individual SCFA are very important as each SCFA impacts host metabolism differently. Propionate has been demonstrated to lower cholesterol synthesis, both *in vitro* in isolated rat hepatocytes (Nishina and Freeland, 1990; Wright *et al.*, 1990; Demigné *et al.*, 1995) and *in vivo* in rats (Illman *et al.*, 1988; Chen *et al.*, 1984) and in humans (Wolever *et al.*, 1995), likely by inhibiting gluconeogenesis, stimulating glycolysis and inhibiting biosynthesis of fatty acids. Conversely, acetate stimulates gluconeogenesis (Rémésy *et al.*, 1992b), inhibits glycolysis, and is a well-known precursor of cholesterol (Nilsson and Belfrage, 1978).

Butyrate and, less efficiently, propionate affect colonocytes at various stages of the adenoma-carcinoma sequence. Butyrate has also been shown to be the preferred energy substrate for the colonocyte, accounting for about 70% of total energy consumption, and to be a potent differentiating agent in cell culture (Roediger, 1980; Rémésy *et al.*, 1992b; Scheppach, 1994). Butyrate and, to a lesser extent, propionate may have a role in preventing certain types of colitis (Scheppach, 1994, 1998) and may inhibit proliferation of colon cancer cells (Scheppach, 1998).

The SCFA ratio resulting from colonic fermentation of inulin appears

quite favorable for modulating carbohydrates and lowering cholesterol. Botham and others (1998) working *in vitro* with human feces showed inulin fermentation provided the highest propionate/acetate ratio of NDOs tested and a relatively high butyrate level. Further, this ratio may be somewhat dose dependent as shown *in vitro* using rat hepatocytes. Levrat and coworkers (1991) showed that higher inulin concentrations, up to 10% of the diet, favored higher propionate levels and a SCFA ratio of approximately 42:38:20 acetate, propionate and butyrate, respectively.

In terms of carbon units, the overall balance from fermentation of 1 mol fructosyl unit of inulin produces about 40% SCFA (mol-ratio: acetate 81:propionate 13:butyrate 6), 15% L(+)-lactate and 5% CO_2 and up to 40% bacterial biomass, predominately bifidobacteria (Roberfroid and Delzenne, 1993).

It has been shown that the activity of microbial inulinases, which are the necessary enzymes for inulin fermentation and SCFA production and gas production, is influenced *in vitro* by the degree of polymerization (Roberfroid *et al.*, 1998). Inulin is reported to produce much more

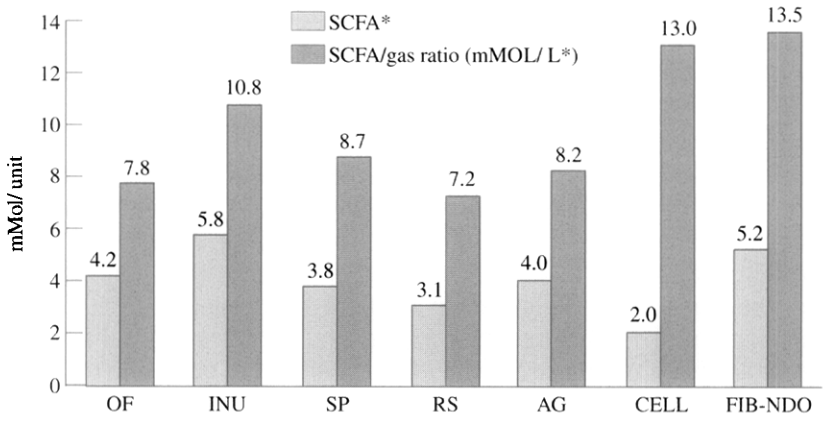

FIG. 5. Short-chain fatty acid and gas production of individual NDO, polysaccharides, and their mixtures.[1] (Adapted from Van Hoeij *et al.*, 1997.)

* Higher values for SCFA are preferred. Higher values for SCFA/gas ratios are preferred, showing greater overall intestinal tolerance.

[1] *In vitro* fermentation using human fecal suspensions, predigested to remove mono- and disaccharides. Only inulin and fiber-NDO mixture continued to produce SCFA after 24 hrs. Gas production did not rise after 24 hrs for any fiber/NDO studied.

[2] OF = oligofructose (GF_n- and F_m-type molecules; avg. DP 4–5), INU = inulin (GF_n-type molecule, 2–60, avg. DP 10 hexose units), SP = soy polysaccharide, RS = resistant starch, AG = arabic gum, CELL = cellulose, FIB-NDO = mixture of both individual non-digestible oligo- and polysaccharides in amounts proportional to those present in a Western diet.

favorable mean SCFA/gas volume ratio (10.8) than shorter-chain FOS (7.8) or non-preferential NDOs [soy polysaccharide (8.7), resistant starch (7.2), or arabic gums (8.2)]. Fiber-NDO mixtures have been shown *in vitro* to provide the highest SCFA/gas volume ratios (13.5), while inulin produced the highest concentration of total SCFA over the fermentation period (5.8 mMol/g inulin vs. 5.2 mMol/g fiber-NDO mixture, see Fig. 5) (Van Hoeij *et al.*, 1997). Favorable SCFA/gas ratio indicates that inulin only results in modest gas production while producing relatively high quantities of the SCFA, an important factor in patient tolerance for supplemented enteral clinical nutrition formula (Van Hoeij *et al.*, 1997). The rate of fermentation also defines intestinal tolerance and SCFA-mediated systemic responses such as mineral absorption, carbohydrate and lipid effects, and osmotic laxation (Roberfroid *et al.*, 1998).

IV. NUTRIENT METABOLISM

A. CARBOHYDRATES AND DIETARY FIBER

The 1,2- and 2,6-β-linkages making up inulin are resistant to mammalian digestive enzymes, such as the disaccharidases (sucrase, maltase, isomaltase or lactase) of intestinal mucosa and α-amylase of pancreatic homogenates (Oku *et al.*, 1984). Consequently, inulin reaches the colon virtually unaltered. Shorter chains of fructooligosaccharides are also neither hydrolyzed nor absorbed from the small intestine (Tsuji *et al.*, 1986).

Glucose tolerance tests conducted on human subjects who consumed either 50 g of sucrose or 25 g of fructooligosaccharide after a 15-hour overnight fast showed no change in either blood insulin or glucose levels when the fructooligosaccharides were used (Hidaka *et al.*, 1986). The mechanism of this effect may be explained by the effect of inulin on glucose uptake itself (Kim and Shin, 1996). An intestinal perfusion technique with rats measured both jejunal and ileal uptake of glucose when inulin and chicory extract was also present. Over 30 minutes, jejunal and ileal segments were perfused with an isotonic electrolyte solution (pH 7.4) which contained glucose (10 mmol/L) and inulin or chicory extract (10 g/L). Both chicory extract and inulin reduced the absorption rate of glucose from the rat jejunum ($p < 0.05$) but not the ileum. The percentage of overall glucose absorption was also significantly reduced from the rat jejunum when inulin or chicory extract was present as compared to controls ($p < 0.05$).

Inulin is compared with soluble-viscous-fermentable dietary fibers since it is not hydrolyzed by the human digestive system but is hydrolyzed and

fermented by colonic microflora which affects systemic physiological functions. Since inulin does not possess the typical physical effects of soluble-viscous dietary fibers it does not significantly reduce digestive transit time. It is defined as a "functional food", a food which when consumed in the course of the daily diet has specific physiological benefits. According to Roberfroid (1993) and Prosky (1999), these characteristics should allow inulin to be classified as a unique soluble dietary fiber.

Because the Food and Drug Administration (FDA) and the United States Department of Agriculture (USDA) have defined dietary fiber as a material that precipitates in 78% ethanol, inulin and FOS are not currently classified as dietary fiber. This definition is under review, however, and may include inulin and FOS in the future. Official AOAC analytical methods (Nos. 997.08 and 999.03) have been approved to quantify the β-(2→l) fructans as part of the soluble dietary fiber complex in foods and food products (Hoebregs, 1997; Kennedy, 1999). To date, inulin and oligofructose have been accepted as dietary fiber in 15 countries (Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Netherlands, Norway, Portugal, South Africa, Sweden and Switzerland) with approvals pending in four other countries besides the United States (Australia, Canada, Spain and the United Kingdom) (Prosky and Hoebregs, 1999; Tungland, 1999).

A physiological definition of dietary fiber combines the nutritional criteria of non-digestibility with the physiological effects that are associated with regular intake of dietary fiber. Potential physiological effects attributed to nondigestible carbohydrates include both local and systemic effects (Jenkins *et al.*, 1999) as outlined in Table IV.

TABLE IV

POTENTIAL EFFECTS OF NONABSORBABLE CARBOHYDRATES: PHYSIOLOGIC EFFECTS[a]

Local	Systemic
↑ Fecal bulk	↓ (↑) Cholesterol
↑ Bacteria	↓ TG (↓ insulin; ↓ glucose)
Selective ↑ bacteria	↓ NH_3
↑ SCFA production	↓ Urea
Selective ↑ in SCFA	↑ B vitamins
↑ Mineral absorption	↑ Immune function
↑ B vitamin synthesis	(↑ Glutamine?)

[a] Abbreviations: SCFA, short-chain fatty acids; TG, triglycerides.

Reference: Jenkins *et al.*, 1999.

B. CALORIC VALUE

Longer-chain oligosaccharides (inulin) and fructooligosaccharides (GF_2, GF_3, GF_4) reach the large intestine virtually intact and, as such, were considered not to be a major source of energy (Oku *et al.*, 1984). Furthermore, in the rat model, there appear to be no hydrolytic enzymatic adjustments in the small intestine to long-term ingestion of these factors. Nilsson and others (1988) used oral intubation to give fructans with a DP of about 9 or DP 16 to rats and found that both proceeded as undigested material through the gastrointestinal tract to the colon. However, due to the bacterial fermentation that occurs in the colon, these oligosaccharides do contribute to the energy pool. The caloric value of a fructosyl unit of oligofructose is calculated at 30 to 40% of a digested fructose molecule or between 1–1.5 kcal/g (Roberfroid *et al.*, 1993). Ranhotra and coworkers (1993) reported a caloric value for oligofructose of 1.48 kcal/g. They determined usable energy value based on efficiency of conversion of gross food energy to net energy (carcass energy) using young rats as the test model. Molis and others (1996) further defined the energy value of fructooligosaccharides (44% GF_2; 46% GF_3; and 10% GF_4) working with six healthy human subjects. Calculated mean energy value of the fructooligosaccharide was 9.5 ± 0.6 kJ/g (range: 8.3–11.7 kJ/g) or about 2 kcal/g. For nutrition labeling purposes, Roberfoid (1999) recommended that inulin and oligofructose, as well as all nondigestible oligosaccharides that are mostly fermented in the colon, be assigned a caloric value of 1.5 kcal/g (6.3 kJ/g).

C. LIPIDS

A commonly referenced systemic effect of inulin is that related to lipid metabolism. Most studies have shown that inulin has an effect on blood lipid levels in animals. Daily feeding of oligofructose (mean DP of 4.8) to rats at a 10% dose level resulted in significant serum triglyceride lowering after just one week of feeding (Fiordaliso *et al.*, 1995). Serum triglycerides in the oligofructose-fed rats continued to remain significantly lower than control-fed rats for an additional 12 weeks of feeding. The decrease in serum triglyceride levels was apparently not due to increased fecal excretion since fecal levels remained unchanged throughout the study.

Tokunaga and coworkers (1986) had also shown that rats fed 10% or 29% fructooligosaccharide (GF_2-GF_4) diets experienced lowered serum triacylglycerol levels. In contrast, however, they failed to show reduced serum cholesterol levels whereas Fiordaliso's group was able to show significantly reduced fasting serum phospolipids and total cholesterol levels in oligofructose-fed rats. Reduced serum triglycerides, phospholipids and

total cholesterol were mainly due to a decreased number of very low density lipoprotein (VLDL) particles, and not the low density (LDL) or high density lipoprotein (HDL) fraction. Trautwein and coworkers (1998) have reported similar findings in hamsters fed 16% inulin diets for five weeks. These works have suggested that oligofructose feeding alters hepatic lipid metabolism which results in less VLDL production.

In confirmation of the work by Fiordaliso and others (1995), Kok and others (1996b) were able to demonstrate a significant decrease in serum triglyceride-VLDL when 10% oligofructose (DP = 4.8) was given in a standard diet for rats. Since liver enzyme activity was also reduced for two of the four enzymes assayed, it was probable that oligofructose decreased liver capacity for *de novo* triglyceride and fatty acid synthesis through inhibition of key enzyme activities, particularly glycerol-3-phosphate acyltransferase and fatty acid synthase.

It has been suggested that oligofructose may have the ability to protect against or modify liver lipid accumulation that was induced by another nutrient such as fructose when fed at the same time (Kok *et al.*, 1996a). An additional study indicated that oligofructose supplementation could reduce postprandial hypertriglyceridemia when fed with a high fat diet (Kok *et al.*, 1998a). The effect, however was only on circulating triglycerides and phospholipids, rather than liver lipids. Feeding oligofructose with a high fat diet did not prevent the hepatic accumulation of triglycerides, phospholipids and cholesterol that is normally prompted by a high fat diet but the livers of rats fed high fat-oligofructose diets displayed smaller lipid droplets as compared to rats fed a high fat diet only.

Fructooligosaccharide effect on lipid metabolism in humans may vary, depending on the presence or absence of chronic disease. A human study with diabetic subjects was conducted over a 14-day period in which 8 g of fructooligosaccharides were ingested in a coffee drink or coffee jelly. Over the course of the study mean serum total cholesterol levels were reduced by 19 mg/dL (242 ± 43 mg/dL vs. 223 ± 27 mg/dL, $p < 0.01$) and LDL-cholesterol levels by 17 mg/dL (164 ± 33mg/dL vs. 147 ± 32 mg/dL, $p < 0.02$) (Yamashita *et al.*, 1984). Serum HDL-cholesterol, triglycerides and free fatty acid levels in these diabetic subjects were not significantly affected by the consumption.

In a double-blind crossover human study involving 12 slightly hypercholesterolemic men, Causey and others (2000b) showed serum triglyceride reduction of 40 mg/dL (282.92 mg/dL vs. 243.24 mg/dL, $p = 0.024$) when 20 g/day of chicory inulin (DP 2 to 60; average 9 units) was consumed. Total serum cholesterol and LDL-cholesterol were numerically but not significantly reduced. No change in HDL-cholesterol was noted.

Another crossover study, involving 21 hypercholesterolemic men and

women ingesting 18 g/day inulin on a low-fat diet, showed statistically significant ($p < 0.05$) reductions for LDL-cholesterol (–4.4%) and total cholesterol (–8.7%), respectively (Davidson *et al.*, 1998). No effects on HDL cholesterol or serum triglyceride were noted. Yet, in another study, Davidson and Maki (1999) reported on the serum lipid profile of 25 adults with mild-to-moderate hypercholesterolemia who were fed 18 g/day of inulin as a substitute for the sugar content of study foods. The study was a random, double-blind, crossover design with six-week study periods and a six-week washout period. At the end of both study periods, serum lipids (LDL-C, HDL-C, total cholesterol and triglycerides) were not significantly different between experimental and control groups. Because serum total cholesterol and LDL-C were higher at the beginning of the control phase than at the beginning of the inulin phase, the results of this study were difficult to interpret. The study was unable to add more evidence regarding the lipid-lowering effects of inulin. It did, however, provide some evidence indicating that daily consumption of 18 g of inulin resulted in more mild gastrointestinal discomfort than during the control food phase (Davidson and Maki, 1999). The mild discomfort was experienced and reported throughout the entire six weeks of inulin treatment.

In *healthy* human populations the effects of inulin and oligofructose are more mixed. Canzi and others (1995) observed that the intake of inulin (9 g/day) from a rice-based ready-to-eat cereal by normolipidemic men resulted in significant reductions ($p < 0.05$) in serum triacylglycerol and LDL-cholesterol levels, 20.4 mg/dL and 8 mg/dL, respectively. However, Pedersen and coworkers (1997) observed no effect when inulin in a low-fat spread (14 g/day) was consumed by a group of 64 healthy normolipidemic women over a four-week double-blind crossover study. Williams (1999) reported on a study by Jackson and others (1999, in press), in which healthy adults consumed 10 g/day of chicory inulin (DP 2 to 60; average 10 units) for an eight-week period. At eight weeks, fasting triglyceride values were 19% lower than baseline in the inulin group and were significantly lower than the control group ($p < 0.05$) but returned to baseline levels within four weeks after the feeding period stopped. Total, LDL and HDL cholesterol levels remained unchanged in both inulin-fed and control groups. These results were similar to those reported by Luo and others (1996) when healthy male subjects consumed 20 g short-chained fructo-oligosaccharides for four-week periods in a double-blind crossover design. Serum triglycerides, total and HDL cholesterol remained unchanged after four weeks of feeding inulin in that study.

Possible mechanisms for the lipid-lowering effects have been proposed. Ellegård and coworkers (1997) found that neither cholesterol absorption nor excretion from the small intestine was affected in ileostomy patients

when either 17.1 g inulin or 15.5 g oligofructose was fed. They proposed that a lipid-lowering effect may happen by another route, such as propionate absorption from the colon which could suppress hepatic synthesis. As previously mentioned, small chain fatty acids, particularly propionate, influence carbohydrate and lipid metabolism. Propionate has been demonstrated to lower cholesterol synthesis (Chen *et al.*, 1984; Illman *et al.*, 1988; Nishina and Freeland, 1990; Wright *et al.*, 1990; Demigné *et al.*, 1995; Wolever *et al.*, 1995). Others propose that some bifidobacteria and lactobacilli in fermented products are able to remove cholesterol (Van Poppel and Schaafsma, 1996).

Like other NDOs, inulin could suppress serum cholesterol through an enhanced secretion of bile acids (Mazur *et al.*, 1990; Kim and Shin, 1998; Trautwein *et al.*, 1998). In addition, inulin could decrease the serum cholesterol level by reducing hepatic cholesterol synthesis through inhibition of HMG-CoA reductase activity, with subsequent effects on concentration of β-hydroxy-β-methylglutaryl CoA (HMG-CoA), the key cholesterol intermediate.

As for the triglyceride-lowering effect Delzenne and Kok (1999), in confirmation of earlier *in vivo* and *in vitro* rat studies, again reinforced the concept that oligofructose exerts a triglyceride-lowering action primarily due to a reduction of *de novo* fatty acid synthesis in the liver, through inhibition of all lipogenic enzymes. This suggested that inulin decreases lipogenic enzyme gene expression.

Yet another route by which inulin and oligofructose may decrease serum lipid levels is via lowered serum insulin and glucose, both known to regulate lipogeneis. Kok and coworkers (1998b) postulated that the lower glucose and insulin levels that were found after feeding a dose of oligofructose of 10 g/100 g to rats contributed to the reduced hepatic fatty acid and triglyceride synthesis, and are part of the mechanism of the hypolipidemic effect of oligofructose. Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1(7–36)amide (GLP-1) are affected but the exact contribution of those two hormones in the antilipogenic effect requires clarification.

While the effects of inulin and oligofructose on lipid metabolism in animals tend to be more concrete and consistent, the determination of consistent lipid-lowering effects for inulin and oligofructose in humans is yet to be confirmed. It appears that hyperlipidemic subjects are more likely to experience a reduction in serum cholesterol levels when inulin is ingested but normal subjects are more prone to reductions in serum triglyceride levels. A number of factors need to be considered including: individual variation, duration of administration of inulin or oligofructose, intakes of dietary fat and carbohydrate in the background diet; and prior

serum lipid levels (Williams, 1999). Part of the reason for the discrepancy in results between animal and human studies may be that the inulin dose level used in animal studies is much higher than the level used in human studies. The dose levels used in animal studies are not likely to be well tolerated by humans.

D. MINERALS

Although cereal fiber and its associated phytate content have been found to depress the absorption and retention of several minerals (Greger, 1999), it has been hypothesized that fermentable carbohydrates, like inulin, could possibly improve the metabolic absorption of various ions, including calcium, magnesium and iron (Scharrer and Lutz, 1990; Schulz *et al.*, 1993; Campbell *et al.*, 1997; Lopez *et al.*, 1998.). The mechanism for this improvement in bioavailability is speculated to be the production of SCFA and lactate from inulin fermentation in the colon. The reduction in luminal pH would increase the free ionic form and solubility of minerals and thus increase bioavailability. The lower luminal pH would raise the concentration of ionized minerals and accelerate passive diffusion (Rémésy *et al.*, 1992a).

In particular, the accumulation of calcium phosphate in the large intestine and the solubilization of minerals by SCFAs are likely to play an essential role in the enhancement of mineral absorption (Rémésy *et al.*, 1993; Kashimura *et al.*, 1996). In experiments conducted by Levrat and others (1991) using rats fed a diet supplemented with a 10% inulin fraction, the cecal pool for calcium, magnesium and phosphate was improved. Furthermore, feeding of fructooligosaccharides to animals has led to reduced fecal excretion of minerals. As an example, Ohta and others (1994a) fed fructooligosaccharides to male Sprague-Dawley rats that also were consuming two levels of magnesium while maintaining constant and sufficient levels of calcium and phosphorous. Under low magnesium intake, fructooligosaccharides were able to increase magnesium absorption and reduced the occurrence of auricular and facial peripheral hyperemia and hemorrhage, both signs of magnesium deficiency. When 5% fructooligosaccharides were fed to rats on low magnesium diets, they began to absorb magnesium at approximately 3.0 mg per day which was similar to rats fed magnesium-sufficient diets.

Further work on the question of fructooligosaccharide effect on mineral absorption has also demonstrated improved calcium (Morohashi *et al.*, 1998), and calcium and magnesium absorption (Ohta *et al.*, 1994b; 1995) in normal rats. Even following gastrectomy, rats fed FOS were found to have greater net calcium absorption than rats fed a control diet (Ohta *et al.*,

1998b). In cecectomized rats, however, only magnesium absorption was improved (Ohta *et al.*, 1994b). This finding, in particular, suggested that colonic fermentation is important for calcium absorption. In addition, relative amounts of calcium binding protein (calbindin-D9k) were increased in the large intestine and decreased in the small intestine in rats fed FOS (Ohta *et al.*, 1998a). Since calcium binding protein concentration and calcium absorption were positively correlated in large intestine segments, observations from this latter study suggested FOS exerted an independent stimulatory effect on calcium absorption from the large intestine via a transcellular route involving calcium binding protein as well as a diffusive, paracellular route.

When focused on iron, Ohta *et al.* (1995) investigated the effects of fructooligosaccharides on mineral absorption in rats that were made anemic prior to the study. Male Sprague-Dawley rats were fed 5% fructooligosaccharides with either 15 mg Fe/kg diet (low iron intake) or 30 mg Fe/kg diet. Magnesium, calcium and iron absorption were measured. Fructooligosaccharide feeding reduced the cecal pH, increased iron, calcium and magnesium solubility in the cecal contents, and increased iron, calcium and magnesium absorption. Furthermore, iron levels did not affect calcium or magnesium absorption when fructooligosaccharides were included in the diet. Thus, fructooligosaccharides may be helpful in correcting iron deficiency anemia while also increasing the absorption of calcium and magnesium. Correction of iron-deficiency anemia in postgastrectomized rats fed a 7.5% FOS diet for six weeks has also been demonstrated (Ohta *et al.*, 1998c).

Delzenne and others (1995) concluded rats fed a diet containing higher levels of inulin (10% inulin) also decreased the fecal excretion of calcium, magnesium, iron and zinc indicating improved absorption of those minerals. The rat study further showed inulin increased fecal excretion and decreased urinary excretion of nitrogen. Thus, inulin may provide a good means to counteract dysfunctions resulting from hyperammonemia or disturbed iron, calcium, magnesium and zinc homeostasis (Delzenne *et al.*, 1995).

In human studies Coudray *et al.* (1997), using nine healthy men, showed significant improvements of calcium absorption and balance (+91.8 mg/day) compared to control ($p < 0.05$) but no apparent altering of magnesium, iron and zinc absorption. Ellegård *et al.* (1997) showed that in ileostomy subjects consuming 17 g/day inulin the NDO did not influence the absorption of calcium in the small intestine, making it likely that, in humans as in animals, a positive effect on calcium balance is dependent on colonic activity. Van den Heuvel and others (1999) found that calcium absorption was increased 11% ($p = 0.05$) with no effect on urinary

excretion in healthy adolescent males who were fed 15 g/day of oligofructose, in contrast to an earlier study when both calcium and non-heme iron absorption were unaffected by the presence of inulin or fructooligosaccharides (Van den Heuvel *et al.*, 1998). The possible explanation was that the period of observation in the earlier study may have missed the colonic contribution.

Food fibers are suspected of interfering with mineral bioavailability. When resistant starch or oligosaccharides were fed, however, phytic acids' impact on mineral absorption was lessened (Lopez *et al.*, 1998), possibly due to the intestinal fermentation that occurs in the large intestine when oligosaccharides are present. This was especially true for calcium, magnesium, zinc, iron and copper. Although precise mechanisms for absorptive action on minerals still require clarification, it will be important to consider that absorptive mechanisms for minerals may vary with the mineral and the type or amount of nondigestible carbohydrate (Greger, 1999).

E. VITAMINS

Bifidobacteria are generally believed to synthesize many of the B vitamins (thiamin, folic acid, nicotinic acid, pyridoxine and vitamin B_{12}) (Deguchi *et al.*, 1985). Since bifidobacteria are present in human intestinal flora, they may be a significant source of the B complex vitamin supply. Whether or not the stimulation of bifidobacterial growth by inulin and oligofructose results in further synthesis and availability of the B vitamins in humans is yet to be confirmed.

V. HEALTH IMPLICATIONS

A. CANCER

In a review of possible mechanistic effects for colon cancer inhibition, Reddy (1999) emphasized the importance of examining both probiotic and prebiotic activity, and the possible synergistic effect when both are used together. Lactic cultures used in the fermentation of milk products are examples of probiotics. These products have been shown to possess antimutagenic and anticarcinogenic properties (Goldin and Gorbach, 1980; Bodana and Rao, 1990; Lidbeck *et al.*, 1992). However, when fed, it is uncertain whether the viability is maintained throughout the gastrointestinal system, until the lower gut is reached. Lactic acid-producing bacteria, like bifidobacteria, are a predominant bacterial species in the gut (Gallaher and Khil, 1999). In addition to lactic acid production these bacteria produce

acetic acid and the combination of the two helps prevent growth of putrefactive bacteria (Rasic, 1983).

Prebiotic factors such as inulin and oligofructose were also able to selectively stimulate growth of bifidobacteria at the expense of more putrefactive bacteria (Gibson and Roberfroid, 1995). Furthermore, fermentation of these oligosaccharides in the colonic regions resulted in production of small-chain fatty acids (acetate, propionate and butyrate) and lactic acid (Roberfroid *et al.*, 1993; Wang and Gibson, 1993). Butyric acid has been shown to increase apoptosis in human colonic tumor cell lines (Hague *et al.*, 1993). Lactic acid production can lower intestinal pH. There is evidence that increasing the numbers of bifidobacteria in the colon and reducing intestinal pH can have a direct impact on carcinogenesis in the large intestine (Goldin and Gorbach, 1980; Hill, 1988; Koo and Rao, 1991).

Possible mechanisms for the anticarcinogenic and antitumorigenic effect are not completely understood. Among all of the possible mechanisms for the tumor inhibitory and anticarcinogenic effect, more work needs to be done to establish which are essential to the process (Taper and Roberfroid, 1999). It is possible that all or some are involved in a metabolic chain reaction for the inhibitory effect to occur.

Modulation of the microflora through the stimulation of bifidobacteria while keeping *E. coli* or clostridia at low levels is one possibility. Rumney and Rowland (1995) suggested that production of toxic metabolites may be reduced by increasing the proportion of more healthy colonic microflora which compete with pathogenic and putrefactive bacteria to reduce the levels of toxin and carcinogenic-producing enzymes. Toxin- and carcinogenic-producing enzymes have relatively low activity in bifidobacteria and lactobacilli compared to other more harmful colonic microflora (Rowland, 1995). These alterations in bacterial enzymes can interfere with the conversion of procarcinogens to its carcinogenic form and thus reduce cancer risk. For example, Hidaka *et al.* (1986) fed rats a diet supplemented with tyrosine and tryptophan as precursors to phenolic products. Fructooligosaccharides were administered at 0.4 to 10% by weight in the diet. Results indicated that p-cresol levels were reduced in the fecal material. Buddington *et al.* (1996) further noted significantly reduced nitroreductase activity while using four grams of a daily dietary supplement of fructooligosaccharides. In addition, the study showed reductive enzymes β-glucuronidase and glycoholic acid hydroxylase were decreased 75% and 90%, respectively. β-glucuronidase has implications in carcinogenesis through the release of aglycones from glycosides. Glycoholic acid hydroxylase is involved with the production of secondary bile acids which may be linked to the increased risk of cancer associated with high-fat diets (Buddington *et al.*, 1996). Rowland and others (1998) also have

demonstrated decreased ammonia concentration and β-glucuronidase activity in cecal contents in the presence of bifidobacterium, inulin, or both. As noted in Table V, such components are known contributors to carcinogenesis of the colon in experimental animal models.

Another manner in which a change in the microflora could exert an anticarcinogenic effect is that bifidobacteria may actually bind carcinogens and physically remove them through the feces. In a study by Gibson *et al.* (1995) in which inulin and oligofructose were administered, there were greater fecal bifidobacteria and reduced levels of putrefactive bacteria in animals fed the oligosaccharides compared to animals fed a basal diet.

The hypothesis that cancer risk is affected through modulation of the gut microflora is supported in other ways. Consumption of *Lactobacillus casei* and *L. acidophilus* was shown to reduce mutagenicity of urine and feces associated with the ingestion of carcinogens in cooked meat (Lidbeck *et al.*, 1992). Mizutani and Mitsuoka (1980), Hashimoto (1985), and Mizota and others (1987) noted that reducing or inhibiting the growth of certain pathogenic and putrefactive bacteria reduced the amount of N-nitroso compounds, phenolic products of tyrosine and tryptophan, metabolites of biliary steroids, and other potential carcinogens in the colon. In addition, Rowland and Grasso (1975) identified bifidobacteria and lactobacilli as effective in reducing the conversion of secondary amines and nitrite to nitrosamine.

TABLE V

METABOLIC PRODUCTS FORMED VIA ENZYMATIC ACTIVITY OF COLONIC MICROFLORA

Enzyme activity	Metabolic product	Toxicity
Urease	Ammonia	Liver toxin, carcinogen
Tyrosinase	ρ/(Gk rho) cresol	Cancer promoter
Tryptophanase	Indole	Carcinogen
Decarboxylase	Amines	Liver toxin
Azoreductase	N-nitroso compounds	Carcinogens
Deaminase	Hydrogen sulfide	Carcinogen
Nitrate reductase	Hormonal substances	Cancer promoters
N-nitroreductase	Aromatic amines	Carcinogens
Nitrification	Secondary amines	Carcinogens/liver toxins
N-dealkylation	Neutral steroids	Carcinogens
Deconjugation	Acid steroids	Carcinogens
β-glucosidase	Aglycones	Often mutagenic
β-glucuronidase	Aglycones	Often mutagenic
Glycocholic acid hydroxylase	Secondary bile acids	Carcinogens/colon cancer promoters

Asano and coworkers (1986) further showed *L. casei* inhibited the growth and metastasis of a transplantable bladder tumor cell line. Additionally, a study by Fujiwara and others (1990) showed a significant anti-cancer effect on BALB/c male mice (6 weeks on day 0, 16/group) inoculated intraperitoneally with Meth A fibrosarcoma (l×10E+05/mouse) on day 0 and treated with intraperitoneal injections of lactic acid bacteria (0.1 mg/treatment) on days 0, 2, 4, 6 and 8.

It is possible that lactic acid producing bacteria might protect against cancer by preventing DNA damage and mutations which are considered early events in carcinogenesis in cell cultures or in animals. Pool-Zobel and others (1993b) demonstrated that several species of *Lactobacillus* inhibited the induction of mutations in *Salmonella typhimurium* by 60–85%. A related study also provided evidence that administration of lactobacilli can decrease DNA damage induced by N-methyl-N-nitro-N-nitrosoguanidine (MNNG) in gastric and colonic mucosa in rats (Pool-Zobel *et al.*, 1993a).

Antitumoral immunity may also be affected when the colonic environment is altered (Pierre *et al.*, 1997). A study by Perdigòn and coworkers (1998) utilizing BALB/c mice investigated the effects of yogurt, containing stock cultures of *L. delbrueckii* subsp. *bulgaricus* CRL 423 and *S. salivarious* subsp. *thermophilus* CRL 412, on the inhibition of 1,2-dimethylhydrazine (DMA)-induced colon tumors. Results showed 70% of the control group ingesting a conventional diet and no yogurt developed colon tumors, while tumor growth was inhibited in the yogurt-eating group. The control group developed substantial influx of mononuclear cells into the lamina propria of the large intestine with an increase in IgG-producing cells, a slight increase in the IgA-secreting cells and of CD8+ but not CD4+ T lymphocytes. Further, the control group had a higher level of β-glucuronidase activity in the intestinal fluid and leucocytosis with neutrophilia in the blood. The inflammatory immune response was reduced in the test group, with an increase in the IgA secreting cells (but not IgG) and no significant increase in either CD8+ or CD4+ T lymphocytes. The authors suggested one of the mechanisms by which the yogurt exerted antitumor activity was through its immunomodulator activity which reduced the inflammatory immune response, a characteristic that was markedly increased when the carcinogen was administered.

Inulin and oligofructose added to animal diets have resulted in reductions in the number of aberrant crypt foci (ACF) (Reddy *et al.*, 1997). Aberrant crypt foci are recognized as early preneoplastic lesions in the colon and, as such, are considered predictive of eventual tumor incidence. Koo and Rao (1991) showed that oral administration of indigenous bifidobacteria and the incorporation of a 5% inulin-fraction in the diet of CF_1 mice significantly reduced the incidence of aberrant crypts and foci in the

process of 1,2-dimethylhydrazine-induced colonic carcinogenesis. The aberrance also appeared to be confined to the more distal end of the colon in the animals fed the bifidogenic diet. Since Reddy and Rivenson (1993) have provided direct evidence of the effect of *Bifidobacterium longum* in preventing induction of colon, liver and mammary tumors in rats that were exposed to a common dietary mutagen, continuing study of the effects of prebiotics and/or probiotics on early pre-cancerous lesions or cancer initiation and growth seems justified.

Verghese and others (1998), while working with Fisher 344 male rats, showed that a diet containing 10% inulin caused a 400% increase in cecal weights and a decrease in cecal pH from 6.04 to 3.74 as compared to controls. In addition, the inulin group showed over 50% reduction of azoxymethane- (AOM) induced preneoplastic aberrant crypt foci (ACF) as compared to the control group. Reddy *et al.* (1997) found that both 10% oligofructose (average DP 4.5) and 10% inulin (average DP 25) added to a control diet at the expense of starch and fed to male weanling rats for 12 weeks resulted in fewer aberrant crypt foci per colon than control rats. The number of aberrant crypt foci was inhibited to a greater degree in rats fed inulin than in those fed oligofructose. However, others suggest that actual tumor growth may be more affected by oligofructose than inulin (Taper *et al.*, 1997).

In another rat study involving AOM-induced aberrant crypt foci, Rowland *et al.* (1998) studied the effects of the consumption of *Bifidobacterium longum* and/or a 5% weight/weight inulin diet (average DP 22 units). The combined administration of both bifidobacterium and inulin resulted in 80% inhibition of aberrant crypt foci (ACF) which was more potent than administration of the two separately. The combined administration also significantly decreased the incidence of large aberrant crypt foci (>4 aberrant crypts per focus) by 59%. Thus, an additive or synergistic relationship between inulin or oligofructose acting as prebiotics and added probiotics is supported in this study and the work of others (Gallaher and Khil, 1999).

Work has also been completed to suggest that oligosaccharides have a role in reducing actual tumor cell initiation and growth (Taper and Roberfroid, 1999). Since both decreased numbers of tumors and decreased numbers of rats with tumors were detected following administration of methylnitrosourea to induce mammary tumors in rats, an antipromoting or antiprogessing effect was proposed which requires confirmation in a larger study. In contrast, Menanteau and others (1998) have shown that diets containing resistant starch and oligofructose (average DP 4) were associated with reduced numbers of precancerous lesions in rats but had no effect on the later stages of carcinogenesis.

Work by Taper and others (1997) demonstrated the effects on tumor

growth when 15% oligofructose, inulin or pectin were added to a basal diet and fed to mice that were transplanted with two tumor lines (EMT6, a mammary carcinoma; and TLT, transplantable liver tumor). Results indicated the solid TLT tumor grew more slowly in mice fed oligofructose, inulin or pectin than in those fed basal diet alone. Likewise, mice fed 15% oligofructose, inulin or pectin also demonstrated inhibited EMT6 tumor growth compared to control mice. With EMT6 tumors, oligofructose appeared to be slightly more active than either inulin or pectin. Several mechanisms were proposed by the authors. They included the active fermentation by colonic bacteria, selective promotion of bifidobacterial growth, and alteration of colonic microflora which could inhibit the number and growth of tumors. Also, since tumor cell proliferation may be dependent on the availability of glucose and insulin levels (Cay *et al.*, 1992; Basserga, 1995; Giovanucci, 1995), and endogenous fatty acid synthesis (Kuhajda *et al.*, 1994), dietary oligosaccharides could exhibit an inhibitory effect by reducing these factors.

Collectively, these findings indicated that both probiotics (bifidobacteria) and prebiotics (oligosaccharides) might stem carcinogenic activity. Differences in initial colonic microflora populations, diet, age or sex of the rats, species or strain of the probiotic, number of viable organisms reaching the colon, and initial body weight at time of introduction of the carcinogen are all critical factors to consider when determining the effectiveness of either the probiotic or prebiotic (Gallaher *et al.*, 1996).

Human studies to investigate the effect of inulin and oligofructose on cancer risk are limited. Bouhnik and others (1996b) found that 12.5 g/day FOS fed to 20 healthy volunteers in two-week periods resulted in increased colonic bifidobacteria but there were no beneficial changes in factors that are potentially involved in the pathogenesis of colon cancer. In this study, fecal total anaerobes, pH, enzymatic activities for azoreductase, β-glucuronidase, and nitroreductase, and the concentrations of bile acids were unaffected. However, under unique conditions, Vanurikhina and others (1997) have been able to demonstrate a possible relationship between inulin/oligofructose and cancer risk. This study involved 25 people exposed to radiation as a result of the Chernobyl accident in 1986. Patients who ingested 10 g/day inulin for two months had reduced frequency of metaphases with chromosomal aberrations due to decreased pair fragments and translocations. In these exposed patients, inulin was associated with an expressed antimutagenous effect.

B. DIABETES MELLITUS

One of the earliest recorded uses of plants high in inulin as a hypoglycemic agent was by the Greek physician, Theophrastus, who used the dandelion

plant (*Taraxacum officinalis*) for this purpose (Bolyard, 1981). The dandelion is also used by various cultures in Eurasia to balance sugar metabolism (Morton, 1981). In North America, elecampane (*Inula helenium*) has been used to lower blood sugar while the North American Squamish, Kwakwaka'waka, Nuu-chah-nulth and Salish tribes of the Pacific Northwest used camas lily (*Camassia quamash*) as a sugar replacement (Foster and Duke, 1990).

The first reported attempt to study the fate of inulin in man was by Külz (1874) who investigated its metabolism in diabetic subjects. In the twentieth century Root and Baker (1925) used inulin-containing Jerusalem artichokes in the diets for diabetic patients. When artichokes were added to the diets glycosuria was not increased if it was already present, nor was glycosuria detected if it had been previously absent. When Jerusalem artichokes were substituted for other carbohydrate-rich foods, glycosuria was reduced. Feeding of Jerusalem artichokes resulted in increased blood sugar but it was less than when an equivalent amount of fructose was fed. These findings offered some promise for the practical use of inulin-containing foods in diabetic diets.

In more recent years, others also have suggested that inulin-containing food products may be beneficial to persons with diabetic disease because of the effect on reducing glucose uptake and thereby reducing postprandial hyperglycemia (Kim and Shin, 1996). Yamashita and others (1984) fed 8 g of fructooligosaccharide (Neosugar) for 14 days to 18 diabetic subjects. By the end of the study the diabetic subjects experienced 15 mg/dl decline in fasting blood glucose levels while control subjects showed no change. The implications from this study suggested that inulin-containing products may be a useful carbohydrate substitute in diabetic diets. A more recent study, however, revealed that feeding 15 g/day of FOS for 20 days to 20 men and women with Type 2 diabetes did not favorably affect either serum glucose or lipid concentrations (Alles *et al.*, 1999).

In eight healthy human subjects, Rumessen and others (1990) also examined the effects of fructans from Jerusalem artichoke on blood responses. After a 20 g load, these subjects demonstrated a lower glycemic response and insulin peaks than when fructose was fed but a glucose response was experienced, nonetheless. The short-term nature of this study hinted that further long-term studies would be needed to further assess the physiological and nutritional benefits of using inulin products in both healthy and diabetic persons.

In 1996, Luo and others worked with 12 healthy male subjects in a double-blind crossover study. In separate four-week periods either 20 g of fructooligosaccharide or sucrose were included in the diets. Results indicated that fasting levels of plasma glucose and insulin did not differ

significantly between the periods. However, production of small-chain fatty acids was noted which could have an effect on liver glucose production. Further studies of the role of small chain fatty acids in regulating hepatic glucose and lipid metabolism were encouraged.

The health benefits of including oligosaccharides in diet may go beyond a direct effect on serum glucose or insulin levels. Some researchers suggest that magnesium deficiency increases the risk for both diabetes and myocardial infarction. Since fructooligosaccharides help to increase magnesium absorption (Ohta *et al.*, 1994a; 1995) it might be helpful in decreasing the risk of both of these diseases.

C. HEART DISEASE

As noted in the earlier discussion on the effect of inulin and oligofructose on lipid metabolism, a definitive role in alterations in serum lipid levels seems to be dependent on pre-existing lipid levels, individual variations, and duration of administration of inulin and/or oligofructose, and previous level of dietary fat intake. If serum total cholesterol and LDL-cholesterol can be reduced without also reducing HDL-cholesterol, there should be a positive impact on long-term risk for heart disease, especially in those who are at risk due to high serum lipid levels.

D. IMMUNE SYSTEM

Systematic studies to fully assess the role of inulin and oligofructose on lymphocyte activity or other tests of immune function are needed. Part of the hypothesis that inulin and oligofructose may play a role comes from work with yogurt as a bearer of lactic acid producing microbes (Hitchins and McDonough, 1989; Van de Water *et al.*, 1999). According to the hypothesis, ingestion of a lactobacillus culture may stimulate an immune response. Since lactobacillus bacteria can be stimulated when inulin and oligofructose are ingested, a similar immune response might be generated.

Of additional interest is the wide historic use of inulin-containing medicinal herbs, used for their immunostimulatory effects. The most widely recognized of these herbs is *Echinacea angustifolia,* containing about 6% inulin. Inulin, the water-soluble polysaccharide of *Echinacea,* is promoted as having significant stimulatory effects on the cellular immune system (Bauer and Wagner, 1991). Even though a number of other immunostimulatory and mild anti-inflammatory polysaccharides have been isolated from *Echinacea* specie (Rawls, 1996), inulin is the most notable of the *Echinacea* polysaccharides. The herb is the best-selling herbal medicine in

the US, claiming 10% of the market for herbal medicines in 1995, and is an important herbal drug in Europe as well (Rawls, 1996).

The alternative pathway of complement (APC) plays a central role in the immune response. Finely divided, insoluble gamma form inulin, but not more soluble forms, has been shown to activate the alternative pathway (Cooper and Carter, 1986a) and, when injected intraperitoneally in mice, has an anti-tumor effect (Cooper and Carter, 1986b). Using keyhole limpet hemocyanin (KLH) as an immunogen, intraperitoneal injections of gamma inulin also increased IgG responses (5 to 28-fold, $p < 0.001$) (Cooper and Steele, 1988) and was determined to have vaccine adjuvant action (Cooper and Steele, 1988; Cooper, 1995).

Feeding inulin has also been demonstrated to impact the immune system. Kelly-Quagliana and others (1998) used B6C3F1 mice to examine the immunomodulating properties of a longer-chain inulin with an average DP of 22 units. By measuring natural killer (NK) cell activity in spleenocytes and quantifying phagocytosis by peritoneal lavage macrophages, they determined that inulin-fed mice had an increased percentage of NK cells ($p < 0.0005$) and/or an increased speed of macrophage response. Causey and others (1998) provided further evidence that long-chain inulin (average DP 22 units) stimulated the human immune system by binding to specific lectin-like receptors on leukocytes. Macrophage proliferation was stimulated without a concomitant rise in the inflammatory marker, leukotriene B4 (LT-B4). Although long-chain inulin did not stimulate interleukin-1α (IL-1α) production at 25, 50 or 100 μg/mL, it was suggested that higher concentrations of inulin may enhance production of this cytokine.

E. GASTROINTESTINAL HEALTH

Fructan-containing plants have been used for centuries to promote gastrointestinal health and treat problems of the GI tract (Culpeper, 1814; Potter, 1907; Bolyard, 1981; Boulos, 1983; Caius, 1986; Hsu *et al.*, 1986). Campbell *et al.* (1997) suggested that gastrointestinal health can be improved by feeding oligosaccharides because of their effect on small-chain fatty acid production, lowering pH, and increasing bifidobacteria. However, since oligosaccharides reach the large intestine largely intact, the possibilities exist that gastrointestinal discomfort will be experienced and might be a factor with which to contend (Rumessen *et al.*, 1990). As an example, rats have developed diarrhea after starting FOS feeding which persisted for two or three weeks (Tokunaga *et al.*, 1986). The FOS levels used would be comparable to more than 10 g per kg of body weight. Saunders and Wiggins (1981) have indicated the human colon is capable of removing appreciable amounts of single doses of poorly absorbed

carbohydrates. When capacity is exceeded, however, increased diarrhea can result. Some human studies have shown that intestinal discomfort, particularly flatulence, is present (Pedersen *et al.*, 1997; Davidson and Maki, 1999) while others show no gastrointestinal side effects up to 20 g/day of fructooligosaccharides, especially when dosages are increased gradually (Molis *et al.*, 1996). Longer, more polydispersed inulin molecules (DP 2 to 60; average 10 units) have been shown to be better tolerated, with only mild discomfort (Kleessen *et al.*, 1997). Because inulin has a somewhat laxative effect, it might be helpful in reducing constipation with only mild discomfort (Hidaka *et al.*, 1991; Kleessen *et al.*, 1997).

It has been proposed that irritable bowel syndrome is a common gastrointestinal disorder that also may be aided by supplementation with inulin or oligofructose due to its effects on bifidobacterial numbers. Further, Scheppach (1994; 1998) has suggested that butyrate, one of inulin's fermentation products, could potentially be an important variable in ulcerative colitis and malabsorption disorders. Hunter and others (1999) found that supplementing 6 g of oligofructose (2 g three times daily) was insufficient for achieving differences in fecal weight, pH, whole-gut transit time, and fasting breath hydrogen concentrations in individuals who were suffering from irritable bowel syndrome. The dose level used in the study may have been too low to achieve differences but higher levels may also prove difficult to use with this population due to fears of provoking undesirable responses such as diarrhea, pain or flatulence.

F. DENTAL HEALTH

Use of inulin/FOS as a replacement for other carbohydrates may have a role in dental health. Using *in vitro* experiments with dental plaque, researchers have shown that low molecular weight fructans (DP < 5) may serve as substrates to oral microorganisms (Nilsson *et al.*, 1988). However, the *in vitro* acid production rate was low compared to glucose.

G. SKELETAL HEALTH AND MENOPAUSAL SUPPORT

Use of inulin, particularly in combination with other nutraceutical ingredients such as soy isoflavones, may play a role in prevention of osteoporosis by increasing calcium absorption, increasing recycling of estrogen-like compounds, and increasing bone density. Inulin and oligofructose have been shown in animal and human studies to enhance dietary calcium absorption and balance thus providing a means to improve bone density (Coudray *et al.*, 1997; Morohashi *et al.*, 1998; Van den Heuvel *et al.*, 1999). Further, inulin's growth-promoting effects on probiotic bacteria

populations could potentially enhance estrogen recycling which can also affect bone health (Chaitow and Trenev, 1990). As summarized by Chaitow and Trenev, 60% of circulating female hormones such as estrogen are excreted via bile into the GI tract. Under normal conditions, the hormones are converted by bacterial enzymes to a recycled form which is mostly resorbed into the bloodstream and converted to a biologically active form. Under conditions such as broad-spectrum antiobiotic use when probiotic bacterial populations could be reduced, estrogen recycling may be minimized. If FOS/inulin, as a prebiotic, can boost the appropriate bacterial populations, the recycling effect might be maintained. Further study is required to elucidate the mechanism and provide clinical evidence as to the influence of prebiotics in this area.

H. OPPORTUNISTIC INFECTIONS (URINARY TRACT HEALTH AND CANDIDIASIS)

Probiotics, particularly *Lactobacillus acidophilus* strains, have been used with some success in helping to maintain a healthy balance of colonic microflora (Kageyama *et al.*, 1984; Pochart *et al.*, 1992; Elmer *et al.*, 1996). In particular, these bacteria are used as aids in treating and preventing pathogenic microorganism overgrowth, especially following periods of antibiotic therapy, or used with individuals having suppressed immune systems or those people on immunosuppressive drugs (Elmer *et al.*, 1996).

As mentioned in the bifidogenic section, probiotics resist opportunistic pathogen colonization by aggressively competing for attachment sites on the colonic epithelial wall and by producing pathogen antagonistic agents, i.e. acetic, lactic acid, hydrogen peroxide and several natural antibiotic substances. However, since probiotic effects may be transient (Bouhnik *et al.*, 1992), use of prebiotics such as inulin may provide another means to selectively modify colonic microflora populations, and establish a healthy colonic balance. Relative to lactic acid producing bacteria, opportunistic pathogenic microorganisms such as *Candida* (yeast infections), *Cl. difficile* (antibiotic-associated diarrhea) and *E. coli* (urinary tract infections) are intolerant of low pH levels (5.0 and 4.5) (Barnett *et al.*, 1990; Borriello, 1990; Wang and Gibson, 1993, Hopkins *et al.*, 1997); and normal pathogenic gut microorganisms do not have highly active intracellular 2,1-β-d-fructan-fructohydrolase enzymes (Pudjono *et al.*, 1993; McKellar *et al.*, 1993). Further, only five of the yeast species can use inulin to any extent as a growth substrate (Barnett *et al.*, 1990). As a consequence, the overgrowth of opportunistic pathogenic microorganisms may be selectively reduced and maintained with the use of probiotics and selective prebiotic agents, like inulin. However, selective prebiotic agents function

effectively only when there are populations of probiotic bacteria present to nourish. In certain situations, where yeasts have overgrown and they are the predominant species, such as following intense antibiotic therapy, it may also be necessary to get control of the overgrowth situation before working towards microflora modification, and further prevention.

Opportunistic *E. coli* overgrowth can affect urinary tract (UTIs) as well as respiratory infections. The National Institutes of Health (NIH) estimate that UTIs account for about 9.6 million doctor visits per year (NIH, 1999). Although *E. coli* found in the colon and rectal areas is responsible for most UTIs, other colonic microflora involved are *Staphylococcus saprophyticus* (as high as 10%), Klebsiella (5%) and occasionally *Proteus mirabilis* or *Enterococcus faecalis, Enterobacter* sp. and *Pseudomonas* sp. (Graber and Martinez-Bianchi, 1995). Consumption of inulin has been shown to significantly reduce concentrations of these bacteria, by mechanisms defined in the bifidogenic section, minimizing the potential for their overgrowth into other areas of the body.

Inulin consumption in itself should not be viewed as a single dietary component or mechanism for any disease prevention system. The best mechanism for prevention of UTIs is likely a systems approach, using good common sense, hygiene, diet and active nutritional components.

VI. FUNCTIONAL CHARACTERISTICS AND FOOD APPLICATIONS

Inulin possesses unique physical and physiological characteristics making it widely useful for adding texture in food applications. For example, when inulin replaced corn syrups in reduced-fat ice cream formulations a chewier texture was created. However, ice crystal formation was reduced when 50% of the corn syrup was replaced with inulin during thermally abusive storage conditions (Schaller-Povolny and Smith, 1999). In consumer tests, plain unsweetened yogurt containing inulin was preferred over samples without inulin. Yogurt with inulin was identified as being creamier in appearance, having a less chalky and more creamy texture, and was sweeter with a less sour/fermented taste and aftertaste (Spiegel *et al.*, 1994). Yogurt made with 10% inulin with a DP of 12–16 was found to increase firmness and decrease syneresis compared to yogurt made with shorter-chained inulin (DP 5–8) and controls with no inulin (Terry *et al.*, 1999).

Purified, analytical-grade inulin occurs as spherical crystals with radial striation. Its average molecular weight is between 5600 and 6300 – fluctuations depending on the degree of polymerization of the molecules used in the measurement. However, refined native inulin powder from chicory is white, amorphous and hygroscopic; it has a specific gravity of about 1.35

and an average molecular weight of about 1600. It is neutral in odor and taste. Commercial inulin contributes a marginally sweet taste due to a small amount of naturally occurring mono- and disaccharides.

Native chicory inulin is soluble in water with the solubility dependent on the temperature of the water, degree of polymerization, distribution of the molecular chains, and how the molecule is processed. Typically, the native molecule is soluble up to 60 g/L at 10°C, while at 90°C it is soluble to about 330 g/L. Under normal conditions inulin is dispersible in water but may have a tendency to clump during hydration due to its hygroscopic character. Dispersability may be improved either through mixing with sugar and/or starch or by instantizing the final product. Inulin has a water-binding capacity of about 1:1.5.

Inulin has a unique ability to add rheological and textural properties to food due to its ability to form discrete highly stable particle gels. Inulin gel characteristics are dependent on a number of factors including inulin solids concentration, which becomes more viscous and fat-like as inulin solids are increased. In addition to concentration, inulin chain length distribution also affects gel characteristics. Higher degrees of polymerization lower the inulin level required to form a gel. Increasing amounts of monomer and dimer content decrease viscosity. Inulin gels are very creamy and fat-like, and as such can be used in fat reduction and fat-replacer systems.

VII. SAFETY AND TOLERANCE

Estimates of current daily inulin consumption from various natural foods range from 1 to 4 g for Americans and up to 12 g for Europeans (Marchetti, 1993b). Historically, the dietary intake of inulin has been significantly higher than current-day consumption estimates, as stated previously in other sections. Estimates of inulin intake from consumption of these foods include approximately 25 to 32 g inulin per day by European populations substituting Jerusalem artichokes for potatoes and approximately 160 to 260 g inulin per day from murnong consumption by the Australian aborigines.

Human tolerance to inulin, as a class of compounds, appears to be dictated by chain length and dosage (Rumessen and Gudmand-Høyer, 1998). Abdominal symptoms, primarily gas problems and some abdominal pain, increased with increasing dose and decreasing chain length. Osmotic diarrhea associated with ingestion of unavailable or unabsorbable oligosaccharides is also notably increased as the molecular weight of the molecule decreases and as such is the most significant factor in determining tolerance

in humans (Tokunaga *et al.*, 1986; Nilsson and Björck, 1988). Therefore, human tolerance to native chicory inulin (DP of 2 to greater than 60, modal DP 9 units) is greater than the tolerance to FOS (DP 3 to 7, average 4.8), which, in turn, is greater than the tolerance to shorter-chain FOS (DP 3 to 5, average 3.7). In both historic times and contemporary times, dietary exposure to the entire range of chain lengths comprising inulin has been orders of magnitude greater than exposure to any specific subset of hydrolyzed or shorter-length inulin-type compounds (fructooligosaccharides). Consequently, human and animal gut microflora utilizing these non-digestible carbohydrates as fermentation substrates have evolved active inulinase enzymes.

Similar to other dietary fibers, human tolerance to inulin has been demonstrated to be greater when inulin is part of the regular diet and spread out over the course of the day, as opposed to a bolus dose. Absolonne and others (1995) observed an increase in tolerance to FOS (DP 3 to 7) when the initial, single dose was split into two doses administered in the morning and afternoon. Under those conditions, the maximum daily dose which did not cause reactions was 27 to 31 g for men and 33 to 37 g for women. They determined the lowest laxative dose (not causing liquid stools) to be 41 g for men and 40 g for women.

Shorter-chain FOS (DP 3 to 5), which caused adverse effects such as diarrhea when initially consumed in large amounts, were more readily tolerated with continued consumption (Oku, 1986). The maximum dose to not cause diarrhea was approximately 21 to 24 g per day (Hata and Nakajima, 1985; Takahashi *et al.*, 1986). However, lower doses of 10 to 15 g FOS (DP < 10; median DP = 3) have resulted in flatulence and other intestinal discomforts (Stone-Dorshow and Levitt, 1987; Rumessen and Gudmand-Høyer, 1998).

Wilpart (1993) reported a rat study involving various indigestible and/or unabsorbable dietary fibers, including inulin. They found that diarrhea incidence was higher with many polyols such as maltitol, mannitol, sorbitol or xylitol compared to most fructooligosaccharides. Wilpart reported adaptation to inulin took place within one week, resulting in no untoward effects. In a human clinical study, Kleessen *et al.* (1997) have shown that intakes up to 40 g per day of inulin produced no untoward effects, especially when divided over the course of a single day. For those who reported milk to be their cause of gastrointestinal symptoms (pseudohypolactasia), a 25 g dose of FOS was found to cause significantly more symptoms than in a control group. This 25 g level of intake may not be initially well tolerated by individuals who already experience gastrointestinal problems (Teuri *et al.*, 1999).

A. LEGAL AND REGULATORY STATUS

Inulin derived from various natural sources, such as chicory root, dahlia and Jerusalem artichoke is legally classified as a food or natural food ingredient, and has non-additive status. In the United States, inulin has food ingredient status and is Generally Recognized as Safe (GRAS). As stated in GRAS policy, inulin can be used without any significant restrictions for all intended food categories, unless the food is standardized and the standard does not permit its use. Canada also accepts inulin as a food ingredient without restriction on use level or the foods in which it can be used, provided there are no limitations on standard of identity for a specific food.

Inulin is classified as a food ingredient according to European Directive 95/002 on Food Additives and is excluded from additive status. All the European Union (EU) countries list inulin as having food ingredient (non-additive) status. Other countries who have given food ingredient status to inulin are Norway, Finland, Denmark, Ireland, United Kingdom, Switzerland, Israel, South Africa, Australia, New Zealand and Japan. In addition to its food ingredient status, inulin is considered as an agricultural product in Europe as part of the EC Treaty (Article 38, Annex II). In other countries specific evaluation must be made for its intended use and be in compliance with the policy of the country.

B. NUTRITION LABELING

In the United States, inulin must be declared following policy written in 21 CFR § 101.4 describing that "ingredients declared shall be labeled by their common or usual name so as to accurately identify or describe the basic nature of the food or its ingredients." Acceptable declarations are "inulin, polyfructose, oligofructose, fructooligosaccharide (FOS)", or "natural extract of chicory root" or other descriptors of their plant origin, when used in combination with any of the common chemical descriptors above. In countries other than the US, the terms "dietary fiber, vegetable fiber, chicory root fiber" may be used as single entities on the declaration, but require review by each country's regulating agency.

Inulin and oligofructose are dietary fiber by their definition and their nutritional properties. In the United States, Canada, Australia, and some other countries that define dietary fiber by prescribing a specific analytical method to be used, such as an official AOAC-type, inulin and oligofructose cannot be labeled as a dietary fiber on the nutrition or supplemental facts panel.

In Europe, according to the European Nutrition Labeling Directive

90/496, inulin and oligofructose can be designated as being either carbohydrates or as dietary fiber. Inulin has been accepted as a food ingredient for labeling as dietary fiber in all the EU countries as well as Norway, Finland, South Africa, Ireland, Switzerland and Portugal. The United Kingdom, a country requiring specific methods be used for dietary fiber labeling, accepts inulin and oligofructose as labeled dietary fiber based on official AOAC method numbers 985.29 (total dietary fiber in foods enzymatic-gravimetric method) and 997.08 (fructans in food products, ion exchange chromatographic method).

In the United States inulin and oligofructose are classified as "other carbohydrates" for nutrition facts labeling and have a defined caloric value of approximately 1.5 kcal/g. The Canadian government allows an energy value of 2 kcal/g to be used on product labels. Caloric values in Europe are specified in the Nutrition Labeling Directive 90/496. Under this directive, dietary fiber has no specified caloric value and should be calculated at zero kcal/g. In certain European countries (Italy, Switzerland, Sweden and Norway) this principle applies. However, since nutritional research shows the caloric value of inulin and oligofructose is not zero several countries have assigned a caloric value that more closely approaches the scientific evidence. For example, Belgium and the Netherlands use 1 kcal/g; Denmark, France and Finland use 2 kcal/g; and Germany uses 1.5 kcal/g. In countries not having an assigned caloric value, the value of 1.5 kcal/g is generally used.

Nutrition claims refer to claims that are made regarding the nutritional make-up of the product or ingredient and their effects on the body such as nutrient content claims, comparative or relative claims, and structure/function claims. No reference can be made to the dietary fiber level associated with inulin in the United States and Canada, due to current dietary fiber labeling issues. In Europe, the rules permitting the use of specific nutrient claims vary, but generally, fiber claims such as "with added fiber, fiber-enriched, source of fiber, high in fiber, with fiber function" are acceptable when dietary fiber labeling is approved.

Comparative claims are acceptable in the US, Canada and in most European countries, but can vary somewhat by country. Generally, claims such as, "fat/sugar reduced, low in fat/sugar, x% fat/sugar, sugar free, no sugar added" are acceptable.

Structure/function claims generally refer to the effect inulin has on the structure or function of the body. The claims, however, cannot suggest that the food is useful in the diagnosis, cure, treatment, prevention or mitigation of a disease or health-related condition, which are health or drug claims. However, structure/function claims may be used to describe non-disease states (e.g. effects on aging, menopause and bone care). Structure/function

claims are acceptable under DSHEA 21 CFR § 101.93 in the US, but are not approved currently in Canada. They are generally allowed in varying degrees in European countries. Bifidobacteria-related claims may be made such as "bifidogenic, stimulates natural Bifidus flora, improves microflora balance/equilibrium, and prebiotic." Examples of structure/function claims in the US for inulin are: "bifidogenic, improves microflora balance, helps maintain a healthy cholesterol level, helps promote urinary tract health, helps maintain cardiovascular function and a healthy circulatory system, helps maintain normal bowel function and aids with constipation, helps maintain regularity, helps promote the immune system, helps with mineral or calcium absorption, helps support the effects of menopause and helps promote bone health."

Health claims refer to disease prevention and medical claims refer to the treatment or cure of a specific disease. Neither of these types of claims can be made for inulin at this time.

VIII. CONCLUSIONS

Inulin-fructooligosaccharides (FOS) belong to the group of carbohydrates known as non-digestible oligosaccharides (NDO) and have a long history of human consumption. Inulin has a number of dietary advantages, which are mainly involved in the promotion of bifidobacteria, as confirmed by *in vitro* and *in vivo* studies. Inulin has all the characteristics and health benefits common to non-digestible polysaccharides (NDP) and resembles those attributed to dietary fiber. However, inulin does not possess the typical physical effects of dietary fiber, such as dramatic viscosity building, intense water holding, large increases in osmotic pressure and intestinal bulking effects. Several of the more pronounced health contributions of inulin arise from its ability to selectively stimulate *in vivo* in humans the growth of bacterial genera and species known to be beneficial for health, such as *Bifidobacterium* (except *B. bifidum*) and Lactobacillus, at the expense of potential pathogenic microorganisms (*Cl. perfringens, C. difficile, E. coli,* and *K. pneumoniae).* Therefore, inulin is generally considered a prebiotic with a bifidogenic factor. In contrast to probiotics, prebiotics like inulin are not unduly affected by their environment, but rather have the advantage of inducing the selective growth of endogenous bacteria in their normal environment.

Effects on gut microflora, blood glucose attenuation, lipid homeostasis, mineral and nitrogen bioavailability, immunomodulation effects, along with the ability to add texture and improve rheological characteristics and nutritional properties of food, allows inulin to be termed a "physiologically

functional food" or food ingredient, or more simply, a food with potential health-promoting effects.

Since interest in inulin as a functional food ingredient with health-promoting properties has been more intense in recent years, further research needs to be completed to understand more fully the health implications of inulin consumption. Additional data to determine effective levels of intake need to be obtained. Studies to determine differences in the physiological effects of short-, medium- and long-chained FOS/inulins and their blends need to be conducted. Before food companies accept inulin as an ingredient, more work to further understand human intestinal sensitivity and tolerance is important. More studies with healthy populations and populations with acute and chronic disease states are also needed.

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