Prebiotics: an update

Christina S Venter

Opsomming

Een van die opwindendste tendense in die hedendaagse voedselbedryf is die soeke na funksionele voedsels, oftewel voedsels wat gesondheid bevorder en/of die risiko vir chroniese siektes verlaag, bo en behalwe die voedingswaarde daarvan. In hierdie opsig speel probiotika en prebiotika 'n belangrike rol. *Probiotika* (voedsels wat lewendige mikrobes bevat) is alombekend en word geassosieer met 'n hele aantal gesondheidsvoordele. *Prebiotika* (selektief gefermenteerde voedselbestanddele wat die samestelling en/of aktiwiteit van gastro-intestinale mikroflora verander tot voordeel van die gasheer se gesondheid en welsyn) is 'n meer onlangse konsep, gedefinieer in 1995. Hierdie artikel gee 'n oorsig van die jongste navorsing oor die gesondheidsvoordele van prebiotika en daar word kortliks verwys na die tegnologiese voordele daarvan in voedselformulerings. Die meeste van die navorsing is gedoen met die fruktosepolimere inulien en frukto-oligosaggariede, wat in hierdie artikel as voorbeelde van prebiotika gebruik word. Inulien en frukto-oligosaggariede is funksionele voedselbestanddele wat 'n unieke kombinasie van voedingkundige eienskappe en tegnologiese voordele bied. Dit kom voor in verskeie groentes en vrugte en word industrieel uit sigoreiwortels en sukrose vervaardig. Selektiewe fermentering daarvan moduleer gastro-intestinale mikroflora in so 'n mate dat dit hardlywigheid kan verlig, mineraalabsorpsie kan verbeter, kanker kan voorkom en lipiedmetabolisme en die immuunstelsel voordelig kan beïnvloed. Verdere navorsing is egter nodig om sodanige aansprake te bevestig en meganismes van werking op te klaar.

— Dr CS Venter
School for Physiology, Nutrition and Consumer Sciences
North-West University
Potchefstroom Campus
Potchefstroom

INTRODUCTION

The search for functional foods or functional food ingredients that can enhance health, is one of the leading trends in today's food industry (Saarela *et al*, 2002). In this context, probiotics (i.e., living microbial food supplements) and prebiotics (i.e., non-digestible food ingredients which stimulate the growth of intestinal probiotic bacteria) receive much attention. Both popular concepts target the gastrointestinal microbiota. While in the Western world intake of probiotics has been recommended for many years, prebiotics have only recently received attention.

Functional foods or functional food ingredients exert a beneficial effect on host health and/or reduce the risk of chronic disease beyond their nutritive value (Ziemer & Gibson, 1998; Saarela *et al*, 2002). A food can be made functional by addition of a potential health-promoting entity, reducing or removing concentrations of harmful components and/or modifying the nature or the bioavailability of one or more components (Ziemer & Gibson, 1998; Saarela *et al*, 2002). The first generation of functional foods was based on enrichment/ fortification with vitamins and minerals (mainly calcium). However, the concept has recently moved towards food ingredients exerting a positive effect on the gut microbiota, introducing probiotics and prebiotics (Ziemer & Gibson, 1998).

Probiotics are living microbial food components that beneficially affect the host by improving its intestinal microbial balance (Gibson & Roberfroid, 1995). The most common probiotics currently used belong to the genera *Bifidobacterium* and *Lactobacillus* (Duggan *et al*, 2002). These genera have a considerable safety record, both with the fermented food industry, where they have been used for many years, and more recently in probiotic foods. Intake of probiotic foods has been associated with a number of health benefits (reviewed by Sanders, 2003). Because probiotics do not permanently colonise in the intestine, they must be taken in sufficient quantities (>1 X 10^{10}/day) to maintain adequate amounts in the colon (Duggan *et al*, 2002). It is important that the ingested bacteria reach the large intestine in an intact and viable form. However, most common products containing probiotic bacteria, namely yogurt and probiotic drinks (milks, fruit juices), have a short shelf life and bacterial numbers are often not stated (Hamilton-Miller, 2004). Even if bacterial numbers are claimed on the label, which has been found not to be the case in several products in South Africa (Elliott & Teversham, 2004; Brink *et al*, 2005), it refers to live bacteria at the time of manufacturing and not the time of purchase. Another source of probiotics is supplements consisting of freeze dried bacteria in capsule, tablet or powder form. Some of these brands contain probiotics with proven beneficial

effects and have a long shelf life; however, many brands may contain contaminants and lower than claimed numbers and/or strains with no proven beneficial effects (Hamilton-Miller, 2004).

Prebiotics are a more recent concept, first defined about ten years ago (Gibson & Roberfroid, 1995). Here, the selective growth of indigenous gut bacteria is required. Prebiotics are indigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a number of health-promoting colon bacteria and thus improve host health (Gibson & Roberfroid, 1995). Prebiotics have become news mainly as an alternative to probiotics, which are difficult to handle in foodstuffs, but whose benefits to health in terms of prevention of diarrhoea and immunomodulation are increasingly well established and because prebiotics currently in use, especially inulin and its derivates as well as galactooligosaccharides, are cheap to manufacture or extract and, in addition to having probable health beneficial effects on the gut flora, are also valuable functional ingredients in foods, with the property of giving fat-based spreads and dairy products a creamy mouth feel (Macfarlane *et al*, 2006).

Gibson and colleagues (2004) have recently reviewed their original prebiotic concept in the light of much research that has been published in the past decade, and in particular the three key aspects of their definition: 1) resistance to digestion; 2) fermentation by the large intestinal microflora; and 3) a selective effect on the flora that promote health. Their new definition is: "A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health". The key words in both definitions are "selective" and "benefit/improve…host …health". Therefore, a prebiotic substrate must be particularly readily available to some groups of bacteria (of which lactobacilli and bifidobacteria are considered indicator organisms) that are beneficial to intestinal health but less available to potentially pathogenic bacteria, such as toxin-producing *Clostridia*, proteolytic *Bacteroides* and toxogenic *Escherichia coli* (Manning & Gibson, 2004). In this manner, a "healthier" microbiota composition is obtained whereby the bifidobacteria and/or lactobacilli become predominant in the intestine and exert possible health-promoting effects.

POTENTIAL PREBIOTICS

An exclusively breastfed baby has flora dominated by lactobacilli and bifidobacteria, which are part of the baby's defence against pathogens and which is an important primer for the immune system (Newburg, 2005; De Morais & Jacob, 2006). These flora are nurtured by the oligosaccharides of breast milk, which is considered to be the original prebiotics. While some peptides, proteins and certain lipids are potential prebiotics, non-digestible carbohydrates, in particular non-digestible oligosaccharides, have received most attention (Ziemer & Gibson, 1998). Any dietary component that reaches the colon intact is a potential prebiotic. However, the prebiotic property has been demonstrated adequately for only a few food ingredients. According to Gibson *et al*. (2004) and Roberfroid (2005) to date only the inulin-type fructans, galactooligosaccharides and lactulose are proven prebiotics. However, the latter is considered a therapeutic ingredient rather than a food ingredient.

Inulin is a group of fructose polymers (or fructans) linked by ß (2-1) bonds, which limit their digestion by upper intestinal enzymes. Naturally occurring fructans exist with a varied degree of polymerisation (DP) from 2 to 60 (Duggan *et al*, 2002). Oligofructose is defined as any fructose oligosaccharide containing 2 to a maximum of less than 10 monosaccharide residuals connected by glycosidic linkages (Roberfroid, 2007). Oligofructose is always present in inulin, a blend of fructose oligomers and polymers, and which differs from inulin in regard to its DP ($2 < DP < 10$ for oligofructose and $2 < DP < 60$ for inulin) (Watzl *et a*l, 2005). Fructans are found in many plant species, including chicory (from which inulin is extracted) and to a lesser extent in onion, garlic, banana, asparagus, leek and Jerusalem artichoke, as depicted in Table 1. For cereal grains, wheat is the best source, providing on average about 2,5 g fructans per 100 g raw bran and baked flour (van Loo *et al*, 1995). Wheat is the major food source of naturally occurring inulin and fructooligosaccharides, providing about 70% of these compounds in American diets (Moshfegh *et al*, 1999).

The daily per capita intake of inulin and fructooligosaccharides in the USA varies between 1 and 4 g and in Europe between 2 and 12 g (van Loo *et al*, 1995). These levels are probably too low to elevate bifidobacteria significantly (about one $\log^{10}$ value) in the human gut. Fructooligosaccharides have been found to be effective in humans at doses of 4 g/day, although the initial counts of bifidobacteria and not just the dose of fructooligosaccharides determine the relative increase in bifidobacteria (Rao, 1999). Hence, much interest exists in the approach of fortification of commonly ingested foodstuffs with prebiotics (Manning & Gibson, 2004) extracted from chicory or commercially produced through hydrolysis of polysaccharides (e.g. dietary fibres, starch) or through enzymatic synthesis from sucrose (Roberfroid *et al*, 1998).

As prebiotics exploit the use of non-viable dietary components to improve gut health, the range of foods to which they can be added is much wider than that for probiotics, where culture viability needs to be maintained. This has the advantage that heat stability or exposure to oxygen is not an issue. As such, virtually all carbohydrate-containing food can be fortified/enriched. Potential applications for prebiotics as food ingredients to improve the gastrointestinal health of the consumer include beverages, bakery products, table spreads, sauces, infant formulae and weaning foods, cereals, confectionary, snack bars, soups, salad dressings and dairy products (Franck, 2002). Inulin-type fructans are used as sugar substitutes and fat replacers (inulin only), and as a means of providing texture, stabilising foam and improving mouth feel in various food products (reviewed by Franck, 2002).

TABLE 1: FRUCTAN CONTENT OF FOODS*

Foods containing fructans	Serving size (g)	Fructan content	
		g / 100 g	g / serving**
Wheat-based foods:			
Bran – raw	4 g	1,4 – 4,0	0,1
White bread	2 slices (65 g)	0,7 – 2,8	1,8
Pasta	1 c. cooked (165 g)	1,4 – 4,1	2,5
Whole-grain breakfast cereal	1 c. (60 g)	0,8 – 3,2	1,9
Muffins	1 (65 g)	0,6 – 2,2	1,4
Cracker	2 biscuits (40 g)	0,8 – 3,4	1,4
Crispbread	2 biscuits (30 g)	1,0 – 3,8	1,1
Rye			
Flour	100 g	0,5 – 1,0	0,1
100% rye bread	2 slices (65 g)	0,35 – 0,63	0,4
Rye crispbread	2 biscuits (30 g)	0,4 – 0,72	0,2
Barley grain	100 g	0,3 – 0,7	0,7
Chicory roots	½ c. (75 g)	35,7 – 47,6	30,4
Chicory greens (witlof, Belgian endive)	½ c. (75 g)	none***	none
Chicory, root-based coffee-substitute beverages	1 – 2 tsp (7 g)	35,7 – 47,6	3,0
Onion	2 T. (35 g)	1,1 – 10,1	2,1
Leek	½ c. (85 g)	3,0 – 10,0	5,6
Asparagus	6 spears (90 g)	1,4 – 4,1	2,6
Jerusalem artichoke	½ c. (75 g)	16,0 – 20,0	15,0
Globe artichoke	1 medium (120 g)	2,0 – 6,8	5,5
Garlic	1 clove 3 (g)	9,0 – 16,0	0,5
Banana	1 medium (90 g)	0,3 – 0,7	0,6

C: cup, T: tablespoon (15 ml), tsp: teaspoon (5 ml)
* Adapted from Shephard & Gibson, 2006. Main source: Van Loo *et al,* 1995
** Upper end of the range
*** Van Loo, 2006 (personal communication)

NUTRITIONAL AND ASSOCIATED HEALTH PROPERTIES OF PREBIOTICS

The nutritional properties of prebiotics are related directly to the physiological changes they induce in the host. Bacterial metabolites are probably the main effectors of most observed effects. The most important metabolites are the short-chain fatty acids (SCFA) acetate, propionate and butyrate (Cummings *et al*, 2001). Prebiotic consumption can double the pool of SCFA in the gastrointestinal tract. These SCFA acidify the colon environment, which is beneficial for the development of bacteria such as bifidobacteria and lactobacilli, and detrimental to the growth of potential pathogenic species (Brandt, 2001; Blaut, 2002). All the SCFA are rapidly absorbed from the colon and then metabolised by various tissues: butyrate by the colonic epithelium, propionate and acetate (partly) by the liver and acetate (partly) by muscle and other peripheral tissues (Gibson & Roberfroid, 1995; Blaut, 2002). Acetate is the principal SCFA in the colon, and after absorption it has been shown to increase choles-

terol synthesis (Jenkins *et al*, 1991; Wolever *et al*, 1991). However, propionate, a gluconeogenerator, has been shown to inhibit cholesterol synthesis in vitro and in animal models (Chen *et al*, 1984; Venter *et al*, 1991). Therefore, it has been hypothesised that substrates that can decrease the acetate:propionate ratio may reduce serum lipids and possibly cardiovascular disease risk (Wong *et al*, 2006). Butyrate has been studied for its role in nourishing the colonic mucosa and in preventing colon cancer by regulating differentiation of colonic epithelial cells (Wollowski *et al*, 2001; Guarner & Malagelada, 2003; Manning & Gibson, 2004).

In the past decade, a large number of studies investigated the health-promoting effects of prebiotics. Although some of the postulated effects have not been fully demonstrated, the data suggest clinically significant effects that warrant further study and explanation. Demonstrating a direct clinical or health benefit of prebiotics is proving difficult. Small changes in lipid metabolism, calcium absorption or immune function may not give rise to evident improvements in health for many years. However, resistance to pathogen invasion through increased colonisation resistance of the gut microbiota, brought about by stimulation of bifidobacteria and lactobacilli, should in principle be easier to show *in vivo* (Cummings & Macfarlane, 2002). The postulated beneficial effects of prebiotics are summarised below.

Alleviation of constipation

All carbohydrates that reach the large intestine have a laxative effect on bowel habit. The mechanism works via stimulation of microbial growth, increase in bacterial cell mass and thus stimulation of peristalsis by the increased bowel content (Cummings, 1994). It can be predicted, therefore, that prebiotics will be laxative. In carefully controlled studies it has indeed been shown that prebiotics that are fermented completely increase bowel frequency (den Hond *et al*, 2000), bringing relief from constipation in chronically constipated subjects, and induce a faecal bulking effect of 1,5 to 2 g of faeces per gram of prebiotic consumed (Gibson *et al*, 1995). However, this is less than seen with non-starch polysaccharide sources such as wheat bran (5.4 g) or fruit and vegetables (4.7 g), but similar to that produced by more rapidly fermented polysaccharides such as pectin (1.2 g) (Cummings, 1993).

Increased mineral absorption

There has been an increased interest in recent years in the possibility of increasing mineral (particularly calcium) absorption through the consumption of prebiotics. Although the small intestine is the principal site of calcium absorption in humans, significant amounts may be absorbed throughout the length of the gut, consequently maximising of colonic effects is desirable. Numerous animal studies have indicated that prebiotics increase calcium, zinc and magnesium absorption from the colon (Delzenne *et al*, 1995; Younes *et al*, 2001; Coudray *et al*, 2003; Raschka & Daniel, 2005a), resulting in increased bone density and bone trabecular structure (Ohta *et al*, 2002; Scholz-Ahrens *et al*, 2002; Raschka & Daniel, 2005b). Raschke and Daniel (2005b) reported increased absorption, retention and femur content of calcium, zinc and magnesium in a rat model only when the basic diet of the control group contained no intrinsic fructans and when the mineral demand was particularly high, as during growth.

Several studies have investigated the effect of prebiotics on mineral absorption in humans, but with opposing results. In postmenopausal women fructooligosaccharides increased magnesium absorption and status after five weeks (Tahiri *et al*, 2001). However, when women were stratified by time of postmenopause, a trend was observed ($P < 0,1$) of a beneficial effect of short-chain oligofructose on calcium balance in women >6 years postmenopause but not in women <6 years postmenopause (Tahiri *et al*, 2003). In healthy young men, the consumption of 40 g inulin/day for 28 days resulted in a significant increase in calcium absorption (Coudray *et al*, 1997). A lower dose, 15 g of inulin, fructooligosaccharide or galacto-oligosaccharide per day, when fed to adults for 21 days, resulted in no effect on absorption of calcium or iron as measured by stable isotope methodology (van den Heuvel *et al*, 1998). In a later study, adolescent boys (aged 14-16) consumed 15 g of fructooligosaccharides/day for 9 days (van den Heuvel *et al*, 1999). An increase (10,8%) in calcium balance was found, with no significant effect on urinary excretion. Griffin *et al* (2002) reported an increase in calcium absorption when inulin plus a fructooligosaccharide mixture were fed to healthy girls (aged 11-13.9), but not with fructooligosaccharide alone.

In a follow-up study, Griffin *et al* (2003) used a dual-tracer stable isotope method to determine the effects of long-chain inulin enriched with oligofructose on calcium absorption in girls. Calcium absorption increased mainly in girls with lower calcium absorption during the placebo period. Other researchers also found that not all prebiotics promote mineral absorption to the same extent, and that the effects were more pronounced when long-chain fructans were present (Abrams *et al*, 2005). Abrams *et al* (2005) reported an increase not only in calcium absorption, but also in the whole-body mineral content and density when a combination of short- and long-chain inulin-type fructans were consumed by pubertal adolescents for a period of one year. These observations have led to the hypothesis that short-chain fructans (DP 2-8) (which are very soluble and are fermented quickly) are able to modify the composition of the intestinal microbiota in the proximal part of the colon and that the long-chain fructans (DP 12-65) (which are fermented slowly) keep the improved microbiota metabolically active for a prolonged period of time and hence in the more distal parts of the intestine (van Loo, 2004). In summary, the most convincing data in humans, until now, have been obtained in adolescents and in postmenopausal women. Furthermore, the effects were more pronounced when long-chain fructans were present.

Several mechanisms have been proposed to explain the effect on mineral absorption and metabolism

(reviewed recently by Scholz-Ahrens *et al,* 2007). The intake of prebiotics acidifies the intestinal contents, which aids the solubilisation of minerals (Coudray *et al*, 2003). Bacterial fermentation products, predominantly lactate and butyrate, enlarge the absorption surface by promoting proliferation of enterocytes (reviewed by Scholz-Ahrens *et al*, 2001). Other mechanisms that have been proposed include an increased capacity of the calcium transporters (calbindin) in the colon (Ohta *et al*, 1998), suppression of bone resorption rate relative to bone formation rate (Zafar *et al*, 2004), release of bone-modulating factors such as phytoestrogens (Ohta *et al*, 2002) and improvement in gut health and gut-associated immune defence (Scholz-Ahrens *et al*, 2007). Raschka and Daniel (2005a) studied the effects of fermentation of inulin-type fructans on transepithelial calcium transport in the large intestine of rats. They found that fructan feeding altered transcript levels of several mucosa genes that can be linked to transcellular and paracellular calcium transport processes. They also reported a decreased luminal pH in the caecum, with markedly increased caecal pools of total, soluble and ionised calcium, and concluded that inulin-type fructans increase large intestinal calcium absorption by various mechanisms, including enhanced pools of calcium, an increase in the absorptive surface, increased concentrations of SCFA, and by direct interaction with the intestinal tissue (Raschke & Daniel, 2005a).

Anticarcinogenic effects

It is known that several species of bacteria commonly found in the colon produce carcinogens and tumour promoters from the metabolism of food components. There have been several studies on anti-carcinogenic effects of dietary prebiotics in various experimental animal models, recently reviewed by Pool-Zobel (2005). Inulin-type fructans have been shown to inhibit the formation of aberrant crypt foci (ACF), a biomarker of colon cancer, and reduce tumour incidence. More pronounced effects were achieved by synbiotics (mixtures of probiotics and prebiotics) and long-chain inulin-type fructans compared to short-chain derivates (sustained activity of the saccharolytic fraction of the intestinal microbiota), especially in the more distal parts of the colon (van Loo, 2004; Pool-Zobel, 2005).

However, very few human studies have been carried out to date. Human studies tend to focus on faecal markers of carcinogenesis rather than being epidemiological in nature. Fructooligosaccharides and galactooligosaccharides have been investigated in this regard. Fructooligosaccharides have been found to reduce genotoxic enzymes concomitant with increasing bifidobacteria (Bouhnik *et al*, 1996), but a study on galactooligosaccharides found no significant changes in bifidobacteria or in markers of carcinogenesis (Alles *et al,* 1999). The lack of effect may be explained by the rather high starting bifidobacteria populations in the volunteers. It has been noted previously (Rycroft *et al*, 2001) that the magnitude of the response to prebiotics by bifidobacteria depends on the starting levels. Recently, the European Union sponsored a human intervention trial (European SYNCAN project, www.syncan.be) to evaluate the potency of a mixture of short- and long-chain fructooligosaccharides (10 g/d) given together with probiotics (*Lactobacillus rhamnosus* GC and *Bifidobacterium bifidum* Bb; 12^{10} – 10^{10} colony-forming units/day) to modulate several criteria associated with a high risk of developing colon cancer (van Loo *et al*, 2005). Preliminary results showed that the symbiotic treatment in polyp patients reduced DNA damage, cell proliferation in colonocytes and faecal water genotoxicity (Pool-Zobel, 2005). These findings are strikingly similar to the data from *in vitro* cell culture experiments and *in vivo* animal studies, thus indicating a potential risk preventing property of this intervention in humans (Pool-Zobel, 2005).

At least two mechanisms have been proposed to explain the effect of prebiotics on the development of cancer:

- Production of protective metabolites. Butyrate is a common fermentation end product and is known to stimulate apoptosis in colonic cancer cell lines, and it is also the preferred fuel for healthy colonocytes (Prasad, 1980).
- Shift of colonic metabolism away from protein and lipid metabolism towards more benign end products (saccharolysis, Manning & Gibson, 2004).

It is thought that lactic acid bacteria have inhibitory effects on several bacteria that produce carcinogenic enzymes and are themselves non-producers. Moreover, prebiotics may indirectly modify the activities of enzymes produced by the lactic acid bacteria that are involved in carcinogenesis (Reddy, 1998).

Modulation of the immune system

Research with prebiotics in this field of interest is very recent and mostly from experimental models. It has been observed that consumption of inulin-type fructans increases the phagocytic capacity of macrophages (Kelly-Quangliana *et al*, 2003). There is increasing evidence from animal studies that the addition of fermentable fibre to the diet can modulate the type and function of cells from different regions of the gut-associated lymphoid tissue (GALT) (Schley & Field, 2002), increase the number of Peyer's patches (Pierre *et al*, 1997) and alter leucocyte and lymphocyte numbers in the intestinal mucosa (Gaskins *et al*, 1996). Human studies have only recently begun. A study in healthy children, supplemented with fructooligosaccharides, demonstrated an impact on the occurrence of febrile illness, either associated with diarrhoea or upper respiratory illness (Saavedra & Tschernia, 2002). Furthermore, prebiotics improved the manifestations of atopic dermatitis in children above two years (Passeron *et al*, 2006) and reduced the incidence of atopic dermatitis during the first six months of life in high-risk infants (Moro *et al*, 2006).

The proposed mechanisms underlying the immunomodulating effects may include the following (Schley & Field, 2002):

- direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine;
- production of SCFA from fermentation; and
- modulation of mucin production.

Although further work is needed to better define the mechanisms for immunomodulation and the ultimate impact on health, there is convincing preliminary data to suggest that the consumption of fermentable fibres can modulate immune parameters in GALT, secondary lymphoid tissues and peripheral circulation (Swanson, *et al,* 2002; Gibson *et al*, 2004).

Regulation of lipid metabolism

There is interest in the food industry in developing functional foods to modulate blood lipids, such as cholesterol and triglycerides. Although convincing lipid-lowering effects of inulin and fructooligosaccharides have been demonstrated in animal models, attempts to reproduce similar effects in humans have produced conflicting results. In some studies no effect on lipid metabolism were noticed (Pederson *et al*, 1997; Alles *et al*, 1999, Kruse *et al*, 1999; van Dokkum *et al*, 1999), while in some studies total serum cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) decreased in comparison with placebo (Hidaka *et al*, 1991; Davidson *et al*, 1998). In some studies prebiotics decreased fasting triglyceride levels (Brighenti *et al*, 1999; Jackson *et al*, 1999; Causey *et al*, 2000). A variety of results may be attributed to the complexity of the biochemistry of lipid metabolism in humans (van Loo, 2004).

Three mechanisms have been put forward to explain a hypolipidaemic effect of prebiotics. The first is the modification of glucose or insulin concentrations. Non-digestible carbohydrates reduce peak levels of blood glucose after a meal and consequently the induction of lipogenic enzymes via an increased gene transcription (Roberfroid, 2000). The second is the production of SCFA in the colon. As mentioned earlier, the ratio of acetate to propionate reaching the liver is a putative intermediate marker predicting the potential lipid-lowering properties of prebiotics (Delzenne & Williams, 2002; Letexier *et al*, 2003; Wong *et al*, 2006). The third mechanism proposes that serum cholesterol is reduced because of precipitation and excretion of bile acids to the intestine, which requires the liver to utilise cholesterol for further bile acid synthesis (Pedersen *et al*, 1997). Because animal studies have identified inhibition of hepatic fatty acid synthesis as the major site of action for the triglyceride-lowering effects of inulin and fructooligosaccharides, and because this pathway is relatively inactive in humans unless a high-carbohydrate diet is followed, variability in response of animals and humans may be a reflection of differences in background diet or experimental foods used (Williams & Jackson, 2002).

Adverse side-effects

Generally, oligosaccharides are well tolerated. Some individuals report increased flatulence initially. Tolerance depends on the dose and individual sensitivity factors. Because fructans of small degree of polimerisation are osmotically active and rapidly fermented, Shepherd and Gibson (2006) recently reported improvement in symptoms of irritable bowel syndrome (IBS) in 62 patients with IBS or fructose malabsorption when they limited the total dietary fructose load (free fructose and short-chain fructans).

DISCUSSION

The purpose of this review is to show that carbohydrate prebiotics interact with physiological processes such as bowel habit, mineral absorption, cancer, immunology and lipid metabolism. This was observed by monitoring biomarkers of these physiological processes. The way in which prebiotics modify these physiological processes may result in a reduced risk of disease or an improved health status. Because the physiological effect is dependent on the type of intestinal fermentation induced by a specific prebiotic, it can be concluded that all other physiological processes are a consequence of these fermentation processes, either directly from the bacteria or indirectly from the metabolites that they produce.

With respect to the prebiotic effect itself, the selectivity of the prebiotic cannot be described in terms of the names of bacterial species that increase in numbers and the species that decrease in numbers (van Loo, 2004). The problem is that more than 80% of the bacterial species present in the intestine are unknown (they cannot be grown *in vitro*, therefore cannot be classified taxonomically). The selectivity is recognised in terms of groups of bacteria (e.g. saccharolytic *vs* proteolytic), each with representative marker organisms (e.g. *Bifidobacteria* and *Clostridia*) (van Loo, 2004).

Prebiotics can be used in a wide variety of foodstuffs such as dairy, meat and bakery products, drinks, spreads and confectionary (Franck, 2002). They are legally classified as food or food ingredients (not as additives) in many countries, most of which have agreed that inulin and fructooligosaccharides may be labelled as 'dietary fibres' (Coussement, 1999). The proposed South African regulations in the Foodstuffs, Cosmetics and Disinfectants Act (Act No. 54 of 1972, www.doh.gov.za) require statements on the amounts and source of prebiotics on the labels of food items claiming to contain these substances (at least 3 g of prebiotic per daily serving). Brink *et al* (2005) evaluated the content claims regarding prebiotic type and concentration of South African products and found that, although the concentration claims were in line with the proposed regulations, the labels of three products investigated did not specify the type of prebiotic.

The classical methods for analysis of dietary fibre (Association of Official Analytical Chemists (AOAC) and Englyst methods) do not analyse prebiotics (because they are either too soluble in ethanol or degraded in the acid hydrolysis steps). Therefore, AOAC International has adopted the 'Fructan method' (method number 997.08), which specifically allows the accurate quantitative determination of inulin and fructooligosaccharides in foods (Hoebregs, 1997). Inulin and fructooligosaccharides can be used for either their nutritional advantages or technological prop-

erties (Franck, 2002), but they are often applied to offer a dual benefit: an improved organoleptic quality and a better-balanced nutritional composition.

In conclusion, the microbiota of the gastro-intestinal tract is of key importance to the nutrition and health of the host. Modulation of the microbiota can occur through diets that contain prebiotics. It is still early days for prebiotics but the approach of using diet to induce microbial change offers a very straight-forward approach towards improved health. In terms of new developments, it is important to determine the definite health bonuses associated with prebiotic intake in humans and the mechanisms of action.

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