

REVIEW ARTICLE

Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics

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Summary

Most studies involving prebiotic oligosaccharides have been carried out using inulin and its fructo-oligosaccharide (FOS) derivatives, together with various forms of galacto-oligosaccharides (GOS). Although many intestinal bacteria are able to grow on these carbohydrates, most investigations have demonstrated that the growth of bifidobacteria, and to a lesser degree lactobacilli, is particularly favoured. Because of their safety, stability, organoleptic properties, resistance to digestion in the upper bowel and fermentability in the colon, as well as their abilities to promote the growth of beneficial bacteria in the gut, these prebiotics are being increasingly incorporated into the Western diet. Inulin-derived oligosaccharides and GOS are mildly laxative, but can result in flatulence and osmotic diarrhoea if taken in large amounts. However, their effects on large bowel habit are relatively minor. Although the literature dealing with the health significance of prebiotics is not as extensive as that concerning probiotics, considerable evidence has accrued showing that consumption of GOS and FOS can have significant health benefits, particularly in relation to their putative anti-cancer properties, influence on mineral absorption, lipid metabolism, and anti-inflammatory and other immune effects such as atopic disease. In many instances, prebiotics seem to be more effective when used as part of a synbiotic combination.

Introduction

There is still a widespread belief that while food intake may regulate certain metabolic activities associated with intestinal micro-organisms, changing diet has little effect on the overall composition and structure of microbial communities in the human gut (Macfarlane and Macfarlane 2003). However, the introduction of prebiotics into the diet in recent years has raised a serious challenge to this concept, and it is increasingly being recognized that the species composition of the microbiota, as well as many of its physiological traits, can be modified by relatively small changes in food consumption.

The original definition of a prebiotic was put forward by Gibson and Roberfroid (1995) as 'a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves

health'. This has recently been amended to 'A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health.' In practice, the beneficial bacteria that serve as targets for prebiotics have been almost exclusively bifidobacteria and lactobacilli (Gibson *et al.* 1999; Bouhnik *et al.* 2004). Unlike the situation with probiotics, where allochthonous micro-organisms are being introduced to the gut, and have to compete against established colonic communities, an advantage of using prebiotics to modify gut function is that the target bacteria are already commensal to the large intestine. As a consequence, prebiotics are arguably a more practical and efficient way to manipulate the gut microflora than probiotics. However, the other side of the coin is that if for any reason, such as disease, ageing, antibiotic or drug therapy, the appropriate health-promoting species are not

present in the bowel, the prebiotic is unlikely to be effective.

Potential prebiotic oligosaccharides can be classified according to their chemical constituents and degree of polymerization (d.p.), and include manno-oligosaccharides (Zentek *et al.* 2002), pectic-oligosaccharides (Olano-Martin *et al.* 2003), soybean-oligosaccharides (Saito *et al.* 1992), isomalto-oligosaccharides (Morgan *et al.* 1992), xylo-oligosaccharides (Campbell *et al.* 1997) and lactulose (Tuohy *et al.* 2002). However, as reviewed recently (Macfarlane *et al.* 2006), the vast majority of studies on prebiotics have focused on inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides/transgalactosylated-oligosaccharides (GOS/TOS). This latter group of carbohydrates in particular, has a history of safe commercial use, and are not classified under the category of novel foods.

FOS and GOS are not sensitive to gastric acid and do not serve as substrates for hydrolytic enzymes in the upper digestive tract. Japan was a pioneer in adding short-chain FOS to commercially available foodstuffs, and 'Neosugar' can be found in more than 500 food products (Guarner 2005). GOS are stable at high temperatures in acidic environments and the calorific value of these oligosaccharides is only 1.7 kcal g^{-1} , which makes them of particular interest to the food and drink industry, for both their prebiotic properties, and their use as sweeteners, especially in confectionary, acidic beverages and fermented milks (Watanuki *et al.* 1996). The calorific energy value of FOS is similarly low, at 1.5 kcal g^{-1} (Roberfroid 2005). The consumption of 8 g of oligofructose per day in 20 healthy subjects has been shown to promote satiety and reduce food intake (Cani *et al.* 2006). Numerous health claims have been made on behalf of prebiotics from investigations undertaken *in vivo* and *in vitro*. Much of this work has been carried out with FOS alone, or in combination with inulin, FOS and GOS in combination, and to a lesser extent, with GOS alone (Table 1). Moreover, a number of studies have been carried out using synbiotics, which are combinations of a probiotic and prebiotic. The rationale here is that the prebiotic stimulates growth of the probiotic in the gut, giving the organism a competitive advantage over indigenous species.

While most research interest has focused on the fermentability and bifidogenicity of oligosaccharide prebiotics, *in vitro* studies have shown that a number of these substances mimic eukaryotic cell surface receptors that virulent bacteria adhere to as part of the pathogenicity process. For example, GOS was shown to inhibit attachment of EPEC (enteropathogenic *Escherichia coli*) to HEP-2 and Caco-2 cells, and to be more effective than either inulin or FOS (Shoaf *et al.* 2006). Moreover, TOS was found to enhance the protective abilities of *Bifidobacterium breve* in mice infected with *Salmonella enterica* serovar

Typhimurium (Asahara *et al.* 2001). In addition to the anti-adherent effects of some oligosaccharides, short-chain chito-oligosaccharides have been reported to have specific anti-bacterial properties (Sekiguchi *et al.* 1994). If manifested *in vivo* in humans, these qualities could in principle enable prebiotic oligosaccharides to be protective in the small intestine, as well as in the colon; however, it is unclear at the moment whether sufficient amounts could be delivered to have any significant therapeutic effects.

Galacto-oligosaccharides

GOS in human milk

One of the earliest sources of GOS was human milk, which contains approx. 7% carbohydrates, 90% of which is lactose, and a variety of oligosaccharides based on lactose (ESPGAN Committee on Nutrition. 1977; Miller *et al.* 1994). Milk contains a greater proportion of neutral (1%) compared to acidic (0.1%) oligosaccharides (Thurl *et al.* 1996; Boehm *et al.* 2005). Oligosaccharides are the third largest component of human milk (Newburg 1997), and high levels are found in the colostrum where these substances constitute up to 24% of total colostrum carbohydrates. Concentrations of these substances in breast milk steadily decrease to between 19% and 15% in the first 2 months after birth (Miller *et al.* 1994). Oligosaccharides in breast milk can reach concentrations as high as $8\text{--}12 \text{ g l}^{-1}$ (Kunz and Rudloff 1996; Kunz *et al.* 2000), which is 100 times greater than in cow's milk. The principal sugar components of oligosaccharides in human milk are sialic acid, *N*-acetylglucosamine, *L*-fucose, *D*-glucose and *D*-galactose. This results in a complex mix of over 130 different oligosaccharides, because of the great variety of different sugar combinations that are possible (Brand-Miller and McVeagh 1999). The type of oligosaccharides produced is influenced by the mother's Lewis blood group. Human milk contains a large amount of galactose with the backbone structure based on lactose (galactose-glucose) plus a further external galactose residue. This leads to the formation of three galactosyl-lactoses, 3', 4' and 6'-galactosyl-lactose (Boehm *et al.* 2005). 6'-Galactosyllactose is found in amounts ranging between 2.0–3.9 mg l^{-1} (Yamashita and Kobata 1974), and the total concentration of GOS in human milk is approx. 1 g l^{-1} (Angus *et al.* 2005).

At the beginning of the last century, it was thought that there were factors in human milk that could promote the growth of certain types of intestinal bacteria (Moro 1900). Bifidogenic nucleotides have been detected in human milk (Gil and Rueda 2000), and many studies using conventional culture and molecular techniques for bacterial identification have shown that breast-fed infants

Table 1 Microbiological changes reported in human feeding studies with galacto-oligosaccharides (GOS)

Substrate	Type of study	Delivery	Effect on microbiota	Reference
GOS	Placebo controlled study involving 12 subjects	10 g prebiotic fed daily for 8 weeks	Faecal excretion of bifidobacteria and lactobacilli increased	Ito <i>et al.</i> (1990)
GOS	Feeding study with 12 subjects	2.5 g prebiotic given per day for 3 weeks	Increased bifidobacteria in faeces, reductions in numbers of clostridia and bacteroides	Ito <i>et al.</i> (1993)
Transgalactosylated-oligosaccharides (TOS)	Feeding study with 8 volunteers	Subjects given 10 g TOS for 3 weeks	Significant increases in faecal bifidobacteria, enterobacteria unaffected	Bouhnik <i>et al.</i> (1997)
TOS	Parallel study involving 40 healthy subjects	7.5 or 15 g prebiotic fed per day for 3 weeks	No effects on bifidobacteria. Lactobacilli increased in 15 g day ⁻¹ group, no effects on clostridia, small reductions in enterobacteria	Alles <i>et al.</i> (1999a)
GOS/fructo-oligosaccharide (FOS)	Placebo-controlled study with 90 term infants	Infant formula supplemented with either 4 or 8 g l ⁻¹ low molecular weight GOS and high molecular weight FOS for 28 days	Significantly increased lactobacilli and bifidobacteria in the two GOS feeding groups. Bifidogenic effects were dose-dependent.	Moro <i>et al.</i> (2002)
GOS	Placebo-controlled study involving 30 subjects	Volunteers given 8.1 g GOS syrup, 8.1 g GOS plus 3×10^{10} <i>Bifidobacterium lactis</i> Bb-12, or 3×10^{10} <i>Bif. lactis</i> without GOS for 3 weeks	Little change in faecal bifidobacteria seen with GOS alone. GOS plus <i>Bif. lactis</i> and <i>Bif. lactis</i> on its own resulted in faecal excretion of the organism, and reduced numbers of <i>Bif. longum</i>	Malinen <i>et al.</i> (2002)
GOS/FOS (9 : 1 ratio)	Double-blinded, randomized-controlled trial (DBRCT) involving 20 infants aged 28–90 days	Infant formula supplemented with 0.8 g 100 ml ⁻¹ GOS/FOS for 6 weeks	Increased total bifidobacteria counts in stools. Reduced <i>Bif. adolescentis</i> compared to standard infant formula controls. Bifidobacteria species composition in prebiotic infants similar to that found in breast-fed babies, with <i>Bif. infantis</i> , <i>Bif. breve</i> and <i>Bif. longum</i> predominating	Haarman and Knol (2005)
GOS/FOS (9 : 1 ratio)	Feeding study involving 42 preterm infants, 15 placebos, and a reference group given fortified mothers milk	Formula food supplemented with 10 g l ⁻¹ prebiotic mixture	Bifidobacteria numbers greatly increased from initially low levels, compared to un-supplemented controls. No significant effects on bacteroides, clostridia, enterobacteria or yeasts	Boehm <i>et al.</i> (2005)

have an intestinal microbiota that is dominated by bifidobacteria, which differs from that of infants fed on cow's milk. These microbiotas are characterized by slightly lower counts of bifidobacteria, with greater numbers of more potentially harmful organisms such as clostridia and enterococci (Harmsen *et al.* 2000; Macfarlane *et al.* 2004). Higher levels of ammonia, amines and phenols and other

potentially harmful substances have also been found in babies fed powdered milk products (Heavey *et al.* 2003). The preponderance of bifidobacteria in breast-fed babies is thought to result from their abilities to utilize oligosaccharides in breast milk, including GOS (Harmsen *et al.* 2000; Newburg 2000), which infants cannot digest in the upper gut (Engfer *et al.* 2000). While it would be difficult

to completely replicate all of the constituents of human milk to fortify infant formulas, the incorporation of manufactured prebiotics such as GOS can be a useful addition to formula feeds to replicate some of the functional attributes associated with breast milk, particularly its bifidogenic effects. However, the ability of GOS to resemble glycoconjugate structures on cell surface receptors used by pathogens for adherence in the gut may also protect babies from infection in early life (Kunz and Rudloff 1996; Newburg 1997). Based on the analysis of human milk and the high concentration of galactose, a mixture of 10% long-chain FOS (inulin) and 90% GOS has been developed to simulate human milk for use in infant formulas (Boehm *et al.* 2002; Weaver 2003). When this mixture was fed to term and preterm infants, it resulted in an increase in intestinal bifidobacteria and lactobacilli, with a gut microbiota and faecal fermentation product composition more resembling that of breast-fed infants (Haarman and Knol 2005; Moro and Arslanoglu 2005). Similar results have been obtained in several other studies involving young children using the same prebiotic combination (see later).

Production and structure of GOS

Galacto-oligosaccharides is principally formed by enzymic treatment of lactose by β -galactosidase to produce several oligomers of different chain lengths (Prensil *et al.* 1987). They usually have a d.p. between 2 and 10 with a terminal glucose. GOS can be produced from lactose in cow's milk, but the main raw material for its production for commercial products is usually whey-derived lactose (Yanahira *et al.* 1995). This is formed in large amounts as a by-product of the dairy industry, and as cheese production increases there is a need for more efficient and practical methods of reducing this waste. One way this could be performed is by the production of GOS. GOS is produced by β -galactosidases that have transgalactosylation activities (Fig. 1), which results in the formation of 4'- or 6'-galactosylactose, longer oligosaccharides, transgalactosylated disaccharides and nonreducing oligosaccharides (Angus *et al.* 2005). For GOS production, β -galactosidases from various fungi, yeasts and bacteria are usually immobilized on microparticle carriers such as ion exchange resins, chitosan, cellulose and agarose beads, or fibrous supports such as cotton cloths, which leads to the formation of different GOS products (Table 2). They usually contain 24–55% oligosaccharides, and smaller amounts of lactose, glucose and galactose. The enzymes and conditions used determine the various glycosidic linkages in the final products. $\beta(1,2)$, $\beta(1,3)$ and $\beta(1,4)$ linkages and branched glucose residues occur, while (1,4) and (1,6) linkages are present in the galactan fragment. This vari-

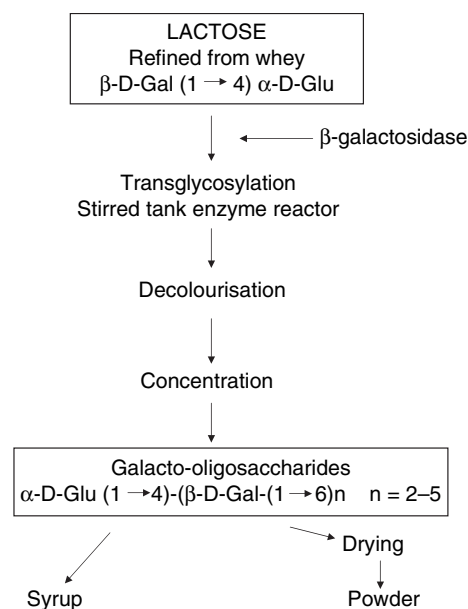


Figure 1 Schematic of diagram showing production of galacto-oligosaccharides from lactose (adapted from Angus *et al.* 2005).

ability in glycosidic linkages may be one of the reasons why GOS possess increased resistance to acid digestion (Tomomatsu 1994). The amount of GOS produced from lactose has also been shown to depend on the initial concentrations of lactose present in the reaction mixture, and not on the concentration of β -galactosidase. The resulting products can then be purified by activated carbon, filtered and concentrated before being added to foodstuffs.

One way of increasing the specificity of GOS products may be by using β -GOS synthesized by bifidobacteria in the manufacture of the product, as this could preferentially increase numbers of the specific organisms used to produce the substrate. Rabiou *et al.* (2001) demonstrated that the oligosaccharide profiles of five bifidobacterial species (*Bif. angulatum*, *Bif. infantis*, *Bif. pseudolongum*, *Bif. angulatum*, *Bif. bifidum*) differed from the commercial GOS product oligomate 55. With the exception of *Bif. adolescentis*, all of the organisms displayed increased growth rates on GOS prepared by their own β -galactosidase, compared to the commercial product.

Properties of GOS

Galacto-oligosaccharides preparations are usually transparent and more viscous than high-fructose corn syrups. They show good moisture retention and have a high solubility. GOS preparations only have about one third of the sweetness of sucrose (Oku 1996) and *in vitro* studies with oral bacteria have shown these substances to have a low

Table 2 Studies using various micro-organisms for the production of galacto-oligosaccharides (GOS) from lactose

Micro-organism	System	Yield	Outcome of study	Reference
<i>Aspergillus oryzae</i>	Enzyme immobilized on cotton cloth membrane in a plug flow reactor	GOS production of 27% (w/w) of initial lactose at 50% lactose conversion. 70% of GOS produced trisaccharides	Cotton cloth did not affect the characteristics of the β -galactosidase. Thermal stability of the enzyme increase 25-fold after immobilization. Yield several times higher than previously reported	Albayrak and Yang (2002)
<i>Talaromyces thermophilus</i>	β -galactosidase on insoluble carrier Eupergit C	Maximum yield 34% with 80% lactose conversion	Immobilization increased the thermostability of the enzyme	Nakkharat and Haltrich (2006)
<i>Lactobacillus reuteri</i>	Not stated	38% GOS produced at approx. 80% lactose conversion. Majority of products disaccharides other than lactose	No major products with β 1–4 linkages formed	Splechtna <i>et al.</i> (2006)
<i>Bacillus stearothersophilus</i>	Comparison of enzyme embedded in sodium alginate, chitosan or gelatin	Maximum yield of GOS 31.2% in stirred reactor with 60% lactose concentration. In the packed bead continuous reactor, the yield was 17.4–31.5%	Gelatin proved the best carrier for immobilization as demonstrated by increased yields. Only 20% of the original yield of the packed bed reactor was lost after 140 h of reaction time	Chen <i>et al.</i> (2001)
<i>Escherichia coli</i>	GOS produced under controllable water concentration in reverse micelles	Maximum 29.7% production of GOS (w/w) allolactose and 14% production of other oligosaccharides	Increasing the molar ratio of water to surfactant. W_o , decreased production of GOS	Chen <i>et al.</i> (2003)
<i>Sterigmatomyces elviae</i>	Toluene treated resting bacterial cells used in batch culture or in a new fermentation system with cell growth after the enzymatic reaction to decrease inhibitory glucose	37% yield in toluene treated cells. Increased yield to 64% because of new fermentation method	Major structure found 4'-galactosyl-lactose. Yeast high capacity for GOS production but low galactose production. β -galactosidase had high levels of transgalactosylation activity but low levels of lactose-hydrolysis. High optimal temperature of production, 80°C	Onishi <i>et al.</i> (1995)
<i>Aspergillus candidus</i>	Immobilized recombinant β -galactosidase in a continuous packed-bed reactor	Maximum yield 32% GOS over 20 days	Recombinant β -galactosidase has potential for GOS production	Zheng <i>et al.</i> (2006)

potential for the development of dental caries (Hartemink *et al.* 1997). Because of their stability, GOS can be incorporated into a wide variety of foods, where they have a pleasant taste, and can increase the texture and mouth-feel of foods, as well as acting as bulking agents. Because of this, GOS and FOS are currently used in a wide range of commercial commodities, including infant formulas, dairy products, sauces, soups, breakfast cereals, beverages, snack bars, ice creams, bakery products, animal feeds, and as sugar replacements (Yang and Silva 1995).

Physiological effects of prebiotics

To function most effectively, prebiotics need to be resistant to digestive processes in the stomach and small bowel, and to come into contact with bacteria growing in the large intestine. Breath hydrogen excretion generally increases following ingestion of prebiotics, indicating that these substances are fermented by gut micro-organisms, but these measurements do not provide information on how much substrate actually escapes digestion in the

upper gut. This is highlighted by the study of Bouhnik *et al.* (1997), where eight volunteers taking 10 g TOS per day apparently showed reduced breath H_2 excretion. In another investigation involving 40 subjects, feeding up to 15 g of TOS per day had no significant effects on faecal pH, short-chain fatty acid (SCFA) excretion, faecal bile acid profiles, or concentrations of putrefactive metabolites; however, faecal nitrogen was increased by 8.5%, and breath hydrogen by 130% (Alles *et al.* 1999a).

Together with SCFA, H_2 and CO_2 are major products of fermentation in the gut, and excess H_2 formation, and osmotic diarrhoea caused by high levels of SCFA production, provide a barrier to the unfettered use of prebiotics. Undesirable symptoms caused by fermentation gases have often been reported in human feeding studies involving prebiotics (Hartemink and Rombouts 1997; Pedersen *et al.* 1997). Stone-Dorshow and Levitt (1987) gave 12 volunteers 15 g of FOS per day for 12 days. Eructation, abdominal pain, bloating and flatulence were all significantly more severe in the FOS group compared to controls, while there was no adaptation over the study period. After 12 days, breath H_2 measurements following a 10-g challenge of prebiotic indicated that there was no difference between the FOS and control groups. Other investigations with FOS in humans, involving doses of 5 and 20 g day⁻¹, have indicated that breath H_2 increased with substrate challenge, with mild flatulence and borborygmi occurring generally, although some individuals reported greater discomfort (Kawaguchi *et al.* 1993; Luo *et al.* 1996). Other evidence suggests that feeding 10 g FOS per day can be well tolerated, but that the consumption of 14 g can lead to the development of gastrointestinal symptoms (Gibson *et al.* 1995; Nadeau 1999).

With respect to GOS, evidence obtained so far from European studies indicates that GOS is safe to add to infant feeds (Moro *et al.* 2006), and a recent report by the EU Scientific Committee on Food has approved the addition of up to 0.8 g 100 ml⁻¹ of a prebiotic mixture comprising 90% GOS and 10% FOS in infant formula feeds (Scientific Committee on Food (SCF) 2003). Most of the studies with GOS to date have used a recommended dosage of 8 to 15 g day⁻¹, because, like FOS, the consumption of higher levels can lead to abdominal discomfort, cramping, flatulence and diarrhoea (Deguchi 1997; Teuri *et al.* 1998; Saavedra and Tschernia 2002). The recommended dose to detect a bifidogenic effect is thought to be at least 10 g of GOS per day, but studies in Japan have shown that in healthy adults and the elderly, doses as low as 2.5 g of 6'-GOS can elicit a bifidogenic effect (Ito *et al.* 1993; Ishikawa *et al.* 1995; Teuri and Korpela 1998).

In one recent study, the safety of a commercial GOS syrup (Vivinal; Barculo Domo, Zwolle, Netherlands) containing 45% GOS was determined by orally feeding

the prebiotic to rats for 90 days. FOS supplemented with lactose and glucose and reverse osmosis-deionized (RODI) water were used as controls. Although significant reductions were found in food consumption (7–13%) in animals fed the GOS syrup, and in the FOS group, compared to the RODI controls, no toxicological manifestations were observed (Anthony *et al.* 2006). These data indicate that the choice of prebiotic substances to use in food products should be made with the ultimate aim of maximizing potential health benefits, and minimizing unwanted side effects.

Fermentation reactions in the large intestine

Quantitatively, and in terms of their significance in host physiology, SCFA, particularly acetate, propionate and butyrate, are the major end products of microbial fermentation processes in the large gut. These metabolites are formed predominantly from polysaccharide, oligosaccharide, protein, peptide and glycoprotein precursors (Macfarlane and Macfarlane 2002). Polysaccharides are the most important SCFA progenitors, and many different types of bacterial hydrolytic enzymes are synthesized by the microbiota to digest these substances and facilitate uptake of their component sugars. In quantitative terms, resistant starches and plant cell wall polysaccharides (celluloses and noncellulosic polysaccharides such as arabino-galactans, xylans, pectins, gums and mucilages) are the principal fermentation substrates in the large bowel (Cummings and Englyst 1987).

Many nutritional, microbiological and host physiological factors affect fermentation reactions in the large intestine, particularly colonic transit time. Other host-related factors that influence metabolic reactions in the microbiota and the types of SCFA that are formed in the gut, include ageing, neuroendocrine system activity, stress, pancreatic and other secretions in the digestive tract, mucus production, disease, drugs and antibiotic therapy. From a microbiological viewpoint, the chemical composition, physical form and amount of substrate available also significantly affects the end products of fermentation, which are further dependent on the types and numbers of different bacterial populations in the gut, as well as competitive and cooperative interactions between different groups of bacteria in the microbiota.

Short-chain fatty acid production is one of the most important physiological processes mediated by colonic micro-organisms. The vast majority of these metabolites are absorbed from the gut, enabling the host to salvage energy from food not digested in the upper bowel. SCFA affect colonic epithelial cell transport processes, energy transduction in colonocytes, growth and cellular differentiation, hepatic control of lipid and carbohydrate metabo-

lism, and provide energy to muscle, kidney, heart and brain (Cummings 1995).

Because >95% of SCFA are absorbed from the gut, measurements of these metabolites in stools does not really tell us very much about the fermentability of different carbohydrates. However, *in vitro* studies, particularly those involving faecal microbiotas, are useful models for studying fermentation processes. At concentrations of 10 g l⁻¹, FOS and GOS were shown to increase acetate and butyrate formation in pH controlled fermenters, with transient accumulation of lactate and succinate (Hopkins and Macfarlane 2003). Acetate and lactate formation are consistent with bifidobacterial and lactobacillus metabolism, but not butyrate production. However, the butyrogenicity of FOS has been reported by other workers (Topping and Clifton 2001), and recent work by Belenguer *et al.* (2006) has shown how butyrate-producing species such as *Anaerostipes caccae* and *Eubacterium halli* can cross-feed on lactate produced by *Bif. adolescentis* growing on FOS, while a nonlactate utilizing, butyrate-forming *Roseburia* sp. could assimilate carbohydrate fragments formed when the *Bifidobacterium* hydrolysed complex polymeric substrates. Indeed, it is important to realize that while prebiotics are often used to stimulate the growth of beneficial bacteria in the gut, they are not entirely specific for these target species, and other organisms have been shown to be able to ferment these carbohydrates. For example clostridia can utilize FOS (Duncan *et al.* 2003), whereas bacteroides and clostridia have been reported to ferment GOS (Ohtsuka *et al.* 1989).

Biological significance of bifidobacteria and lactobacilli in the digestive tract

Lactobacilli do not occur in great numbers in the human large intestine under normal circumstances; however, significant bifidobacterial communities are usually present in the colon, although their community sizes and species composition can vary greatly in different human population groups (Finegold *et al.* 1983). Evidence suggests that *Bif. adolescentis* and *Bif. longum* predominate in most adults, whereas *Bif. breve* and *Bif. infantis* are the main species colonizing the infant gut (Mitsuoka 1984; Scardovi 1986). Bifidobacteria, together with some lactobacillus species play an important role in the eco-physiology of the colonic microbiota. These organisms have been linked to increased resistance to infection and diarrhoeal disease (Yamazaki *et al.* 1985; Gorbach *et al.* 1987; Tojo *et al.* 1987; Saavedra *et al.* 1994), stimulation of immune system activity (Sekine *et al.* 1985; Kirjavainen *et al.* 2002), as well as protection against cancer (Reddy and Rivenson 1993; Sekine *et al.* 1994). Some bifidobacteria and many lactobacillus species manifest strong anti-mutagenic and

anti-tumour properties, and in animal models, have prophylactic and therapeutic benefits (Hosono *et al.* 1997). This may be related, in part, to immune enhancement effects resulting from the chemical composition and structure of their cell wall components.

Bifidobacteria are also known to excrete a range of water soluble vitamins, though these processes generally appear to be species and strain dependent (Deguchi *et al.* 1985). Thus, some strains of *Bif. bifidum* and *Bif. infantis* produce large quantities of folate, nicotinic acid and thiamine, while *Bif. longum* and *Bif. breve* only form small amounts of these substances. These vitamins do not seem to be made at all by *Bif. adolescentis*. Pyridoxine and vitamin B₁₂ are also produced, but not excreted by *Bif. breve*, *Bif. longum*, *Bif. bifidum*, *Bif. adolescentis* and *Bif. infantis*.

Bacteria growing in the large bowel play an important role in colonization resistance. This can be defined as the mechanisms through which the colonic microbiota protects itself against incursion by invading micro-organisms, thereby playing an important role in the maintenance of homeostasis. Comparatively little is known in detail about this phenomenon, but it is likely that many different species, including lactobacilli and bifidobacteria (Lievin *et al.* 2000) are involved. *In vitro* fermentation studies with mixed cultures of faecal bacteria growing on inulin and GOS (oligomate 55) have demonstrated that the oligosaccharides, especially GOS, inhibited growth and toxin production by *Clostridium difficile* (Hopkins and Macfarlane 2003). However, while the GOS preparation, in particular, was shown to be bifidogenic, stimulating growth of *Bif. adolescentis*, *Bif. angulatum* and *Bif. bifidum*, these organisms were not responsible for suppressing the pathogen, indicating that other species in the microbiota were protective.

In contrast, recent studies have shown that *Bif. breve* was more effective in protecting mice infected with *Escherichia coli* O157 H7, than strains of *Bif. bifidum* and *Bif. catenulatum* (Asahara *et al.* 2004). Some lactobacillus and bifidobacterial species form substances that are antagonistic to other organisms, such as organic acids, hydrogen peroxide, diacetyl and bacteriocins. Bacteriocin secretion is growth-associated in some species, and is dependent on carbon availability (Lejeune *et al.* 1998). Some of these small proteins and peptides are highly species-specific. In one study, of 13 bifidobacterial isolates tested for bacteriocin formation, only one strain formed a protease-sensitive inhibitory substance, with broad spectrum activity against other bifidobacteria, streptococci, lactobacilli and clostridia, but not Gram-negative organisms such as proteus, klebsiella, *E. coli* or pseudomonas (Meghrouss *et al.* 1990). Gibson and Wang (1994) reported that eight different bifidobacterial species produced similar antagonistic substances that were inhibitory

to several gut pathogens, including listeria, shigella, campylobacter, vibrio and shigella. Unlike the antibiotics formed by lactic acid bacteria, bifidobacterial anti-microbial secretory products have not been studied extensively, although they generally appear to exhibit broader activity spectra than is associated with conventional bacteriocins.

Feeding studies with human volunteers clearly demonstrate that bifidobacterial numbers in the colon can be increased by FOS and GOS (Ito *et al.* 1990; Gibson *et al.* 1995). There are reports of these prebiotics also stimulating the growth of lactobacilli (McBain and Macfarlane 2001; Smiricky-Tjardes *et al.* 2003), but bifidobacteria generally compete better for these substrates. The ability to target specific groups of organisms in the large intestine through dietary means is increasingly seen as being of significant health value. Two factors that are especially important in this respect are the rate at which an organism can grow on a particular carbon source, as this will influence its ability to compete with other bacteria in the colon, the other is the extent to which the substrate is converted into bacterial cell mass, because cell population size will affect the degree of both prebiotic and probiotic activity.

Metabolism of carbohydrates in bifidobacteria

Many different types of carbohydrate are present in the human colon at any given time, particularly in the proximal bowel. Consequently, nutritionally versatile bacteria that can grow on a variety of carbon sources and adapt rapidly to changing substrate availability have a competitive advantage in the ecosystem. Carbohydrate utilization in colonic micro-organisms is to a large extent controlled by catabolite regulatory mechanisms, which determine the synthesis of hydrolytic enzymes and substrate transport systems, as well as their physiological activities (Macfarlane and Degnan 1996). Catabolite regulatory mechanisms enable colonic micro-organisms to selectively use certain substrates to the exclusion of others, enabling some species to compete more efficiently for a restricted range of preferred substrates in relatively specialized metabolic niches. Because different bacteria form distinct patterns of metabolic end products, catabolite regulatory mechanisms ultimately affect the types and amounts of these substances that can be produced from individual substrates. Consequently, these control processes are ecologically important, and are of considerable physiological significance to the host.

Studies on carbohydrate utilization by bifidobacteria have shown that individual strains have specific substrate preferences (Degnan and Macfarlane 1991). For example, when eight different species were grown on various mixtures of arabinose, mannose, galactose, glucose and

xylose, *Bif. bifidum* sequentially utilized the carbohydrates in the order glucose > galactose > mannose, with inhibition of pentose uptake. *Bif. longum* co-utilized glucose and xylose, while galactose assimilation was repressed. In *Bif. catenulatum*, glucose repressed galactose uptake. Glucose and galactose were assimilated simultaneously by *Bif. adolescentis*, with mannose and arabinose utilization being repressed. Glucose and galactose were also preferred substrates in *Bif. infantis* and *Bif. angulatum*, whereas sequential sugar uptake (galactose > glucose > xylose) was observed in *Bif. pseudolongum*. Conversely, glucose and arabinose were co-utilized in *Bif. breve*, while mannose uptake was repressed. These manifestations of catabolite regulation of carbohydrate assimilation were not directly related to specific growth rates of the organisms on the individual substrates; however, six of the eight species tested showed the highest specific growth rates with galactose. These data suggest that the operation of catabolite regulatory processes in bifidobacteria favouring galactose uptake may allow these organisms to compete favourably for this substrate in the colon (Macfarlane and Macfarlane 2002).

Hopkins *et al.* (1998) compared the growth of several bifidobacterial strains on a range of oligosaccharide substrates. Results showed that as with monosaccharide utilization, considerable inter-species differences existed in their abilities to utilize these substrates (Table 3). GOS and oligofructose supported the best growth of the test bacteria, while pyrodextrins and xylo-oligosaccharides were poor substrates for the test strains. Growth was often found to be better on oligosaccharides in comparison to their component monosaccharides, supporting previous suggestions that some bifidobacterial species may be particularly adapted to growth on oligosaccharides in the large gut (Degnan and Macfarlane 1995).

Ohtsuka *et al.* (1989) found that several pure cultures of bifidobacteria (*Bif. bifidum*, *Bif. adolescentis*, *Bif. longum*, *Bif. infantis*, *Bif. breve*) grew well on GOS, but only one of four lactobacillus species tested (*Lactobacillus acidophilus*) utilized the substrate. No growth was seen with *L. gasseri*, *L. salivarius* or *L. casei*. Substrate preferences occur in the utilization of GOS by intestinal bacteria. This was shown in a study where *Bif. adolescentis* was able to degrade oligosaccharides with a d.p. > 3, while *Bif. infantis* and *L. acidophilus* could only use those with a d.p. < 3. The ability of *Bif. adolescentis* to use high concentrations of TOS more efficiently was thought to be due to the production of a novel membrane bound β -galactosidase II by the bacterium, with activity towards TOS, but not lactose (van Laere *et al.* 2000). Similarly, in another study, substrate preferences were seen with respect to GOS utilization, where *Bif. lactis*, unlike *L. rhamnosus* was able to metabolize tri and tetrasaccharide

Table 3 Bifidobacterial growth on different oligosaccharide substrates*

Organism	Specific growth rate (h^{-1})							
	Galacto-oligosaccharides (GOS)†	Oligomate 55	Raftilose LS	Raftilose L60	Raftilose P95	Pyrodextrins	Soya-oligosaccharides	Xylo-oligosaccharides
<i>Bifidobacterium adolescentis</i> NCFB 2230	0.20	0.19	No growth	0.21	0.19	No growth	0.13	No growth
<i>Bif. catenulatum</i> NCFB 2246	0.20	0.21	0.07	0.20	0.13	0.09	0.24	No growth
<i>Bif. pseudolongum</i> NCFB 2244	0.23	0.21	0.29	0.40	0.38	0.27	0.28	0.40
<i>Bif. longum</i> NCFB 2259	0.37	0.43	0.30	0.44	0.17	0.45	0.43	0.38
<i>Bif. infantis</i> NCFB 2205	0.12	0.28	No growth	0.20	0.23	No growth	0.22	No growth
<i>Bif. bifidum</i> NCFB 2203	0.20	0.29	No growth	0.19	0.22	No growth	0.17	0.24

*Adapted from Hopkins *et al.* (1998).

†GOS: 85% oligogalactose, small amounts of lactose, glucose and galactose. Oligomate 55: predominantly galactosyllactose, degree of polymerization (d.p.) 2–5 (55% pure). Raftilose LS: inulin d.p. 10–12 (99% oligosaccharides). Raftilose L60: fructo-oligosaccharides (60%). Raftilose P95: fructo-oligosaccharides, d.p. 2–8 (95%). Pyrodextrins: complex mixture of glucose oligosaccharides. Soya-oligosaccharides: mixture of raffinose (fructose, galactose, glucose) and stachyose (fructose, galactose, galactose, glucose). Xylo-oligosaccharides: xylose containing oligosaccharides, d.p. 2–4 (70% pure).

fractions (Gopal *et al.* 2001). As mentioned earlier, organisms other than bifidobacteria and lactobacilli have been shown to be able to grow on GOS, including species of clostridia and bacteroides, which can utilize the trisaccharides 4' galactosyllactose and 6' galactosyllactose (Ohtsuka *et al.* 1989; Sako *et al.* 1999), and it seems that the pattern of glycosidic linkages in different GOS products influences the abilities of individual bacteria to grow on them.

Immunomodulatory properties of prebiotics

The immune system

The colonic microbiota is important for development and maturation of the immune system, and mediating tolerance to bacterial and dietary antigens. Diet is one of the major factors that can influence the immune system in the gastrointestinal tract (Roller *et al.* 2004b), as well as intestinal microbial composition and metabolic product formation. Currently, there is increasing interest in the use of functional foods to modulate the gut immune system, with the aim of improving health and well-being.

Immunity comprises both innate and adaptive responses, and the innate immune system is the first line of defence for the body. It manifests nonspecific immediate responses to antigens, and is mediated through

phagocytic, inflammatory, dendritic and natural killer (NK) cells, as well as other soluble components such as complement and the cytokine network. Acquired or adaptive immune system responses occur after activation of the innate system. They are more antigen-specific, and are primarily based on the T- and B-lymphocytes. Lymphocytes play a major role in this component of the immune system, either modulating the function of other immune cells, or directly destroying infected cells. To assess the effects of prebiotics such as FOS and GOS on the immune system, a large number of immunological parameters need to be assessed. Recently, a collaborative study was made of the suitability of various immunological markers to measure outcomes in human intervention studies. The markers were grouped into three categories depending on whether they were of high, medium or low suitability (Albers *et al.* 2005). Markers with high suitability included the delayed-type hypersensitivity response, vaccine-specific or total secretory immunoglobulin A (IgA) in saliva, and vaccine-specific serum antibody production. Patterns of cytokines produced by activated immune cells, NK cell toxicity and lymphocyte proliferation were some of the markers considered to be of medium suitability. However, the study also noted that choice of immune markers needs to be correlated with the particular condition that is being assessed, the relevant clinical end points, and whether any immune markers are differentially expressed in disease and control populations.

Measurements of these markers should also take into consideration the fact that they can be affected by age and gender, and that they might vary because of other external factors such as stress, smoking and alcohol intake, which necessitates careful selection of the control subjects.

Prebiotics and the immune system

The gut contains a major part of the body's immune system, termed the gut-associated lymphoid tissue. Experimental-obtained data so far suggest that immune modulation of the gastrointestinal tract can occur through the use of functional foods such as prebiotics. To date, few studies have been made on interactions between fermentable carbohydrates and the immune system, or whether they exert direct or indirect modulatory effects. More CD4⁺ T cells have been found in mesenteric lymph nodes in rats fed a diet containing 5% pectin, compared to less fermentable cellulose (Lim *et al.* 1997). Other immunological effects have been observed after adding prebiotics such as FOS and lactulose to the diet, including increases in mucosal immunoglobulin production, mesenteric lymph nodes, Peyer's patches, and altered cytokine formation and lymphocyte numbers in the spleen and intestinal mucosa (Schley and Field 2002).

Methods by which prebiotics can exert their effects on the immune system, and attenuate inflammation in the colon, include increased SCFA production, and increases in immunogenic bacteria such as lactobacilli and bifidobacteria. As outlined earlier, several studies have shown that GOS and FOS fermentation can lead to the production of butyrate, the principal fuel for colonic epithelial cells. This SCFA also stimulates apoptosis (Rowland 1998) and may be a protective factor in carcinogenesis (Scheppach and Weiler 2004). Propionate is also produced from GOS, and has been shown to be anti-inflammatory with respect to colon cancer cells (Nurmi *et al.* 2005). A number of studies have indicated that SCFA have direct immunomodulatory properties (Pratt *et al.* 1996; Bohmig *et al.* 1997). For example, butyrate has been shown to suppress both cytokine-induced and constitutive expression of the transcription factor NF- κ B in HT-29 cell lines (Inan *et al.* 2000), while intravenous administration of acetate increases peripheral blood antibody production and NK activity in cancer patients, compared to controls (Ishizaka *et al.* 1993). Glutamine is used by immune cells in the body and increased production of butyrate may reduce the requirement of epithelial cells in the gut for this amino acid, thereby enhancing immune system reactivity (Jenkins *et al.* 1999).

The mucosal immune system

Prebiotics also influence the mucosa-associated immune system. The intestinal mucosa contains large amounts of sIgA which has a protective role against adherence and invasion by harmful bacteria and viruses. Levels of sIgA in faeces have been found to correlate with an increased ability to neutralize and clear viruses. Infants have an immature immune system, and because of the lack of transfer of immunoglobulins from breast milk, formula-fed babies have low levels of sIgA, which can lead to increased risk of gastrointestinal infection. The colonic microbiota plays a major role in immune system development in the newborn, and bifidobacteria in the digestive tract have been shown to be important for protection against viral pathogens by stimulating IgA production (Moreau and Gaboriau-Routhiau 2000). Lactobacilli can enhance both the innate and adaptive immune defences, resulting in increased production of phagocytes and immune effector molecules such as sIgA. Moreover, experiments with bifidobacteria have shown that these organisms are able to induce formation of large amounts of IgA in Peyer's patches of mice (Yasui *et al.* 1991).

Thus, the addition of prebiotics such as GOS alone or in combination with probiotic bifidobacteria or lactobacilli with immunomodulatory abilities that are able to utilize the substrate, has potential to increase production of sIgA in the body. Several investigations have demonstrated an increased sIgA response resulting from the use of FOS in animal models (Hosono *et al.* 2003; Nakamura *et al.* 2004), and in one study, feeding mice yogurt containing a mixture of bifidobacteria and *L. acidophilus* significantly increased the IgA response to cholera toxin (Tejada *et al.* 1999). Roller *et al.* (2004b) showed that local sIgA concentrations in the ileum could be increased in rats by feeding a synbiotic combination consisting of oligofructose enriched inulin together with *L. rhamnosus* GG and *Bif. animalis* BB-12 for 4 weeks. In one recent randomized double-blinded trial (Bakker-Zierikzee *et al.* 2006), 57 formula-fed infants were split into three groups, which were fed either standard formula alone, standard formula containing 6.0×10^9 probiotic *Bif. animalis* Bb-12, or formula supplemented with a prebiotic mixture containing a commercial prebiotic (Vivinal), which consists of a combination of 90% GOS and 10% FOS. Measurements were made of faecal IgA at intervals up to 32 weeks. Levels of IgA were found to be variable in babies receiving the probiotic formula, whereas children given the prebiotic mixture demonstrated a trend towards higher levels of IgA excretion in their stools compared to the controls.

Apart from children, another group in the community that could benefit from the immunomodulatory proper-

ties of prebiotics is the elderly, because the functional capacity of the immune system has been shown to decline with age. This is particularly associated with age-related T-cell changes, and reductions in antibody production, and a decline in cell-mediated immunity, resulting in increased susceptibility to infection and disease (Ginaldi *et al.* 1999; Effros 2001). However, while several studies with children have suggested that there may be an enhanced vaccine response with prebiotics, it is unclear whether a similar effect occurs in older people. In one study, 43 healthy subjects were randomized to FOS or placebo for 28 days. After 14 days, they were vaccinated with influenza and pneumococcal vaccines, and antibody titres, proteins and IgA were measured as a baseline, at the end of the feeding and a further 6 weeks later (Bunout *et al.* 2002). No immunological benefits were observed.

Allergy

There has been an increase in the incidence of allergic disease in developed countries over the last two decades, and studies have indicated that there may be evidence for a link between the colonic microbiota and allergy. For example, lower cell numbers of bifidobacteria with reduced adhesive properties have been found in the faeces of allergic infants compared to control children (He *et al.* 2001; Kalliomaki *et al.* 2001a; Kirjavainen *et al.* 2002). These infants have IgE-mediated food allergies, and a Th2-biased immune response. The babies have a skewed Th2 response at birth, because of high levels of Th2 cytokines produced by the mother, which become re-balanced after appropriate stimulation by bacteria colonizing the gut. Studies so far with prebiotics such as raffinose and alginate-based oligosaccharides, have resulted in a reduced Th2 response in these children (Nagura *et al.* 2002; Yoshida *et al.* 2004). This could be due to prebiotics influencing the gastrointestinal microflora by increasing numbers of commensal bifidobacteria thereby re-balancing the immune response by stimulating Th1 reactivity, and increasing the anti-inflammatory cytokines interleukin 10 (IL-10) and TGF- β (Kelly *et al.* 2005).

Atopic dermatitis (AD) is one of the early signs of allergy in infancy, and can affect 10–25% of children in Western countries. In a double-blind placebo-controlled trial, 102 infants at risk of atopy were fed a prebiotic mixture composed of 90% GOS and 10% FOS, and compared with a placebo group who were given maltodextrins (Moro *et al.* 2006). The faecal microbiotas of 98 children were examined for changes in bacterial species composition, and all were examined for clinical features of AD. AD developed in 24 children in the control group, and in 10 fed the prebiotic. Significantly higher numbers of bifi-

dobacteria were found in the prebiotic group, compared to controls, but no increases in lactobacilli were observed. While this investigation demonstrated that the prebiotic mix was able to modulate the immune system in young children, a significant limitation of the study is the fact that the bacterial counts were carried out on stools that had been frozen prior to cultural analysis, which has previously been shown to significantly reduce cell viability (Bonten *et al.* 1997).

Lactobacillus rhamnosus has been shown to induce production of Th1-promoting cytokines in mouse dendritic cells (Christensen *et al.* 2002), and in a randomized placebo-controlled trial of children at high risk of allergy, to be useful in primary prevention of AD (Kalliomaki *et al.* 2001b, 2003). In one recent double-blinded, randomized-controlled trial (DBRCT), 1223 pregnant women carrying children at high risk of allergy, were given either a mixture of 4 probiotic bacteria (*L. rhamnosus* GG, *L. rhamnosus* LC705, *Bif. breve* BB99, *Propionibacterium freudenreichii* ssp. *shermanii* JS) twice daily for 2–4 weeks before delivery. The same probiotic mix in combination with 0.8 g GOS, or a placebo, was also fed once daily to the infants from birth to 6 months (Kukkonen *et al.* 2007). The cumulative incidence of allergic disease, immunoglobulin E (IgE) sensitization and levels of faecal bacteria were measured over 2 years. It was found that the synbiotic combination significantly reduced IgE-associated diseases compared to the placebo group, eczema (32–26%) and atopic eczema (18–12%), but no difference occurred in the cumulative incidence of allergic disease. Increased gut colonization by lactobacilli and bifidobacteria were found in the synbiotic group; however, samples of meconium and faeces, as in the earlier study, were frozen before analysis.

Inflammatory bowel disease (IBD)

Ulcerative colitis (UC) and Crohn's disease (CD) are both inflammatory conditions that are thought to result from inappropriate immune responses to the normal commensal gut microbiota (Cummings *et al.* 2003). Reduced numbers of bifidobacteria and increased counts of other organisms such as *E. coli* and peptostreptococci have been found in faecal samples, and on the colonic mucosa in IBD (Favier *et al.* 1997; Cummings *et al.* 2003; Macfarlane *et al.* 2004). Mucosa-associated lymphoid tissue normally maintains gut integrity by tightly regulating immune responses to commensal and pathogenic bacteria and dietary antigens, by a balance of pro-inflammatory and anti-inflammatory cytokines. Normally, tolerance is mediated to the commensal microflora by release of the immunoregulatory cytokines IL-10 and TGF- β (Kelly *et al.* 2005). The response profiles associated with UC and CD are

different in that a Th1 pattern of cytokine formation develops in CD, with increased production of tumour necrosis factor- α (TNF- α), interferon- γ , IL-12 and IL-1 β (Shanahan 2002) whereas UC more resembles a modified Th2 profile, with up-regulation of a different cytokine set, including IL-5 and IL-10 (Farrell and Peppercorn 2002). TNF- α is expressed in the intestinal mucosa of patients with IBD and triggers inflammation via an NF- κ B-dependent signalling cascade. Studies involving the use of prebiotics in human IBD are distinctly lacking; however, inulin and FOS have been shown to ameliorate inflammation in animal models of colitis, and in combination with probiotics in human trials (Cherbut *et al.* 2003; Schultz *et al.* 2004; Furrie *et al.* 2005).

The use of GOS as a therapy for immune modulation in IBD has been tested in one animal study. Holma *et al.* (2002) used the trinitrobenzene sulphonic acid (TNBS) model of colitis, in which rats were fed 4 g kg⁻¹ body mass of either whey or lactose-derived GOS per day, 10 days before the induction of colitis, or dexamethasone at colitis induction, as a control. Faecal bifidobacterial levels, myeloperoxidase damage, and macroscopic damage were assessed 72 h after the induction of colitis, and it was found that while there was an increase in bifidobacterial numbers in the animals, there was no reduction in inflammatory processes.

Other health benefits associated with prebiotics

Neonates and infants

Babies provide a unique opportunity to investigate the effects of prebiotics on the gut microbiota, as they are born with what is an essentially sterile gastrointestinal tract. These infants become progressively colonized over a series of weeks and months by different bacteria, which results in the establishment of an increasingly complex and stable microbiota. For the first 2 years of life, bifidobacteria predominate in the colonic microbiota in breast-fed infants, constituting up to 95% of culturable bacteria (Tissier 1900, 1906; Bullen and Willis 1971). As discussed earlier, naturally occurring oligosaccharides in mother's milk are thought to go some way to explaining the predominance of bifidobacteria in breast-fed babies.

So far, it has only been possible to study faecal bacterial populations in very young children, rather than epithelial communities, because of the invasive nature of obtaining mucosal samples. Consequently, we have an incomplete picture of the gastrointestinal microbiota in these individuals. Nevertheless, many studies have established that prebiotics increase bifidobacterial numbers in infant stools, to levels comparable with breast-fed infants.

Prebiotics have been used in infant formulas in Japan for over 20 years, and have been available in infant formulas in Europe for the last 5 years. Infant and neonate studies are probably the area most extensively researched using DBRCT, 15 of which are listed in Table 4. The majority of these trials have focused on demonstrating the abilities of oligosaccharides to increase faecal bifidobacteria populations. Relatively few studies have been made on disease prevention, or possible treatment benefits of prebiotic use in infants. Atopy is an area that has been looked at in some detail, and the immunological aspects of this have been described in the previous section. There is also an *in vivo* study that investigated the effects of prebiotics on the hypersensitivity response, a DBRCT measuring faecal sIgA and three DBRCT, one using a prebiotic, the other two using synbiotics, looking at prophylactic and therapeutic effects on atopic disease in childhood. Despite these studies, the failure to find a satisfactory mechanism of action means that more investigations will be needed before prebiotics can begin to play a role in the routine management of atopic disease.

Effects of prebiotics on bone mineralization

Adult bone mass is dependent on both the adequate supply and bioavailability of calcium. A deficit in either can result in osteoporosis, which has high morbidity associated with increasing age and postmenopausal conditions (Melton *et al.* 1993; Snelling *et al.* 2001). Currently, treatment and prevention of osteoporosis is limited to increasing calcium uptake, or by stimulating bone formation and reducing bone resorption.

Although the majority of studies on calcium metabolism have so far been performed on rats (Table 5), experimental evidence indicates that prebiotics have a role to play in increasing bioavailability of this mineral, but as yet, these results have been translated into only a few human trials, many of which are powered by small numbers. The human trials have had mixed results, with the majority supporting data from the rat studies suggesting that prebiotic consumption increases calcium absorption. Only one investigation has taken this a step further and examined bone mineral density and content (Abrahms *et al.* 2005). This investigation needs to be repeated, particularly in subject groups at risk of osteoporosis, such as elderly women, postmenopausal women, chronic liver disease patients or IBD patients. GOS has only been used in the smaller human trials, with mixed effects, and they have not been adequately powered for any definitive conclusions to be drawn. In contrast, FOS has been used more widely in powered trials with more definitive results. In summary, therefore, results obtained to date with prebiotics in relation to osteoporosis offer promise

Table 4 Infant studies with prebiotics and synbiotics*

Type	Dose	Subjects	No	Length of study	Objectives/methods	Result	Statistical significance	Reference
<i>In vitro</i>	4 g l ⁻¹ inulin	<i>In vitro</i>	–	–	Study Ca availability from inulin supplemented infant formula compared to standard formula	Increased Ca, Fe, Zn availability	–	Bosscher <i>et al.</i> (2003)
<i>In vivo</i>		Murine	–	–	Analysis of prebiotic properties in relation to modulation of vaccine-specific delayed hypersensitivity responses as a marker of Th1 immunity	Prebiotic group increased proportion of bifidobacteria and lactobacilli. Prebiotic group enhanced delayed-type hypersensitivity response in dose dependent manner	$P < 0.01$ $P < 0.05$	Vos <i>et al.</i> (2006)
Double-blinded, randomized-controlled trial (DBRCT)	10 g l ⁻¹ , 0.8 g dl ⁻¹	Preterm 31 weeks	27 infants	28 days	Study effects of prebiotic on faecal bifidobacteria	Increase in faecal bifidobacteria excretion	$P = 0.0008$	Boehm <i>et al.</i> (2002, 2004)
DBRCT		Preterm 31 weeks	30 infants	28 days	Spot plasma and urinary calcium and phosphorus measurements measured from placebo and prebiotic supplemented group	Trend towards similar urinary Ca/P ratio so prebiotic may improve Ca absorption. Increased bifidobacteria and lactobacilli populations. Similar stool consistency and frequency between prebiotic group and breast-fed group		Marini <i>et al.</i> (2003)
DBRCT	0.4 g dl ⁻¹ 0.8 g dl ⁻¹	Term	90	28 days	Study effect of different doses of prebiotic vs placebo on faecal bifidobacteria and lactobacilli populations and stool pH, consistency and frequency	Faecal bifidobacteria increased in dose dependent manner. Increased numbers of lactobacilli – not dose dependent. Reduced stool pH. Increased stool consistency and frequency. No change in crying, regurgitation or vomiting	$P < 0.001$ $P < 0.001$	Moro <i>et al.</i> (2002, 2003)
DBRCT		Term	150	10 weeks	Compare faecal bifidobacteria, stools, serum amino acids and biochemistry of prebiotic and standard formula babies	Prebiotic group had increased faecal bifidobacteria. Softer stools.No difference in growth, serum biochemistry or amino acid profiles		Schmelze <i>et al.</i> (2003)

Table 4 Continued*

Type	Dose	Subjects	No	Length of study	Objectives/methods	Result	Statistical significance	Reference
DBRCT	0.8 g dl ⁻¹	Term	259	6 months	Randomize infants at risk of atopy to prebiotic or standard formula	Faecal flora dominated by bifidobacteria. Reduced incidence of atopic dermatitis (AD) in first 6 months	$P = 0.014$ 95% CI 5.4–17.1	Moro et al. (2006)
DBRCT	0.6 g dl ⁻¹	Term	57	4 months	Study faecal bifidobacteria and faecal secretory immunoglobulin A (IgA) in three different groups, prebiotic, probiotic and standard formula	No difference in faecal bifidobacteria. Increased faecal acetate ratio in probiotic group. Increased faecal sIgA in prebiotic group. Reduced stool pH in prebiotic group	$P < 0.05$ $P < 0.05$ $P < 0.05$	Bakker-Zierikzee et al. (2005)
DBRCT	0.8 g dl ⁻¹	Term		6 weeks	Study faecal bifidobacteria, stool pH, stool acetate levels and short-chain fatty acid	After 6 weeks, proportion of bifidobacteria in prebiotic group significantly higher. Infants in prebiotic group had lower pH, an increased proportion of acetate and decreased proportion of propionate	$P < 0.05$ $P < 0.001$	Knol et al. (2005)
DBRCT	0.4 g dl ⁻¹ <i>Bifidobacterium longum</i> 999	Term	138	7 months	Study effects of synbiotic on weight gain, gastrointestinal symptoms, growth and morbidity	Mean weight gain was equivalent in both groups. No statistical difference in head circumference, recumbent length or incidence of adverse effects. Infants in synbiotic group had fewer incidences of constipation and a trend toward fewer respiratory tract infections		Puccio et al. (2007)
DBRCT	0.45 g dl ⁻¹	Infants 1–2 years	140	21 days	Study effect of prebiotic on bifidobacteria populations in children treated with amoxicillin for bronchitis	Amoxicillin significantly reduced bifidobacterial populations and increased <i>Escherichia coli</i> . Prebiotic significantly increased bifidobacterial numbers. No GI's in either group		Brunser et al. (2006)

Table 4 Continued*

Type	Dose	Subjects	No	Length of study	Objectives/methods	Result	Statistical significance	Reference
DBRCT	4.5 g day ⁻¹	4–6 months	35	6 weeks	Testing the effect of prebiotic supplemented solid food on fully bottle fed infants during the weaning period	Percentage of faecal bifidobacteria significantly increased in prebiotic group. No significant change to control group and significant difference between control and prebiotic groups	$P = 0.031$ $P = 0.387$ $P = 0.026$	Scholten <i>et al.</i> (2006)
DBRCT	Oligofructose supplemented cereal	7–9 months	50	28 days	Cereal given prior to measles vaccine	Significant rise in postvaccine anti-measles immunoglobulin G in treatment group. No difference to GI tolerance of vaccine		Firmansyah <i>et al.</i> (2001)
DBRCT	0.55 g 15 g ⁻¹ cereal	6–36 months	282	14 days	Study effect of prebiotic and zinc effects on prevalence of diarrhoea in Peru	No effect on prevalence of diarrhoea	$P = 0.66$	Duggan <i>et al.</i> (2003)
DBRCT	Galacto-oligosaccharides (GOS) 2.5 g 100 g ⁻¹ , plus <i>Bif. lactis</i>	1–3 years	634	12 months	To look at the prophylactic effect of this synbiotic on childhood diarrhoea	Significant reduction in incidence of dysentery in synbiotic group. Significant reduction in prevalence of dysentery in synbiotic group. Nonsignificant 10% reduction in all diarrhoea events. Significant reduction in prevalence of severe illness days, fever and ear infections. 35% reduction in proportion of Fe deficient children. Significantly better growth and higher weight gain	OR 0.78 95% CI 0.61–1.00 OR 0.85 95% CI 0.71–1.01 OR 0.84, OR 0.68. OR 0.93 $P = 0.03$ $P = 0.02$	Sazawal <i>et al.</i> (2004)
DBRCT	Prebiotic +/- <i>Lactobacillus rhamnosus</i>	>2 years	48	3 months	Study effects of prebiotic and synbiotic on children with AD	Both groups had significantly reduced AD scores, with no statistical difference between the two groups	$P < 0.0001$ $P < 0.0001$ $P = 0.535$	Passeron <i>et al.</i> (2006)

Table 4 Continued*

Type	Dose	Subjects	No	Length of study	Objectives/methods	Result	Statistical significance	Reference
DBRCT	GOS plus probiotic combination: <i>L. rhamnosus</i> , <i>Bif. breve</i> , <i>Propionibacterium freudenreichii</i> ssp. <i>shermanii</i>	4 weeks prior to delivery until age 6 months	925	6 months	Randomized high risk women for atopic babies to trial and looked at incidence of atopy, skin prick antigen immunoglobulin E and faecal bacteria	Reduced incidence of IgE-associated diseases Probiotic and GOS reduced eczema. No reduction in cumulative incidence of allergic disease. Probiotic and GOS reduced atopic eczema. Lactobacilli and bifidobacteria colonized gut	$P = 0.52$ CI 0.50–1.00 $P = 0.035$ CI 0.55–0.98 $P = 0.025$ CI 0.46–0.95 $P < 0.001$	Kukkonen <i>et al.</i> (2007)

*All prebiotics were a combination of inulin (10%) and GOS (90%), unless otherwise stated.

for a new, inexpensive and safe way of preventing this disease.

Cardiovascular effects of prebiotics

Obesity and diet have long been linked to atherosclerosis, cardiovascular disease and type 2 diabetes (Wong *et al.* 1989; Singh *et al.* 1992), and there have been several animal experiments indicating the potential of prebiotics to positively influence serum lipid levels (Fiordaliso *et al.* 1995; Rault-Nania *et al.* 2006). On the whole, there have been mixed results in human trials (Table 6). Six of these studies have focused on normolipidaemic patients, two on type 2 diabetics and only 1 on 12 patients with hyperlipidaemia, but again, the majority of these investigations are underpowered. Only a few of the studies looked at blood glucose changes, and only the oldest trial in the table, by Yamashita *et al.* (1984), has demonstrated a reduction in fasting glucose with FOS. None of the other investigations replicated that result. However, one of the larger studies, involving 54 subjects, demonstrated reduced insulin concentrations with inulin, and so the possible role of oligosaccharides in glucose metabolism in humans certainly warrants further investigation.

The results of total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and serum triacylglycerol levels from all of the prebiotic trials make it difficult to draw firm conclusions. Some studies showed no differences at all, one demonstrated significant reductions in LDL, while a few indicated reductions in total cholesterol and triacylglycerol concentrations. Nonetheless, despite these confusing results it is possible, that with further investigation, prebiotics may be found to be involved in control of lipid metabolism in the future. What that function is, and whether we should focus on targeting specific groups of patients needs to be determined. Once again, GOS has been under-investigated in this area, and only one trial has used it as a prebiotic in 12 normolipidaemic subjects. The study failed to show any significant changes in either blood lipids or glucose absorption.

Gastroenterological effects of prebiotics

Prebiotics such as lactulose, FOS and GOS have laxative effects (Roberfroid 1993), with lactulose in particular being well established as a treatment for constipation (Petticrew *et al.* 1997; de Schryver *et al.* 2005). Table 7 shows some of the available data from prebiotic studies. Prebiotic trials in the elderly have been more specifically designed to assess the effects of prebiotics on constipation, although the methods of measurement can be crude, such as frequency of defecation rather than mean daily stool weight, whereas trials in younger people seem to

Table 5 Mineralization studies involving prebiotics

Prebiotic	Dose per 100 g	Study type	Objectives/methods	Result	Reference
Inulin and fructo-oligosaccharide (FOS)	Unknown	Model – large intestine	Investigate effects of prebiotic on Ca and Mg absorption	Increased Ca and Mg absorption in large intestine. Increased Ca uptake into bone and improved mineral density	Franck (2000)
Galacto-oligosaccharides (GOS)	5 g	Rats	Study Ca absorption in low and normal dietary levels with GOS	Ca absorption stimulated in rats by GOS if dietary concentrations normal and not at low concentrations	Chonan and Watanuki (1995, 1996)
GOS	5 g	Rats	Assess effects of GOS on ovariectomized rats	Stimulated Ca absorption.	Chonan (1995)
GOS	5 g	Rats	Study Ca and Mg absorption in rats of a prebiotic diet and the effects of neomycin	Reduced loss of bone mass and calcium content Rats on the GOS diet absorbed Ca and Mg more efficiently, but the increment was reduced by neomycin sulphate	Chonan <i>et al.</i> (2001)
FOS and inulin	10 g	Rats	Investigate effects of prebiotic on Ca and Mg absorption	Increased Ca and Mg absorption	Delzenne <i>et al.</i> (1995)
FOS	5 g	Rats	Study effects of prebiotic on Ca absorption	Increased Ca absorption	Brommage <i>et al.</i> (1993)
FOS	2.5 g, 5 g	Rats	Assess effects of prebiotic on Ca and Mg absorption in ovariectomized rats	Increased Ca and Mg absorption	Taguchi <i>et al.</i> (1995)
FOS	2.5 g, 5 g	Rats	Study effects of different doses of prebiotic on Ca absorption and bone mineralization in ovariectomized rats	Dose-related increase in Ca absorption and bone mineralization of the femur and lumbar vertebrae	Scholz-Ahrens <i>et al.</i> (1998)
Isoflavones with or without FOS		Rats	Isoflavones in various concentrations were administered to ovariectomized rats. Half of each group received FOS. Femoral mineral bone density was measured	Isoflavones exhibited a bone-sparing effect at 20 µg g ⁻¹ day ⁻¹ . FOS significantly increased the efficacy of isoflavone protective effects on femoral bone mineral density	Matthey <i>et al.</i> (2004)
GOS inulin FOS	15 g	Humans (n = 12)	Study of Ca and Fe absorption in healthy young male subjects using different types of prebiotic. Using 24 h urine collections for Ca isotopes	No change in Ca or Fe absorption*	van den Heuvel <i>et al.</i> (1998)
GOS	20 g	Humans (n = 12)	Study of GOS effects on Ca absorption in menopausal women, using urine collections for administered Ca isotopes	Increased Ca absorption in menopausal women. P = 0.04	van den Heuvel <i>et al.</i> (2000)
FOS	15 g	Humans (n = 7)	Study for changes in mineral excretion in ileostomy effluent	No change in mineral excretion (cholesterol, Ca, Mg, Zn, Fe or bile acid)†	Ellegard <i>et al.</i> (1997)
FOS inulin	8 g	Humans (n = 59)	Crossover, randomized study of prebiotic effect on Ca absorption in girls near menarche, using dual stable isotope technique	A statistically significant trend of increased Ca absorption. P = 0.01	Griffin <i>et al.</i> (2002)

Table 5 Continued

Prebiotic	Dose per 100 g	Study type	Objectives/methods	Result	Reference
Inulin-type fructan	8 g	Humans (n = 100)	Double-blind randomized controlled trial for young adolescents to receive prebiotic for 1 year and to measure their Ca absorption, bone mineral content and density	Calcium absorption was significantly higher in the prebiotic group at 8 weeks $P < 0.001$ and at 1 year $P = 0.04$. At 1 year the prebiotic group had a greater increment in bone mineral content $P = 0.03$ and bone mineral density, $P = 0.01$	Abrahms <i>et al.</i> (2005)

*Measured using 24 h urine collection for content of Ca isotopes; may not have been for long enough.

†Model studies suggest increased mineral absorption occurs in short-chain fatty acid rich colonic environment, so unlikely to see altered absorption in this study.

suggest that prebiotics have only a mild laxative effect at best. One of the clearest demonstrations of the laxative effects of prebiotics was the controlled feeding study of Gibson *et al.* (1995), where it was shown in a group of eight volunteers that 15 g of FOS per day significantly increased stool output from 136 to 154 g day⁻¹, and in a smaller group of subjects ($n = 4$), 15 g of inulin was also found to be laxative, increasing faecal output from 92 g day⁻¹ in controls, to 123 g in the test group. However, three other feeding studies did not show an increase in faecal excretion (Ito *et al.* 1990; Alles *et al.* 1996; Bouhnik *et al.* 1997), but diet was not controlled in these investigations, which would make small changes difficult to detect. Moreover, in the investigation reported by Alles *et al.* (1996), the volunteers started with unusually high faecal weights on the control diet (272 ± 26 g day⁻¹). Bouhnik *et al.* (1997) fed 10 g of TOS per day for 3 weeks to healthy subjects without any significant effects on bowel habit, while Ito *et al.* (1990), who fed 4.8–19.2 g oligomate daily to 12 volunteers, were also unable to demonstrate a change in bowel habit, despite the subjects noting an increase in abdominal symptoms. In three other investigations, which only provided qualitative data, FOS and inulin were reported to improve constipation in small groups of hospitalized patients (Sanno 1986; Hidaka and Hirayama 1991; Kleessen *et al.* 1997).

As far as can be ascertained, there have been no full trials published using prebiotics alone in patients with irritable syndrome (IBS), while the probiotic studies that have been carried out have had mixed results. In a DBRCT using 'Prescript-Assist' (a combination of probiotic and prebiotic components containing a proprietary blend of bacteria, fungi and Leonardite), IBS patients reported significant reductions in general feelings of ill health and nausea ($P = 0.042$), indigestion and flatulence ($P = 0.008$), and diarrhoea ($P = 0.003$) after 14 days (Bittner *et al.* 2005). Currently, the diagnosis of IBS covers a broad spectrum of pathophysiologies and it is possible that there are subsets of IBS patients who may benefit from prebiotics, but as many of these individuals do not tolerate gas in the gut, or have greatly increased gas production, the side effects of prebiotic fermentation may worsen their symptoms (King *et al.* 1998; Cummings *et al.* 2001; Serra *et al.* 2001).

Trials in infectious diarrhoea (Table 8) have been essentially limited to FOS, and have been made almost entirely in animals, with the exception of one promising study reported by Cummings *et al.* (2001) involving 244 people at increased risk of acquiring traveller's diarrhoea. This investigation showed that travellers who received 10 g of FOS per day had a reduced incidence of diarrhoeal events (11.2%) compared with the placebo group (19.5%), although the reduction was not significant

Table 6 Prebiotic effects on lipid metabolism

Trial	Subjects	Prebiotic	Dose per day	No	Objectives/methods	Results	References
<i>In vivo</i>	Rats	Fructo-oligosaccharide (FOS)	10 g	–	Faecal and serum samples used for lipid analysis over 16 weeks	No differences in weight gain ($P > 0.05$). Significant reductions in serum triglycerides ($P < 0.05$) after 1 week that lasted up to 16 weeks. Reductions in serum phospholipids and total cholesterol, reduction in VLDL (very low-density lipoprotein) only, no change in low-density lipoprotein (LDL) or high-density lipoprotein (HDL)	Fiordaliso <i>et al.</i> (1995)
<i>In vivo</i>	Rats	FOS	100 g kg ⁻¹	–	Investigated FOS feeding on blood and direct hepatic metabolism of lipids using enzyme assays	Significant reductions in triacylglycerol and phospholipids in liver and blood ($P < 0.05$, $P < 0.01$). Decrease in acylglycerol synthesis. No effects on blood nonesterified fatty acid concentrations. Reduced capacity of isolated hepatocytes to secrete triacylglycerols	Kok <i>et al.</i> (1996)
<i>In vivo</i>	Murine	Inulin, FOS, FOS enriched inulin	10 g 100 g ⁻¹	32	Investigated the effect of different fructans on atherosclerotic plaques in apo E-deficient mice	Inulin reduced plasma cholesterol ($P < 0.001$). Inulin and FOS-enriched-inulin reduced hepatic cholesterol ($P < 0.05$). Triacylglycerol concentrations reduced in all 3 groups ($P < 0.0001$)	Rault-Nania <i>et al.</i> (2006)
Double-blind randomized controlled trial (DBRCT) latin square	Human normo-lipidaemic	Inulin FOS, galacto-oligosaccharides (GOS), controls	15 g	12	Patients had 4 treatment periods of 3 weeks for each substance and stool, lipids and glucose absorption were analysed	Higher concentrations of faecal acetate in inulin and GOS treatment periods ($P < 0.05$). Lowered β -glucuronidase activity in inulin and GOS periods ($P < 0.05$ and $P < 0.02$). No significant changes in blood lipids or glucose absorption	van Dokkum <i>et al.</i> (1999)

Table 6 Continued

Trial	Subjects	Prebiotic	Dose per day	No	Objectives/methods	Results	References
DBRCT crossover	Human normo-lipidaemic	Inulin	14 g	64	Investigation of fasting blood lipids in healthy young women	No significant differences between total cholesterol, HDL, LDL and triacylglycerol concentrations. Flatulence and GI symptoms associated with inulin period ($P < 0.05$)	Pedersen <i>et al.</i> (1997)
SBRCT crossover	Human type 2 diabetes	FOS	15 g	20	Investigate effect of FOS (20 days) in diabetics on cholesterol and blood glucose	No changes in blood glucose. No effects on total, HDL, LDL cholesterol or serum triacylglycerol	Alles <i>et al.</i> (1999b)
DBRCT	Human normo-lipidaemic	Inulin	10 g	54	8 weeks of inulin or placebo in healthy middle aged volunteers using fasting blood samples to analyse lipids and glucose	Insulin concentrations significantly lower in inulin group ($P < 0.01$). Trends for lower triacylglycerol levels in inulin group ($P < 0.08$), returned to baseline 4 weeks after end of study	Jackson <i>et al.</i> (1999)
Prospective	Human normo-lipidaemic	Inulin	22–34 g	11	64 days with prebiotic replacing some fat in diet using blood and stool samples	Increased bifidobacteria concentrations in faeces. No changes in blood lipids and short-chain fatty acid	Kruse <i>et al.</i> (1999)
DBRCT crossover	Human normo-lipidaemic	FOS	2.0 g	12	4 weeks on FOS-enriched biscuits	No changes in blood lipids or glucose	Luo <i>et al.</i> (1996)
DBRCT crossover	Human hyperlipidaemic	Inulin	18 g	21	6 weeks on inulin diet and 6 weeks on standard diet	Reductions in LDL ($P < 0.05$). Mild gastrointestinal discomfort	Davidson <i>et al.</i> (1998)
DBRCT	Human type 2 diabetes	FOS	8 g	28	14 days of FOS or placebo in diabetic patients and monitoring serum cholesterol	Significantly reduced fasting glucose in FOS group ($P < 0.05$). Significant decreases in total cholesterol in FOS group, and the reduction was greater in hypercholesterolaemic patients ($P < 0.01$). No change in LDL or HDL	Yamashita <i>et al.</i> (1984)

Table 6 Continued

Trial	Subjects	Prebiotic	Dose per day	No	Objectives/methods	Results	References
DBRCT crossover	Human hyperlipidaemic	Inulin	20 g	12	21 days on each diet of ice cream supplemented with or without inulin	Significant reduction in serum triglycerides ($P = 0.05$). Trends towards reduction in cholesterol	Causey <i>et al.</i> (2000)
SB sequential trial	Normo-lipidaemic	Inulin and FOS	9 g	12	4 weeks on placebo then prebiotic cereal diet	Significant reductions in total cholesterol ($P < 0.05$). Significant reductions in triacylglycerol ($P < 0.05$). Increased bifidobacteria ($P < 0.05$). No changes in glucose tolerance or body weight	Brighenti <i>et al.</i> (1999)

($P = 0.08$). However, the authors noted that many gut infections occur in the small bowel, where prebiotic effects might be reduced, while some forms of traveller's diarrhoea do not have a microbial aetiology, and can be caused by travel, stress and changes in diet. Clearly this area of prebiotic research has been seriously under-investigated, but in view of the anti-adhesive properties of some oligosaccharides discussed earlier, it potentially has promise to lead to the development of prophylactic strategies for the prevention of infection.

Table 8 also shows that few studies have been made on prebiotics and antibiotic-associated diarrhoea (AAD). In the investigation by Brunser *et al.* (2006), 140 children who had received amoxicillin for bronchitis had significantly reduced faecal bifidobacteria concentrations and increased numbers of *E. coli*. None of the children had any gastrointestinal side effects so it is not possible to say whether treatment with GOS or FOS actually altered the outcome of AAD. In the other two large studies, by the same group, the authors focused specifically on *C. difficile*, in the first instance with the aim of preventing the incidence of infection, and in the second, in preventing relapse (Lewis *et al.* 2005a,b). Faecal bifidobacteria populations were increased, but there was no reduction in the incidence of *C. difficile*. However, there was a significant reduction in the rate of relapse in the prebiotic group ($P < 0.001$). It might be worth exploring this further in a large trial using GOS as the prebiotic, but as many of these large trials have failed to demonstrate any consistent benefits, it seems more likely that the next avenue for research in this area is to see what effects synbiotics have in preventing or treating AAD.

Human IBD research involving functional foods has mainly centred on the effects of probiotics and synbiotics. As mentioned earlier, IBD involves dysbiosis in the microbiota, particularly evidenced by lower levels of bifidobacteria and increased concentrations of potentially pathogenic enterococci, enterobacteria and other Gram-positive cocci (Favier *et al.* 1997; Cummings *et al.* 2003; Macfarlane *et al.* 2004). Other putative mechanisms of pathogenesis include genetic predisposition, dysregulation of the inflammatory response leading to a cell-mediated cytotoxic cascade (Blumberg *et al.* 1999; Romagnani 1999). Promising results have been obtained with probiotics and synbiotics in reducing inflammation and down-regulating the inflammatory response, and this has led people to consider the use of prebiotics alone, which would be appealing to patients and financially cost. Table 9 shows investigations that have been carried out using different types of prebiotics, with and without probiotics. In almost all of these studies, a significant reduction in inflammation was demonstrated. There are only four human studies though that were adequately

Table 7 Effect of prebiotics on constipation

Substrate	Dose	No	Methods	Results	Significance	Reference
Galacto-oligosaccharides (GOS)	9 g	14	Double-blind randomized controlled trial (DBRCT) – crossover using GOS containing yoghurt or control yoghurt, 2 weeks on each type in chronically constipated elderly women	Defecation frequency per week was higher during GOS period, mean 7.1 v 5.9.	$P = 0.07$	Teuri <i>et al.</i> (1998)
GOS	4.8 g, 9.6 g, 19.2 g	36	Mean daily stool weight measured	No statistical difference on use of laxatives and consistency of faeces. Statistically easier to defecate		
				When data normalized to per gram of prebiotic ingested no significant difference		Ito <i>et al.</i> (1990)
Transgalactosylated-oligosaccharides	10 g	8	Mean daily stool weight measured	No significant change		Bouhnik <i>et al.</i> (1997)
Inulin, fructo-oligosaccharide (FOS), GOS	15 g	36	Mean daily stool weight measured. Looking at effect on glucose and lipid metabolism	No significant change		van Dokkum <i>et al.</i> (1999)
FOS	15 g	24	Mean daily stool weight measured	No significant change		Alles <i>et al.</i> (1996)
	5 g					
FOS, lactose	20–40 g	25	Lactose or inulin randomized to elderly patients suffering from constipation and dose gradually increased from 20 g to 40 g. Study lasted 19 days	FOS increase bifidobacteria and reduced enterococci and enterobacteria significantly.	$P < 0.01$ $P < 0.001$ $P < 0.05$	Kleessen <i>et al.</i> (1997)
				Lactose patients had rises in enterococci and reductions in lactobacilli and clostridia.		
FOS, inulin	15 g	8	Mean daily stool weight measured	Inulin had better laxative effect, but no significance data given		
	5 g	4		Significant increase 1.3 g g^{-1} in FOS group	$P < 0.05$	Gibson <i>et al.</i> (1995)
Inulin	31 g	9	Mean daily stool weight measured	No significant change in inulin group		
				Significant increase 2.4 g g^{-1} .	$P < 0.05$	Castiglia-Delavaud <i>et al.</i> (1998)
Lactitol, guar gum, wheat bran	150 ml	33	DBRCT of elderly hospitalized patients, measuring mean faecal output over 2 weeks	Significant increase in microbial mass	$P < 0.01$	
				Mean faecal output increased in patients receiving the fiber yoghurt 1.6-fold.	$P < 0.05$	Rajala <i>et al.</i> (1998)
				No significant change in blood glucose, serum cholesterol or triglyceride, body weight or faecal pH		
Isomalt	30 g	19	Mean daily stool weight measured	No significant change		Gostner <i>et al.</i> (2006)

Table 8 Trials using prebiotics to prevent or treat diarrhoeal disease

Trial	Subjects	Substrate	Dose	No	Methods	Results	Significance	Reference
Infectious diarrhoea								
–	Murine	Fructo-oligosaccharide (FOS), inulin	100 g kg ⁻¹	75	Mice challenged with a colorectal promoter and an enteric pathogen (<i>Candida albicans</i> , <i>Listeria monocytogenes</i> or <i>Salmonella Typhimurium</i>)	Fructan diet mice had 50% less <i>C. albicans</i> . Systemic infection with <i>L. monocytogenes</i> and then with <i>S. Typhimurium</i> killed more control mice than inulin mice, with FOS mice having intermediate death rates	(<i>P</i> < 0.05) (<i>P</i> < 0.05)	Buddington <i>et al.</i> (2002)
–	Rats	FOS	3 g 100 g ⁻¹ , 6 g 100 g ⁻¹	23	Male Wistar rats fed diet for 2 weeks then infected with <i>Salmonella enteritidis</i>	FOS dose dependently increased caecal contents and mucosal <i>Salmonella</i> . FOS caused increase in infection-induced diarrhoea		Ten Bruggencate <i>et al.</i> (2003)
–	Pigs	FOS			Diarrhoea induced with cholera toxin and pigs randomized to oral electrolyte solution with or without FOS. Bacteria and diarrhoea monitored	Increased lactobacilli in FOS group. Enterobacteria higher in placebo group		Oli <i>et al.</i> (1998)
Double-blind randomized controlled trial (DBRCT)								
	Human	FOS	10 g	244	All travellers going to medium or high risk destinations and randomized for 2 weeks prior to departure and their 2-week holiday. Assessed by questionnaire and bowel habit diary	Prevalence of diarrhoea less in FOS group 11.2% vs 19.6%. No significant difference between bowel frequency or consistency. FOS group experienced less severe diarrhoea	(<i>P</i> = 0.08)	Cummings <i>et al.</i> 2001
Antibiotic-associated diarrhoea DBRCT								
	Human	Oligofructose	12 g	435	In-patients over 65 on broad spectrum antibiotics were randomized to sucrose (placebo) or oligofructose within 24 h of receiving antibiotics and took them for duration of course and 7 days after. Patients were followed-up for 7 more days	Bifidobacteria concentrations increased in prebiotic group. No difference in incidence of <i>Clostridium difficile</i>	(<i>P</i> < 0.001) CI 0.69–1.72	Lewis <i>et al.</i> (2005b)
DBRCT								
	Human	Oligofructose	12 g	142	In-patients with <i>C. difficile</i> randomized to oligofructose or placebo in addition to antibiotic treatment for 30 days	Bifidobacteria increased in prebiotic group. Relapse of <i>C. difficile</i> more common in placebo group	(<i>P</i> < 0.001) CI 0.45–0.94 (<i>P</i> < 0.001)	Lewis <i>et al.</i> (2005a)
DBRCT								
	Human	Galacto-oligosaccharides and FOS	0.45 g dl ⁻¹	140	Study effect of prebiotic on bifidobacteria populations in children treated with amoxicillin for bronchitis	Amoxicillin significantly reduced bifidobacteria populations and increased <i>Escherichia coli</i> . Significant increase in bifidobacteria populations. No GI side effects in either group		Brunser <i>et al.</i> (2006)

Table 9 Studies and trials using prebiotics in inflammatory bowel disease

Trial	Subjects	Substrate	Dose	Methods	Results	Reference
–	Rats	Galacto-oligosaccharides (GOS), whey	4 g kg ⁻¹	Rats given placebo or GOS diet for days before colitis induced. Assessed 72 h later	GOS increased levels of bifidobacteria No reduction in inflammation severity	Holma <i>et al.</i> (2002)
–	Rats	Goat's milk oligosaccharides	5% of diet	DSS (dextran sodium sulphide)-induced colitis in 20 rats. Clinical and inflammatory markers monitored	Oligosaccharide fed rats did not lose weight ($P < 0.05$). Inflammation and clinical symptoms less severe in oligosaccharide fed rats ($P < 0.05$)	Lara-Villoslada <i>et al.</i> (2006)
–	Rats	Lactulose	2.5% (w/v)	Trinitrobenzene sulphonic acid (TNBS) induced colitis treated for 14 days	Lactulose significantly reduced myeloperoxidase, colonic tumour necrosis factor- α (TNF- α), and leukotriene B ₄ production Increased levels of lactobacilli and bifidobacteria	Camuesco <i>et al.</i> (2005)
–	Rats	Lactulose, 5-ASA	300–1000 mg kg ⁻¹	DSS-induced colitis in Wistar rats given lactulose or 5-ASA for 6 days	Lactulose ameliorated colitis in dose-dependent manner. 5-ASA reduced the severity of colonic lesions	Rumi <i>et al.</i> (2004)
–	Rats	Inulin	400 mg day ⁻¹	DSS-induced colitis in 60 rats	Inulin prevented inflammation ($P < 0.05$) and resulted in reduced release of mediators ($P < 0.05$), and lower tissue myeloperoxidase ($P < 0.05$)	Videla <i>et al.</i> 2001
–	Rats	Synergy I (inulin/oligofructose)	Unknown	HLA-B27 transgenic rats given oral prebiotic prior to clinical detection of colitis and assessed at 7 weeks	Prebiotic treatment significantly reduced gross caecal scores and inflammatory histological scores. Prebiotic treatment reduced caecal IL-1 β , but increased caecal TGF- β . Prebiotic significantly increased caecal lactobacillus and <i>Bifidobacterium</i> populations and reduced tissue proinflammatory cytokines	Hoentjen <i>et al.</i> (2005)
–	Rats	Fructo-oligosaccharide (FOS), resistant starch	60 g	DSS-induced colitis in 72 Sprague-Dawley rats that were fed basal diet, FOS or resistant starch	No change in body weight. FOS and resistant starch diet significantly reduced histological score $P < 0.05$ in caecum and resistant starch reduced score in colon ($P < 0.05$)	Moreau <i>et al.</i> (2003)
–	Rats	FOS, lactic acid bacteria	1 g day ⁻¹ , 10 ¹¹ CFU	TNBS-induced colitis in 82 rats that were given intragastric infusions of either prebiotic or probiotic for 14 days	FOS reduced the gross score of inflammation ($P < 0.001$), myeloperoxidase activity ($P < 0.001$), pH ($P < 0.001$) and increased lactate ($P = 0.02$) and butyrate concentrations ($P < 0.001$). LAB had similar effects	Cherbut <i>et al.</i> (2003)

Table 9 Continued

Trial	Subjects	Substrate	Dose	Methods	Results	Reference
–	Rats	Synbiotic comprising inulin, lactobacilli and bifidobacteria (SIM)	Unknown	HLA-B27 transgenic rats with colitis randomized to synbiotic SIM or placebo	Histological improvement in inflammation of SIM rats at 4 months ($P < 0.03$). SIM increased biodiversity especially <i>Bifidobacterium animalis</i> . No detection of any of the added probiotic	Schultz <i>et al.</i> (2004)
Double-blind randomized controlled trial (DBRCT)	Human	Inulin	24 g	Ileal pouch-anal anastomosis patients (20) with inflamed pouches were randomized for 3 weeks and assessed endoscopically, clinically and histologically	Ileal pouch-anal anastomosis patients (20) with inflamed pouches were randomized for 3 weeks and assessed endoscopically, clinically and histologically. Inulin group had lowered pH, increased butyrate, reduced <i>Bacteroides fragilis</i> . Endoscopically and histologically there was a reduction in inflammation	Welters <i>et al.</i> 2002
Open label/crossover	Human	FOS + inulin	Unknown	Children (10) with active Crohn's disease (CD) were randomized to the NG prebiotic supplement or placebo for 3 weeks, then changing over	Weight gain in prebiotic group ($P < 0.0001$). Reduced paediatric CDAI scores in prebiotic group ($P < 0.0001$). Reduced erythrocyte sedimentation rates in prebiotic group ($P < 0.01$) Harvey Bradshaw index significantly reduced ($P < 0.01$)	Hussey <i>et al.</i> (2003)
Open label	Human	FOS	15 g	Patients (10) with active CD all received FOS and disease activity was measured	Significant increase in bifidobacteria concentrations ($P < 0.001$). Percentage of IL-10 +ve dendritic cells increased ($P = 0.06$). Percentage dendritic cells expressing TLR2 and TLR4 increased ($P = 0.08$ and $P < 0.001$)	Lindsay <i>et al.</i> (2006)
DBRCT	Human	Synbiotic comprising <i>Bif. longum</i> and Synergy I	12 g	Ulcerative colitis patients (18) with active disease were randomized to placebo or synbiotic	Sigmoidoscopy score reduced in synbiotic group ($P = 0.06$). Human beta defensins significantly reduced in synbiotic group ($P = 0.016$). TNF- α and IL- α significantly reduced in synbiotic group ($P = 0.018$)	Furrie <i>et al.</i> (2005)

powered. Welters *et al.* (2002) demonstrated an improvement in pouchitis, using inulin over a relatively short experimental period. In two small investigations looking at children and adults, significant improvements were seen in clinical scores, while in the pilot study reported by Furrie *et al.* (2005), synbiotic feeding significant improvements were found in sigmoidoscopy scores and in specific immunological markers such as inducible human beta defensins, TNF- α and IL-1 α . Following on from this promising start, what are now needed are randomized, placebo-controlled intervention trials that are adequately powered.

Rheumatology

The obvious physiological links indicating the potential usefulness of prebiotics in rheumatology are those between arthritic symptoms and IBD, and the sharing of some inflammatory cytokines in both conditions (Gross *et al.* 1992; Feldmann *et al.* 1996; Woywodt *et al.* 1999). Furthermore, the success of sulfasalazine preparations and immune modulation using chimeric monoclonal antibodies in the treatment of rheumatoid arthritis and IBD to reduce levels of TNF- α and other pro-inflammatory cytokines, and disrupt inflammatory networks (Smolen *et al.* 1999; Bathon *et al.* 2000; Travis *et al.* 2006), is also indicative of a linkage between these conditions.

Experimental data purely assessing the benefits of prebiotics in rheumatoid disease are few and far between, which makes this a massive area for potential research. Animal models of colitis using HLA-B27 transgenic rats, treated with prebiotics, have been able to demonstrate reduced colitis and arthritis (Hoentjen *et al.* 2005; Lories 2006). Arthritis is a common feature of HLA-B27 rats (Hammer *et al.* 1990), in which the normal gut microbiota is thought to be involved, because like colitis, it does not occur in germ-free animals (Taurog *et al.* 1994). In the study by Hoentjen *et al.* (2005), 16 rats were split into two groups, one of which was fed the prebiotic Synergy 1 (inulin and oligofructose). A significant increase in caecal lactobacilli and bifidobacteria was detected, together with reductions in inflammatory scores and pro-inflammatory cytokines. One study used adjuvant-induced arthritis Wistar rats, and type II collagen-induced arthritis in DBA/1 J mice. The animals were given oral alpha-GOS and dose-dependent benefits were seen in erythema and swelling of limbs, as well as histological findings in the hind paw joints. Plasma nitrite/nitrate levels were reduced, but there was an enhancement in IL-1 production from peritoneal macrophages (Abe *et al.* 2004).

These results indicate that there is potential to use prebiotics on their own or in combination with probiotics to immunomodulate the inflammatory cascade and alleviate

some of the suffering of patients with joint inflammation, but animal studies aimed at understanding the immunomodulatory mechanisms of action in this area will first be needed, so that specific targeted human trials can be designed.

Cancer

The immunomodulatory properties of prebiotics discussed earlier provide an opportunity to prevent the development or progression of neoplasia, by reducing inflammation, enhancing immune function, binding carcinogens, reducing the synthesis of enzymes that metabolize pro-carcinogens into carcinogens, and anti-tumourigenic activity.

However, there are few epidemiological data concerning the specific anti-cancer effects of fermentable carbohydrates and the colonic microbiota in humans, although diet itself has long been implicated in the pathogenesis of colorectal cancer (CRC). Perhaps not unsurprisingly, all of the experimental data on carcinogenesis inhibition comes from *in vitro* studies, or work in animals (Table 10). While early investigations used lactulose, and then GOS to demonstrate reduced activities of genotoxic enzymes produced by colonic micro-organisms that have been linked to CRC, the majority of more recent studies have used FOS as the prebiotic of choice.

The animal data is encouraging, consistently demonstrating an ability of prebiotics to reduce the numbers of precancerous aberrant crypt foci in 10 of the 11 listed animal studies. However, translating these results into human clinical trials designed to retard or prevent tumour growth, directly or indirectly, is a major challenge for the future. The one human study involving prebiotic effects on cancer was actually designed to look at lipid levels in young healthy men, but did note a significant lowering of β -glucuronidase (see below). With a suggested mechanism of action and the animal data, prebiotics alone, or more probably in combination with probiotics, may provide protection against the development or re-occurrence of cancer.

Effects of prebiotics on bacterial enzyme activities associated with genotoxicity

Studies in human microflora-associated rats have shown that TOS increased faecal bifidobacteria and lactobacilli, and reduced numbers of enterobacteria when included at 5% w/w in the diet (Rowland and Tanaka 1993). TOS lowered caecal pH and reduced the activities of enzymes involved in the formation of toxic metabolites such as β -glucuronidase and nitrate reductase; however, β -glucosidase was increased. Kikuchi *et al.* (1996) found that

Table 10 Studies on the effects of prebiotics and cancer

Subject	Prebiotic	Dose	No	Objective/method	Results	References
Rats	Galacto-oligosaccharides (GOS)	5% or 20% w/w GOS	102	Fischer rats injected twice with AOM to induce colorectal tumours and fed low or high concentration GOS. At 28 days, 18 rats in each group were killed and scored, half of each remaining group swapped to the other diet. 6 weeks later 9 rats in each group killed. The rest were killed after 10 months	At first count no of aberrant crypt foci less in 20% GOS than in the 5% treatment. After diet change total no of aberrant crypt foci increased, but less increase in animals changed to 20% GOS than those switched to 5% GOS	Wijnands <i>et al.</i> (2001) $P < 0.05$ $P < 0.05$
Humans	GOS, fructo-oligosaccharide (FOS), inulin	15 g	12	Healthy young men fed 3 weeks GOS, FOS or inulin then control diet each, originally aimed at assessing blood lipid levels	Significantly lower β -glucuronidase activity with GOS and inulin	van Dokkum <i>et al.</i> (1999) $P < 0.02$ $P < 0.05$
Murine	FOS, corn starch, wheat bran	5.8% of dietary fibre	40	Heterozygous mice for nonsense mutation in Apc gene, divided into groups and fed for 42 days then colon examined and tumours counted	Growth was unaffected by altered diet. Significant reductions in colon tumours in FOS fed mice. 4 FOS mice were tumour-free, which did not occur with any of the other diets. No significant differences in small intestinal tumours	Pierre <i>et al.</i> (1997) $P < 0.01$
Murine	FOS	5.8%	45	All Min mice given FOS diet for 42 days. 15 mice depleted of CD4+ and CD8+ lymphocytes, 15 injected as controls. Colon tumours counted at end of the study	Immunocompromised mice developed twice as many tumours as immunocompetent mice. Food supplementation with FOS stimulated a mechanism of immunosurveillance that otherwise remains inefficient	Pierre <i>et al.</i> (1999) $P = 0.02$
Murine	FOS, inulin	100 g kg ⁻¹	75	Mice challenged with a colorectal promoter and an enteric pathogen (<i>Candida albicans</i> , <i>Listeria monocytogenes</i> or <i>Salmonella Typhimurium</i>)	Lower incidence of aberrant crypt foci in mice of FOS or inulin diets compared to control. Not reduce incidence of lung cancer. Fructan diet mice had 50% less <i>C. albicans</i> . Systemic infection with <i>L. monocytogenes</i> and then with <i>S. Typhimurium</i> killed more control mice than inulin mice, with FOS mice intermediate	Buddington <i>et al.</i> (2002) $P < 0.05$ $P < 0.05$ $P < 0.05$
Rats	FOS, Inulin, AIN-76A (control)	10% of diet	18	Animals fed diet for 14 days before injection with AOM weekly for 2 weeks. Animals examined 7 weeks later	Significantly less aberrant crypt foci in FOS group 24%. Significantly less aberrant crypt foci in inulin group 35%. Crypt multiplicity was also significantly inhibited in inulin and FOS groups	Reddy <i>et al.</i> (1997) $P < 0.024$ $P < 0.006$ $P < 0.02$ $P < 0.04$

Table 10 Continued

Subject	Prebiotic	Dose	No	Objective/method	Results	P	References
Murine	FOS, Inulin, pectin	15 g	22	Mice fed 7 days of diet before transplanting tumours intramuscularly (mammary and liver) and mean tumour surface was measured	Liver tumour growth was significantly less in all 3 treatment groups than the control group. Mammary tumour growth was significantly less in the FOS group and less in the inulin and pectin groups	$P < 0.01$ $P < 0.01$ $P < 0.05$	Taper et al. (1997)
Rats	Inulin, <i>Bifidobacterium longum</i> synbiotic	50 g kg ⁻¹	60	Animals injected with AOM and then commenced on diet	Significantly less aberrant crypt foci in inulin group 21%. Significantly less aberrant crypt foci in probiotic group 29%. Significantly less aberrant crypt foci in synbiotic group 74%	$P < 0.05$ $P < 0.05$ $P < 0.001$	Rowland et al. (1998)
Rats	FOS, Bifidobacteria, FOS + bifidobacteria, soybean + bifidobacteria, wheatbran + bifidobacteria	2% of whole diet	80	Animals given carcinogen DMH and skim milk was the control diet	Significantly less aberrant crypt foci in synbiotic group. No other significant differences	$P = 0.039$	Gallaher and Khil (1999)
Rats	Synergy I <i>L. rhamnosus</i> plus <i>Bif. lactis</i> synbiotic	10%	129	Rats fed the diet for 10 days then treated with AOM twice. Examined 31 weeks after injections	Prebiotic and synbiotic groups had significantly lower numbers of adenomas and tumours than controls and probiotics alone. Small, nonsignificant effect of probiotics reducing tumours. Caecal short-chain fatty acid higher in prebiotic and synbiotic groups. COX-2 expression increased in tumours of control rats, but not in other groups. Expression of i-NOS lower in prebiotic and synbiotic group. Expression of GST-P expression lower in prebiotic and synbiotic group	$P < 0.001$ $P = 0.079$ $P < 0.001$ $P < 0.01$ $P = 0.06$ $P < 0.05$	Femia et al. (2002)
Rats	<i>L. rhamnosus</i> plus <i>Bif. lactis</i> Synergy I synbiotic	100 g kg ⁻¹	129	Rats fed for 10 days then treated with AOM twice. Fed experimental diet for 33 weeks and then examined. AIN-76A control diet	No difference between mean weights at end of experiment. Prebiotic and synbiotic group had lower no of adenomas and carcinomas than control and probiotic group. Rats in probiotic and synbiotic group had significantly less interferon- γ	$P < 0.001$ $P < 0.05$	Roller et al. (2004a)
Rats	FOS celecoxib, FOS plus celecoxib	6%		On diet for 3 weeks then injected twice with AOM and aberrant crypt foci were counted after another 15 weeks	The association of FOS and celecoxib resulted in a 61% reduction in aberrant crypt foci	$P < 0.01$	Buecher et al. (2003)

Table 10 Continued

Subject	Prebiotic	Dose	No	Objective/method	Results	References
Humans	Synergy 1, <i>L. rhamnosus</i> GG, <i>Bif. lactis</i> Bb12	12 g prebiotic, probiotics numbers unknown	80	37 colon cancer and 43 polypectomized patients enrolled in a 12-week double-blind randomized controlled trial	Significant changes in faecal microflora. Increased bifidobacteria and lactobacilli, <i>C. perfringens</i> decreased. Significant reductions in cell proliferation rates and improved epithelial function in polypectomized subjects	Rafter <i>et al.</i> (2007)

GOS (5% or 10%) increased β -galactosidase and inhibited β -glucuronidase formation in rats. Kleessen *et al.* (1997) showed that no changes occurred in faecal β -glucosidase or β -glucuronidase activities when constipated elderly volunteers were given inulin, while Bouhnik *et al.* (1996) reported that the consumption of 12.5 g of FOS per day had no effect on azoreductase, nitroreductase or β -glucuronidase. van Dokkum *et al.* (1999) observed that feeding either 15 g GOS or inulin per day to healthy young volunteers significantly reduced β -glucuronidase, though FOS had little effect.

In vitro studies using a three-stage continuous culture of the large gut (McBain and Macfarlane 2001) indicated that 5% GOS (85% pure) was weakly bifidogenic, but strongly increased numbers of lactobacilli in the fermentation vessel simulating the caecum. No significant effects were observed in the other two fermenters representing the distal bowel. GOS strongly suppressed bacterial synthesis of arylsulphatase, β -glucosidase and β -glucuronidase in all three culture vessels; however, nitroreductase and azoreductase activities were increased, making it difficult to assess the substrate's anti-mutagenicity potential. In parallel experiments, the microbiological and enzymic effects of FOS were shown to be similar, although there was no significant suppression of either β -glucosidase or β -glucuronidase.

It can be seen from the foregoing that it is difficult to arrive at a consensus regarding the effects of prebiotics on the regulation of production of enzymes associated with the formation of genotoxic substances in the gut, based on the results from animals, humans and *in vitro* modelling studies. This may be due to the fact that many prebiotic effects occur mainly in the proximal bowel, and depending on the length of gut transit time, may be obscured by the time faecal material is examined. This would be particularly significant in humans. With respect to *in vitro* studies using complex gut models, varying results can be obtained depending on the nature of the original faecal inoculum, and prebiotic substrate used. Thus, the recent study by Tzortzis *et al.* (2005) indicated that bifidobacterial numbers were stimulated by 1% GOS in a three-stage colon simulator, in vessels corresponding to the proximal and transverse colons, while no effects were seen with lactobacilli. GOS was shown to be bifidogenic in the first two fermentation vessels of the three-stage gut model, and the lack of bifidogenicity in vessel 3 was ascribed to complete utilization of the substrate by faecal bacteria in the first two vessels.

Miscellaneous effects of prebiotics

The full potential of health benefits conferred by prebiotics have probably just been touched on by researchers,

and so far, the majority of investigations have focused on more obvious gut-related diseases, but there are a few isolated papers in other areas of health, which will undoubtedly expand in the future, and are worth discussing here.

Critical illness is an area where nutritional manipulation appeals as an avenue of treatment, particularly one that is relatively free of side effects, that might provide some nutritional stability and benefit to patients who are essentially in a catabolic state. Burns patients, who will be receiving high doses of antibiotics, are particularly known to have dramatically altered intestinal microbiotas, and animal studies using probiotics have shown improvements in microbiota composition, and reduced symptoms of diarrhoea (Deitch 1990). A relatively recent study looking at the effects of prebiotics in burns patients, has shown that these individuals have increased intestinal permeability, and are at risk of bacterial translocation, septicaemia and intestinal failure (Olguin *et al.* 2005). Forty-one patients were admitted into this trial, 10 of whom failed to complete it. The subjects were randomly assigned into a control group or a group that received 3 g of oligofructose twice per day, and gut permeability studies were undertaken. The results confirmed that gastrointestinal permeability was increased fourfold, which supports evidence from other authors (Ziegler *et al.* 1988; Deitch 1990). No difference was seen between the two groups with respect to the lactulose/mannitol excretion ratio or in urinary sucrose excretion, showing that there was no improvement in intestinal permeability. One reason for human data being different from the animal results is that the animal studies did not use antibiotics (Maejima *et al.* 1984; Nieuwenhuijzen and Goris 1999), secondly, the human gastrointestinal studies that had bifidogenic effects (Lindsay *et al.* 2006; Kleessen *et al.* 1997) used significantly higher doses of prebiotic than was employed in the investigation by Olguin *et al.* (2005), and thirdly, the average inulin consumption per day in Europe is 3–11 g, so that study would have barely replaced what would be expected from an ordinary diet (van Loo *et al.* 1995).

The elderly are a population group where malnutrition is common, as is constipation and diarrhoea. Ageing can greatly affect human gut function, and this is often associated with an increase in the incidence of gastrointestinal infections (Lovat 1996), which may be linked to changes in diet, host physiology and immune system reactivity, but also to bacterial population shifts in the large bowel. For example, a number of investigations have shown that colonic bifidobacterial populations decline in older people, while there are increased numbers of potentially harmful clostridia and enterobacteria (Gavini *et al.* 2001; Hopkins *et al.* 2001; Bartosch *et al.* 2005). These findings would suggest that this is a particularly good target group in the

human population, where there is scope for improving the composition of their gut microbiotas. Synbiotic use (*Bif. lactis*, *Bif. bifidum*, Synergy 1, $2 \times 6 \text{ g day}^{-1}$ for 4 weeks) in nine healthy elderly volunteers (mean age 73) years resulted in strong bifidogenic effects (Bartosch *et al.* 2005). Throughout feeding, both probiotic bifidobacteria were detected in stools from all individuals receiving the synbiotic. At least one of the probiotic bifidobacteria remained detectable in stools up to 3 weeks postfeeding, indicating significant persistence. Indigenous bifidobacterial species (particularly *Bif. adolescentis* and *Bif. angulatum*) and some lactobacilli (e.g. *L. cateniforme*), increased greatly during synbiotic feeding compared to the placebo group. These results showed that synbiotic consumption increased the size and diversity of protective faecal bifidobacterial communities in older people, which might confer health benefits to these individuals. Given that the World Health Organization has suggested that probiotic yoghurts be used for nutritional recovery in children (World Health Organization 2000), there is considerable scope for more detailed studies to determine the health benefits of prebiotics and probiotics in older people.

Concluding remarks

The consumption of GOS and inulin-derived prebiotics has unquestionably been shown to have a wide variety of metabolic consequences in humans and animals. Many of the physiological traits attributed to prebiotics are related to their effects on the gut microflora, and its biochemical activities. Despite this, there are very few reports in the literature that contain detailed analyses of the effects of prebiotics on microbiota composition. The vast majority of these investigations have looked at only a few bacterial groups, which have usually been characterized to genus level. To date, few investigators have taken advantage of the advanced analytical technologies that have become available over the last few years for detailed community analysis. Because of this, we have little or no idea of the global effects of prebiotic use on microbial community structure. Following on from these remarks, many *in vitro* studies on prebiotic utilization have been carried out using pure cultures of bacteria, but the growth effects seen in these experiments may not necessarily be replicated in the gut, where many different carbon sources are potentially available for the bacteria. As it is unlikely that all of the bifidobacteria and lactobacillus species colonizing the colon have distinct health-promoting properties, simply measuring changes in their overall numbers does not actually tell us very much. There is now a considerable prebiotic literature in relation to animal studies and *in vitro* fermentation studies;

however, future work with established prebiotics needs to focus more on well designed human investigations. More information is needed concerning the effects of prebiotics on infants with respect to growth, body composition, nutrient availability and water balance. In particular, further studies are needed into the potential role of dietary manipulation for allergy prevention, the immune modulating effects of prebiotics in babies and in older people, and to ascertain whether they are long lasting. The optimal doses of different prebiotics for specific subject groups needs to be determined, and while caution should be used when applying the results from animal investigations to humans, *in vitro* studies may be useful in helping to select novel prebiotics prior to their employment in controlled interventional trials. *In vitro* trials with prebiotics have suggested that there may be inhibitory effects on some pathogenic bacteria, which have been associated with sudden infant death syndrome and diarrhoea, and this needs further investigation. Human trials using prebiotics to improve calcium and other mineral bioavailability are also needed, together with trials to identify the most appropriate population groups for lipid targeting, and to discover the underlying mechanisms of action. Prebiotics undoubtedly have a role to play in the treatment of IBD and other causes of diarrhoea, though few quality studies have been reported to date. Given the essentially positive results that have arisen from animal experiments, it is important that systematic studies are instigated to test for prebiotic benefits in human cancer prevention.

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