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The Role of Prebiotics and Probiotics in Human Health

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Abstract

There has recently been a significant increase in research on the potential health benefits associated with probiotics and prebiotics. Some effects attributed to selected probiotics or prebiotics have been proved by clinical trials, while others have been acquired on the basis of *in vitro* tests which need to be replicated *in vivo* in order to be validated. Clinical reports in the literature for the application of probiotics have been done for the treatment of infectious diseases including viral, bacterial or antibiotic associated diarrhea, lowering of serum cholesterol, decreased risk of colon cancer, improved lactose digestion, and altered intestinal microbiota. However, information on probiotic species, a specific strain-therapeutic application, and adequate dosages, is not yet sufficient to allow rational consumption. Moreover, prebiotic oligosaccharides are poorly understood in regard to their fermentation profiles and dosages required for health effects. The present review summarizes some of the literature in regard to clinical or therapeutic trials of probiotics and prebiotics.

Bacteria of the Human Gastrointestinal Tract

The microflora in the Gastrointestinal (GI) tract of human infants begins establishment immediately after delivery. Gram-negative aerobic bacteria together with Gram-positive and Gram-negative anaerobic bacteria can be recovered from fecal specimens within 3 days after birth [1]. The vaginal and fecal flora of the mother, and also the environment (air and food, for instance) are important sources of bacteria [2]. After approximately 2 years of age the microbial composition resembling that of an adult becomes established [3]. Furthermore, it has been found that changes in the diet can modify the microbial composition in the colon [4]. For example, infants fed breast milk have mainly *Bifidobacteria* in their gut, but infants fed formula have more complex microflora which are predominantly *Bacterioides*, *Bifidobacteria*, *Clostridia* and *Streptococci* [3,5].

In the GI tract the large intestine is the most colonized area and can harbor up to 10^{12} bacteria per gram of waste contents [6,7] with hundreds of species represented [7]. The large intestine contains mostly strict anaerobes and they may be categorized as bacteria that are either harmful or beneficial [5]. The pathogenic effects of harmful bacteria include diarrhea, systemic infections, liver damage and carcinoma. In contrast, the beneficial bacteria promote the host's health by stimulating the immune system, synthesizing vitamins, inhibiting the growth of harmful bacteria and improving digestion and absorption of essential nutrients [3]. Furthermore, the microflora in the large intestine derives a proportion of their energy from dietary components that have not been degraded in the upper intestinal tract and reach the large intestine [8]. These dietary components consist mainly of polysaccharides, oligosaccharides, proteins, peptides and glycoproteins that are fermented by the gut bacteria and subsequently produce short chain fatty acids (SCFA) as their major end products; the majority of these SCFA are acetate, propionate and butyrate [9].

Probiotics

Probiotics are live microorganisms which when consumed may confer a health benefit to the host [10]. Certain species of Lactic Acid Bacteria (LAB) and *Bifidobacteria* spp. are commonly used in the manufacture of probiotic products because of their well-known beneficial effect to host health and they are Generally Recognized as Safe (GRAS) [11-13]. Many studies have shown that probiotics can stimulate the immune system, decrease serum cholesterol, alleviate lactose intolerance, decrease diarrheal incidence, control infections, act as antibiotics, suppress tumors and protect against colon/bladder cancer [14].

Role of probiotics in lactose intolerance

For most mammals, including humans, lactase activity declines after weaning; however, in some humans, lactase activity persists at a high level throughout adult life due to the development of lactase persistence [15]. This lactase persistence among the population of the world varies depending on ethnicity and habit of consuming dairy products; consequently, the northern European population has as little as 2% of the population deficient in lactase [16]. The lactase deficiency is prevalent in Hispanic people (80%), black and Ashkenazi Jewish people (60 to 80%) and almost 100% of Asian and Native American Indian people [15].

In persons lacking lactase, when lactose reaches the large intestine, it is metabolized by the colonic microflora to produce methane, carbon dioxide, and hydrogen and altering the osmotic balance in the colonic lumen, causing symptoms such as cramping, diarrhea, flatulence, and abdominal bloating [17]. However, a number of human studies have shown that high-lactose milk products supplemented with starter cultures containing *Lactobacilli* and/or *Bifidobacteria* can be tolerated by lactose-intolerant individuals, possibly because these fermented products contain the microbial β-galactosidase which functions in the small intestine to support lactose hydrolysis [18]. Additionally, it has been demonstrated that in mice *Streptococcus thermophilus* or *Lactobacillus casei* subsp *defensis* are able to hydrolyze lactate during transit through the gut [19]. Independent of easing lactose intolerance, probiotics also seem to relieve some gastrointestinal complaints such

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as flatulence or diarrhea most probably by their influence on the gut microflora [20,21].

Probiotics effects on diarrheal diseases

Infectious diarrhea caused by bacteria or viruses: The World Health Organization (WHO) estimates 8.1 million deaths occur yearly in children (<5 years of age) with diarrhea accounting for 14% of those deaths [22]. In addition to causing mortality, diarrhea elicits serious long-term effects with multiple episodes and persistent diarrhea affecting growth, nutrition, and cognition [23]. Rotavirus is the most common cause of diarrhea in children [24]. The beneficial effects of certain probiotic strains on preventing rotavirus-caused diarrhea have been studied. In one study, human infants aged 5 to 24 months who were admitted to a chronic medical care hospital were randomized to receive a standard infant formula or the same formula supplemented with *Bifidobacterium bifidum* (1.9×10^8 CFU/g) and *Streptococcus thermophilus* (1.4×10^7 CFU/g) for 14 days. The results demonstrated significantly lower numbers of children experiencing diarrhea who received the supplemented formula compared to un-supplemented control groups [25]. Other beneficial effects recorded from clinical studies include shortening the duration of diarrhea by as much as 1.5 days and less shedding of rotavirus [26,27]. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) and the European Society for Pediatric Infectious Diseases recommend that only probiotic strains with proven clinical efficacy and in appropriate dosages be used as an adjunct to rehydration therapy for the management of children with acute diarrhea [28]. Proven clinical efficacy was determined from formal systematic reviews of the literature; treatments were highly recommended if there was strong evidence from more than one systematic review of well-designed randomized control trials that a probiotic was more effective than a placebo [28]. It was determined that probiotics *L. reuteri* (ATCC 55730), *L. acidophilus LB*, *S. boulardii* and a mixture of *S. thermophilus*, *L. acidophilus* and *L. bulgaricus* significantly reduced the duration of diarrhea when compared with placebo, particularly in rotaviral gastroenteritis [28]. Effects of probiotics were also determined to be dose dependent dose-dependent, that is the effects were greater for doses higher than 10^{10} to 10^{11} colony-forming units [28]. The World Gastroenterology Organization (WGO) included *L. casei* DN-114 001 in their evidence-based recommendation and pointed out that timing of administration (at the very first sign of illness) is considered highly important [29].

Antibiotic associated diarrhea: Many patients taking antibiotics that disturb the gastrointestinal flora experience diarrhea as a side effect [30,31]. Symptoms range from mild and self-limiting to severe, and this antibiotic-associated diarrhea (AAD) is an important reason that patients refuse to take antibiotics or curtail their treatment regimen [32]. Many studies indicate the ingestion of certain probiotic strains, primarily *Lactobacilli* either alone or in combination with other genera, before and during antibiotic treatment reduces the frequency, duration and severity of antibiotic induced diarrhea [33].

Lowering of cholesterol

There have been several mechanisms proposed for the purported cholesterol lowering effects of probiotics, including deconjugation of bile acids by bile-salt hydrolase enzymes of probiotics [34], assimilation of cholesterol by probiotics [35], co-precipitation of cholesterol with deconjugated bile [36], cholesterol binding to cell walls of probiotics [37], incorporation of cholesterol into the cellular membranes of probiotics during growth [38], conversion of cholesterol into coprostanol [39] and production of short-chain fatty acids upon fermentation by probiotics in the presence of prebiotics [40].

Numerous studies on the effects of probiotics on cholesterol have used animals as models. Shinnick et al. [41] studied the effect of processing on the ability of oat fiber to lower plasma and liver cholesterol concentrations in rats. Rats were fed diets containing 6% dietary fiber as cellulose, oat bran, high fiber oat flour or one of four processed high fiber oat flours for 3 wk; all diets also contained 1.0% cholesterol and 0.2% cholic acid. All of the oat products significantly lowered plasma and liver cholesterol without depressing food intake or weight gain. A study was conducted to determine the effect of glucomannan, chitosan), or an equal mixture of the two on cholesterol absorption and fat and bile acid excretion in rats [42]. Total liver cholesterol was significantly reduced in all groups compared with the control group. Cholesterol absorption was significantly reduced from 37.5% in the control group to 20.2% in the glucomannan group, 18.2% in the combination and 9.4% in chitosan. Mice deficient in the LDL receptor have also been widely used as a model to mimic human atherosclerosis by feeding them Western diets with or without insulin-sensitizing agents; however, the time-course of atherosclerotic lesion development and distribution of lesions at specific time-points are yet to be established [43]. Lin et al. [44] conducted a study aimed to investigate whether the combination of plant sterol esters with soy protein or soy isoflavones would have extra cholesterol-lowering effects. Hamsters were fed diets containing casein (control), plant sterol esters, intact soy protein, soy isoflavones, plant sterol esters plus soy protein, or plant sterol esters plus soy isoflavones for 5 wk. All diets contained 0.08 g cholesterol/100 g diet. The plant sterol esters and the soy protein diets significantly lowered the plasma total cholesterol concentration by 13% and 9%, respectively as compared to the control, whereas the isoflavone diet had no effect. The combination of plant sterol esters and soy protein decreased plasma total cholesterol by 26%. In conclusion, the combination of PSE and soy protein more dramatically lowers plasma lipids than the individual ingredients [44]. Guinea pigs [45] and pigs [46] have also been used as models for humans in studies of cholesterol lowering drugs. Because these animals share some attributes with humans in terms of cholesterol and bile acid metabolism, plasma lipoprotein distribution, and regulation of liver cholesterol enzymes [47] and digestive anatomy and physiology [46] they are useful experimental models for research. Therefore the cholesterol lowering effects seen in these animal studies suggest a similar potential in humans.

Although numerous studies have demonstrated convincing cholesterol-lowering effects of probiotics in both animals and humans, controversial results have surfaced. Hatakka et al. [48] reported that the administration of *L. rhamnosus* LC705 (10^{10} CFU/g per capsule; two capsules daily) for 4 weeks did not influence blood lipid profiles in thirty-eight men with mean cholesterol levels of 6.2 mmol/L (240 mg/dL). In another study involving forty-six volunteers (aged 30 to75 years old), Simons et al. [49] found that the consumption of *Lactobacillus fermentum*, (2×10^9 CFU per capsule; four capsules daily) did not result in any lipid profile changes after 10 weeks. Lewis and Burmeister [50] conducted a randomized, placebo-controlled double blind and crossover designed study to determine the effect of *Lactobacillus acidophilus* on human lipid profiles. In the study, eighty volunteers 20 to 65 years of age with a baseline total cholesterol of more than 5.0 mmol/L (greater than 193 mg/dL) and a mean Body Mass Index of 27.8 kg/m^2 consumed two capsules containing freeze-dried *L. acidophilus* (3×10^{10} CFU/2 capsules) three times daily for six weeks, and crossed over for another six weeks after a 6-week washout period. The authors

found that *L. acidophilus* capsules did not significantly change plasma total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides of the subjects.

Even *in vivo* trials using human subjects are also affected by external factors such as experimental design, lack of adequate sample size for statistical purposes, failure to control for other nutrient intake and energy expenditure during the experiments, and different strains and dosages of probiotics [51,52]. There is a need for large-scale clinical trials with controlled dosing, determination of viability, definition of strains and other critical variables to provide the necessary scientific evidence required to determine efficacy of the ever-increasing use of probiotics.

Cancer Prevention

Yogurt and other fermented milk products have been promoted as cancer preventing, especially for colon cancer [53]. Epidemiological studies suggest that colon cancer is associated with a high-fat diet because of the large international variations in rate are strongly correlated with apparent per capita consumption; the high fat diet potentially stimulates bile acid turnover and can lead to an increase of bile acids in the colon which affects the metabolism of the bacterial flora [54]. The bile acids sodium cholate and sodium chenodeoxycholate were studied as promoters of colon carcinomas by comparison of conventional rats to germ free rats and these researchers concluded that indigenous microflora in the intestine could produce enzymes such as glucuronidase, nitroreductase and azoreductase which can convert pro-carcinogens into carcinogens [54]. This has prompted research on the use of probiotic bacteria to reduce the risk of colon cancer. However, the factors involved in the initiation and promotion of colon cancer are separated in time from actual tumor development, making it difficult to find definitive indicators of usefulness of probiotics. Brady et al. [55] analyzed animal studies over a 10 year period that looked most directly at the relationship between probiotic consumption and colon cancer development and found that apparently probiotics do have an inhibitory effect on the development of precancerous lesions and tumors in animal models. Several mechanisms have been proposed for the anti-cancer effects of probiotics including preventing the growth of bacteria that produce enzymes responsible for converting pro-carcinogens into carcinogens [56,57], inhibition of tumor growth by metabolites of the *Lactobacilli* stimulating the immune system [58], and production of tumor necrosis factor by macrophages [59]. Goldin and Gorbach [56] recruited 21 people for a study designed to investigate the effect of oral supplements of *Lactobacillus acidophilus* on the fecal bacterial enzymes beta-glucuronidase, nitroreductase, and azoreductase, which can catalyze procarcinogen conversion to carcinogen. The sequence of feeding studies and fecal enzyme assays was the same for all subjects: 4 wk of a control period; 4 wk of plain milk feeding; 4 wk of control, without any dietary supplements; 4 wk of milk containing 2×10^6/ml viable *L. acidophilus*; and 4 wk of control, without any supplements. Reductions in the activities of the three fecal enzymes were noted in all subjects and were highly statistically significant, but were observed only during the period of *Lactobacilli* feeding. During the final control period, after *Lactobacilli* feeding, fecal enzyme levels returned to normal after 4 wk. Fujiwara et al. [57] investigated the fate *L. gasseri* SBT2055 (LG2055SR) and the influence of its oral administration on the composition and metabolism of the intestinal microflora. They confirmed that *L. gasseri* became established in the human intestine fecal and noted that populations of *Staphylococcus* and fecal contents of p-cresol were reduced [57]. A double-blind trial was conducted in 138 human patients with carcinoma of the bladder after surgery to evaluate the prophylaxis of recurrence by an oral *L. casei* preparation, wherein the *L. casei* preparation showed a better prophylactic effect than placebo [58]. Roller et al. [59] fed probiotics (*L. rhamnosus* GG and *Bifidobacterium lactis* Bb12), prebiotics (inulin enriched with oligofructose), and synbiotics (combination of probiotic and prebiotic) to rats for 4 wk as supplements to a high fat diet. Functions of immune cells isolated from peripheral blood mononuclear cells, spleen, mesenterial lymph nodes and Peyer's patches were investigated. The synbiotic supplement increased secretory immunoglobulin A production in the ileum compared with controls fed the high fat diet alone, and decreased the oxidative burst activity of blood neutrophils compared with rats fed probiotics alone. The prebiotic supplement enhanced the production of interleukin-10 in Peyer's patches as well as the production of sIgA in the cecum, compared with controls [59].

For probiotics to be effective they must be alive in the food or supplement as well as being able to survive the harsh conditions of the GI tract while still maintaining activity [60]. A multitude of factors affect viability and survivability including the strain of bacterium, pH of the food or supplement, nutrients in the food or supplement and heat treatment [61-66]. A myriad of methods have been developed to increase survivability and viability of the probiotics ranging from pre-exposure to sublethal stresses, use of immobilized cell technology, microencapsulation, genetic modification, combining different synergistic strains, to incorporation of nutrients and prebiotics to the matrix [64,67-70].

Prebiotics

Prebiotics are employed to promote both beneficial bacteria which are already established in the colon as well as externally administered probiotic bacteria. The original definition of prebiotics was that they are food ingredients which are indigestible in the upper GI tract and reach the colon to beneficially influence the host by selectively promoting the growth and/or activity of certain bacteria in the colon [3]. Given the large number of bacterial strains present in the GI tract, some of which are non-cultivable, the definition was revised to "a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health" [71,72]. Consumers often take in moderate levels of prebiotics naturally from many fruits and vegetables including leeks, Jerusalem artichokes, chicory, onion, garlic, banana, and asparagus, but the levels of prebiotics in these food sources are generally too low to exhibit any significant effect on the composition of intestinal microflora [73]. Thus, prebiotics are commercially extracted and concentrated from fruits and vegetables through the hydrolysis of polysaccharides from dietary fibers or starch, or through enzymatic generation. Prebiotics are mixtures of indigestible oligosaccharides, except for inulin which is a mixture of fructooligo- and polysaccharides [73,74]. All currently known prebiotics are carbohydrates, and there are many different carbohydrates marketed world-wide as prebiotics, the only four that are well supported by good quality data from human trials are the fructans (inulin and fructo-oligosaccharides), galacto-oligosaccharides, and the synthetic disaccharide, lactulose.

Fructans

A fructan is composed of fructose polymers which are generally linked to the moiety of a terminal glucose [75]. Fructans are resistant to enzymes in the digestive tract of humans and pass through the upper portion of the human GI tract, reach the colon and are fermented by colonic microflora producing SCFA [76].

Inulin: Inulin is a naturally occurring storage carbohydrate commonly found in leeks, onions, wheat, asparagus, garlic, Jerusalem artichoke and chicory [77,78]. Most of the inulin commercially produced is from Jerusalem artichokes and chicory [79]. The effect of daily intake of 10 g inulin on fasting blood lipid, glucose and insulin levels in healthy middle-aged men and women with moderately raised total plasma cholesterol and triacylglycerol levels was studied in a double-blind randomized placebo-controlled parallel study in which subjects received either inulin or placebo for a period of 8 weeks [78]. Fasting blood samples were collected before the supplementation period and at weeks 4 and 8, with a follow-up at week 12. Compared with baseline values, insulin concentrations were significantly lower at 4 weeks in the inulin group, and there was a trend for triacylglycerol values to be lower in the inulin group at 8 weeks returning to baseline concentrations at week 12 [78]. Nair et al. [80] reviewed the literature on functional aspects of inulin and reported that several animal and human studies have shown inulin to promote optimal digestive health, positively influence lipid metabolism, decrease the risk of osteoporosis by increasing calcium absorption, as well as reduce the risk of colon cancer, breast cancer, and tumor growth.

Fructo-oligosaccharides: Fructo-oligosaccharides (FOS) are composed of a mixture of oligosaccharides consisting of glucose linked to fructose units by β-(1,2) links with a degree of polymerization (DP) between 1 and 5. They occur naturally in several plants such as asparagus, wheat, Jerusalem artichokes, and rye. Onion is especially rich in FOS which ranges from (25 to 40% of dry matter [81]. The commercial production of FOS is mainly based on two processing methods which are either a continuous process using immobilized cells in calcium alginate gel or enzyme on an insoluble carrier or a batch conversion of sucrose by fungal fructosyltransferase [82]. In the human colon the FOS are completely fermented mostly to lactate, SCFA (acetate, propionate and butyrate), and gas [83]. The FOSs stimulate *Bifidobacteria*l growth and suppress the growth of potentially harmful species in the colon [83]. Other effects of FOS include a decrease in fecal pH, an increase in fecal or colonic organic acids, a decrease in fecal bacterial enzymatic activities and a modification in fecal neutral sterols [83]. They have been demonstrated to enhance magnesium absorption in humans and, in animal models, have demonstrated reduction in colon tumor development due to enhancement of both colon butyrate concentrations and local immune system effectors [83].

Galacto-oligosaccharides

Galacto-oligosaccharide (GOS) is a collective term for a group of carbohydrates composed of oligo-galactose with some lactose and glucose, and which are produced commercially from lactose by β-galactosidase [84]. There are oligosaccharides that resemble GOS naturally found in human milk which may be one of the factors that protect breast fed infants from gastrointestinal pathogenic bacteria [85]. Several animal studies, mainly in rats, evaluated GOS and have generally been shown to promote growth of the beneficial *Bifidobacteria* [86-88]. The effect of feeding a diet containing GOS with or without *Bifidobacterium breve* (administered in the drinking water) was investigated in rats colonized with a human fecal microflora [86]. The GOS or GOS plus *B. breve*, given for 4 weeks, induced increases in cecal concentration of total anaerobic bacteria, *Lactobacilli* and *Bifidobacteria*, and decreases in numbers of enterobacteria; cecal pH was significantly reduced, as were the activities of beta-glucuronidase and nitrate reductase [86]. Rats inoculated with a human fecal flora and fed a diet containing GOS exhibited non-specific induction of beta-galactosidase, beta-glucosidase and alpha-glucosidase activities and increased CH_4 excretion, a decrease in cecal pH and total SCFA concentration as compared to control diet [87]. Morishita et al. [88] studied the effect of 5% GOS on the population of *Clostridium perfringens* during the development of primary intestinal microflora in gnotobiotic mice. *B. breve* increased and *C. perfringens* decreased in the feces of the GOS-fed animals, but not in the controls [88]. There have also been several human trials of GOS with different methods and study designs which have generated rather mixed results. While several studies failed to show any significant changes in fecal microbiota of volunteers [89-91], others did show a response [92-94]. In one study strictly controlled experimental diets were supplied to 3 intervention groups in a parallel design of 2 consecutive 3-wk periods during which each participant consumed a run-in diet followed by an intervention diet that differed only in the amount of GOS: 0 (placebo), 7.5, and 15 g/d; breath samples and fecal samples were collected at the end of both the run-in and intervention periods [89]. The number of *Bifidobacteria* increased after both placebo and GOS ingestion, but the differences between the increases were not significantly different. Additionally, GOS did not significantly affect bowel habits, stool composition, the concentration of short-chain fatty acids or bile acids in feces, the concentration of ammonia, indoles, or skatoles in feces, fecal pH or the composition of the intestinal microflora [89]. A feeding trial was conducted on healthy human volunteers to study the effect of GOS-containing syrup (60% GOS) alone or combined with the probiotic strain *B. lactis* Bb-12 on selected components of the fecal flora, and the effect of GOS supplementation on colonization of *B. lactis* Bb-12 [90]. Mean numbers of *Bifidobacteria* increased slightly in all study groups during the feeding period and there was good survival of *B. lactis* Bb-12 through the gastrointestinal tract, but no differences in the prevalence or numbers of isolates with *B. lactis* Bb-12 genotype could be observed between groups suggesting that GOS-containing syrup did not enhance the survival or persistence of *B. lactis* Bb-12 in the gut [90]. A culture-independent approach based on genus-specific PCR and denaturing gradient gel electrophoresis (DGGE) was used to monitor qualitative changes in fecal *Bifidobacteria*l communities in the same type of human feeding trial [91]. The DGGE profiles revealed that administration for two weeks of GOS and/or *B. lactis* Bb-12 (8 g and 3×10^{10} CFU/day, respectively) did not affect the qualitative composition of the indigenous *Bifidobacterium* population, while *B. lactis* Bb-12 transiently colonized the gut [91]. Gopal et al. [92] studied the effects of GOS and *B. lactis* HN019 on the microbial composition of the gastrointestinal tract of human subjects using a randomized, double blind, placebo-controlled study design. Volunteers either consumed a reconstituted milk containing 2.4 g of GOS/day, reconstituted milk containing 3×10^{10} CFU of *B. lactis* HN019/ day, or reconstituted milk without additions. Subjects receiving either GOS or probiotic bacteria had a significant increase in the fecal counts of both *Lactobacilli* and *Bifidobacteria* [92]. Other studies using human volunteers have also found GOS to be bifidogenic [93,94].

Due to the stability of GOS at high temperature and in acidic environments, they are used in many food applications such as infant formulas, dairy products, sauces, soups, breakfast cereals, snack bars, ice creams, beverages, bakery products, animal feeds, and as sugar replacements to increase texture and mouthfeel of foods, and act as bulking agents [95]. Also, the mixture of 90% GOS and 10% inulin are used in infant formulas to simulate human milk [96,97].

Lactulose

Lactulose is a synthetic disaccharide which was used originally as a laxative [98]. Lactulose has also been shown to increase *Lactobacilli*

and *Bifidobacteria* and significantly decrease *Bacteroides* in mixed continuous fecal culture [99]. In some clinical trials lactulose has been demonstrated to have potential for use as a prebiotic. Terada et al. [100] studied the effect of dietary supplementation with lactulose (3 g/d for 2 wk) on the fecal flora and fecal bacterial metabolism in healthy human volunteers. During the intake of lactulose, the number of *Bifidobacteria* increased significantly, whereas the numbers of *Clostridium perfringens* and *Bacteroidaceae* decreased; fecal indole, skatol and phenols, and fecal P-glucuronidase, nitroreductase and azoreductase activities were also decreased significantly [100]. In another study involving human volunteers, lactulose (2×10 g/d) increased probiotic bacteria and decreased putrefactive bacteria and potential pathogens [101]. In another human feeding trial a dose of 10 g per day, half the pharmacological dose, was fed to healthy adult volunteers and *Bifidobacteria* showed a statistically significant increase and a simultaneous decrease in *Clostridia* as compared to the control group with placebo [102]. A single administration of lactulose significantly decreased urinary nitrogen-excretion in a dose-dependent way in healthy volunteers, and long-term administration of lactulose produced a significant reduction of the urinary nitrogen-excretion accompanied by a significant increase in the fecal nitrogen-output; a significant rise in the *Bifidobacterium* population was also observed [103]. In spite of these promising results lactulose is not yet widely used as a prebiotic, although it remains an established product in the medical market.

Emerging and Novel Prebiotics

Several other oligosaccharides have been proposed as prebiotics that do not have sufficient data from human studies to support the claim. There are other carbohydrates also currently being studied for their prebiotic potential that have data mostly from *in vitro* studies or animal studies but do not yet have human data to support them.

Xylo-oligosaccharides

Xylo-oligosaccharides (XOS) are chains of xylose molecules linked by β1–4 bonds [104,105] which are produced enzymatically by hydrolysis of xylan from birch wood, oats, or corn cobs [105]. They have been studied using *in vitro* batch culture systems where they have proven to be very selective for *Bifidobacteria* [106,107], and the prebiotic potential has also been confirmed in animal studies [108-110]. Lecerf et al. [111] performed a randomized, parallel, placebo-controlled, double-blind study of XOS in healthy humans and found that it increased the fecal concentrations of *Bifidobacterium* and butyrate and activities of α-glucosidase and β-glucuronidase, while decreasing the concentrations of acetate and p-cresol, showing a clear prebiotic effect.

Resistant starch

Resistant starch is the term used for the fraction of starch that escapes digestion in the upper GI tract and that reaches the colon to be fermented by the colonic microbiota [112]. A few animal studies on the probiotic properties of resistant starch have been carried out and in general show an increase in *Bifidobacteria* and SCFA in rats [113-115], human-flora-associated rats [116], and pigs [117]. The effect of prolonged feeding of resistant potato starch on butyrate production in rats was studied and it was determined that butyrate production was promoted by long-term ingestion from the cecum towards the distal colon, suggesting a slow adaptive process within the digestive tract in response to a chronic load of indigestible carbohydrates [113]. In another rat study resistant starch increased the rate of fermentation accompanied by a decrease of pH in cecum, colon, and feces; also bile acids became bound to the resistant starch and thus were not reabsorbed, resulting in a higher turnover through the large bowel [114]. Silvi et al. [116] studied the effect of resistant starch on human gut microflora and associated parameters in human flora-associated (HFA) rats, colonized with microflora populations from UK or Italian subjects. In both the UK and Italian flora-associated rats, numbers of *Lactobacilli* and *Bifidobacteria* were increased 10 to 100-fold, and there was a concomitant decrease in enterobacteria [116].

Pectic oligosaccharides

Pectin is a complex galacturonic acid-rich polysaccharide which occurs naturally in the cell walls of higher plants and acts as a cement-like material for the cellulosic components of the plant cell wall [118]. The major polysaccharides forming pectin include homogalacturonan (HGA), rhamnogalacturonan-I (RG-I), and rhamnogalacturonan-II (RG-II) which are believed to covalently link together [118].

Enzymatic or physical methods can be used to manufacture pectic oligosaccharides. Enzymatic hydrolysis of citrus and apple pectins in membrane reactors produces oligosaccharides of 3-4 kDa molecular weight [119]. A nitric acid hydrolysis of citrus peel produces low molecular weight arabinose-based oligosaccharides [120]. Both of these materials have been evaluated in fecal batch cultures and have both been shown to promote growth of *Bifidobacteria* [121,122].

Conclusions

There is adequate evidence of benefit of probiotics in specific circumstances, such as acute diarrhea of viral or bacterial origin and AAD. When using or prescribing probiotics, consideration must be given to the probiotic formulation, including live, dead, compounded preparations or their products, the effective dose to use and the type of disease targeted. It is not possible to extrapolate specific actions or doses of a given probiotic and generalize these properties to other doses or strains of probiotic bacteria. The United States Food and Drug Administration does not currently regulate probiotic products, providing no government oversight for quality control, and the actual number of viable organisms in commercial products may be quite different from what is being advertised. In summary, future large-scale clinical trials controlling dosing, viability and other critical variables will be crucial to provide the necessary scientific evidence required to determine efficacy of the ever-increasing use of probiotics.

Oligosaccharides are gaining increasing recognition as prebiotics capable of modulating the colonic microbiota in humans and animals. The currently defined prebiotics have a wide range of degrees of robustness of data to support their prebiotic status, and there is a need for more large scale, well designed human trials with clearly defined outcomes using modern molecular microbiological methods. There is much data that supports the role of prebiotics in dietary intervention in several disease conditions, including colon cancer, acute infections, and inflammatory bowel disease.

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References

1. Rotimi VO, Duerden BI (1981) The development of the bacterial flora in normal neonates. J Med Microbiol 14: 51-62.

2. Mevissen-Verhage EA, Marcelis JH, de Vos MN, Harmsen-van Amerongen WC, Verhoef J (1987) *Bifidobacterium*, Bacteroides, and Clostridium spp.

in fecal samples from breast-fed and bottle-fed infants with and without iron supplement. J Clin Microbiol 25: 285-289.

3. Gibson GR, Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 125: 1401-1412.
4. Drasar BS, Roberts AK (1989) Control of the large bowel microflora. In Hill MJ, Marsh PD, eds, Human Microbial Ecology, Boca Raton, FL: CRC Press. 87-106.
5. Yoshiota M, Fujita K, Sakata H, Murono K, Iseki K (1991) Development of the normal intestinal flora and its clinical significance in infants and children. *Bifidobacteria* Microflora 10: 11-17.
6. Cummings JH (1981) Short chain fatty acids in the human colon. Gut 22: 763-779.
7. Moore WE, Holdeman LV (1974) Human fecal flora: the normal flora of 20 Japanese-Hawaiians. Appl Microbiol 27: 961-979.
8. Macfarlane GT, Gibson GR, Macfarlane S. (1994) Short chain fatty acid and lactate production by human intestinal bacteria grown in batch and continuous culture. In Binder JH, Cummings HJ, Soergel HK, eds, Short Chain Fatty Acids London: Kluwer Publishing. 44-60.
9. Macfarlane S, Macfarlane GT (1995) Proteolysis and amino acid fermentation. In Gibson GR, Macfarlane GT, eds, Human colonic bacteria: role in nutrition, physiology and pathology, Boca Raton, FL: CRC Press. 75-100.
10. Fuller R (1989) Probiotics in man and animals. J Appl Bacteriol 66: 365-378.
11. Goldin BR, Gorbach SL (1992) Probiotics for humans. In Fuller R, ed, Probiotics the scientific basis, London: Chapman and Hal*L*. 355-376.
12. Chomarat M, Espinouse D (1991) *Lactobacillus rhamnosus* septicemia in patients with prolonged aplasia receiving ceftazidime-vancomycin. Eur J Clin Microbiol Infect Dis 10: 44.
13. Oakey HJ, Harty DW, Knox KW (1995) Enzyme production by *Lactobacilli* and the potential link with infective endocarditis. J Appl Bacteriol 78: 142-148.
14. Scheinbach S (1998) Probiotics: functionality and commercial status. Biotechnol Adv 16: 581-608.
15. Swallow DM (2003) Genetics of lactase persistence and lactose intolerance. Annu Rev Genet 37: 197-219.
16. Itan Y, Powell A, Beaumont MA, Burger J, Thomas MG (2009) The origins of lactase persistence in Europe. PLoS Comput Biol 5: e1000491.
17. Suarez FL, Savaiano DA (1997) Diet, genetics, and lactose intolerance. Food Tech 51: 74-76.
18. de Vrese M, Sieber R, Stransky M (1999) Lactose malabsorption and eating yogurt. Schweiz Med Wochenschr 129: 253-254.
19. Oozeer R, Goupil-Feuillerat N, Alpert CA, van de Guchte M, Anba J, et al. (2002) *Lactobacillus casei* is able to survive and initiate protein synthesis during its transit in the digestive tract of human flora-associated mice. Appl Environ Microbiol 68: 3570-3574.
20. de Vrese M, Stegelmann A, Richter B, Fenselau S, Laue C, et al. (2001) Probiotics--compensation for lactase insufficiency. Am J Clin Nutr 73: 421S-429S.
21. Zhong Y, Priebe MG, Vonk RJ, Huang CY, Antoine JM, et al. (2004) The role of colonic microbiota in lactose intolerance. Dig Dis Sci 49: 78-83.
22. Black RE, Cousens S, Johnson HL, Lawn JE, Rudan I, et al. (2010) Global, regional, and national causes of child mortality in 2008: a systematic analysis. Lancet 375: 1969-1987.
23. Guerrant RL, Oriá RB, Moore SR, Oriá MO, Lima AA (2008) Malnutrition as an enteric infectious disease with long-term effects on child development. Nutr Rev 66: 487-505.
24. Claeson M, Merson MH (1990) Global progress in the control of diarrheal diseases. Pediatr Infect Dis J 9: 345-355.
25. Saavedra JM, Bauman NA, Oung I, Perman JA, Yolken RH (1994) Feeding of *Bifidobacterium bifidum* and *Streptococcus thermophilus* to infants in hospital for prevention of diarrhoea and shedding of rotavirus. Lancet 344: 1046-1049.
26. Huang JS, Bousvaros A, Lee JW, Diaz A, Davidson EJ (2002) Efficacy of probiotic use in acute diarrhea in children: a meta-analysis. Dig Dis Sci 47: 2625-2634.
27. Nixon AF, Cunningham SJ, Cohen HW, Crain EF (2012) The effect of *Lactobacillus* GG on acute diarrheal illness in the pediatric emergency department. Pediatr Emerg Care 28: 1048-1051.
28. Guarino A, Albano F, Ashkenazi S (2008) European society for paediatric gastroenterology, hepatology, and nutrition/European society for paediatric infectious diseases evidence-based guidelines for the management of acute gastroenteritis in children in Europe. J Paediatr Gastroenterol Nutr 46: S81-S122.
29. http://www.worldgastroenterology.org/assets/export/userfiles/Probiotics_FINAL_20110116.pdf
30. McFarland LV (1998) Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 16: 292-307.
31. Barbut F, Meynard JL (2002) Managing antibiotic associated diarrhoea. BMJ 324: 1345-1346.
32. Szajewska H, Mrukowicz J (2005) Meta-analysis: non-pathogenic yeast *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea. Aliment Pharmacol Ther 22: 365-372.
33. Hempel S, Newberry SJ, Maher AR, Wang Z, Miles JN, et al. (2012) Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. JAMA 307: 1959-1969.
34. Lambert JM, Bongers RS, de Vos WM, Kleerebezem M (2008) Functional analysis of four bile salt hydrolase and penicillin acylase family members in *Lactobacillus plantarum* WCFS1. Appl Environ Microbiol 74: 4719-4726.
35. Pereira DI, Gibson GR (2002) Cholesterol assimilation by lactic acid bacteria and *Bifidobacteria* isolated from the human gut. Appl Environ Microbiol 68: 4689-4693.
36. Liong MT, Shah NP (2006) Effects of a *Lactobacillus casei* synbiotic on serum lipoprotein, intestinal microflora, and organic acids in rats. J Dairy Sci 89: 1390-1399.
37. Liong MT, Shah NP (2005) Acid and bile tolerance and cholesterol removal ability of *Lactobacilli* strains. J Dairy Sci 88: 55-66.
38. Lye HS, Rusul G, Liong MT (2010) Removal of cholesterol by *Lactobacilli* via incorporation and conversion to coprostano*L*. J Dairy Sci 93: 1383-1392.
39. Lye HS, Rusul G, Liong MT (2010) Mechanisms of cholesterol removal by *Lactobacilli* under conditions that mimic the human gastrointestinal tract. Int Dairy J 20: 169-175.
40. De Preter V, Vanhoutte T, Huys G, Swings J, De Vuyst L, et al. (2007) Effects of *Lactobacillus casei* Shirota, *Bifidobacterium breve*, and oligofructose-enriched inulin on colonic nitrogen-protein metabolism in healthy humans. Am J Physiol Gastrointest Liver Physiol 292: 358-368.
41. Shinnick FL, Longacre MJ, Ink SL, Marlett JA (1988) Oat fiber: composition versus physiological function in rats. J Nutr 118: 144-151.
42. Gallaher CM, Munion J, Hesslink R Jr, Wise J, Gallaher DD (2000) Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats. J Nutr 130: 2753-2759.
43. Lichtman AH, Clinton SK, Iiyama K, Connelly PW, Libby P, et al. (1999) Hyperlipidemia and atherosclerotic lesion development in LDL receptor-deficient mice fed defined semipurified diets with and without cholate. Arterioscler Thromb Vasc Biol 19: 1938-1944.
44. Lin Y, Meijer GW, Vermeer MA, Trautwein EA (2004) Soy protein enhances the cholesterol-lowering effect of plant sterol esters in cholesterol-fed hamsters. J Nutr 134: 143-148.
45. Madsen CS, Janovitz E, Zhang R, Nguyen-Tran V, Ryan CS, et al. (2008) The Guinea pig as a preclinical model for demonstrating the efficacy and safety of statins. J Pharmacol Exp Ther 324: 576-586.
46. Patterson JK, Lei XG, Miller DD (2008) The pig as an experimental model for elucidating the mechanisms governing dietary influence on mineral absorption. Exp Biol Med (Maywood) 233: 651-664.
47. Fernandez ML, Roy S, Vergara-Jimenez M (2000) Resistant starch and cholestyramine have distinct effects on hepatic cholesterol metabolism in Guinea pigs fed a hypercholesterolemic diet. Nutr Res 20: 837-849.
48. Hatakka K, Mutanen M, Holma R, Saxelin M, Korpela R (2008) *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp

shermanii JS administered in capsules is ineffective in lowering serum lipids. J Am Coll Nutr 27: 441-447.

49. Simons LA, Amansec SG, Conway P (2006) Effect of *Lactobacillus fermentum* on serum lipids in subjects with elevated serum cholesterol. Nutr Metab Cardiovasc Dis 16: 531-535.

50. Lewis SJ, Burmeister S (2005) A double-blind placebo-controlled study of the effects of *Lactobacillus acidophilus* on plasma lipids. Eur J Clin Nutr 59: 776-780.

51. Greany KA, Bonorden MJ, Hamilton-Reeves JM, McMullen MH, Wangen KE, et al. (2008) Probiotic capsules do not lower plasma lipids in young women and men. Eur J Clin Nutr 62: 232-237.

52. Liong MT (2007) Probiotics: a critical review of their potential role as antihypertensives, immune modulators, hypocholesterolemics, and perimenopausal treatments. Nutr Rev 65: 316-328.

53. Challa A, Rao DR, Chawan CB, Shackelford L (1997) *Bifidobacterium longum* and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. Carcinogenesis 18: 517-521.

54. Reddy BS, Watanabe K, Weisburger JH, Wynder EL (1977) Promoting effect of bile acids in colon carcinogenesis in germ-free and conventional F344 rats. Cancer Res 37: 3238-3242.

55. Brady LJ, Gallaher DD, Busta FF (2000) The role of probiotic cultures in the prevention of colon cancer. J Nutr 130: 410S-414S.

56. Goldin BR, Gorbach SL (1984) The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. Am J Clin Nutr 39: 756-761.

57. Fujiwara S, Seto Y, Kimura A, Hashiba H (2001) Establishment of orally-administered *Lactobacillus gasseri* SBT2055SR in the gastrointestinal tract of humans and its influence on intestinal microflora and metabolism. J Appl Microbiol 90: 343-352.

58. Aso Y, Akaza H, Kotake T, Tsukamoto T, Imai K, et al. (1995) Preventive effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer in a double-blind tria*L*. The BLP Study Group. Eur Urol 27: 104-109.

59. Roller M, Rechkemmer G, Watzl B (2004) Prebiotic inulin enriched with oligofructose in combination with the probiotics *Lactobacillus rhamnosus* and *Bifidobacterium lactis* modulates intestinal immune functions in rats. J Nutr 134: 153-156.

60. de Vrese M, Schrezenmeir J (2008) Probiotics, prebiotics, and synbiotics. Adv Biochem Eng Biotechnol 111: 1-66.

61. Randheera RDCS, Baines SK, Adams MC (2010) Importance of food in probiotic efficacy. Food Res Int 43: 1-7.

62. Bazrafshan M, Homayouni Rad A (2010) Effect of environmental stress factors on the survival of *Lactobacillus acidophilus*: A review. Microb Biotechnol J Islamic Azad Univ 2: 47-56.

63. Homayouni Rad A, Ehsani MR, Azizi A, Razavi SH, Yarmand MS (2008) Growth and survival of some probiotic strains in simulated ice cream conditions. J Appl Sci 8: 379-382.

64. Sleator RD, Hill C (2008) New frontiers in probiotic research. Lett Appl Microbiol 46: 143-147.

65. Kailasapathy K, Chin J (2000) Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 78: 80-88.

66. Kolida S, Tuohy K, Gibson GR (2000) The human gut flora in nutrition and approaches for its dietary modulation. Br Nutr Found Nutr Bull 25: 223–231.

67. Farnworth ER (2008) Handbook of fermented functional foods (functional foods and nutraceuticals). (2ndedn), London: Taylor and Francis, UK.

68. Homayouni Rad A, Azizi A, Ehsani MR, Yarmand MS, Razavi SH (2008) Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chem 111: 50-55.

69. Alizadeh A, Homayouni Rad A, Ehsani MR (2008) Probiotic survival in yogurt made from ultrafiltered skim milk during refrigerated storage. Res J Biol Sci 3: 1163-1165.

70. Homayouni Rad A, Ehsani MR, Azizi A, Razavi SH, Yarmand MS (2008) Spectrophotometric evaluation of probiotic growth in liquid media. Asian J Chem 20: 2414-2420.

71. Gibson GR, Probert HM, Loo JV, Rastall RA, Roberfroid MB (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 17: 259-275.

72. Roberfroid M (2007) Prebiotics: the concept revisited. J Nutr 137: 830S-7S.

73. Manning TS, Gibson GR (2004) Microbial-gut interactions in health and disease. Prebiotics. Best Pract Res Clin Gastroenterol 18: 287-298.

74. Gibson RG, Berry OP, Rastall RA (2000) Prebiotics: New Developments in Functional Foods. Oxford, U.K.: Chandos Publishing Limited.

75. López GM, Urías-Silvas EJ (2007) Agave fructans as prebiotics In Shiami N, Benheblia N, Ondera S, eds, Recent Advances in Fructooligosaccharides Research. Kerala: Research signpost. 297-310.

76. Cummings JH, Macfarlane GT, Englyst HN (2001) Prebiotic digestion and fermentation. Am J Clin Nutr 73: 415S-420S.

77. Carpita NC, Kanabus J, Housley TL (1989) Linkage structure of fructans and fructan oligomers from *Triticum aestivum* and *Festuca arundinacea* leaves. J Plant Physiol 134: 162-168.

78. Jackson KG, Taylor GR, Clohessy AM, Williams CM (1999) The effect of the daily intake of inulin on fasting lipid, insulin and glucose concentrations in middle-aged men and women. Br J Nutr 82: 23-30.

79. Kaur N, Gupta AK (2002) Applications of inulin and oligofructose in health and nutrition. J Biosci 27: 703-714.

80. Nair KK, Kharb S, Thompkinson DK (2010) Inulin dietary fiber with functional and health attributes- a review. Food Rev Int 26: 189-203.

81. Jaime L, Martín-Cabrejas MA, Mollá E, López-Andréu FJ, Esteban RM (2001) Effect of storage on fructan and fructooligosaccharide of onion (Allium cepa L.). J Agric Food Chem 49: 982-988.

82. Vega R, Zúniga-Hansen ME (2011) Enzymatic synthesis of fructooligosaccharides with high 1-kestose concentrations using response surface methodology. Bioresour Technol 102: 10180-10186.

83. Bornet FR, Brouns F, Tashiro Y, Duvillier V (2002) Nutritional aspects of short-chain fructooligosaccharides: natural occurrence, chemistry, physiology and health implications. Dig Liver Dis 34 Suppl 2: S111-120.

84. Macfarlane S, Macfarlane GT, Cummings JH (2006) Review article: prebiotics in the gastrointestinal tract. Aliment Pharmacol Ther 24: 701-714.

85. Fanaro S, Boehm G, Garssen J, Knol J, Mosca F, et al. (2005) Galacto-oligosaccharides and long-chain fructo-oligosaccharides as prebiotics in infant formulas: a review. Acta Paediatr Suppl 94: 22-26.

86. Rowland IR, Tanaka R (1993) The effects of transgalactosylated oligosaccharides on gut flora metabolism in rats associated with a human faecal microflora. J Appl Bacteriol 74: 667-674.

87. Djouzi Z, Andrieux C (1997) Compared effects of three oligosaccharides on metabolism of intestinal microflora in rats inoculated with a human faecal flora. Br J Nutr 78: 313-324.

88. Morishita Y, Oowada T, Ozaki A, Mizutani T (2002) Galactooligosaccharide in combination with *Bifidobacterium* and Bacteroides affects the population of *Clostridium perfringens* in the intestine of gnotobiotic mice. Nutr Res 22: 1333-1341.

89. Alles MS, Hartemink R, Meyboom S, Harryvan JL, Van Laere KM, et al. (1999) Effect of transgalactooligosaccharides on the composition of the human intestinal microflora and on putative risk markers for colon cancer. Am J Clin Nutr 69: 980-991.

90. Alander M, Mättö J, Kneifel W, Johansson M, Kögler B, et al. (2001). Effect of galacto-oligosaccharide supplementation on human faecal microflora and on survival and persistence of *Bifidobacterium lactis* Bb-12 in the gastrointestinal tract. Int Dairy J 11: 817-825.

91. Satokari RM, Vaughan EE, Akkermans AD, Saarela M, De Vos WM (2001) Polymerase chain reaction and denaturing gradient gel electrophoresis monitoring of fecal *Bifidobacterium* populations in a prebiotic and probiotic feeding trial. Syst Appl Microbiol 24: 227-231.

92. Gopal PK, Prasad J, Gill HS (2003) Effects of the consumption of *Bifidobacterium lactis* HN019 (DR10TM) and galacto-oligosaccharides on the microflora of the gastrointestinal tract in human subjects. Nutr Res 23: 1313-1328.

93. Bouhnik Y, Raskine L, Simoneau G, Vicaut E, Neut C, et al. (2004) The capacity

of nondigestible carbohydrates to stimulate fecal *Bifidobacteria* in healthy humans: a double-blind, randomized, placebo-controlled, parallel-group, dose-response relation study. Am J Clin Nutr 80: 1658-1664.

94. Depeint F, Tzortzis G, Vulevic J, I'Anson K, Gibson GR (2008) Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of *Bifidobacterium bifidum* NCIMB 41171, in healthy humans: a randomized, double-blind, crossover, placebo-controlled intervention study. Am J Clin Nutr 87: 785-791.

95. Yang ST, Silva EM (1995) Novel products and new technologies for use of a familiar carbohydrate, milk lactose. J Dairy Sci 78: 2541-2562.

96. Boehm G, Lidestri M, Casetta P, Jelinek J, Negretti F, et al. (2002) Supplementation of a bovine milk formula with an oligosaccharide mixture increases counts of faecal *Bifidobacteria* in preterm infants. Arch Dis Child Fetal Neonatal Ed 86: F178-181.

97. Weaver LT (2003) Improving infant milk formulas: near the end of the trail for the holy grail? J Pediatr Gastroenterol Nutr 36: 307-310.

98. Saunders DR, Wiggins HS (1981) Conservation of mannitol, lactulose, and raffinose by the human colon. Am J Physiol 241: G397-402.

99. Fadden K, Owen RW (1992) Faecal steroids and colorectal cancer: the effect of lactulose on faecal bacterial metabolism in a continuous culture model of the large intestine. Eur J Cancer Prev 1: 113-127.

100. Terada A, Hara H, Kataoka M, Mitsuoka T (1992) Effect of lactulose on the composition and metabolic activity of the human faecal flora. Microb Ecol Health Dis 5: 43-50.

101. Ballongue J, Schumann C, Quignon P (1997) Effects of lactulose and lactitol on colonic microflora and enzymatic activity. Scand J Gastroenterol Suppl 222: 41-44.

102. Tuohy KM, Ziemer CJ, Klinder A, Knöbel Y, Pool-Zobel BL, et al. (2002) A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic microbiota. Microb Ecol Health Dis 14: 165-173.

103. De Preter V, Vanhoutte T, Huys G, Swings J, Rutgeerts P, et al. (2006) Effect of lactulose and *Saccharomyces boulardii* administration on the colonic urea-nitrogen metabolism and the *Bifidobacteria* concentration in healthy human subjects. Aliment Pharmacol Ther 23: 963-974.

104. Vázquez MJ, Alonso JL, Domínguez H, Parajó JC (2000) Xylooligosaccharides: manufacture and applications. Trends Food Sci Technol 11: 387-393.

105. Moure A, Gullón P, Domínguez H, Parajó JC (2006) Advances in the manufacture, purification and applications of xylo-oligosaccharides as food additives and nutraceuticals. Process Biochem 41: 1913-1923.

106. Rycroft CE, Jones MR, Gibson GR, Rastall RA (2001) A comparative *in vitro* evaluation of the fermentation properties of prebiotic oligosaccharides. J Appl Microbiol 91: 878-887.

107. Zampa A, Silvi S, Fabiani R, Morozzi G, Orpianesi C, et al. (2004) Effects of different digestible carbohydrates on bile acid metabolism and SCFA production by human gut micro-flora grown in an *in vitro* semi-continuous culture. Anaerobe 10: 19-26.

108. Campbell JM, Fahey GC Jr, Wolf BW (1997) Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J Nutr 127: 130-136.

109. Hsu CK, Liao JW, Chung YC, Hsieh CP, Chan YC (2004) Xylooligosaccharides and fructooligosaccharides affect the intestinal microbiota and precancerous colonic lesion development in rats. J Nutr 134: 1523-1528.

110. Santos A, San Mauro M, Díaz DM (2006) Prebiotics and their long-term influence on the microbial populations of the mouse bowel. Food Microbiol 23: 498-503.

111. Lecerf JM, Dépeint F, Clerc E, Dugenet Y, Niamba CN, et al. (2012) Xylo-oligosaccharide (XOS) in combination with inulin modulates both the intestinal environment and immune status in healthy subjects, while XOS alone only shows prebiotic properties. Br J Nutr 108: 1847-1858.

112. Topping DL, Fukushima M, Bird AR (2003) Resistant starch as a prebiotic and synbiotic: state of the art. Proc Nutr Soc 62: 171-176.

113. Le Blay G, Michel C, Blottière HM, Cherbut C (1999) Enhancement of butyrate production in the rat caecocolonic tract by long-term ingestion of resistant potato starch. Br J Nutr 82: 419-426.

114. Dongowski G, Jacobasch G, Schmiedl D (2005) Structural stability and prebiotic properties of resistant starch type 3 increase bile acid turnover and lower secondary bile acid formation. J Agric Food Chem 53: 9257-9267.

115. Jacobasch G, Dongowski G, Schmiedl D, Müller-Schmehl K (2006) Hydrothermal treatment of Novelose 330 results in high yield of resistant starch type 3 with beneficial prebiotic properties and decreased secondary bile acid formation in rats. Br J Nutr 95: 1063-1074.

116. Silvi S, Rumney CJ, Cresci A, Rowland IR (1999) Resistant starch modifies gut microflora and microbial metabolism in human flora-associated rats inoculated with faeces from Italian and UK donors. J Appl Microbiol 86: 521-530.

117. Brown I, Warhurst M, Arcot J, Playne M, Illman RJ, et al. (1997) Fecal numbers of *Bifidobacteria* are higher in pigs fed *Bifidobacterium longum* with a high amylose cornstarch than with a low amylose cornstarch. J Nutr 127: 1822-1827.

118. Ridley BL, O'Neill MA, Mohnen D (2001) Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry 57: 929-967.

119. Olano-Martin E, Mountzouris KC, Gibson GR, Rastall RA (2001) Continuous production of pectic oligosaccharides in an enzyme membrane reactor. J Food Sci 66: 966-971.

120. Fishman ML, Chau HK, Hoagland P, Ayyad K (2000) Characterization of pectin, flash-extracted from orange albedo by microwave heating, under pressure. Carbohydr Res 323: 126-138.

121. Olano-Martin E, Gibson GR, Rastell RA (2002) Comparison of the *in vitro* bifidogenic properties of pectins and pectic-oligosaccharides. J Appl Microbiol 93: 505-511.

122. Manderson K, Pinart M, Tuohy KM, Grace WE, Hotchkiss AT, et al. (2005) *In vitro* determination of prebiotic properties of oligosaccharides derived from an orange juice manufacturing by-product stream. Appl Environ Microbiol 71: 8383-8389.