Prebiotics: A role for dietary fibre and resistant starch?

Anthony R Bird MAgrSc, PhD

CSIRO Human Nutrition, Adelaide, South Australia, Australia

Diet influences the composition and metabolic activity of the colonic microflora, which in turn may modulate host susceptibility to a wide range of infectious and non-infectious diseases. Efforts to lower bowel disease risk can involve changes to the microflora. Bifidobacteria have been a major target of dietary-based manipulations, particularly by prebiotics, to favourably alter the intestinal microbial balance. Most research has focused on non-digestible oligosaccharides, especially fructo-oligosaccharides, and has shown that these substrates selectively promote intestinal populations of *Bifidobacterium* and are therefore effective prebiotics. Recent experimental research suggests that certain polymerized carbohydrates may have similar potential. Resistant starches (RS) are utilized by numerous groups of enteric bacteria; however, *Bifidobacterium* species appear more efficient in using RS as a substrate for proliferation. Consumption of RS augments intestinal populations of *Bifidobacterium* in some animal models. *In vivo* studies also indicate that the type of RS has a profound influence on the bacterial population structure of the hindgut. The beneficial effects of various oligosaccharides and/or RS can be further improved by combining them with probiotics (synbiotics). The limited data on dietary fibre indicate that certain non-starch polysaccharides apparently do not stimulate faecal densities of beneficial bacteria; however, by suppressing populations of pathogenic strains, they may nevertheless improve intestinal microbial balance. There are, therefore, clear opportunities for the food industry to develop ingredients and formulate foodstuffs with specific functional properties and potential for lessening risk of bowel diseases such as colorectal cancer.

Key words: prebiotics, diet, dietary fibre, resistant starch, microflora, bowel disease.

Introduction

Bacteria are a major cause of infectious gastrointestinal diseases that account for considerable morbidity and mortality worldwide. Perhaps less well known is the suspected role of gut bacteria in the pathogenesis of several major non-infectious bowel disorders, including inflammatory bowel disease,[1] irritable bowel syndrome,[2] and colorectal cancer.[3] Various strains of intestinal bacteria have been linked to initiation or promotion of carcinogenesis through their ability to transform apparently benign luminal substrates to potentially harmful metabolites.[4] Putrefactive bacteria in particular have long been the focus of attention because they catabolise proteins and amino acids to N-containing compounds, especially ammonia and amines, that are possible aetiological agents in neoplastic transformation.

Components of the colonic microflora, however, confer benefits on the host. Endproducts of enteric bacterial metabolism, especially short-chain fatty acids (SCFA), are vital for sustaining large bowel mucosal integrity and ensuring normal physiologic function.[5] The established (normal) microflora also provide a highly effective barrier against colonization of the bowel by potential microbial pathogens.[6] In effect, the beneficial components of indigenous microflora are thought to create a micro-environment in the large bowel that is unfavourable to the growth and/or survival of undesirable bacteria, thereby suppressing their metabolic activity, as well as their establishment and proliferation in the bowel. Furthermore, the physicochemical properties of the luminal micro-environment are such that mucosal exposure to mutagens and other toxins is lessened when beneficial bacterial species are predominant members of the colonic microflora. Thus, as various bacteria appear to promote or afford protection against diseases such as cancer, the microbial profile of the colon may be an important determinant of bowel health.

Taxonomic composition and metabolic activity of the microflora of the large bowel are determined by several factors, of which exogenous substrate supply is of particular importance. Therefore, dietary modulation to create a balance in favour of beneficial bacteria conceivably offers potential for developing and maintaining an optimal intestinal flora with respect to minimizing risk for acute and chronic intestinal disease. However, such an approach rests entirely on the premise that the target colonic bacteria possess clearly defined health-promoting attributes.

Beneficial intestinal bacteria

Species of the genera bifidobacteria and lactobacilli, which are predominant and subdominant members, respectively, of the colonic ecosystem, are deemed to be beneficial for the host.[7,8] Their positive association with host health derives from various favourable properties, namely, antibacterial, antimutagenic and anticarcinogenic activity, that have been demonstrated largely by studies in experimental animals and *in vitro*.[9–11]

The health benefits of elevated levels or relative proportions of either *Bifidobacteria* or *Lactobacilli* in the colonic microflora have yet to be comprehensively established.

Correspondence address: Dr Anthony Bird, CSIRO Human Nutrition, PO Box 10041, Adelaide BC 5000, SA, Australia.
Tel: 61 8 8303 8902: Fax; 61 8 8303 8899
Email: tony.bird@dhn.csiro.au

Kubota[12] described an inverse association between colon cancer incidence and colonic populations of bifidobacteria; however, in a recent study, faecal bifidobacteria level was positively correlated with greater colon cancer risk, whereas there was a negative association for some lactobacillus species.[13] Other studies also suggest that faecal Lactobacilli counts are greater in populations with a comparatively lower risk for colon cancer.[14] However, in general, epidemiological studies examining relationships between faecal microflora and colon cancer risk have produced ambivalent outcomes.

Intestinal populations of *Bifidobacterium* and/or *Lactobacillus* can be increased directly by administering live cultures of these bacteria, thereby improving intestinal microbial balance and, supposedly, host health. Indeed, this probiotic strategy has proved very effective for selective manipulation of the gut microflora, and regular ingestion of large doses of viable micro-organisms elicits substantial qualitative and quantitative changes in the human faecal flora. Purported health benefits of probiotics are extensive, and include prevention and amelioration of diarrhoea, as well as other antipathogenic properties, promotion of intestinal motility and laxation, anticarcinogenesis, and stimulation of the enteric and systemic immune systems.[15]

However, results of numerous clinical trials evaluating efficacy of probiotics in the prevention or treatment of various human disorders are equivocal. Nevertheless, studies involving experimental animal models have yielded largely consistent results demonstrating that oral administration of specific probiotic strains under well-controlled experimental conditions augments local and systemic immune responses, reduces intestinal and faecal densities of potential pathogens and has antitumour actions.[15,16]

Prebiotics

Although species composition of the colonic microflora can be modulated by probiotic bacteria, the gut ecosystem has evolved highly effective defence mechanisms specifically designed to prevent foreign organisms from colonizing or invading its luminal surface. An alternative to probiosis for stabilizing or improving the host's intestinal microbial balance is provision of exogenous substrates that selectively promote proliferation and/or activity of beneficial bacteria indigenous to the intestinal tract. This concept, known as prebiosis, was introduced by Gibson and Roberfroid, and prebiotics were defined as 'nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and (or) activity of one or a limited number of bacterial species already resident in the colon, and, thus, attempt to improve host health'.[8] A dietary-based strategy to manipulate the gut microbiota for the express purpose of improving host health and bolstering disease resistance offers opportunities either alone or in addition to a microbiological approach.

The major source of nourishment for colonic bacteria is fermentable carbohydrates that have escaped digestion and absorption in the upper gut.[5] Consequently, it is not surprising that saccharolytic species, principally of the genera *Bacteroides, Eubacterium, Bifidobacterium, Ruminococcus* and *Lactobacillus*, are numerically dominant in the large bowel ecosystem. Recent investigations suggest that the colonic microflora are amenable to changes in supply of certain dietary indigestible carbohydrates, and their incorporation into the diet results in distinctive and consistent changes in faecal microbial profile and metabolic activity.

Non-digestible oligosaccharides

Of the possible prebiotic carbohydrates, naturally occurring and synthetic non-digestible oligosaccharides (NDO), especially those containing fructose (fructo-oligosaccharides; FOS), have attracted most attention. Chicory or inulin-type fructans (a mixture of oligo- and polysaccharides) are considered the archetypal prebiotic.[17] When consumed in sufficient quantity, FOS dramatically and consistently stimulate bifidobacteria proliferation, often to such an extent that this organism usually becomes the major bacterium of the faecal flora.[18–20] Although numerous types of enteric bacteria other than bifidobacteria have the capacity to ferment NDO *in vitro*, it appears that, in the complex ecosystem of the large bowel, bifidobacteria outcompete other species.[21] Non-digestible oligosaccharides, therefore, selectively promote bifidobacteria proliferation largely at the expense of other bacterial groups, such as *Escherichia coli* and clostridia.[19,21,22]

Although most attention has been focused on FOS, recent investigations have shown that NDO-containing galactose, xylose, mannose and glucose moieties are also selectively bifidogenic.[23,24] Studies in pigs have demonstrated that GOS and FOS appear to be equally effective in acidifying the lumen of the proximal colon, and in their ability to elicit absolute and relative changes in individual SCFA (DL Topping *et al.*, unpubl. data, 1997).

Studies using chemically induced cancer rat models have indicated that NDO supplementation may lower the risk of bowel cancer, as indicated by improvements in biomarkers including aberrant crypt foci, and tumour multiplicity and burden.[16] Studies in humans showed that FOS supplementation (neosugar 4 g/day for 25 days) substantially reduced β-glucuronidase and glycocholic hydroxylase activities in faeces,[25] which is suggestive of a reduced cancer risk. In addition to possible anticancer effects, NDO may have other health and nutritional advantages, including enhancing intestinal nutrient absorption.[7]

Dietary NDO provide a supply of readily fermentable substrates for the colonic microflora. Consequently, the metabolic alterations induced by rapidly fermentable substrates, such as oligofructose, may be limited to the proximal colon.[24] Indeed, numerous *in vivo* experiments in animals, such as that conducted by Brown *et al.*, have confirmed this hypothesis.[26] Because the predominant intestinal site for cancer and several other degenerative bowel diseases is the distal bowel,[27] consumption of oligosaccharide prebiotics may not equate to a lowered risk for malignant disease of the distal colon.

As with probiotics, prebiosis is also transient, and any favourable alteration of the intestinal flora persists for not much longer than the period of prebiotic supplementation. However, as noted by Buddington *et al.*, the return to baseline faecal bifidobacterial (and total anaerobe) densities may be more rapid following withdrawal of probiotics, than with prebiotics.[25] Brown *et al.* showed that synbiotics may enhance persistence of the improved microflora state in pigs

supplemented with resistant starch and *Bifidobacterium longum*.[26]

Resistant starch

Large amounts of undigested and indigestible starch and starch residues (resistant starch; RS) reach the colon and, quantitatively, are the most important substrate for the colonic microflora.[5] Starch may be resistant to digestion because it is physically entrapped (RS1, for example, in coarsely milled grains); consists of resistant granules (RS2, for example high-amylose maize starch); or has undergone retrogradation (RS3; resulting from cooking and cooling starchy foods). The fourth category comprises chemically modified starches (RS4) which are used extensively by the food industry to improve physical properties of manufactured foodstuffs. Diets rich in RS have been shown to increase intestinal fermentation and improve indices of bowel function and health in humans, including increased stool mass and SCFA excretion, and faecal acidification. Resistant starch qualifies as a colonic food, but is not considered to fulfil the criteria for classification as a prebiotic because its effect on the microflora may lack specificity.[8] However, *in vitro* and recent *in vivo* studies suggest that RS may be preferentially utilized by beneficial colonic bacteria, resulting in production of putative beneficial metabolites, particularly butyrate.

Many different types of colonic (saccharolytic) bacteria, including *Eubacterium, Bacteroides* and *E. coli*,[28,29] ferment starch; also several species of *Bifidobacterium* and *Clostridium butyricum* were shown to be particularly efficient *in vitro* utilizers of certain starch preparations.[28] A high-amylose maize starch has been shown to be a potent bifidogenic agent in mice, and chemical modification of this RS had profound effects on its bifidogenicity.[28] Similarly in rats, resistant potato starch was shown to be bifidogenic and various forms of RS (RS2 and RS3) had differential effects on faecal and caecal microflora.[30] However, in a preliminary report, consumption of foods containing high amylose starch (RS2) by volunteers resulted in lower faecal densities of bifidobacteria, and higher butyrate levels, compared with a cornflake control diet.[31] In young pigs fed rice-based diets designed to increase the quantity of starch (RS1 and RS3) flowing into the caecum, counts of bifidobacteria and lactobacilli in the proximal colon were similar to those in pigs fed a digestible starch diet (AR Bird *et al.*, unpubl. data, 1998). However, numbers of coliforms, especially *E. coli*, were several log units lower in pigs offered the RS-rich diet.

Certain forms of RS may also promote intestinal lactobacilli populations. Kleessen *et al.* have shown that diets containing retrograded potato starch (RS3), but not native potato starch (RS2), increased total lactobacilli numbers in faeces and caecal digesta in rats.[30] In addition to the quantitative changes in total lactobacilli, the type of RS that was ingested produced substantial differences in species composition of the caecal *Lactobacillus* population.

Although the data is limited to a few nutritional studies in animal models and humans, it suggests that various starches, upon reaching the large bowel, have a favourable effect on the composition and metabolic activity of colonic microbiota. The data also suggest that these effects are dependent not only on the amount, but also in particular on the chemical and physical properties, of starches that reach the colon.

Non-starch polysaccharides

Non-starch polysaccharides (NSP), the primary constituent of dietary fibre, are important fuels for many different groups of colonic bacteria. Consequently, because NSP apparently have general, rather than selective, effects on colonic microflora, they are not considered prebiotics.[8] However, studies examining the effects of specific NSP on the composition of the human microflora are few. A further complication in determining the prebiotic potential of NSP is that they rarely occur in homogenous form in foods, and are often present with various other fermentable substrates, for instance starch, that may interfere with their fermentation.[32]

In a preliminary nutritional study in humans, supplementation of the habitual diet of volunteers with high fibre foods prepared from wheat bran or wheat aleurone concentrate tended to slightly reduce faecal densities of bifidobacteria, while numbers of lactobacilli were unaffected by dietary fibre treatments (AR Bird *et al.*, unpubl. data, 1998). There were also indications that the high-fibre supplements improved the microbial balance in the colon by reducing coliform populations. Rao *et al.* have shown that consumption of foods rich in insoluble and soluble fibres initially increased faecal anaerobes and bifidobacteria densities; however, after several weeks, values had returned to their baseline level.[33] In both studies, consumption of the fibre-rich diets improved putative metabolic and physiologic indices of bowel health, despite little apparent change in faecal populations of beneficial bacteria.

In rats, consumption of guar gum was associated with higher caecal numbers of bifidobacteria relative to fibre-free controls.[34] However, there were also increased numbers of several other bacterial groups and, therefore, the non-selective response precludes this fibre from fulfilling the requirements of a prebiotic. In the same study caecal lactobacilli levels were remarkably similar, regardless of whether the diet was fibre-free or contained 10% guar gum or pectin.

Prebiotic and probiotic combinations: Synbiotics

Synbiotics are a mixture of pre- and probiotics. When these ingredients are combined in a single product the efficacy of either or both may be enhanced. For instance, in the chemically induced cancer rat model, the reduction in colonic preneoplastic lesions in response to the ingestion of the disaccharide, lactulose, or inulin (FOS/polysaccharide), together with *B. longum*, was greater than when either of these dietary treatments was given alone.[35–37] Indeed, it is possible that in order to reduce colon cancer risk, only the combined treatment of FOS and bifidobacteria may prove effective.[36] Further evidence of the promotional effects of prebiotic carbohydrates on probiotic activity is provided by *in vitro* experiments. Kullen *et al.* have demonstrated that the inhibitory actions of bifidobacteria against the growth of *Clostridium perfringens* is enhanced by certain oligosaccharides.[38]

In pigs supplemented with *B. longum*, faecal excretion of total bifidobacteria was greater, and the rate of decline in faecal numbers of bifidobacteria after probiotic withdrawal was markedly reduced, when the diet contained high-

amylose maize starch.[26] Inclusion of high-amylose maize starch (RS2) in a probiotic preparation maintained higher densities of viable probiotic micro-organisms compared with products devoid of RS.[28] Synbiotics may be more effective than prebiotics because the prebiotic affords a degree of protection for probiotic bacteria as they pass through the upper gut, thereby enhancing delivery of large numbers of viable probiotic cells to the colon as well as, possibly, assisting in their implantation and proliferation in the colon.

Conclusion

There is increasing scientific evidence to suggest that ingestion of various NDO, dietary fibres and RS significantly alters the microflora profile of the large bowel, and that such changes are perceived to benefit the health of the host. Although the focus to date has been on bifidobacteria and NDO, and it has been clearly shown that these exogenous substrates elicit specific and consistent changes in hindgut microflora, results of recent studies suggest that other non-digestible carbohydrates have similar potential for improving the microbial balance of the gut. Specific types of resistant starch, for instance, may reduce bowel disease risk by selective repression of pathogens, or by effecting positive quantitative changes in populations of potentially beneficial bacteria. Therefore, dietary intervention using a range of dietary fibres offers potential for promoting large numbers of beneficial or benign bacteria in the large bowel while suppressing the growth and metabolic activity of, and possible epithelial colonization by, potentially deleterious species. Combinations of different prebiotics (including chemically modified novel RS), especially when administered together with probiotics, appear particularly effective agents for favourably manipulating the composition and metabolic activity of the large bowel ecosystem.

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