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REVIEW ARTICLE

Prebiotics and health: Clinical implications

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ABSTRACT

Prebiotic agents have been shown to have significant clinical beneficial effects in the prevention and management of gastrointestinal and non- gastrointestinal conditions. Prebiotics are short chain fatty carbohydrates that alter the composition or metabolism, of the gut microbiota in a beneficial manner. It is therefore expected that prebiotics will improve health in a way similar to probiotics, whilst at the same time being cheaper, and carrying less risk and being easier to incorporate into the diet than probiotics. These observations have led to work demonstrating that an important mechanism of these agents in their close interaction with the gut associated lymphoid tissue [GALT]. The preliminary finding of several recent human clinical trials reviewed in this article indicates that prebiotics may indeed prove to be beneficial dietry supplement, in the context of novel nutritional strategies for the management and systemic conditions.

Key words: Humans, Prebiotics, Health effects

Introduction

The term "*prebiotic*" was coined in 1995 by Prof Glenn Gibson and Prof Marcel Roberfroid. They defined it as a "non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health" [1].

Prebiotics are important because of: [i] the growing belief that there is such a thing as a healthy or balanced gut microbiota, [ii] the demonstration that prebiotics can alter the composition of the microbiota towards this more healthy profile, [iii] as an alternative to probiotics, which can be difficult to handle in some foodstuffs, but whose benefits to health in terms of diarrhea prevention and immunomodulation are becoming increasingly well established and [iv] because prebiotics currently in use, especially inulin and its derivatives, and galacto-oligosaccharides [GOS] are relatively cheap to manufacture or extract from plant sources. In addition to having beneficial effects on the gut microbiota and host, they are also valuable functional ingredients in foods with the potential to give fat-based spreads and dairy products with improved organoleptic properties. Gibson et al [2] reviewed their original prebiotic concept in the light of research published over the past 10 years, particularly the three key aspects of the original definition: [i] resistance to digestion, [ii] fermentation by the large intestinal microbiota and [iii] a selective effect on the microbiota that has associated health-promoting effects. It is now proposed that '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'. The key ideas in both this and the earlier definition are 'selective' and 'benefit/improve host health' [2].

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Sources and Effective dose

Prebiotics are found naturally in many plants including leeks, onions, wheat, garlic, chicory root and artichokes where they function as storage carbohydrates and soluble fibers in pulses, fruit and some cereal products. A number of poorly digested carbohydrates fall into this category of prebiotics including certain fibers and resistant starch [3] but the most widely described prebiotics are nondigestible oligosaccharides [NDO's]. These are low molecular weight carbohydrates with 2-10 degree of polymerization, which are poorly digested in the small intestine thus reaching the colon largely unaltered and can act as a substrate for the colonic micro flora. Effective doses of oligosaccharides are in the range of 5-10 grams/day for healthy adults. Doses less than 5 grams are generally considered ineffective [2], [4]. Here a review of published evidence on the health effects of prebiotics in humans is given.

Characteristics features of Prebiotics

There are many carbohydrates that are non-digestible, hence called dietary fibers. If fermented they promote bacterial growth but only a few are true prebiotics that meet all the criteria. The definition is important because it clearly outlines the three criteria that must be met before an ingredient can be classified as a *prebiotic*, namely that it should: Besides being non-digestible oligosaccharide, which selectively stimulate the growth and/or metabolic activity of the 'good' some more criteria have been recommended;

- It may not be hydrolyzed or absorbed in the upper gastrointestinal tract.
- It should alter the colonic micro flora in favour of a composition more favorable to the individual's health.
- It must induce luminal or systemic effects which effect a beneficial change in the health of the host organism.
- Be fermented by bacteria in the large intestines.
- It should improve conditions associated with both constipation and diarrhea.
- It should be able to resist pH and enzymatic action conditions found in the human stomach and intestines.

Prebiotic candidates

A team of scientists, led by Prof Glenn Gibson from the University of Reading [UK], evaluated a range of candidate prebiotics in 2004 giving their verdict on each. Only three met the strict criteria [2]:

1. Inulin & Oligofructose
2. Galacto-Oligosaccharides
3. Lactulose

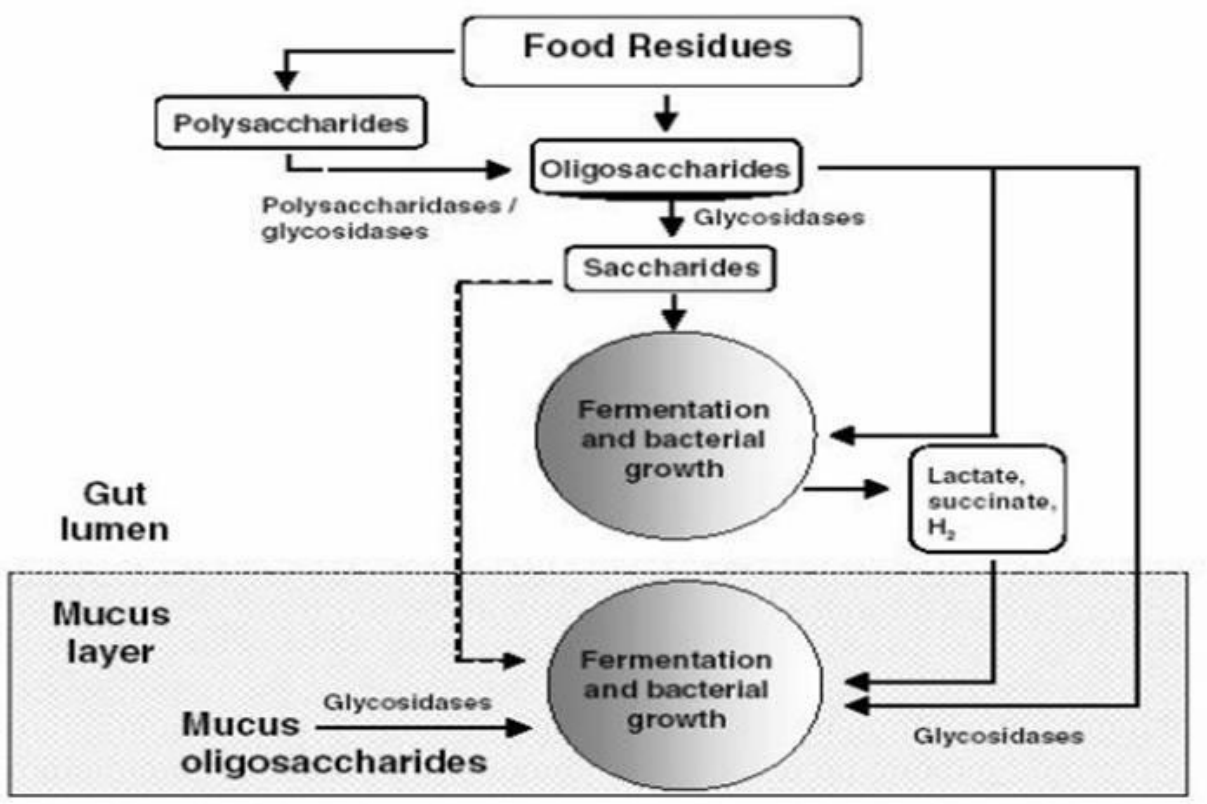
The health effects of FOS studied and reviewed here are change in the microbiota of the gut, bowel habit and constipation, inflammatory bowel disease, antibiotic associated diarrhea, mineral absorption and bones, lipid metabolism, carcinogenesis and its effect on immune response.

Change in the microbiota of the gut

The intake of prebiotics resists the growth of unwanted bacteria in the gut and stimulates the growth of wanted bacteria i.e. probiotics. Inulin, Fructooligosacharides (FOS), trans GOS & Lactulose, when taken in diet in relatively small amounts (5-20g/day) have been clearly shown in human studies to stimulate growth of health promoting species belonging to the genera *Bifidobacterium* and *Lactobacillus*, which ordinarily are not the most numerous organism in the gut except in the breastfed baby [2,4]. Macgillivray used lactulose many years ago as prebiotic formula as food supplement to increase number of *Lactobacilli* in infant intestine [5]. The reduction of the ammonia concentration in human blood and the production of short-chain fatty acids, especially of acetate, have been regarded as important effects after the application of lactulose [6], [7] [8]. Hayakawa et al observed no significant effects on faecal pH or amino acid breakdown products [p-cresol, phenol and indole] despite changes in faecal *Bifidobacteria* numbers in human volunteers, given soy bean oligosaccharides [SOE]; [10g/day] with or without simultaneous consumption of *B. breve* [9]. Cumming *et al* [10] showed that prebiotics effect the growth of *Bifidobacteria* and *Lactobacilli* at the expense of other groups of bacteria in the gut, such as *Bacteriodes, Closteridia, Enterobacterial, Enterococci*, etc. In practice, studies show that such selectivity is variable and the extent to which the microbiota allow a substance to be called prebiotic have not been established, although this may have to be undergone in the near future for food labeling and health claim purposes. For example wide variations are evident in the ratios of *Bifidobacteria* to *Bacteroides* in normal faeces from around .08 to 1.07 & an equally wide range in microbial

growth responses occur in human volunteers follow prebiotic consumption with final ratio of these organism from .40 to 5.0 [10]. In adults, consumption of fructooligosacharides resulted in the numerical predominance of *Bifidobacteria* in faeces [1, 11]. Lindsay et al. in a small open label trial in humans observed that, 10 patients with active ileo-colonic disease were given 15g FOS daily for three weeks. A significant reduction in Harvey Bradshaw Index of disease activity was observed and feacal *Bifidobacteria* increased from $\log_{10}$ 8.8 to $\log_{10}$ 9.4/cell/g/dry faeces [12]. The purposed mechanism whereby dietary substrates become available for mucosa-associated microbiotas in the large intestine is given in [Table/Fig 1] by Macfarlane S. et al [13].

Table/Fig 1



Bowel habit and constipation

Any carbohydrate that reaches the large bowel should have a laxative effect, whether fermented or not. The results of seven published investigations in which mean daily faecal weight was summarized and the response to a prebiotic determined [14-20] reveal this fact. When the extent of change in bowel habit is normalized to per gram of prebiotic ingested, it can be noted that a significant increase in stool output is seen in only two of the seven studies. This is 1.3 g of stool/g of prebiotic for OF [134–154 g of stool/day] in the study of Gibson et al [15] and 2.4 g/g for inulin [129–204 g/day] in the study of Castiglia-Delavaud et al [18]. Therefore prebiotics are only mildly laxative, as these results compare with an increase of stool output of 5.4 g/g for NSP from wheat and 3.7 g/g for gums and mucilages, such as ispaghula, sterculia, etc. [21]. Measuring small changes in mean daily faecal weight is, however, difficult and requires accurate methods by using appropriate faecal markers. At this comparatively early stage in the study of prebiotics, it might be noted, that inulin appears to be a better laxative than OF [22]. This could be due to its higher molecular weight, and the lower solubility of inulin resulting in its slower fermentation, an argument also made by Van Loo et al [23] in respect of several properties of these fructans. The laxative properties of inulin have long been known, and were in fact, first reported in 1912 by Lewis et al [24]. Almost all

studies showed a clear *bifidogenic* effect, so this alone is not sufficient to change bowel habit. They also report increased flatulence and bloating in many volunteers, as well as changes in fermentation patterns. These include an increase in faecal nitrogen, largely due to increased excretion of bacterial cell mass as a result of carbohydrate breakdown, increased faecal energy, lower pH, but no change in SCFA concentrations in faeces, or bile acid profiles. Studies of prebiotics in the management of constipation have mostly been qualitative, relying on bowel habit diaries, and subjective patient reports of symptoms. The efficiency and tolerance of lactulose in constipation in pregnant women [25] and in the treatment of chronic constipation in children [26] have been discussed. Lactulose has been shown to have laxative effects in humans, depending on the individual has been defined by Clausen et al [27]. Kleesen et al [22] found some subject-to-subject variation in their study comparing lactose and FOS (in the form of inulin), given to elderly subjects in dosage of 20g increasing to 40g/day and showed that inulin had more lexative action. Teuri and Korpela [28] found that GOS (9g/day) relieved constipation in some but not in all elderly subjects mainly by making defecation easier. Shitara [29] reported that in japanese subjects GOS was effective as laxative.

Inflammatory bowel disease and antibiotic associated diarrhea

Prebiotics are found to be helpful in the prevention of inflammatory bowel disease and antibiotic associated diarrhea. The anti-inflammatory effect of prebiotics has been reported in pouchitis patients by Welters et al [30]. In a randomized double-blind crossover study, 24 patients with stable asymptomatic pouchitis were given 20g of inulin or placebo daily, for 3 weeks each. At the end of prebiotic period, results showed that there was a reduction in the edoscopic and histological pouchitis disease activity index (PDAI) score, together with lower gut pH, reductions in faecal *Bacteroids fragilis* and secondary bile acids. Butyrate concenteration was increased while symptoms score were low initially and were essentially unchanged. Brunser et al [31] reported a randomised control trial [RCT] in children aged 1-2 years who were given a mixture of FOS and inulin after 1 week of amoxicillin therapy for acute bronchitis. A significant increase in faecal *Bifidobacteria* was seen on day 7th of the prebiotic supplement without any apparent change in diarrheal symptoms. Three RCT of prebiotics and the prevention of of antibiotic-associated diarrhoea (AAD) have been reported in a study by Lewis et al [32] who undertook a large study involving 435 patients aged over 65 years, who were hospitalized and have been prescribed a broad spectrum antibiotic in the 24 h before the study. They were randomized to receive either 12 g of OF daily or placebo, for the duration of the antibiotic treatment, and 1 week beyond. The end points were based on a stool form and defecation frequency diary, and feacal microbiology. Twenty-seven percentage of all patients developed diarrhoea, of which 11% had *C. difficile* toxin-positive stools. Oligofructose made no difference to the risk of diarrhoea, or other aspects of bowel habit, or *C. difficile* infection [32]. *Bifidobacterial* counts increased in the OF group and decreased in the control group. The authors suggested that in the presence of antibiotic, OF does not show such selectivity in changing the microbiota, and may also have stimulated the growth of other anaerobes. However, in another RCT, Lewis et al (2005) successfully observed prevention of diarrhea in patient with *C. difficile* associated symptoms which were treated with metrinidazole and vancomycine. They used 12g of OF and given for thirty days. Follow-up was for a further 30 days. FOS significantly reduced episodes of diarrhea from 34.3% (placebo) to 8.3% (FOS; P< .001). Hospital length of stay was also reduced and *Bifidobacterial* no. increased significantly by the prebiotic [32].

Mineral absorption and bones

Prebiotics can increase mineral absorption which in turn helps in improving bone mineral density. Direct evidence substantiating the effects of FOS and other prebiotics in improving mineral absorption in humans, is increasing. Van den et al [33] observed that dietary Ca absorption increased significantly by 26% from 47.8% to 60.1% in adolescents fed 5g/day of FOS.

In a study of young man the results were conflicting, possibly because two different methods for measuring calcium absorption were used. The double isotope method of Van Den et al [34] carried out at day 21st of the diet period did not show a benefit of either inulin, FOS or GOS, despite a reasonable dose of prebiotic (15g/day) The authors subsequently felt that the

double isotope technique they used did not include the colonic component of calcium absorption [34] because 24h urine was used to calculate isotope enrichment, which would not allow long enough for a colonic phase to be detected. However the double isotope technique has been used successfully in adolescents to demonstrate enhanced absorption although urine collection in these studies was for 36h [34] or 48 hrs [35]

Moreover, the effects of FOS are not confined to calcium. Magnesium absorption has also been shown to increase when ingesting FOS [36]. Ducros et al [37] reported that feeding 10 g of FOS per day for 5 weeks increased the absorption of copper in healthy postmenopausal women. Taken together, these studies give a strong indication that prebiotics can increase calcium absorption and bone mineral density. For the gastroenterologist, this could be a simple, harmless and beneficial adjunct to the management of bone problems in chronic disease (CD), coeliacs and postgastrectomy syndromes. The effect of lactulose on calcium and magnesium absorption: a study using stable isotopes in adult men has also been done [38].

Lipid Metabolism

Various reports reveal that intake of oligosaccharides helps in decreasing the blood cholesterol level. Yamashita et al [39] studied the effect of oligofructose intake on blood_lipid levels of individual with noninsuline dependent diabetes. They reported 8% reduction in total cholesterol and 10% reduction in LDL-cholesterol level after the administration of 8g of a synthetic oligosaccharides for 14 days compared with a control group given sucrose in the same food vehicles. They also observed that the reduction was greater in hypocholesterolemic subject. No effect on circulatory tryacyloglycerols was reported. Canzi et al [40] studied that effect on lipid metabolism of a prolonged ingestion of 9g/day of inulin from chicory incorporated into a ready to eat breakfast cereal in 12 healthy young male volunteers. A market effect of inulin in reducing fasting triglycerides concentration (-27%) and to a lesser extent total cholesterol (-5%) without undue effects of HDL cholesterol was reported. Pedersen et al [41] evaluated the effect of a daily intake of 14g inulin added to a low fat spread, on fasting blood in 64 young women in a randomized, double blind, cross-over trial involving two periods of 4 weeks, no significant differences in plasma total cholesterol HDL and LDL cholesterol and triacylglycerol concentration were observed between the placebo and inulin periods. However the authors reported a significant decrease in the cholesterol HDL and LDL cholesterol ratio at the end of the both the control period and the inuline period. These results were in accordance with those reported in a previous study [42] involving 12 young healthy males who ingested 20g/FOS/ day for 4 weeks in a radom crossover experiment. Davidson et al examined the effect of dietry inuline on serum lipids in hypocholesterolemic subjects [43]. This was a randomized, double-blind crossover study performed using 21 adults with mild to moderate hypocholesterolemic with two 6-week treatment periods separated by a six week washout. During the treatment period the subject consumed three serving per day of inulin containing foods, corresponding to a total of 18g/day. A significant reduction was reported when comparing the response periods either in LDL-cholesterol (-14.4% $P<.05$) or in the total cholesterol level (-8.7%, $P<.05$) when comparing to the values to any of the lipid variables no differences were observed. This was mainly attributed to a significant increase in total cholesterol and LDL-cholesterol observed during the control phase, whereas these values did not change appreciably during the inulin phase. Williams [44] observed that consumption of 10 g per day of inulin or placebo, in a powdered form has no significant changes in concentrations of total, LDL- or HDL-cholesterol in either group over the 8-week intervention. In contrast, it was reported that fasting serum triglycerides were significantly lower (19%) after 8 weeks in the inulin-treated group, returning to baseline values 4 weeks after treatment. Similar results were reported by Causey et al. [45] in a dietary study involving hypocholesterolemic men. In this study 12 male subjects were randomly assigned to two controlled diets that differed only in that the test diet contained a daily intake of 20 g of inulin, while in the control diet that was replaced by sucrose, and consumed each diet for 3 weeks. A significant decrease in serum triglycerides levels (40 mg/dl, 14%) was observed with the inulin diet. A trend toward a reduction in serum cholesterol was also observed, although this was not significant. A meta-analysis on the cholesterol lowering effects of dietary fiber

suggested that soluble fibers (pectin, oat bran, guar gum, and psyllium) had a small but significant decreasing effect on total and LDL-cholesterol levels within the practical range of intake [46]. Inulin appears to have a similar effect on blood lipids when consumed by hyperlipidemic adults. Therefore, preliminary evidence exists for a hypotriglyceridemic effect of FOSs, but, at the present stage of knowledge, it is not possible to conclude a hypocholesterolemic effect.

Carcinogenesis

Review of literature reveals that consumption of oligosaccharides may also help in overcoming cancer also. Potential protective effect of lactulose in colonic carcinogenesis have been observed by Berge Henegouwen et al [47]. The protective role of Lactulose in intestinal carcinogenesis was noticed by Hennigan et al [48]. Genotoxic enzyme activity has been seen to reduce on the administration of prebiotics. An early study on feeding GOS to humans resulted in a decrease in nitroreductase (a metabolic activator or mutagenic/carcinogenic substances) and also decreased levels of indole and isovaleric acid (produced as products of proteolysis and deamination and markers of putrefaction) [14]. When a model system of the human gut was used to investigate the effect of GOS on genotoxic enzymes it was found that glucosidase, glucuronidase and arylsulphatase were strongly inhibited but azo and nitroreductase were stimulated [49]. As these effects occurred rapidly on the addition of GOS to the system, changes attributable to population levels can be ruled out and it is more feasible that direct inhibition by GOS or the production of repressors or deactivators by bacteria was responsible. However, increasing the proportion of *Bifidobacteria* and *Lactobacilli* at the expense of *Bacteroides* and *Clostridia* may also decrease genotoxic enzyme production, as the former produce lower levels of such enzymes than the latter [50]. A further observation important to the reduction of cancer was a high level of caecal butyrate. Not only is butyrate the major source of energy for colonocytes and helps maintain a healthy epithelium [51], it can also play an important role in preventing cancer. Several cellular processes are affected by butyrate, largely by interaction with DNA and its surrounding proteins [52]. These processes include induction of apoptosis, a process which is deactivated in cancer cells which would normally lead to their elimination and an increase in immunogenicity of cancer cells due to an increase in expression of cell surface proteins [35]. Buddington et al [11] observed that when Neosugar (4g/day ; fructooligosacharide) was given to healthy volunteers in the form of chewable tablets, it increased the intestinal *bifidobacteria* and reduced appreciably the faecal activities of enzymes involved in producing genotoxic metabolites such as β glucuouronidase and glycholic acid hydrooxylase, indicating the potential of prebiotics to prevent the carcinogenesis.

Immune response

The review of literature reveals that only a little work has been carried out to see the effect of prebiotics on immune response. Guigoz et al [53] investigated the effects of giving the prebiotic FOS, 8g daily for three weeks, to frail elderly subjects in a nursing. An increase in number of feacal *Bifidobacteria* was accompanied by significant rise in counts of total lymphocytes, CD4+ and CD8+ cells. An unexpected finding was a fall in phagocytic activity of polymorphs and monocytes, as well as reduced expression of inerleukin-6 mRNA in peripheral blood monocytes. The authors attributed these changes to a general decrease in inflammation. However, Bunout et al [54] found that a prebiotic mixture of inuline and oligofructose did not augment the result of vaccination with influenzal and pneuococcal antigens.

Conclusion

The review of literature reveals that prebiotics, the “non-digestible food ingredient beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [1]. Have positive health effects which include anticancerous effect, anticholestereamic effect, helps in change in the microbiota of the gut and mineral absorption and bones overcome bowel habit and constipation, inflammatory bowel disease, and antibiotic associated diarrhea. These effects have been shown due to effect of prebiotics on microflora and environment of the intestine. Work needs to be carried out to understand the mechanism of health effects of prebiotics especially on the effect immune response.

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