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Effects of Prebiotics on Git and Human Health: A Review

Ajaypal Singh[1]*, Omji Porwal[1], Neeraj Sharma[1], Amit Singh[2], Saurabh Kumar[3] and P.K. Sharma[1]

[1]KIET School of Pharmacy Meerut-Road Murad Nagar Ghaziabad. India.
[2]RV Northland College of Pharmacy Dadari. India.
[3]Rajiv Academy for pharmacy Mathura, Chattikara Mathura. India.

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Prebiotics are short-chain 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. Three prebiotics, oligofructose, galacto-oligosaccharides and lactulose, clearly alter the balance of the large bowel microbiota by increasing bifidobacteria and Lactobacillus numbers. These carbohydrates are fermented and give rise to short-chain fatty acid and intestinal gas; however, effects on bowel habit are relatively small. Randomized-controlled trials of their effect in a clinical context are few, although animal studies show anti-inflammatory effects in inflammatory bowel disease, while calcium absorption is increased. It is still early days for prebiotics, but they offer the potential to modify the gut microbial balance in such a way as to bring direct health benefits cheaply and safely.

Keywords: Prebiotics, Microbiota, inulin, bifidobacteria.

'A prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one of 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 diarrhoea prevention and immunomodulation are becoming increasingly well established.

(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, and in addition to having beneficial effects on the gut microbiota and host,

(v) They are also valuable functional ingredients in foods with the potential to give fat-based spreads and dairy products improved organoleptic properties.

Gibson *et al.*[2] recently 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.

They now propose that 'A prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and activity in the gastrointestinal microbiota that confers benefits upon host well-being and health'.

* To whom all correspondence should be addressed.
Mob.: +91-9873945240; E-mail: ajaykansana@yahoo.co.in

Change in the Microbiota of Gut

Inulin, fructo-oligosaccharides (FOS), trans-GOSs and lactulose, when taken in the diet in relatively small amounts (5–20 g/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 organisms in the gut except in the breastfed baby.[2, 3] this change in the microbiota was initially observed by Japanese researchers and reported in the first issue of a new journal, Bifidobacteria and Microflora in March 1982. However, their effects on the global composition of the flora is less well documented at the present time because newly developed molecular methods for identification of individual species are only now demonstrating its true complexity and diversity. Almost any carbohydrate that reaches the large bowel will provide a substrate for the commensal microbiota, and will affect its growth and metabolic activities. This has been shown for non-starch polysaccharides (NSP; dietary fibre)[4], and will occur with other substrates, such as resistant starches, sugar alcohols and lactose. However, stimulation of growth by these carbohydrates is a non-specific, generalized effect, which probably involves many of the major saccharolytic groups, and associated cross-feeding species in the large bowel[5].

The selective properties of prebiotics are supposed to relate to the growth of bifidobacteria and lactobacilli at the expense of other groups of bacteria in the gut, such as Bacteroides, clostridia, eubacteria, enterobacteria, enterococci, etc. In practice, studies show that such selectivity is variable, and the extent to which changes in the microbiota allow a substance to be called prebiotic have not been established, although this may have to be underwent in the near future for food labelling and health claims legislation purposes. For example, wide variations are evident in the ratios of bifidobacteria to Bacteroides in normal faeces, from around 0.08 to 1.07, and an equally wide range in microbial growth responses occurs in human volunteers following prebiotic consumption, with final ratios of these organisms being from 0.40 to 5.01.6 Not only has 'selectivity' not been defined in quantitative terms, but also there are qualitative aspects of the microbiota that also need to be reviewed in this context. Thus, some investigations have shown increases in other bacterial genera, such as Roseburia, Ruminococcus and Eubacterium, with established prebiotics like inulin.[7, 8] do such a changes negate the concept of selectivity? Moreover, it is now recognized that many bacteria inhabiting the large bowel have not yet been identified and are difficult to culture routinely.[9] One consequence of this is that we do not know what the global effects of prebiotics are on the structure of the microbiota.

Another important factor to bear in mind when using prebiotics to selectively modify the composition of the microbiota is that prebiotics on their own can only enhance the growth of bacteria that are already present in the gut. However, different people harbour different bacterial species, while the composition of the microbiota can be affected by a variety of other factors, such as diet, disease, drugs, antibiotics, age, etc.

A Healthy Microbiota

A healthy, or 'balanced' microbiota has been considered to be one that is predominantly saccharolytic and comprises significant numbers of bifidobacteria and lactobacilli.[10] This concept is based on a number of observations. The genera Bifidobacterium and Lactobacillus do not contain any known pathogens, and they are primarily carbohydrate fermenting bacteria, unlike other groups, such as Bacteroides and clostridia which are also proteolytic and amino acid fermenting. The products of carbohydrate fermentation, principally short-chain fatty acids (SCFA) are beneficial to host health, while those of protein breakdown and amino acid fermentation, which include ammonia, phenols, indoles, thiols, amines and sulphides are not.[11] Furthermore, lactic acid-producing bacteria, such as bifidobacteria and lactobacilli are believed to play a significant role in the maintenance of colonization resistance, through a variety of mechanisms.[12] Equally importantly, the exclusively breast-fed neonate has a microbiota containing proportionately higher numbers of bifidobacteria, which is believed to be part of the baby's defence against pathogenic microorganisms, and which may be important primers for their immune system. This microbiota is nurtured by oligosaccharides in breast milk, which can be considered to be the original

prebiotics. While some investigations have reported detailed analysis of the effects of prebiotics on microbial communities in the gut, [13] to date, the majority of microbiological studies carried out on prebiotics have only characterized bacterial populations to group or genus level. Because of this, an important issue is seldom addressed, namely that which relates to the types of bifidobacteria and lactobacilli that ferment, or are affected by prebiotics in the gut. Not all of these organisms are able to utilize or compete for prebiotics, [13] or have any recognized health-promoting properties, therefore unless it is known which species are being stimulated by these substances, we cannot say for certain that specific health benefits will necessarily accrue from prebiotic consumption. This argument applies equally to the lack of knowledge of the effect of prebiotics on the many newly discovered, unculturable, species belonging to other genera, whose effects on health are presently unknown and which prebiotics may affect.

Mucosal Microbiota

Most studies on the colonic microbiota have focused on faecal material. However, increasing evidence suggests that the epithelial surface is also heavily colonized by large and diverse bacterial communities, which are structurally distinct from those that occur in the gut lumen.[14–16] such bacteria, which grow in biofilms on or adjacent to the colonic mucosa, exist in close proximity to the host and are likely to be particularly important in modulating immune system reactivity.[17, 18] Indeed, studies have shown that mucosal communities can change markedly in inflammatory conditions, such as ulcerative colitis (UC) and Crohn's disease (CD).[16, 19] Importantly, the composition of these mucosal communities in humans can be manipulated through the use of prebiotics.

Langlands *et al.*[7] showed that bifidobacterial and eubacterial numbers could be increased more than 10-fold in mucosa of the proximal and distal colons in patients fed 15 g of a prebiotic mixture containing 7.5 g inulin and 7.5 g FOS/day for 2 weeks prior to colonoscopy Potential mechanisms whereby dietary components in the gut lumen can affect bacteria on the mucosal surface are illustrated in Fig. 1. Until this study, it was unclear if mucosal communities could sequester dietary components, or whether they were principally dependent on mucus and other host secretions. However, the fact that small additions to the diet can have profound effects on the mucosal microbiota opens up the possibility of developing therapeutic strategies for tackling bacteria-associated gut diseases.

Fermentation

While the concept of selectivity and changing the composition of the colonic microbiota is essential to the characterization of prebiotics, the suggestion that these substances are characteristically non-digestible but fermentable is probably not. Many dietary carbohydrates and proteins undergo fermentation in the large intestine and thus this cannot be a primary defining quality of prebiotics. Nevertheless, fermentation of carbohydrates is viewed as a beneficial function of the microbiota, and currently recognized prebiotic carbohydrates are probably all fermented. Certainly, faecal recoveries of dietary inulin and oligofructose (OF) have been universally close to zero, and such studies that have been carried out on the upper intestinal digestibility of these substances have suggested recoveries of around 88% at the ileo-caecal junction.[20] Thus, prebiotics will yield SCFA, such as acetate, and butyrate, together with hydrogen, carbon dioxide and biomass, as do other fermented carbohydrates. However, whilst many bacterial species grow well on prebiotic carbohydrates there may be a selective benefit to some types of bifidobacteria and lactobacilli, depending on the sugar composition and molecular size of the prebiotic.[21, 22]

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.[23–29] 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.*[24] and 2.4 g/g for inulin (129–204 g/day) in the study of Castiglia-Delavaud *et al.*[27]

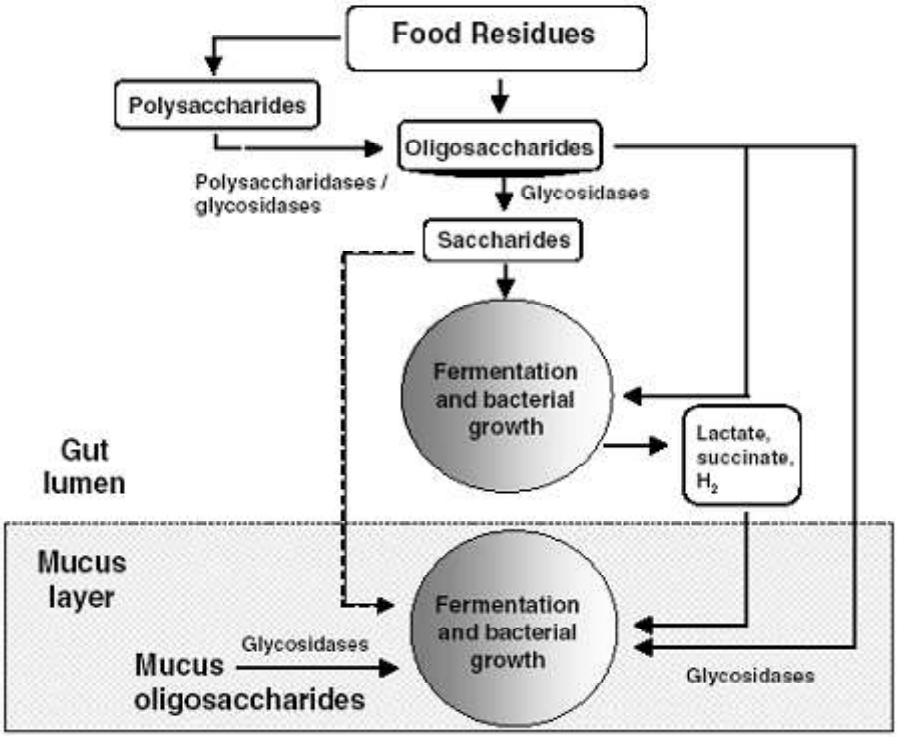

Fig. 1. Mechanisms whereby dietary substrates become available for mucosa-associated microbiotas in the large intestine.

At best, 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.[30] 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). 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[31] 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.*[32] 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.[33–35] Den Hond *et al.*[36] did measure stool output in six healthy volunteers with low stool frequency (4.0 ± 0.4 S.E.M. stools/ week), and showed a non-significant increase from 91 ± 107 to 113 ± 22 g of stool/day with 15 g of inulin (equivalent to 1.5 g of stool/g of

inulin fed), but a significant increase to 6.5 stools/ week. Moreover, Chen *et al.*[37, 38] showed significant increases in stool weight from 32.4 ± 1.8 (S.E.M.) to 69.03 ± 6 g/day in elderly constipated subjects fed 10 g/day OF. This is somewhat surprising in view of the results .Furthermore, a 70% increase in stool output was recorded by these authors in a similar study with isomalto-oligosaccharides.

This latter investigation, the increase in stool weight was due to increased microbial cell mass, which would be the correct mechanism as isomalto-oligosaccharides are not recovered in faeces.[29] The parallels here with lactulose are clear, but in mechanistic terms, we now know that all of these carbohydrates also change the species composition of the microbiota.[2, 39]

Traveller's Diarrhoea

Traveller's diarrhoea (TD) is an ideal model in which to test the benefits of prebiosis. Despite this, only one clinical study has been published[40] in which 244 healthy subjects travelling to high or medium risk destinations for TD were randomized to receive either 10 g of FOS or placebo for 2 weeks prior to their holiday, and then for the 2 weeks they were away. The prevalence of diarrhoea was less in the FOS group, as recorded in a post study questionnaire, at 11.2% FOS vs. 19.5% placebo, but this was not statistically significant (P ¼ 0.08). There were no significant differences in the primary end points of bowel frequency or consistency between the two groups, as recorded in bowel habit diaries, but those subjects taking FOS experienced less severe attacks of diarrhoea than the placebo group (Fig. 2). These results were strongly indicative of a benefit of prebiotics, but not conclusive. This could be because not all cases of TD are due to infection, and other factors contribute to the condition, including exposure to rarely encountered foods, alcohol excess and anxiety. Moreover, many infecting agents that cause TD, such as *Escherichia coli*, campylobacters, Salmonella, giardia and yersinia, mainly affect the small intestine, and the essence of prebiosis is a change in the microbiota of the large bowel.

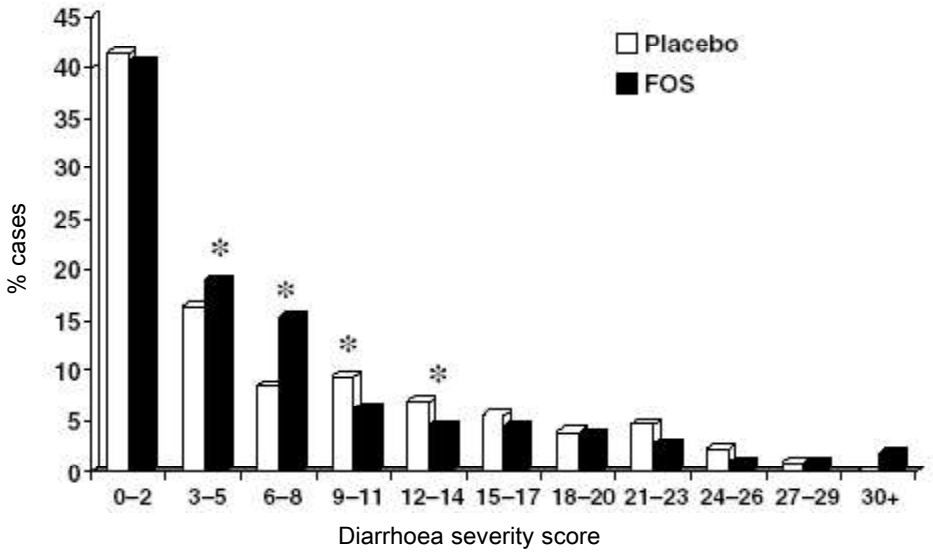

Fig. 2. The severity of episode of diarrhoea in travelers (n=244) taking either placebo or OF 10 gram/day for 14 day,s prior to travel .

Well-being

An unexpected finding from the TD study cited above was the significantly greater proportion of subjects on FOS (12.9% vs. 4.7%, P < 0.04) who responded affirmatively to the post study questionnaire, by ticking the box that said 'whilst taking the sachets, did you experience a general improvement in well-being'?

Well-being is a state of body and mind that is very difficult to define and measure. It is, however, a core principle of the functional food concept that wellness is improved rather than disease or symptoms treated.[10] Food has long been known to induce a sense of well-being, for complex reasons, but little attention has been paid to this key component of quality of life, despite wellness being something to which we all aspire. But as the preamble to the Regulation states 'There are many factors, other than dietary ones, that can influence psychological and behavioural functions. Communication on these functions is thus very complex and it is difficult to convey a comprehensive, truthful and meaningful message in a short claim to be used in the labelling and advertising of foods. Therefore, it is appropriate, when using psychological and behavioural claims, to require scientific substantiation'. The gut is a key organ in the relationship of food to well-being. Many sensations arise from the gut in association with the intake of food, such as satiety, postprandial intestinal sensations, bowel habit, gas production and excretion. The boundary between a pleasant feeling and unwanted sensations, such as nausea, bloating, pain, incomplete rectal evacuation, etc. is not well defined, and is the same boundary as between irritable bowel syndrome (IBS) and health. The large gut is well served by the enteric nervous system, and there is a complex interplay between neural and hormonal regulation and our consciousness. Such perception of our digestive processes can be measured to some extent.[41] However, few studies have been undertaken in humans in which the effects of prebiotics on well-being have been investigated. One recently reported study[42] observed the effect of the intake of 10 g/day inulin on aspects of energy, mood and cognitive function in 142 healthy volunteers, as assessed by a battery of questionnaires. Included in this were six questions relating to the gastrointestinal tract. No significant differences were recorded between placebo and inulin periods in mood, bowel function, sleep quality, memory or performance; however, subjects noticed increased wind, bloating and stomach cramps with inulin, and very slight changes in bowel habit. Clearly, this is an area that deserves more work, especially with objective measures of gastrointestinal function that can be related to changes in brain activity,perhaps employing new imaging technology and reproducible descriptions of well-being using established criteria and questionnaires.

Irritable bowel syndrome

Randomized controlled trials (RCT) concerning the use of prebiotics alone in IBS. A number of studies using probiotics have been carried out with varying benefits[43] but the pathogenesis of IBS may preclude the use of prebiotics in this condition. While it is accepted that IBS is probably not a single syndrome, and may well encapsulate several different pathophysiologies, it is now clear that at least a subset of these patients have increased intestinal gas production,[44, 45] reduced tolerance of gas in the gut[46] and differences in their gut microbiota.[47] Marked variabilities can be seen in the bacterial composition of faeces from IBS patients by using quantitative polymerase chain reaction (PCR), for example, Malinen *et al.*[47] reported reduced numbers of lactobacilli and bifidobacteria in diarrhoea-predominant IBS. The known abilities of some prebiotics to selectively increase numbers of lactobacilli and bifidobacteria in both the faecal microbiota and mucosal populations should, in principle, allow correction of these imbalances in microbial community structure. Bifidobacteria and lactobacilli do not produce gases as end products of metabolism.[48] However, as previously discussed, a well known consequence of feeding even moderate amounts of some of the currently favoured prebiotics is increased gas production in the gut, because of their rapid fermentation in the proximal bowel.[40, 49] This might preclude prebiotic use in diarrhoea-predominant IBS, or where bloating or gas are prominent symptoms, but might allow their mild laxative properties[20] to be useful in constipation predominant IBS. The only preliminary report so far suggests no benefit, even in mainly constipated patients.[50]

Antibiotic Associated Diarrhoea

Probiotics now have an established place in the prevention of antibiotic-associated diarrhoea (AAD), and so it might be expected that prebiotics would also be effective in some circumstances. Changing the composition of the microbiota to one dominated by bifidobacteria and lactobacilli should, in principle, increase

colonization resistance in the gut. Furthermore, many intestinal pathogens utilize monosaccharides or low DP oligosaccharide sequences as receptors, binding to which is the first step in the colonization process.[12] Gibson *et al.*[12] report that there are several pharmaceutical preparations based on these receptor saccharides in clinical trials and suggest they should, by binding to the oligosaccharide receptor on the gut mucosal surface, inhibit adhesion of pathogens and act as 'decoy oligosaccharides'. In vitro modelling of AAD by using clindamycin and *Clostridium difficile* inoculation of human faecal microbiota[51] showed that supplementing cultures with either FOS, GOS or inulin reduced clostridial numbers and increased total bifidobacteria counts. However, when the cultures were supplemented with clindamycin, marked reductions in bifidobacteria occurred, which were augmented by the presence of prebiotics, while FOS actually enhanced growth of *C. difficile* under these conditions. Although these data suggested that stimulation of bifidobacterial growth by the prebiotics was responsible for suppressing the pathogen, subsequent modelling experiments by using chemostats demonstrated that bifidobacteria did not manifest antimicrobial effects against *C. difficile,* indicating that other mechanisms must have been involved. These results are supported in human trials.

Three RCT of prebiotics and the prevention of AAD have been reported. Lewis *et al.*[52] undertook a large study involving 435 patients aged over 65 years, who were hospital in-patients 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 faecal 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. Why did the OF not protect these patients from AAD? The amount of OF was sufficient, and compliance was good. 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' group[53] successfully prevented further episodes of diarrhoea in patients with *C. difficile* associated symptoms who were treated with metronidazole and vancomycin. Again, 12 g of OF was used and given for 30 days. Follow-up was for a further 30 days. FOS significantly reduced episodes of diarrhoea from 34.3% (placebo) to 8.3% (FOS; $P < 0.001$). Hospital length of stay was also reduced and bifidobacterial numbers increased significantly with the prebiotic. In abstract only, Brunser *et al.*[54] reported a 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 7 of the prebiotic supplement without any apparent change in diarrhoeal symptoms. The antipathogenic effects of prebiotics have also been investigated in studies other than those associated with AAD. A investigation in 66 liver transplant patients given various probiotics and prebiotics (but no placebo) post-operatively showed no benefit for FOS, but a major reduction in infections, especially urinary infections, with probiotics.[55] Similarly, synbiotic treatment involving OF and a variety of probiotics was found to be ineffective in preventing systemic inflammation and postsurgical septic complications.[56] A synbiotic is a mixture of a probiotic and a prebiotic, and the rationale for this combination is that the prebiotic is used to stimulate growth of the probiotic in the gut, thereby increasing its effectiveness. Inflammatory bowel disease The enthusiasm with which probiotics have been used in inflammatory bowel disease (IBD)[57, 58] and their apparent benefits has led to the suggestion that prebiotics might also be useful. Certainly, patients would welcome such an approach, which would be inexpensive and without significant side-effects, provided it were effective. Despite this, there are no reports of RCT using prebiotics alone in either UC or CD, although some preliminary work suggests prebiotics have anti-inflammatory properties. Reports of animal studies are quite numerous, and in general, they show a benefit in reducing

symptoms, including inflammation, as seen histologically and biochemically, with appropriate increases in bifidobacteria or lactobacilli, and in some reports, in concentrations of butyrate in the gut. These effects are seen across a wide range of models of IBD, and with varying prebiotics, including the trinitrobenzene sulphonic acid (TNBS) rat treated with either FOS[59] or lactulose,[60] the dextran sulphate sodium (DSS) model with inulin,[61] a mixture of inulin/ FOS[62] or lactulose[63] and the HLA-B27 transgenic rat, treated again with a mixture of inulin/FOS.[64] There are also multiple reports of the use of 'prebiotic-germinated barley foodstuff' in both animals and humans from one research group[65] but this substance is a mixture of NSP (fibre) and glutamine and has not been accepted as a prebiotic.[2] In a small open-label trial in humans, 10 patients with active ileo-colonic CD were given 15 g FOS daily for 3 weeks. A significant reduction in the Harvey Bradshaw index of disease activity was observed, and faecal bifidobacteria increased from log 10 8.8 to log 10 9.4 cells per gram dry faeces. The proportion of dendritic cells expressing Toll-like receptors TLR2 and TLR4 also increased.[66] Furrie *et al.*[18] have reported a double-blinded RCT in which a synbiotic was fed to UC patients for a period of 1 month. Eighteen patients were enrolled in the study, and those receiving the synbiotic were given 12 g of Synergy 1 (OF-enriched inulin) and 2 · 1011 live Bifidobacterium longum per day. Results showed that bifidobacterial numbers on the rectal mucosa increased 42-fold in subjects receiving the synbiotic.

This was accompanied by highly significant reductions in mucosal proinflammatory cytokines (TNF-a, IL-1a) as well as inducible b-defensins 2, 3 and 4. These substances are antimicrobial peptides produced by epithelial cells during inflammatory episodes in the gut, but unlike TNF-a and IL-1a, b-defensins are not formed by inflammatory cells infiltrating the mucosa, so they were important markers of healing events occurring on the epithelial surface. Histology showed marked reductions in inflammatory cells and crypt abscesses in patients receiving the synbiotic, together with regeneration of normal tissue, while sigmoidoscopy scores and clinical activity indices were also improved in these individuals. This short-term pilot study provides the first evidence that synbiotics have the potential to be developed into acceptable therapies for patients suffering from acute UC, but further work is needed to investigate the long-term efficacy of synbiotics in inducing and maintaining remission. Pouchitis patients do well with probiotics, and one successful study has been reported in which prebiotics were used for this condition.[67] In a randomized double- blind crossover study, 24 patients with stable symptomatic pouchitis were given 24 g of inulin or placebo daily, for 3 weeks each. At the end of the prebiotic period, results showed that there was a reduction in the endoscopic and histological pouchitis disease activity index (PDAI) score, together with lower gut pH, reductions in faecal Bacteroides fragilis and secondary bile acids. Butyrate concentrations were increased, while symptom scores were low initially, and were essentially unchanged.

Calcium Absorption and Bones

Lactose has long been thought to enhance dietary calcium absorption, although the effect in healthy humans is not shown consistently.[68] The effects of other carbohydrates have been studied including prebiotics derived from lactose, such as GOS. Much of this work has been carried out in animal models, which show clearly enhanced absorption of calcium, and also magnesium and iron with GOS, FOS and inulin.[69–74] More importantly, this enhancement of absorption leads to increased bone mineral density[75] and prevents osteopenia following gastrectomy or ovariectomy.[72,76,77] Calcium absorption from the gut is mediated by a vitamin D and energy-dependent carrier-mediated transport process, principally in the duodenum and upper jejunum.

However, passive non-saturable paracellular transport also occurs more distally in the gut, which is probably 1,25(OH)2D3 responsive.[78] In the rat, the caecum plays a major role in calcium absorption[72] where calcium-binding protein is expressed and is specifically stimulated by FOS.[7,79,80] The mechanism is not clear, but increased solubility of calcium because of fermentation, which lowers caecal pH and increases SCFA production, or changes intracellular Ca^{2+} concentration, which may enhance paracellular transport, are all

possible.[81-84] The caecal microbiota may be involved, because the stimulatory effect of GOS on calcium absorption is suppressed by neomycin.[85] However, in humans it is not thought that the large bowel has a major role to play in calcium absorption, but it is reassuring to read that prebiotics also enhance this process, especially in adolescents and less certainly in young men and postmenopausal women. Further studies from which it can be seen that both FOS and inulin increase calcium absorption, which in the 1 year investigation of Abrams *et al.*[86] led to a greater bone mineral density in the prebiotic group. In the two studies of young men, the results are conflicting, possibly because two different methods for measuring calcium absorption were used. The double isotope method of van den Heuvel *et al.*,[87] carried out at day 21 of the diet period, did not show a benefit of either inulin, FOS or GOS, despite a reasonable dose of prebiotic (15 g/ day). The authors subsequently felt that the double isotope technique they used 'did not include the colonic component of calcium absorption[88] because 24 h 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 36 h 88 or 48 h.[89] Coudray *et al.*[90] used classical metabolic balance techniques to show increased absorption. Despite the belief that calcium absorption is thought to occur in the proximal gut in humans, a colonic phase may exist. Ellegard *et al.*[91] showed that neither inulin nor FOS when fed to ileostomy subjects had any effect on ileostomy excretion of calcium, magnesium, zinc or iron. As prebiotic carbohydrates pass through the small bowel unchanged, but are fermented in the caecum or colon, a large bowel effect on absorption is possible. Prebiotics have also been reported to increase the uptake of other metal ions from the gut. Ducros *et al.*[92] reported that feeding 10 g of FOS per day for 5 weeks increased the absorption of copper in healthy postmenopausal women. In a randomized double- blind, placebo-controlled trial, however, no effects were seen in relation to zinc and selenium uptake. This selectivity would suggests that factors other than simple acidification of luminal contents were involved. 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 CD, coeliacs and postgastrectomy syndromes.

Future Scenario of Prebiotics

The possible health benefits of prebiotics are now being explored in many situations, facilitated by their safety and ease of use. A substantial literature is accumulating on prebiotics and cancer, but much of the published work is in animals, where the role of prebiotics looks to be beneficial, whereas human studies are mostly concerned with identification of early biomarkers of risk.[93] Prebiotics are now being added to follow- on feeds for infants,[94] a practice which is riding on the back of clear benefits to children of probiotics in preventing and ameliorating the symptoms of acute infectious diarrhoea, and in atopic disease. Their use to prevent necrotizing enterocolitis shows promise in animal models.[95] Prebiotics clearly change the gut microbiota of infants and alter large bowel function, but large clinical trials are awaited. Another area of importance is lipid metabolism where prebiotic studies in animals have shown reduced blood levels of cholesterol and triglycerides and beneficial effects on fatty liver. Clinical trials in humans have not yielded such consistent results, although the effects on hepatic lipid metabolism are worth further study.[96, 97] There is also great interest in prebiotics in the pet food and animal feed industry,[98] where improved control of gastrointestinal infection is reported and enhanced growth performance is seen particularly in poultry. Other areas of interest include prebiotics and immunomodulation of the gut immune system, glycaemic control, behavioural effects, especially cognitive performance and the enhancement of probiotic activity in synbiotics.

CONCLUSION

Prebiotics are short-chain carbohydrates (oligosaccharides) that have unusual effects in the gut. They alter the composition, or balance, of the microbiota, both in the lumen and at the

mucosal surface, to one in which bifidobacteria and lactobacilli come to greater prominence. This, so-called healthier flora, should provide increased resistance to gut infections and may also have immunomodulatory properties. Prebiotics also act as carbon and energy sources for bacteria growing in the large bowel, where they are fermented to SCFA and are energy sources for the gut and other body tissues. For regulatory purposes, the definition of 'prebiotic' needs to be clarified, particularly with respect to the concept of non-digestibility and the exact parameters that constitute selective modification of the gut microbiota. In a clinical context, prebiotics are relatively poor laxatives and have been used without much success to manage constipation, whilst in the prevention of TD, a single study indicates a reduction of diarrhoea severity. There are no published RCT of prebiotics and IBS, and two RCT in the prevention of AAD made no impact on symptoms or risk, unlike probiotics, which are effective in this condition. Animal studies of prebiotics and IBD show benefits across a wide range of models, and with varying prebiotics, but again, there are no RCT in humans. One study of a synbiotic shows anti-inflammatory effects, while pouchitis may also improve. Perhaps surprisingly, a clear benefit of increased calcium absorption is seen and increased bone mineral density in adolescents with prebiotics. It is still early days for prebiotics, but evidence increasingly suggests that they offer the potential to modify the gut microbial balance in such a way as to bring direct health benefits cheaply and safely.

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