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Session: Nutrients contributing to the fibre effect

Resistant starch as a prebiotic and synbiotic: state of the art

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Non-infectious diseases such as CHD and certain cancers have become major causes of death and disability in affluent countries. Probiotics (principally lactic acid bacteria; LAB) may assist in lowering the risk of these diseases. Experimental studies with probiotics have given generally inconclusive outcomes for infectious disease and for biomarkers for non-infectious disease. In part this situation may reflect their inability to colonise the adult human gut effectively. Prebiotics can assist in promoting colonisation, and resistant starch (RS), as a high-amylose starch, is a prebiotic and synbiotic. This starch exerts its synbiotic action through adhesion of the bacteria to the granule surface. Consumption of RS assists in recovery from infectious diarrhoea in man and animals. A rice porridge, high in RS, appears to modify the autochthonous porcine large-bowel microflora favourably through lowering *Escherichia coli* and coliform numbers. Many of the beneficial effects of RS on large-bowel function appear to be exerted through short-chain fatty acids (SCFA) formed by bacterial fermentation. In man LAB are found in relatively highest numbers in milk-fed infants where the profile of fermentation products differs quite markedly from that in adults. It appears unlikely that ingestion of current probiotics will alter either total SCFA or the proportions of the major acids. More emphasis needs to be given to the investigation of the effects of complex carbohydrates, including RS, on the autochthonous microflora of the human large bowel.

Resistant starch: Prebiotics: Probiotics: Synbiotics

There is considerable industry and public interest in the capacity of foods and food components to promote health and lower risk of non-infectious diseases related to diet and lifestyle. The potential of probiotics to deliver such benefits can be traced back to the early work of Metchnikoff (1907) who linked the consumption of specific live organisms in fermented foods (e.g. yoghurts) by certain ethnic groups with an apparently greater longevity. The health-promoting attributes of these exogenous bacteria (mostly species of lactic acid bacteria; LAB) contrasted with the 'putrefactive' actions of the resident flora. With time, the term probiotics has come to describe these live LAB and also some other micro-organisms with the potential to improve health. A critical point, which often escapes attention, is that in Metchnikoff's time the major causes of death for individuals were infections, including food- and water-borne diarrhoeal diseases. Probiotics could be of benefit in these conditions either through direct competition with pathogens or the production of specific antibacterial or antiviral compounds (Naidu *et al*. 1999). There is some experimental support for their effectiveness in man in this connection. For example, probiotic consumption has been reported to lower the duration of infectious diarrhoea (van Niel *et al*. 2002) and the severity of dental caries in children (Nase *et al*. 2001). Consumption of a fermented-milk product supplemented with specific strains of *Lactobacillus acidophilus* or *Bifidobacterium bifidum* by healthy volunteers led to enhanced phagocytosis of *Escherichia coli in vitro* (Schiffrin *et al*. 1997). These and other studies support a role for probiotics in the control of infective pathogenic agents. However, since the concept was first evolved there has been a temporal shift in the causes of death in affluent societies

Abbreviations: cfu, Colony-forming units; FOS, fructo-oligosaccharides; GIT, gastrointestinal tract; LAB, lactic acid bacteria; OS, oligosaccharides; RS, resistant starch; SCFA, short-chain fatty acids.

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through improved hygiene, which has led to a substantial decline in morbidity and mortality from infectious agents. In these populations major causes of death and disability now relate to diet and lifestyle: CHD, obesity, constipation, certain cancers (especially of the colon), diabetes, etc. Their socio-economic impact has led to interest in the potential of probiotics as therapies and preventive agents in these conditions. For example, probiotic consumption has been purported to assist in the lowering of plasma cholesterol and reduction of CHD risk. The mechanism for this lowering is unclear and the evidence from human studies for such a benefit is equivocal (de Roos & Katan, 2000). The same appears to be true for other conditions such as colo-rectal cancer. Among the problems identified in published studies are concerns about the numbers and types of organisms used, the biomarkers that have been followed and the duration of the trials. One particular issue is that of host colonisation with the probiotic and the apparent refractoriness of the autochthonous microflora. It seems that only a relatively small proportion of ingested organisms survives passage through the stomach and small intestine and colonises the large intestine (Bezkorovainy, 2001). When probiotic consumption ceases, it appears that the organism is washed out of the gastrointestinal tract (GIT) due to a capacity of the host's microflora to remain stable in the face of exogenous organisms. For probiotics to exert any sustained effect, either continued ingestion or boosting their residence time seems to be necessary. Enhanced colonisation seems to be a viable option which means promoting the survival of ingested probiotics to ensure that greater numbers reach the large bowel and/or enhancing their residence time in that viscus. The caecum and colon are the major site of bacterial colonisation in the human GIT. Relatively few bacterial species and numbers are found in the oropharynx, oesophagus, stomach or small intestine. An ingested probiotic must survive passage through these viscera and take up active residence in the large bowel. This process can be facilitated by prebiotics which, when combined with probiotics, are synbiotics (Gibson & Roberfroid, 1995).

Prebiotics as enhancers of probiotic effectiveness

Prebiotics are growth substrates directed specifically towards potentially beneficial bacteria already resident in the colon (Gibson & Roberfroid, 1995). These authors enunciated the currently accepted definition of a prebiotic as a non-digestible food ingredient that affects the host beneficially by stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health. The definition does not refer specifically to autochthonous organisms and would not be expected to do so as prebiotic use is predicated, at least partially, on the expectation that there is a defect in the host's microflora. It is implied also that a prebiotic acts to stimulate the growth of organisms within the large bowel alone. However, this concept overlooks the fact that synbiotics (combinations of prebiotics and probiotics) have health potential (Gibson & Roberfroid, 1995) and that increasing the numbers and/or activity of ingested organisms within the colon by enhancing their survival as they pass through the hostile environment of the upper GIT will have the same net effect as stimulating growth of related organisms in the large bowel. On this basis, a capsule containing probiotics could be a prebiotic if it (the capsule) increased the numbers of those organisms in the large bowel *in vivo*. This issue is important as alginates (Hansen *et al.* 2002) appear to have prebiotic potential as indicated by their capacity to maintain LAB numbers in a dairy product when challenged with simulated gastric secretions. In this study the bacteria were microencapsulated with alginate and it would be expected that, on ingestion, their survival through the gut would be enhanced.

Prebiotics are differentiated from colonic foods in that the latter serve as general fuels for the endogenous colonic microflora, thus providing the host with energy, metabolic substrates and essential micronutrients. Much of the previous research focus on prebiotics has concentrated on oligosaccharides (OS), principally fructo-oligosaccharides (FOS) and galacto-oligosaccharides. These compounds are indigestible by human digestive enzymes and have well-documented effects on the large-bowel microflora. Although they are classified as colonic foods, they also meet the criteria for prebiotics (Gibson & Roberfroid, 1995). However, other food ingredients and components regarded formerly as colonic foods, e.g. partially-hydrolysed guar gum (Tuohy *et al.* 2001), appear to promote probiotic numbers. One dietary component showing great potential as a prebiotic and colonic food is resistant starch (RS), which is emerging as a major factor in the bacterial ecology of the human hindgut.

Starch digestion and resistant starch

Starch is a substantial component of many human diets and in traditional agrarian cultures provides > 50 % of the daily energy intake, largely from grains. In affluent westernised societies (i.e. where interest in prebiotics and probiotics is greatest) average consumption is much lower, possibly as little as 25 % of the daily energy intake (Baghurst *et al.* 1996). Starch is the only food polysaccharide occurring naturally which can be digested by the intrinsic enzymes of the human GIT. It was thought that starch digestion in the human small intestine was complete as very little starch is found normally in faeces. However, a substantial body of data from studies *in vitro* in animals and intact human volunteers and those with defunctioning bowel surgery (ileostomates) has shown that a substantial proportion of the starch escapes into the large bowel (for a review, see Topping & Clifton, 2001). This starch is termed resistant starch (RS) and is defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992). The definition has two important corollaries. First, RS is defined exclusively in terms of the large bowel, i.e. the rate of small intestinal digestion is not relevant. Thus, a starch may be digested slowly in the small intestine but if the rate of passage of the food is sufficiently slow, digestion is complete and there is no RS. Second, RS includes oligosaccharides and other products of small intestinal starch hydrolysis so that measurement of undigested starch alone is not a full measure of RS. These factors are important in the general impact of RS on the large bowel, as well as their prebiotic potential.

Table 1. Nutritional classification of resistant starches (RS)

Types of RS	Examples of occurrence
RS_1: Physically inaccessible	Whole or partly-milled grains and seeds
RS_2: Resistant granules	Raw potato, green banana, some legumes and high-amylose starches
RS_3: Retrograded	Cooked and cooled potato, bread and cornflakes
RS_4: Chemically modified	Etherised, esterified or cross-bonded starches (used in processed foods)

Table 2. Potential bacterial substrates of dietary origin reaching the colon in adult human subjects consuming a Westernised diet* (modified from Cummings & Macfarlane, 1991; Baghurst *et al.* 1996)

Substrate	Amount (g/d)
Resistant starch	8–40
NSP	8–18
Oligosaccharides	2–8
Simple sugars	2–10
Proteins	3–9

*Endogenous secretions (6–9 g/d) and sloughed intestinal cells (unquantified) also contribute substrates for the colonic microflora.

RS exists in foods for a variety of reasons (Topping & Clifton, 2001). Raw starches (e.g. in unripe banana) are digested poorly and their digestibility is enhanced through cooking, especially in the presence of water, which gelatinises the starch giving greater access to amylases. Disruption of food structure by milling and other processes (e.g. chewing) also enhances digestion by permitting access to the matrix for the digestive enzymes. While cooking increases starch digestibility, subsequent cooling leads to the formation of crystallites that are resistant to digestion. This process is termed retrogradation and the RS content of foods appears to increase when they are subject to increasing numbers of heating and cooling cycles. Chemical structure is an important factor in starch digestibility, especially amylose:amylopectin. The former is a relatively small essentially linear glucose polymer, whereas the latter has a highly-branched structure. Most food starches are predominantly (about 70 %) amylopectin. The remainder is amylose which occurs within the amylopectin matrix. The greater the content of amylose, the more difficult the starch is to gelatinise and the more susceptible to retrogradation (Colonna & Mercier, 1985). Ungelatinised high-amylose starches (60–70 % total starch) are resistant to amylolysis and are used commercially as an ingredient to raise the RS content of processed foods (Brown *et al.* 2000). Chemically-modified starches also qualify as RS; an important issue for the food industry, as these starches are used widely for their functional attributes in food processing (Brown *et al.* 1995). All these influences on RS have led to their classification into four types: RS_1–RS_4 (Table 1). Physiological factors can also impact on the amount of RS in a food (Topping & Clifton, 2001). For example, chewing can dictate the particle size of an ingested food, with highly-comminuted foods having a smaller particle size. Large particles travel more rapidly through the gut than small ones so greater mastication would be expected to increase digestibility. This proposition is supported by controlled animal studies in which it was shown that the ileal digestibility of finely-divided rice was greater than that of coarse rice (Bird *et al.* 2000*b*). Transit itself is a major physiological determinant of starch digestibility in the small intestine, and in women can be affected by the ovarian cycle, with less RS being found in mid-cycle (McBurney, 1991). This wide range of determinants of RS suggests strongly that all types might not have the same effects on the large-bowel microflora, especially as prebiotics.

Resistant starch as a prebiotic and synbiotic

Interest in RS grew from the appreciation that the fermentation of complex carbohydrates by the large-bowel microflora was important for human health. The metabolic products, especially short-chain fatty acids (SCFA), have emerged as important metabolic fuels for colonocytes as well as having specific actions that promote normal colonic function. It had been assumed that NSP (major components of dietary fibre) were the principal fermentative substrates. NSP intakes documented from population studies are generally < 20 g/subject per d (Baghurst *et al.* 1996). These values are well below the 60–80 g substrate/d required to sustain the 10^{13}–10^{14} organisms found in the normal human large bowel. Evaluation of the candidate substrates showed that RS is most likely to fill this 'carbohydrate gap' (Table 2). Indeed, it appears probable that some populations at low risk of large bowel disease, including cancer, consume relatively little NSP but their diets are high in starch (Topping & Clifton, 2001).

Interest in the potential of RS as a prebiotic grew out of animal and human studies where consumption of high-RS foods and ingredients led to a time-dependent shift in faecal and large-bowel SCFA profiles. This finding suggested an adaptive change in the autochthonous microbial population and also the possibility that RS could interact with ingested bacteria. Experimental studies were initiated to test the prebiotic potential of one form of RS, a high-amylose starch. Much of the initial work has been conducted in pigs as this species seems to be one of the best models for man available currently (Topping & Clifton, 2001). Pigs are free of the disadvantage of selective faecal refection which is practised by rodents and which can greatly influence SCFA production. Further, pigs will eat human foods in quantities close to those consumed by man. When pigs consumed RS as a high-amylose starch, faecal concentrations and excretion of *Bifidobacterium longum* ingested orally were higher than in those consuming a conventional starch (Brown *et al.* 1997). The increase was approximately $0{\cdot}8\ \log_{10}$ colony-forming units (cfu)/g faeces and $1\ \log_{10}$ cfu/d for concentration and excretion respectively. These increases are of a generally similar order to those reported for other prebiotics such as FOS in human studies (for example, see Tuohy *et al.* 2001). Studies in mice have confirmed the prebiotic action, with an increase in faecal LAB numbers in the absence of oral supplementation with

Table 3. Counts of faecal Bifidobacteria (log_{10} colony-forming units/g) in pigs fed diets containing low- or high- amylose starches with (+FOS) and without (–FOS) fructo-oligosaccharides (from Brown *et al.* 1998)

Amylose level**	–FOS**	+FOS**
Low	10·35	11·00
High	11·74	12·02

Independent effects of starch and FOS were significant: $^{**}P<0·01$.

probiotics (Brown *et al.* 1998). Based on these studies, it was concluded that this RS qualifies as a prebiotic and a synbiotic. A study with a FOS and this RS in pigs fed a diet based on human foods showed that both raised faecal bifidobacteria numbers by approximately equal amounts when fed separately. When fed together there was an increase that exceeded the individual increases, suggesting that they operate by different mechanisms (Brown *et al.* 1998; Table 3). Almost certainly, FOS acts as a metabolic substrate for LAB but the high-amylose RS seems to function differently. Studies with the starch *in vitro* suggest that bifidobacteria have a limited capacity to use it as a substrate. Other studies *in vitro* and *in vivo* in pigs (with a caecal cannula) and human subjects (with ileostomy) showed that amylolysis generates a characteristic etching pattern. There is conversion of the irregular granule to a more spherical shape with formation of a pit, which may be the site of initiation of amylase attack (Topping *et al.* 1997). It was thought that the etched granule could confer physical protection on the LAB on passage through the upper GIT. Several *in vitro* studies have confirmed physical adhesion of several Bifidobacteria species not only to this RS but also to chemically-modified RS starches, i.e. RS_4 (Brown *et al.* 1998). An interesting observation from the feeding trials in pigs is that FOS and RS maintain colonisation in pigs when probiotic consumption ceases. Thus, in animals fed the control diet faecal bifidobacteria numbers declined rapidly after withdrawal of the probiotic, but the decline was much slower in those fed the FOS or RS. When FOS and RS were consumed together, there was no decline in faecal numbers. It could be suggested that the frequency of consumption of probiotics can be lowered through eating foods containing RS or FOS. The data also resemble those obtained from *in vitro* incubation studies with yoghurts in which the addition of RS to yoghurt maintained the viable counts of bifidobacteria over several weeks, while those in the control yoghurt declined (Brown *et al.* 1998). The question remains as to whether all types of RS can function as prebiotics? Although relatively few foods have been examined, the answer seems to be that some do not. For example, studies in which weanling pigs were fed a convenience rice baby food have shown that it is high in RS relative to conventional foods. More starch was recovered in the large bowel, and SCFA levels were higher in pigs fed the rice product. However, there was no significant difference between the groups in lactobacilli and bifidobacteria in the large bowel (A Bird, M Jackson, R Rankin and D Topping, unpublished results). Given the range of influences on RS, it is not unexpected that there are differences between different forms of RS. Clearly, there is need for further systematic investigation of the prebiotic and synbiotic potential of various RS types and the reason(s) for any differences between them.

Resistant starch and the colonic microflora

It appears probable that RS has health-promoting actions on the colonic microflora above and beyond the prebiotic effect. Studies in which children with cholera-induced diarrhoea consumed RS (as a high-amylose starch) plus the usual hydration therapy have shown a major reduction in fluid loss and a halving of time to recovery (Ramakrishna *et al.* 2000). This study has been replicated in children with other forms of infectious diarrhoea where it was shown that both RS (as green bananas) and NSP facilitated recovery (Rabbani *et al.* 2001). Accelerated recovery from infectious diarrhoea has also been shown in animals. A specific micro-organism, *Brachyspira hyodysenteriae*, causes substantial economic losses in the commercial pig breeding industry through morbidity and mortality in the weaning period. The effect is expressed as diarrhoeal disease on the introduction of solid food. Feeding with cooked rice, an established source of RS (Marsono *et al.* 1993), lowers the incidence and severity of disease with a consequent reduction in mortality (Hampson *et al.* 2000). Part of the benefit seems to be due to increased fluid absorption through greater SCFA production, as these acids stimulate the uptake of water and cations (Na^+, K^+ and Ca^{2+}), particularly in the proximal colon. This outcome is an obvious mechanism for reversing diarrhoea-induced fluid loss. SCFA also appear to modulate the muscular activity of the large bowel and to promote the flow of blood through the viscera; both these actions could assist in lowering the severity of diarrhoea. In addition to these well-documented effects, it is possible that RS could limit the viability of the cholera organism in the gut. It may be hypothesised that the bacteria could adhere to starch granules, very much in the same way as bifidobacteria, and thus be removed from the site of infection. The same mechanism would explain the effect of the convenience rice-based baby food on coliforms in pigs. In the study mentioned previously (A Bird, M Jackson, R Rankin and D Topping, unpublished results) it was noted that the numbers of total coliforms and of *E. coli* in proximal colonic contents were lowered from about 8 and about 7 log_{10} cfu/g digesta respectively to about 6 log_{10} cfu/g digesta. Both the high-amylose starch and the rice product also lowered gut pH, which is a further means of biocontrol for pathogens and potential pathogens. Given that probiotics also can speed recovery from diarrhoea, there may be potential for a synbiotic that would optimise the benefits of both agents. Maximal effectiveness might be achieved if the prebiotic were a mixture of OS and RS, as these agents seem to have additive effects.

Probiotics, prebiotics and large-bowel short-chain fatty acids

A key issue in probiotic research is how the organisms act to promote the health of the large bowel and lower the risk of infectious and non-infectious disease. It seems reasonable to expect that the production of secondary metabolites (e.g. lactate) and of specific compounds (such as bacteriocins) would inhibit the growth of pathogens (Bird *et al*. 2000*a*). This inhibitory effect could be enhanced through the immune stimulatory effects of probiotics. However, it is hard to see how probiotics could act in some of the other conditions of interest proposed, especially in weaned infants. For example, it seems unlikely that these mechanisms would be effective in conditions such as colo-rectal cancer, constipation or inflammatory bowel disease, which are major socio-economic problems in affluent Westernised countries. The situation seems relatively clear for dietary complex carbohydrates, especially in facilitating bowel emptying where their preventive and therapeutic actions are well established. These carbohydrates influence the health of the large bowel in man either through physical bulking (e.g. NSP) or through the products of fermentation (e.g. OS and RS). Foods high in NSP, especially those containing what has been termed 'insoluble fibre' (e.g. wheat bran), are particularly effective in promoting bowel emptying through greater physical bulking. Published studies suggest that RS fermentation in the large bowel is complete so that it is a much weaker laxative. It appears that, for RS, the SCFA actually mediate its actions. This effect is especially relevant for conditions other than constipation and diverticular disease. Studies *in vitro* or in animals and human subjects suggest that some (but not necessarily all) forms of RS raise butyrate production. Elsewhere in the present symposium there is considerable discussion of the mechanisms whereby butyrate acts to promote a normal phenotype in colonocytes (Williams *et al*. 2003). Nevertheless, there appears to be a consensus that raising butyrate levels in the large bowel could be of positive health benefit. Increasing butyrate levels is particularly important in the distal colon, the site of most organic human large bowel disease. SCFA production and availability predominate in the proximal large bowel where fermentation is greatest, reflecting substrate supply. Fermentation and SCFA levels fall on passage of the digesta stream through a combination of diminished production and uptake and utilisation by colonocytes. Some studies suggest that butyrate falls disproportionately, probably due to its preferential utilisation by colonocytes, and it has been suggested that a combination of RS and insoluble fibre is optimal in terms of ensuring that SCFA supply to the distal colon is optimised (Govers *et al*. 1999). While it is reasonably well established that RS can modify large bowel and faecal SCFA favourably, probiotics do not (for example, see Brown *et al*. 1997) and, on current evidence, seem unlikely to do so. LAB (bifidobacteria and lactobacilli) are found most abundantly in the human GIT in milk-fed infants where a bifidobacterial fermentation appears to predominate (Wolin *et al*. 1998*b*). The SCFA profile in these babies is quite unlike that in adults (Edwards *et al*. 1994). Acetate is the major SCFA in both adults and pre-weaned bottle- or breast-fed infants. However, in the latter propionate is present at much lower concentrations while butyrate is virtually absent. Other products such as ethanol, formate, succinate and lactate can appear in faeces from pre-weaned infants. These are not present in substantial amounts in adults (Wolin *et al*. 1998*a*). Clearly, these metabolites may play an important role in controlling infection, while the absence of butyrate could be important for neonatal gut development, with milk-borne or locally-synthesised growth factors fulfilling this role (Bird *et al*. 2000*a*). The fact that yoghurt does not contain free butyrate at high levels supports the view that probiotics available currently will stimulate its production in the human colon. Probiotics under development could promote butyrate production, while current preparations may exert health benefits through other mechanisms such as non-specific stimulation of the bactericidal action of lymphocytes.

Conclusions

The current state of knowledge of RS as a prebiotic is fragmentary and limited, and there is no substantial 'state of the art'. Much of the experimental work has been done in animals and most of these studies and those in human subjects were of relatively short duration. However, it must be recognised that there is a great deal of promise. One preparation (a high-amylose maize starch) has been shown to function as a prebiotic and synbiotic, probably through physical adhesion of the bacteria to the starch granule. This physical protection is a central issue in prebiotics, as adhesion or encapsulation seems to be an important means of ensuring the viability of ingested organisms. Obviously, this activity may not apply to those organisms already resident in the gut and it is becoming clear that there needs to be a distinction made between autochthonous bacteria and those which are consumed either as supplements or as part of the diet. However, the number of published studies on the prebiotic action of RS is quite small and much more work is required to determine which forms of RS are prebiotics. The interaction of RS and FOS suggests that combinations may be more effective than the ingredients alone and this factor too needs to be investigated. Various forms of RS themselves appear to have positive effects on large bowel function through the SCFA produced by their bacterial fermentation. Indeed, it seems that modulation of the bacterial population and its production of SCFA by RS and other fermentable carbohydrates is of critical importance in the health of the large bowel. It appears equally unlikely that current probiotics, which are mostly LAB, can produce similar changes in SCFA, especially in butyrate. Studies are required to determine whether the stimulation of LAB in adult human subjects through the ingestion of RS leads to any health benefit above and beyond that of the carbohydrate itself. The field of prebiotics is in its infancy and only recently has there been systematic investigation of the health potential of probiotics. Much more work is required to answer the questions raised in the present paper, especially longer-term studies to quantify the health benefits of prebiotics, probiotics and synbiotics.

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