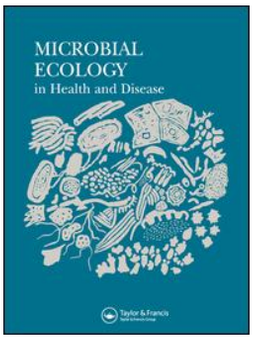

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**EXTENDED ABSTRACT**

# Resistant starches as a vehicle for delivering health benefits to the human large bowel

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**Abstract**

Non-starch polysaccharides (NSP; major components of dietary fibre) have been rather disappointing in the prevention and management of large bowel inflammatory diseases (IBD) or colorectal cancer (CRC). Resistant starch (RS) is that starch which escapes small intestinal digestion and enters the large bowel. RS contributes to total dietary fibre and could be as important as NSP in promoting large bowel health and preventing IBD and CRC. Indeed, it appears that some societies historically at low risk for these conditions eat relatively little NSP but have diets high in RS through their culinary practices. RS acts largely through its large bowel bacterial fermentation products which are, in adults, short chain fatty acids (SCFA). Collectively, SCFA have several non-specific positive actions on large bowel physiology including lowering of luminal pH. Of the major acids, butyrate has attracted the most attention. It is a major metabolic fuel for and promoter of a normal phenotype in colonocytes. Recent data from our laboratory support the latter suggestion. We have shown that, in rats, higher dietary protein (as casein, red meat or soy) increases colonocyte genetic damage and thinning of the colonic mucus barrier. However, feeding of RS as a high amylose maize starch opposed both of these changes in proportion to increased colonic butyrate. These data accord with prospective population data showing lower CRC risk with consumption of total dietary fibre. RS intakes appear to be low in most affluent industrialized countries, so increasing its consumption by modifying consumer foods is one strategy to improve public health. CSIRO and its partners are developing new high amylose cereal cultivars for this purpose. Colonic delivery of specific SCFA could also be useful clinically and we have shown that acetylated, propionylated and butyrylated starches resist small intestinal amylolysis. The bound SCFA are released by the large bowel microflora, raising their digesta levels, with the greatest increase being in the esterified acid. Feeding studies with butyrylated starch in rats have confirmed the opposition of diet-induced genetic damage, supporting a role for this SCFA in lowering risk of CRC and IBD. Further human and animal interventions are planned to determine the potential of these new types of RS in enhancing colonic health.

## Introduction

Diet and lifestyle-related diseases are major, preventable causes of morbidity and premature mortality in many affluent industrialized countries. These illnesses include coronary heart disease, certain cancers (e.g. large bowel), inflammatory bowel diseases (IBD) and diabetes (1). Evidence is accumulating that they are emerging as serious issues in developing countries through greater affluence with industrialization (2). There is broad consensus that they are linked to diet and lifestyle, and dietary modification is an established means of risk reduction (3). One strategy for improving public health is appropriate modification of the food supply to give products that deliver substantiated health benefits while retaining consumer appeal. Foods high in dietary fibre are a ready example. Increased fibre intakes are an effective means for the prompt improvement of laxation (4). Fibre intakes have increased in Australia relative to some other comparable countries, apparently through greater consumption of high fibre foods (not as supplements) (5). It is a reasonable assumption that problems of laxation are less in Australia than in those other countries where fibre intakes are

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lower. However, despite this change in dietary habit, some of the expected health benefits have failed to materialize. This is particularly the case for colorectal cancer (CRC), where morbidity and mortality remain stubbornly high (6). Indeed, a meta-analysis of a large number of cohort studies has failed to show any relationship between fibre intakes and CRC risk (7). A dietary intervention in people with a genetic predisposition to greater risk through familial adenomatous polyposis has not shown any diminution of polyp recurrence with consumption of low fat diet, high in fibre, fruits and vegetables (8). Conversely, a large, multicentre European prospective study showed a substantial protective effect of fibre on risk (9). Part of the reason for this discrepancy may be the measurement methodology for dietary fibre, as this can include other components beside non-starch polysaccharides (NSP). It may also be due to a misinterpretation of the original studies that stimulated interest in the role of dietary fibre in human health.

## NSP, starches and human health

One of the central theses of this review is not that fibre is unimportant to human health, clearly it is. Rather, it is that the focus paid to NSP has tended to diminish the contribution of other 'fibre' components, particularly resistant starch (RS). Much of the current interest in fibre comes from observational studies, which showed that that the consumption of unrefined cereals protected against serious non-infectious disease. Specifically, Dennis Burkitt and his colleagues noted that native Africans consuming foods high in unrefined cereals (principally maize corn) showed a lowered risk of the diseases of affluence affecting the Europeans living in the same region who ate highly refined foods (10). The marked difference in foods led to the natural assumption that the dietary fibre consumption of the Africans was much higher than that of the Europeans. It has become apparent that what is meant by fibre in the traditional sense is principally polysaccharides other than starch, i.e. NSP. These polysaccharides resist human small intestinal digestive enzymes completely (11), which helps to explain their excellent faecal bulking properties, particularly products high in insoluble NSP (such as wheat bran) (12). Indeed, it is possible that the laxating effects of fibre can be explained largely in terms of increased stool bulk (13). It appears likely that, until relatively recently, there were serious problems with the measurement of the fibre content of human foods, with underestimates being the norm due to the destructive nature of the procedures (12). Modern analytical procedures using specific enzymes to degrade starch followed by either weighing of the residue (for total dietary fibre, TDF) or hydrolysis followed by chromatographic analysis of the constituent sugars (for NSP) has given much greater reliability and also a more accurate estimate of their levels in foods. Application of these techniques to staple South African and European foods has revealed that although the former were unrefined, their fibre content was actually lower than those of the Europeans (14). The factors that distinguish the two populations were that the Africans ate considerably more unrefined starch and also that their cooking practices were rather different. Both rural and urban Africans consume as much as 70% of energy as maize starch, largely as cooked foods that have been cooled and then stored for some time. Cooking of starch is necessary to increase digestibility but cooling and storage leads to a re-association of starch chains through a reorganization process known commonly as retrogradation. The latter means that accessibility of starch to amylase is restricted so that its small intestinal digestion is much less than in freshly gelatinized, refined foods, leading to a higher level of what has become known as resistant starch (RS). RS is that fraction of dietary starch and products of starch digestion that is not digested in the small intestine of healthy humans but passes into the large bowel (15). It is possible that modern processing and food consumption practices have led to lower RS consumption, which could contribute to the rise in serious large bowel disease in affluent countries.

RS is, effectively, a contributor to dietary fibre and some is measured in the TDF assay. However, it needs to be recognized that the TDF procedure was designed to remove starch enzymatically, meaning that RS should remain. However, RS occurs for a variety of reasons, some of which are not duplicated in the TDF procedure so that, without a standardized and reliable measurement procedure, the exact contribution of RS to dietary intakes remains to be determined at the population and individual level (12).

## RS, large bowel bacterial fermentation and human health

RS is now attracting widespread attention for its health potential after a period of some neglect. In part, this reflects the uncertainty due to the analytical gap plus the well-known potential of the human small intestine to digest starch completely. It is very rare to find any undigested starch in normal human faeces – consistent with the concept that no dietary starch left the small intestine. However, Levitt and colleagues (16) showed that a potentially metabolically significant fraction of starch escaped

into the large bowel of healthy individuals. This was demonstrated by the substantial evolution of breath $H_2$ following consumption of convenience cereal foods and points to the key difference between small and large intestinal digestion. In the latter, digestion is effected by the large and taxonomically diverse population of resident bacteria (12). This colonization occurs during birth and early life. Post weaning, these bacteria obtain energy through fermenting undigested dietary carbohydrates, proteins and other nutrients and endogenous secretions in a process that resembles that in obligate herbivores with similar end products – gases, some heat and SCFA. The process consumes nitrogen (largely as urea or ammonia) for bacterial protein and nucleotide synthesis. It also provides energy for the host, as more than 95% of SCFA are absorbed and metabolized by the viscera.

The three principal SCFA found in adults (acetate, propionate and butyrate) have a number of general effects in the colonic lumen. For example, their production lowers digesta pH both through direct acidification and also the consumption of $NH_4^+$ to supply nitrogen for bacterial growth. Lower pH is thought to control the overgrowth of potentially pathogenic bacteria and lower the risk of infectious diarrhoea. A more acidic anvironment will also limit the absorption of potentially cytotoxic agents such as $NH_4^+$. SCFA absorption is coupled to that of water and cations. It was thought formerly that the effect was limited to $Na^+$ and $K^+$ but it is becoming apparent that there is also substantial absorption of $Ca^{2+}$ and $Mg^{2+}$. Increasing SCFA supply to the large bowel should improve water salvage. RS (as high amylose maize starch) has been used successfully to treat humans with cholera (17), although SCFA levels were not reported.

Of the major SCFA, butyrate has been credited with quite specific beneficial actions (12,18). It is believed to be the principal metabolic substrate for normal colonocytes, especially in the distal colon – the site of most organic large bowel disease including cancer. Butyrate is believed to modulate colonic muscular activity with relaxation at lower concentrations and contraction at higher levels. This acid also promotes large bowel blood flow through relaxing resistance vessels, improving tissue perfusion and oxygenation. However, it is the potential of butyrate to maintain a normal cell phenotype in colonocytes that has attracted most interest. A wealth of experimental data shows that butyrate inhibits the growth of cancer cells *in vitro*, especially through the induction of apoptosis. Butyrate also stimulates the growth of normal cells and promotes DNA repair in damaged cells. These actions of butyrate are achieved at concentrations that can occur in the colonic digesta. There is also evidence from rat studies which suggests that it can promote a normal colonocyte population when the animals are treated with agents such as azoxymethane (AOM) to induce large bowel cancers. A human intervention showed also that colonic mucosal proliferation (as measured by proliferating cell nuclear antigen immunostaining) was significantly lower when large bowel butyrate was increased by consumption of RS (19). However, it must be recognized that demonstration of a direct inhibitory effect of higher large bowel butyrate concentrations on human colon carcinogenesis is yet to be obtained. Nevertheless, the circumstantial evidence for a positive role for butyrate is promising. Propionate seems to have many of the beneficial effects of butyrate but much higher concentrations of the acid are required.

A key element in the development of what has become known as the 'butyrate hypothesis' linking the higher consumption of RS to lower risk of non-infectious large bowel diseases is the demonstration that those populations were protected by the consumption of fermentable carbohydrate, especially RS. This has been done for native Africans by Segal and colleagues (20). They showed that consumption of staled maize porridge (i.e. the porridge had been boiled and then allowed to cool for some time) by volunteers with ileostomy gave much higher levels of SCFA, including butyrate, in the ileal effluent compared with fresh maize porridge. The difference between the two is that maize porridge left to stand has more RS through retrogradation. Indeed, small intestinal carbohydrate malabsorption has been proposed as a key protective mechanism against colorectal disease in native South Africans (21), who have much lower rates of CRC than African-Americans in the USA (22).

## RS, butyrate and the reversal of protein-induced colonocyte genetic damage and mucus thinning in rats

Of necessity, much of the evidence for a protective role of butyrate in large bowel disease has been derived largely from population studies and animal and *in vitro* experimentation. In the case of CRC, experimental support for a possible protective role of RS via butyrate generation has come from studies in rodents, where it has been shown that feeding of RS lowers the size and number of bowel tumors induced by treatment with genotoxic agents such as azoxymethane (AOM) (23). For IBD, there are data indicating that, in rats, feeding RS raises butyrate and ameliorates colitis induced by the administration of dextran sodium sulphate (DSS) (24). This model uses an agent that works through a mechanism

(disruption of the mucus barrier) that may differ from that operating in human ulcerative colitis (UC). In the latter condition, the lesions are located principally in the distal colon and are thought to arise from an inadequate supply of SCFA (especially butyrate), leading to colonic atrophy. There are limited data showing that butyrate enemas can ameliorate UC, which is consistent with the idea of colonocyte substrate starvation (12).

The experimental approaches used to investigate CRC and IBD both involve treatment with agents that do not necessarily replicate the disease initiation and progression processes in humans. Using epidemiological data as a base, we have explored the interaction between dietary protein and RS to probe their respective potential for disease causation and prevention without any additional agent. Population data showed that higher dietary protein intakes were associated with greater risk while RS (but not NSP) was protective (25). Initially casein was used as a model protein but in subsequent studies the role of red and white meat and other protein sources has been examined. These comparisons were made in light of a meta-analysis of prospective cohort studies that has shown a correlation between consumption of red (and processed) meat and risk of CRC (26). Many of these population studies suggest that there is no relationship between the consumption of white meat (fish or chicken) and CRC. Genomic damage is a precondition for oncogenesis and was assessed using the comet assay, which measures DNA strand breaks.

Initial studies showed that colonocyte genetic damage was increased by 150% by increasing casein from 15% to 25% of the diet in rats that were fed a highly digestible starch (27). Feeding RS as a high amylose maize starch at 48% of the diet opposed the damage completely. It must be recognized that these levels of RS are excessive in the context of human diets and a subsequent study showed that the protective effect of RS was dose-dependent, with significant opposition of the damage at 10% of the diet (28). As postulated from the epidemiological data, cooked red meat also increased genetic damage, an effect that was opposed by RS (29). The mechanism of induction of damage remains to be elucidated but may involve toxic products from protein fermentation. That the effect is not limited to proteins of animal origin was shown when soy protein was fed, with rates of genetic damage that equalled those obtained with red meat or casein (30). Whey protein did not induce any damage above control values. In all cases, any damage was reversed by RS. The mechanism for this apparent protection also remains to be elucidated but appears to be linked to butyrate as the closest negative correlation was with caecal butyrate pools (28). A further observation of relevance to UC was that higher dietary protein led to thinning of the mucus barrier, an effect that was again reversed by RS and correlated with the rise in butyrate. While caution needs to be exercised in extrapolating from animals to humans, these data suggest that RS could exert a protective role in the large bowel against serious diseases.

## Controlling starch digestibility to improve human health

RS occurs in foods for a variety of reasons, leading to its classification into four main types (Table I) (31). $RS_1$ is physically inaccessible starch that is found in foods such as partially milled grains where components such as NSP present a physical barrier between amylase and its substrate. $RS_2$ are the granular (ungelatinized) starches found in raw or partially cooked foods. Cooking is necessary to hydrate the starch granules to allow access of small intestinal amylase. $RS_3$ occurs through the retrogradation of cooked starches through the re-associaton of starch chains on cooling and standing (e.g. in the maize porridge consumed by Africans). This limits access of amylase to the chains. Finally, the $RS_4$ category describes the chemically modified starches used currently in the food industry for their technological (but not nutritional) attributes. Chemical modification leads to substitutions that inhibit amylolysis.

All of these are options to raise the RS content of processed consumer foods which seems, with a few exceptions such as beans and brown rice, to be low in this attribute currently (AR Bird and DL Topping, unpublished observations). One established approach is to develop grains with a high amylose content. Starch comprises two polymers, amylose and amylopectin. The latter is a very large (molecular weight $>10^7$ D), highly branched polymer which is relatively easy to gelatinize and slow to retrograde. In most food starches it represents 20–30% of the total. In contrast, amylose is a relatively small molecule (500–600 glucosyl units)

Table I. Nutritional classification of resistant starches.

| Types of resistant starch | Examples of occurrence |
|---|---|
| RS1 – physically inaccessible | Partly milled grains and seeds |
| RS2 – resistant granules | Raw potato, green banana, some legumes and high amylose starches |
| RS3 – retrograded | Cooked and cooled potato, bread and cornflakes |
| RS4 – chemically modified | Etherized, esterified or cross-bonded starches (used in processed foods) |

with a relatively unbranched structure. This starch variant is relatively slow to gelatinize and quick to retrograde, making it a potential source of RS. There are two basic routes to raise the amylose content of grains, i.e. either by inhibiting the synthesis of amylopectin or by enhancing that of amylose synthesis. One of the first of these high amylose starches to find widespread food use was the high amylose maize (corn) starches (HAMS), which contain $\geq$70% amylose and are made using the amylose extender route. HAMS have found general food use as food ingredients, especially in bakery products (32). Foods containing HAMS have been shown to raise faecal SCFA (including butyrate) in humans when consumed as processed foods (33). However, they are susceptible to loss of resistance on heating, which reduces their RS content appreciably (34) and other sources of RS are being sought. CSIRO has embarked on a program of research and development to meet that need.

Barley is a major cereal crop worldwide but relatively little is used for human food, with the bulk going to either brewing or animal feed. There is interest in the potential for barley foods to promote human health, especially as sources of RS. A novel hull-less barley cultivar (BARLEYmax™) has been produced using conventional breeding techniques. This cultivar shows elevated levels of amylose through inhibition of amylopectin synthesis. It has a single nucleotide change in the gene coding for a key enzyme in starch synthesis (starch synthase IIa), leading to a loss of its activity, yielding a lower content of total starch and relatively more amylose (35). Other changes include increases in total dietary fibre and β-glucan. Animal trials have shown clearly that this new barley is high in RS, with significantly more starch in the large bowel compared with standard barley or oats (36,37). Wheat is a more important global human food crop than barley and wheat products offer a major route for large-scale improvements in public health. Using genetic modification technology, a novel wheat cultivar high in amylose has been produced (38). This wheat was generated using RNA interference (RNAi), whereby a key starch synthetic enzyme was inhibited. A feeding trial in rats has shown that large bowel digesta mass and SCFA were significantly higher when the diet contained high amylose wheat compared with a standard wheat (38). These data are consistent with more RS in the new wheat and further trials are planned to determine its potential nutritional benefits in humans.

Generation of SCFA within the large bowel is a key aspect of RS action and there has been considerable interest in developing a method for their effective delivery to the colon for use in specific (clinical) applications. The $RS_4$ classification encompasses the range of chemically modified starches used industrially in processed food manufacture. Acylated starches fall in this grouping and are potential vehicles to deliver SCFA to the large bowel. These starches have a number of benefits, not least of which is that specific SCFA (i.e. acetate, propionate or butyrate) can be conjugated so that products can be tailor-made for particular applications. Acylated starches have been shown to deliver SCFA to the large bowel of rats (39,40). The degree of acylation needed for SCFA delivery is rather higher ($\sim$20%) than that used currently (2%) by the food industry for ingredient manufacture. This is because the catalytic site of human small intestinal α-amylase spans four glucosyl units, so a high degree of substitution is needed for inhibition. Once in the large bowel bacterial lipases and/or esterases release the acyl groups while the residual starch is fermented. Acylated starches have an additional advantage in that their capacity to deliver SCFA survives the cooking conditions that are used in food processing (41). Studies are under way to determine the effectiveness of these starches in humans.

## Conclusions

Dietary starches are established, important sources of energy for diverse human societies but it is clear that they can also make quite specific contributions to health. In the large bowel these benefits come largely from the fermentation of undigested starch (RS) to SCFA by the resident microflora. Human and animal studies have demonstrated effects of SCFA consistent with enhanced large bowel function and protection against serious large bowel diseases. Many modern convenience foods are low in RS, which offers opportunities for the development of new cereal cultivars and starch-based ingredients for food products that can improve public health. These products can also be applied clinically.

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