Location of colonic fermentation events: Importance of combining resistant starch with dietary fibre

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Dietary carbohydrates which escape digestion may be important in protecting against colon cancer. The two major forms of carbohydrate to reach the colon are non-starch polysaccharide (NSP) and resistant starch (RS). Any carbohydrate that reaches the large bowel is fermented by colonic microflora to products including gases and short chain fatty acids (SCFA). The type of byproduct produced is largely dictated by the substrate undergoing fermentation. Beneficial effects of indigestible carbohydrates include increased faecal bulk; reduced intestinal transit time; lower luminal pH; increased SCFA production (particularly butyrate); and lower concentrations of potentially damaging bile acids, ammonia and phenols. Butyrate appears to have anti-tumour-like properties. Non-starch polysaccharides (the major component of dietary fibre) have a broad range of effects on large bowel physiology. Experimental evidence to date suggests that wheat cereal fibre (rich in slowly fermented, insoluble NSP) may be most protective against colon cancer. Resistant starch has emerged as another major dietary determinant of large bowel function. Resistant starch appears to be rapidly fermented early during transit through the colon, producing SCFA and lowering luminal pH and concentrations of ammonia and phenols. While RS has no great impact on faecal bulking or transit time, RS-fermentation does produce more butyrate than the fermentation of NSP. In humans most colonic tumours occur in the distal portion of the colon. Thus it is important to consider the best combination of indigestible carbohydrate (RS and NSP) needed to produce the most optimal fermentation-dependent parameters at this site. Protective effects may relate to the supply of SCFA (especially butyrate), low luminal pH and dilution of toxic byproducts at the location where neoplasm occurs: the distal colon. Resistant starch provides a rich source of highly fermentable carbohydrate but is degraded too early in its intestinal transit. Thus slowly fermentable, insoluble NSP could act as a carrier to propel RS into the distal colon. The combination of RS and insoluble NSP may contribute to the prevention of colon cancer.

Key words: non-starch polysaccharides, resistant starch, short chain fatty acids, dietary carbohydrates, butyrate, colon cancer.

Introduction

In affluent Western countries such as Australia, the incidence of colon cancer is high, while in developing Asian countries such as India and China the incidence is low (Fig. 1).[1,2] Studies on migration from Africa and Asia to Western countries have provided particularly strong evidence that colon cancer is sensitive to environmental influences, particularly dietary change, with incidence rates sometimes reaching those of the host country within one generation.[1] The major causative dietary factors are fat and meat while plant food provides a range of protective factors.[1–6]

Plants are composed of a range of complex carbohydrates, in addition to factors such as antioxidants, each of which may have a different but protective role to play. Indeed, carbohydrate-enriched plant foods have probably had an important role in protecting against many of the so-called diseases of affluence including cardiovascular disease, non-insulin dependent diabetes as well as colon cancer.[7] In relation to colon cancer protection, indigestible carbohydrate fractions of plants are of particular importance.

This discussion will focus on the role of complex carbohydrates (i.e. non-starch polysaccharides and starch) in promoting colonic health, with emphasis on fermentative events within the colon and their putative relationship to colon cancer in humans.

Major classes of carbohydrate in the human diet

Much research on the protective role of dietary carbohydrates has concentrated on 'dietary fibre'. Population studies on fibre have, however, often produced results that are both difficult to interpret and misleading.[3] While some studies report protective effects for dietary fibre, others do not.[1,3] A better approach in the future may be to use 'markers' of carbohydrate-rich foods because when epidemiological studies are re-evaluated using whole grains, cereals and cereal fibre instead of 'dietary fibre', a more consistently protective effect has been found.[3]

Some of the confusion in this area appears to relate to the continued difficulty in defining and measuring the different classes of carbohydrate. Dietary carbohydrates can be

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broadly divided into digestible and indigestible classes (Fig. 2).[8] The major types of carbohydrate that are digested and absorbed in the small intestine include sugars (glucose, fructose, sucrose and lactose) and starch.[8] The major types of carbohydrate to reach the colon undigested include non-starch polysaccharides (NSP), resistant starch (RS) and short-chain carbohydrates (SC).[8,9] Short-chain carbohydrates, a term proposed by Englyst and Hudson (1996), includes naturally occurring fructans and galactans as well as synthetically produced fructo-oligosaccharides, pyrodextrins and Polydextrose.[9] For example, inulin would fit into the SC category. In addition, some SC will originate from the partial breakdown of RS. As can be seen from Fig. 2, the major component of 'dietary fibre' as measured by the Association of Analytical Chemists (AOAC) method, is NSP. The AOAC method also includes in its estimation for measuring 'dietary fibre' lignin as well as some Maillard reaction products and some forms of RS (which are generated by food processing and treatment of analytical samples).[9]

The measurement of total dietary fibre represents most but not all of the carbohydrate reaching the colon. Similarly, measurement of NSP will represent only one component of the carbohydrate reaching the colon undigested. It is generally agreed that the amount of carbohydrate that reaches the colon undigested is greatly underestimated because while approximately 60–70 g of carbohydrate per day would be necessary to sustain the colonic bacterial biomass,[10,11] a typical affluent Western diet is found by present methods to provide only approximately 15–25 g undigested carbohydrate per day.[3] To date, there is no single analytical method for predicting the quantities of carbohydrate reaching the colon. Clearly this is an important area for future research.

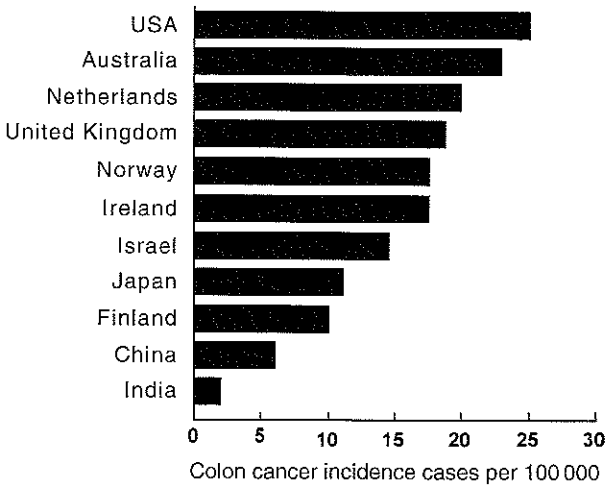

Figure 1. International comparison of incidence of colon cancer.[2]

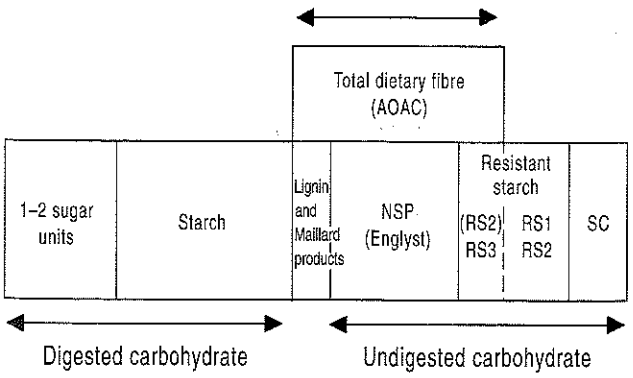

Figure 2. Classification of carbohydrates into digested and undigested categories. SC, Short-chain carbohydrates. RS, resistant starch; RS1, RS type 1; RS2, RS type 2; (RS2), some forms of RS type 2; RS3, RS type 3; NSP, non-starch polysaccharides; AOAC, Association of Analytical Chemists. Adapted from Asp.[8]

Carbohydrates which reach the colon undigested have the potential to affect the process of carcinogenesis in the colon. It is the metabolism of this indigestible carbohydrate by endogenous colonic bacteria which generates a luminal milieu that can influence the development of a wide range of bowel diseases including cancer.

Fermentation of undigested carbohydrate reaching the colon

Carbohyrate which escapes digestion is a potential substrate for colonic fermentation. The products of fermentation include gases (CO_2, CH_4, H_2), short chain fatty acids (SCFA) (acetate, propionate and butyrate), lactate and branched chain fatty acids (isobutyrate, isovalerate).[11,12] The type and amount of by-product is dictated by the substrate undergoing fermentation. Short chain fatty acids are the principal end-products of colonic fermentation and are produced in the approximate molar ratio of 60:25:15 (acetate, propionate and butyrate, respectively).[11,12] The major types of carbohydrate to reach the colon are NSP and RS and the products of starch degradation.

Fermentation of non-starch polysaccharides (dietary fibre)

It is now well established that NSP, the main component of dietary fibre, has a broad range of effects on large bowel physiology. The major classes of NSP are the following: (i) soluble/rapidly fermented NSP; and (ii) insoluble/slowly fermented NSP. Cereal fibres are rich sources of insoluble NSP (e.g. wheat bran), while vegetables and fruit are rich sources of soluble NSP (e.g. pectin). The amount of NSP in the human colon is largely governed by dietary consumption.[13] The most recent Australian National Nutrition Survey, conducted in 1995, indicates that Australian men consume approximately 26 g of dietary fibre per day while women consume 20 g per day.[14] The CSIRO National Dietary Surveys collected in 1993 determined that Australians consumed 21 g of NSP per day, 9 g from soluble NSP and 12 g from insoluble NSP.[15] Experimental evidence involving humans strongly suggests that wheat cereal fibre (rich in slowly fermented NSP) may be the most protective against colon cancer.[16]

Many of the beneficial effects of NSP are linked to the process of colonic fermentation. The products of NSP fermentation include gases (CO_2, CH_4, H_2), SCFA (acetate, propionate and butyrate) and lactate.[11,12] Short chain fatty acids, the major by-products, have several actions that appear relevant to maintaining the health of the large bowel. They are rapidly absorbed by the colonic mucosa, promoting water and sodium absorption, and thereby preventing osmotic diarrhoea.[17] Also, together with lactate, they help to acidify the colonic lumen.[11,12] A low pH reduces the bacterial conversion of primary bile acids into secondary bile acids.[18] In

addition, a low pH can affect other processes including the ionization of SCFA; epithelial proliferation; the balance of bacterial species; bacterial metabolism of ingested carcinogens; bacterial activation of oxygen free radicals; the activity of bacterial enzymes (e.g. β-glucosidase, β-glucuronidase); and faecal water cytotoxicity.[18–20] Some of these actions may provide a degree of protection against bowel cancer.

There is strong evidence that the SCFA butyrate is important for the metabolic welfare of the epithelium of the large bowel. Butyrate is utilized as an important energy source by the colonocyte, the main cell type in the colon.[21] Butyrate also has a range of effects particularly relevant to bowel cancer. It 'stabilizes' DNA by inhibiting histone deacetylase[22] and increasing methylation of DNA;[23] it induces differentiation; and it reduces the growth rate in a range of mammalian cells, including colorectal cancer cell lines.[24] Recent interest has also focused on the effects of butyrate on induction of apoptosis (i.e. programmed cell death) of colonic epithelial cells[25] (see Graeme Young *et al.*).[26]

The capacity of rich sources of insoluble/slowly fermented NSP, such as wheat bran, to increase faecal bulk and reduce transit time are well established.[6,11] The increase in faecal mass is due to an increase in both bacteria and water content. Some water may also be retained within the structure of remaining non-degraded fibre (e.g. lignin in bran).[11] An increase in stool mass is important in relief of constipation and in the prevention of diverticulosis and anorectal disorders such as haemorrhoids. Bulkier stools may also enable a dilution of potential toxic compounds, with important implications for reducing the risk of bowel cancer.[6] The ability of cereal fibre to increase stool bulk and to dilute potential carcinogens and tumour promoters is one of the most thoroughly tested and well established mechanisms of fibre action.[27]

Strong evidence for the importance of wheat cereal NSP in the diet for protection against carcinogenesis can be gained from the recently completed Australian Polyp Prevention Project. This randomized controlled trial in volunteers with previous adenoma established that low fat diets with high fibre intake (from wheat bran) reduced the appearance of large adenoma at 2 and 4 years.[16]

Resistant starch: Another carbohydrate substrate for colonic fermentation

Resistant starch is emerging as another major dietary determinant of large bowel function in humans. For example, RS, like NSP, acts as a substrate for fermentation.[28–35] This area has attracted great research interest because it appears that the fermentation of starch produces significantly more butyrate than the fermentation of NSP.[36]

Three main types of RS occur naturally in the human diet: RS1, physically inaccessible starch (e.g. coarsely ground grains and cereals); RS2, ungelatinized starch granules (e.g. green banana, high amylose starch, raw potato); and RS3, recrystallized or retrograded polymers (amylose and amylopectin) (e.g. gelatinized and cooled high amylose maize starch).[37] A fourth type of RS, RS4, does not occur naturally and represents chemically modified starches.[37]

As seen in Fig. 2, the AOAC method for measuring dietary fibre includes some forms of RS in its estimation, mainly RS3 and some forms of RS2. The recovered forms of RS2 are starch granules requiring super atmospheric temperatures to become fully disrupted (e.g. the starch granules found in high amylose maize). As some of the major types of RS found in the diet are RS1 and RS2 (see Figs 3 and 4), most RS will not be measured by the AOAC method. RS1 is destroyed by grinding while RS2 becomes gelatinized when boiled in the presence of excess water and both grinding and boiling are early steps in the AOAC method. Clearly a separate test was needed for the determination of RS. While a number of methods have now been developed, the most appropriate methods are those that include all forms of RS and that have been validated in an appropriate human model (e.g. human ileotomy or intubation method).[37–41] It is becoming increasingly apparent that is important to identify the various types of RS present in food as recent evidence sug-

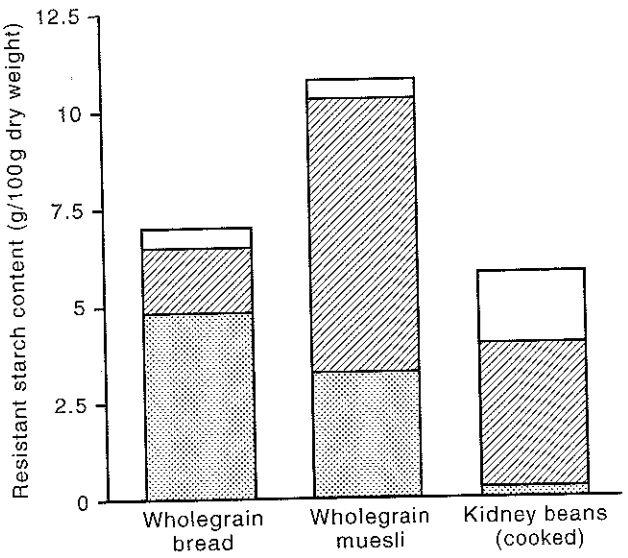

Figure 3. Examples of types of resistant starch (RS; RS1 (▨), RS2 (▨) and RS3 (□)) present in a whole grains and cereals.[42]

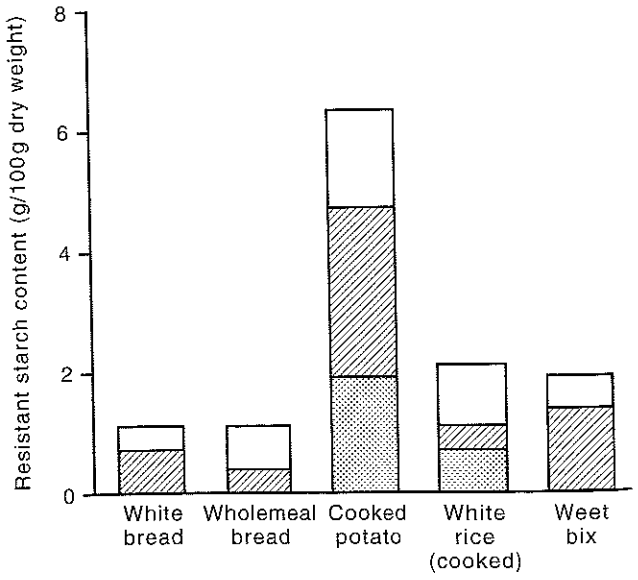

Figure 4. Examples of types of resistant starch (RS; RS1 (▨), RS2 (▨) and RS3 (□)) present in processed foods found in a typical Australian diet.[42]

gests that, like NSP, different types of RS may have different physiological effects.

Unlike NSP, RS levels in foods can be easily manipulated through choice of plant variety (e.g. high or low amylose maize starch) or food processing technique (e.g. grinding/milling or cycles of cooking and cooling). It is this aspect of RS which had made it difficult to develop an analytical test with which to reliably measure levels in foods. The major types of RS present in unprocessed grains and cereals (i.e. whole grains) are RS1 and RS2 (Fig. 3), while in breads and cereals typical of a westernized diet RS2 and RS3 are the major contributors (Fig. 4).[42]

The total amount of RS reaching the human colon is influenced greatly by total starch consumption.[2,43] Around 4–5% of total starch intake will reach the colon undigested.[2,43] From studies where a typical Australian diet was fed to individuals with an ileostomy,[44] we can predict that Australian men consume approximately 6 g RS/day while Australian women consume approximately 4 g/day.[44]

The effects of RS on human health are currently being elucidated; however, it appears that most of the effects of RS come from localized effects in the large bowel. A number of reports, including our own work, now describe the physiological effects of RS on the large bowel of humans.[28–35] Resistant starch raised faecal SCFA (including butyrate),[28,30,31,34] lowered faecal pH,[31] reduced colonic cell proliferation,[30] and lowered secondary bile acid concentration in human faecal water.[30,33] Our group has also observed a clear relationship ($r = 0.84$, $P < 0.01$) between starch content in the faeces and faecal butyrate excretion. This is consistent with previous *in vitro* evidence that starch is a good substrate for the production of butyrate.[31]

Although findings are inconsistent, some reports have indicated that RS has beneficial effects on faecal bulking and gut transit time.[29,31–3] The response may depend on both the quantity and type of RS consumed. Cummings *et al.* recently reported that effects on bulking were greater with RS3 (from retrograded maize and wheat) than with RS2 (potato and green banana).[32] They also found that RS2 (from green banana) delayed whole gut transit, while the bran NSP group increased it.[32] To date, effects of all forms of RS on bulking and transit are considerably less than bran NSP.[32] The most likely explanation for this difference relates to the extent to which RS and bran NSP are fermented; approximately 80–90% of RS but only 40% of bran NSP entering the colon are fermented (i.e. not recovered in faeces).[31–33]

Interactions between undigested carbohydrate and protein in the colon

Protein is also an important, but much less studied, dietary component that may escape digestion.[11] As much as 12 g of undigested protein may enter the colon per day.[11,45] This may derive both from endogenous sources (i.e. enzymes, mucin, shed epithelial cells) and also from the diet.[11] Diet-derived protein reaching the colon generally exceeds that from endogenous sources.[45] To date, little is known of factors affecting protein digestibility in the small intestine of humans, although there is evidence that the physical form (e.g. whole *vs* pureed legumes) can be important.[45]

As with undigested carbohydrate, protein which reaches the colon undigested is metabolized by the colonic microflora to end-products which include iso-derivatives of SCFA (isobutyric and isovaleric acids), phenol, cresol, indoles, amines, ammonia and phenylated SCFA, many of which have adverse effects.[11] Ammonia may promote tumourigenesis by stimulating cell proliferation[11,46] and favouring growth of malignant cells in preference to normal cells.[46] Volatile phenols (*p*-cresol and phenol), produced from the bacterial metabolism of aromatic amino acids, phenylalanine and tyrosine, are promoters of skin cancer and have been implicated in development of both bladder and bowel cancer.[11]

Fermentable carbohydrates may protect against the build-up of the undesirable by-products of undigested protein metabolism.[11,47,48] A number of studies have shown that fermentable NSP can lower levels of faecal ammonia.[11,47–49] A high fibre diet may also lower levels of urinary phenol, cresol[11] and *N*-nitroso compounds.[50] The mechanism for this action may be through the preferred use of carbohydrate as an energy substrate by rapidly replicating colonic bacteria.[11] Proliferating bacteria use undigested protein for their nitrogen requirements and thus act as 'nitrogen sinks'.

We have found evidence for these effects in a study on RS and bowel function.[35] In addition to a reduction in levels of ammonia we also found a decrease of faecal phenols (i.e. phenol and *p*-cresol).[35] Another study has confirmed our observation that high RS diets may be useful in reducing the concentration of faecal ammonia in humans, showing, in addition, that the effect was specific for RS3 (retrograded high amylose maize starch) and was not associated with RS2 (uncooked high amylose starch).[51]

A summary and comparison of the physiological effects of RS and soluble and insoluble NSP is given in Table 1. In general, RS is an indigestible carbohydrate with marked effects on fermentation-dependent parameters in the lumen (particularly increases in the SCFA butyrate). This contrasts with rich sources of insoluble NSP (from wheat) which have most effects on faecal bulking and hastening intestinal transit (Table 1).

Relative effects on faecal markers relevant to colon cancer risk between a high-starch Chinese and low-starch Australian diet

Most dietary studies into the effect of RS on bowel have been conducted using large quantities of relatively pure sources of RS (i.e. high amylose maize starches). Our group decided to

Table 1. Summary of the physiological effects of resistant starch (RS) and soluble and insoluble non-soluble polysaccharides (NSP) on large bowel function

	RS	Soluble NSP (e.g. pectin)	Insoluble NSP (e.g. wheat bran)
Faecal bulking	–/+	–/+	+++
Reduced intestinal transit time	—	–/+	+++
Increased SCFA conc.	+++	++	+
increased butyrate conc.	+++	—	+
Lowered faecal pH	++	+	—
Lowered faecal ammonia conc.	++	++	+
Lowered faecal phenols conc.	++	?	+

SCFA, short chain fatty acids; conc., concentration; +, positive effect; –, negative effect; ?, unknown.

examine the effects of a diet that is naturally high in starch and RS from a country with a low incidence of colon cancer (i.e. China) with one naturally low in starch and RS and from a country with a high incidence of colon cancer (i.e. Australia) on faecal markers relevant to colon cancer risk.[2] The Australian diet, however, is also high in meat protein and fat,[52] while the diet in China is low in both fat and animal protein (Table 2).[53] Both diets include similar intake of total dietary fibre — around 3 g dietary fibre per MJ per day.

We hypothesized that the mix of undigested food reaching the colon from a typical Australian diet would create a milieu detrimental to the maintenance of a healthy epithelium and would generate unfavourable changes in faecal markers (i.e. faecal output, faecal pH and faecal concentrations of SCFA, ammonia and phenols). In contrast, a low-income Chinese diet, high in starch, low in meat protein and fat would be expected to produce a more favourable luminal environment. The results of this study have recently been published.[54]

Two diets, each based on a 7-day menu, were constructed. The simulated Australia diet was based on the results of the 1990 survey of 3000 individuals conducted in Victoria, using the most commonly consumed foods and the mean macronutrient profile reported.[52] The low-income Chinese diet was based on the 1989 China Health and Nutrition Survey involving 16 000 individuals.[53] The composition of the two diets is shown in Table 2. Starch intake was considerably higher on the Chinese diet (370 g/day) than on the Australian diet (108 g/day). Twelve human volunteers followed each diet for 3 weeks in a random cross-over design study for which all meals were provided. Markers were ingested in week 3 for estimation of transit time. Total faecal output for 5 days was collected onto dry ice during week 3. Faeces were pooled and stored at – 70°C before analysis.

Compliance to the low fat, high starch Chinese diet was evidenced by the significantly lower level of serum total cholesterol after this diet than after the Australian diet (mean ± SEM: 4.17 ± 0.3 *vs* 5.04 ± 0.28 mmol/L, respectively; $P < 0.05$). As shown in Table 3, with the exception of faecal pH, the simulated Australian diet produced more favourable changes to faecal markers believed to be relevant to colon cancer risk than the simulated Chinese diet, that is, faster transit through the gut, increased faecal bulk and higher concentration of SCFA (including butyrate). Moreover, the concentrations of the potentially damaging ammonia, phenols[54] and secondary bile acids were lower during the simulated Australian diet (Table 3) (M Govers, unpubl. data, 1997). This was confirmed by a lower faecal water toxicity level (M Govers, unpubl. data, 1997).

These differences in effects on risk markers between the two diets could largely be explained in terms of differences in insoluble NSP. Both intake and faecal excretion of insoluble NSP was greater during the Australian diet (Table 3). Greater levels of insoluble NSP resulted in greater faecal bulking and hence dilution of potentially damaging toxic products (ammonia, phenols) in the luminal environment (Table 3).

Our results did not explain the low occurrence of colorectal cancer in China and tend to provide support for those studies suggesting starch as a causative agent in colon cancer.[3] It is interesting, however, to note comments made by Hill.[3] In a recent review, he suggested that the strongest evidence for dietary protection against colon cancer comes from diets enriched with high-fibre cereals (e.g. wheat and wheat products) and not from diets enriched with low-fibre cereals (i.e. rice and rice products).[3] Moreover, protection by 'cereals' may be less evident in the absence of clear dietary risk (e.g. high animal fat). It is also interesting to note that a study comparing colon cancer risk between the Chinese living in China and the Chinese living in the USA found that high saturated fat and lack of exercise were the major determinants of colon cancer risk.[55]

We suggest that one of the most likely explanations for our failure to observe more apparently beneficial changes in faecal markers during the simulated Chinese diet is that fermentable substrates (particularly RS) may have been broken

Table 2. Macronutrient intake during the simulated average Australian and the low-income Chinese diets (mean ± SEM, $n = 12$)[54]

	Australian	Chinese
Energy (MJ/day)	9.0 ± 0.6	9.0 ± 0.6
Total protein (g/day)	89.1 ± 5.6	63.9 ± 4.0
Animal protein	56.0 ± 3.5	10.6 ± 3.3
Vegetable protein	33.1 ± 2.1	52.6 ± 3.3
Total fat(g/day)	81.9 ± 5.1	38.3 ± 2.4
P:M:S	0.41:0.78:1	0.99:0.82:1
Total carbohydrate (g/day)*	260 ± 16	392 ± 25
Total starch**	108 ± 6.7	318 ± 20
Resistant starch**	7.8 ± 0.5	17.6 ± 1.1
Total sugars**	152 ± 9.5	73.8 ± 4.6***
Total NSP**	24.2 ± 1.5	13.7 ± 0.9
Insoluble NSP**	12.3 ± 0.8	8.7 ± 0.5
Soluble NSP**	11.9 ± 0.7	5.0 ± 0.3

Data represents the average of 7 days. P:M:S, ratio of polyunsaturated to monounsaturated to saturated faty acids. All values were derived from the food composition tables except starch, sugars and non-starch polysaccharides (NSP), which were measured by laboratory analysis of pooled sample diets. *Total carbohydrate = total starch + total sugars; **measured by laboratory analysis of pooled sample diets; ***the high sugar fraction of the Chinese diet was due to a high proportion of maltose (60 g/day), a product of starch breakdown. Data adapted from reference 54.

Table 3. Effect of simulated Australian and Chinese diets on faecal measurements (mean ± SEM, $n = 12$)[54]

	Australian	Chinese
Faecal output (wet weight g/day)	141 ± 20	86 ± 11*
Transit time (h)	56 ± 7	69 ± 6 **
Faecal pH	6.63 ± 0.05	6.51 ± 0.04*
Faecal excretion		
Total SCFA (mmol/L)	98.0 ± 7.6	72.8 ± 7.3*
Acetate	56.1 ± 4.3	39.9 ± 4.3*
Propionate	15.0 ± 1.3	12.8 ± 1.3*
Butyrate	18.4 ± 2.3	12.2 ± 1.3*
Ammonia (mg/L)	450 ± 40	540 ± 50*
Phenols (mg/L)	68.5 ± 12.9	109.2 ± 13.2*
Carbohydrate excretion (g/day)		
Starch	0.83 ± 0.38	0.82 ± 0.42
Total NSP	6.21 ± 0.88	3.74 ± 1.78*
Insoluble NSP	5.56 ± 0.72	3.07 ± 0.45*
Soluble NSP	0.65 ± 0.32	0.67 ± 0.13

SCFA, short chain fatty acids. *$P < 0.05$; **$P = 0.06$. Data adapted from reference 54.

down rapidly high up in the bowel, leaving little intact RS to reach more distal regions. Faecal pH and concentrations of SCFA, therefore, did not differ greatly from those found after the simulated Australian diet.[54] Certainly, these results do not support placing Australians on diets which are high in starch for cancer prevention, unless optimal amounts of insoluble NSP are also present.

Location of fermentation: Relevance to colon cancer

In humans, most colonic tumours occur in the distal portion of the colon.[56] For this reason it is particularly important to focus on fermentation-dependent events in this region.

It is known that diets differ in their degree and site of fermentation. For example, on a typical Western diet, the proximal colon contains the highest levels of undigested carbohydrate and protein.[11] This generates high levels of SCFA but low levels of ammonia and phenols, and a low pH. In contrast, by the time the undigested food reaches the distal colon most available carbohydrate (NSP and RS) has been fermented. Levels of SCFA are low, concentrations of ammonia and phenols are elevated and the pH rises.[11] For this reason, dietary interventions which result in greater amounts of undigested carbohydrate reaching the distal colon could be expected to greatly affect colon cancer risk. This was largely confirmed by our recent study comparing the Chinese and Australian diets on faecal markers (see above).

It is also important to consider the best combination of indigestible carbohydrate (starch and NSP) needed to produce these effects. Animal studies indicate that rich sources of insoluble NSP (e.g. wheat bran) generally reduce distal colonic tumorigenesis while soluble NSP (e.g. guar and pectin) do not.[57,58] This difference appears to be related to the site of fermentation of these nutrients. For example, in the rat, guar gum and oat bran are rapidly and completely fermented in the proximal colon and so have little impact on parameters such as pH and SCFA levels in the distal colon.[57,58] In contrast, wheat bran is more slowly fermented and maintains low pH and high SCFA concentrations along the entire length of the rat large intestine.

It is possible that the protective effects of undigested carbohydrate may relate to its ability to supply firstly, SCFA (especially butyrate) and a low luminal pH and secondly, an energy source for bacterial growth to divert the production of ammonia and phenols from undigested protein. Optimally, this should occur at the location where neoplasm occurs, that is, the distal end of the large intestine. Resistant starch provides a rich source of highly fermentable carbohydrate but it is degraded too early in its intestinal transit. Thus, slowly fermentable insoluble NSP could act as a carrier to propel RS into the distal colon (see Table 1 for comparison of RS and NSP effects).

Altering the site of fermentation in the pig: Implications for colon cancer risk in humans

We have recent evidence to support this hypothesis in the pig.[59] The pig appears to be a good model to study the effects of dietary manipulation on luminal changes which are relevant to colon cancer risk in humans.

In this study, 24 Large White Landrace, male pigs (initial bodyweight 60 kg ± 1.9 kg) were fed for 21 days (40 MJ gross energy (GE)/day) one of four diets, which differed only in RS and insoluble NSP content. Macronutrient composition including total fibre content was otherwise similar to human diets. The Control diet contained 10 g RS and 20 g NSP/10 MJ GE. Coarsely ground (< 3 mm) Hi-maize™ (mainly RS1 and RS2), and wheat bran (ground to < 3 mm) were employed to increase the RS and insoluble NSP content, respectively, giving diets high in RS (30 g RS/10 MJ GE), high in insoluble NSP (35 g NSP/10 MJ GE) or high in both RS and NSP (30 g RS and 35 g NSP/10 MJ GE). Celite™ was added to all diets as an indigestible marker. Pigs were commercially slaughtered on day 21 and intestinal contents sampled. Samples were analysed for starch, NSP and total SCFA including butyrate and ammonia.[59]

The results shown in Table 4 suggest that wheat bran (rich in insoluble NSP) was effective at shifting the fermentative process of RS to the distal colon, thereby improving the luminal conditions as indicated by higher butyrate and lower ammonia levels.[59] As tumours are most common in the distal colon, the combined intake of RS and insoluble NSP may allow dietary modulation of colon cancer risk.

Conclusion

Our work suggests the possible importance of combining indigestible carbohydrates such as RS and NSP (particularly insoluble NSP) to optimize colonic health. This may be particularly important given the location of most human tumours, that is, the distal region of the large bowel.

Table 4. Fermentation of resistant starch (RS) and non-starch polysaccharides (NSP) (g/day) and effects on butyrate and ammonia concentration in the proximal and distal regions of the pig colon ($n = 6$, mean ± SE)

	Control	RS	NSP	RS + NSP
Caecum + proximal colon				
RS fermented* (g/day)	20 ± 2	57 ± 13**	15 ± 5	48 ± 7
NSP fermented (g/day)	19 ± 3	21 ± 5	.34 ± 9	41 ± 6
Butyrate (mmol/L)	10.7 ± 0.8	17.8 ± 1.8**	13.0 ± 1.5	14.1 ± 0.9
Ammonia (mmol/L)	43 ± 3	35 ± 3**	45 ± 2	30 ± 4
Middle + distal colon				
RS fermented (g/day)	5 ± 2	13 ± 2**	9 ± 4	21 ± 2**
NSP fermented (g/day)	9 ± 2	5 ± 3	4 ± 9	5 ± 6
Butyrate (mmol/L)	10.6 ± 0.8	12.9 ± 1.2	11.2 ± 2.1	17.1 ± 1.6***
Ammonia (mmol/L)	46 ± 3	40 ± 2**	44 ± 5	34 ± 1 ***

Table reproduced from reference 59. *Fermentation of RS or NSP (g/day) was determined by disappearance of RS and NSP from the proximal or distal regions of the colon; **main effect of RS or NSP from control group (one-way analysis of variance, $P < 0.05$); ***significant positive interaction between RS and NSP (two-way analysis of variance, $P < 0.05$).

One method to combine RS and insoluble NSP naturally in the diet is by including more unprocessed whole grains and cereals. Our recent studies indicate the efficacy of this approach. We fed two diets to individuals with an ileostomy for 2 days and collected ileal effluent for 24 h on day 2.[44] One diet was a 'typical' Australian diet (low in starch, 88 ± 8 g/day) while the other was a modified Australian diet containing more starch (176 ± 24 g/day) in the form of unprocessed whole grains consumed both as a whole grain bread and as a breakfast muesli (Fig. 3). The results in Fig. 5 show that by using whole grain cereals, both RS and NSP recovered in the effluent were significantly ($P < 0.05$) increased.[44] For comparison, the levels of RS and NSP contained in a Chinese diet is included (Fig. 5).

The major forms of RS that increased in this study were RS1 and RS2 (Fig. 3). These types of RS probably made up the bulk of traditional human diets, that is, those rich in unprocessed grains and cereals. Indeed, population studies are now showing that whole grain consumption is linked to reduced risk of several types of cancers.[60] Also, in a report published very recently by Jacobs *et al.*, post-menopausal women who consumed whole grain cereals (23 serving per week) had a 30% lower risk of ischaemic heart disease compared with women consuming 1.5 servings per week.[61]

Thus, while the protective factor(s) in whole grains are not known they probably include the combination of low saturated fat content, and the presence of lignin, NSP, RS (particularly the trapped starches) and antioxidants (e.g. vitamin E). The benefits derived from whole grain supplementation of the diet emphasizes the importance of whole food consumption rather than relying on single nutrient additives. As nutritionists, we must encourage the replacement of fat in our diet by increasing our total starch intake through consumption of whole grains and cereals (this will automatically increase our intake of both RS and NSP) as well as other protective factors.

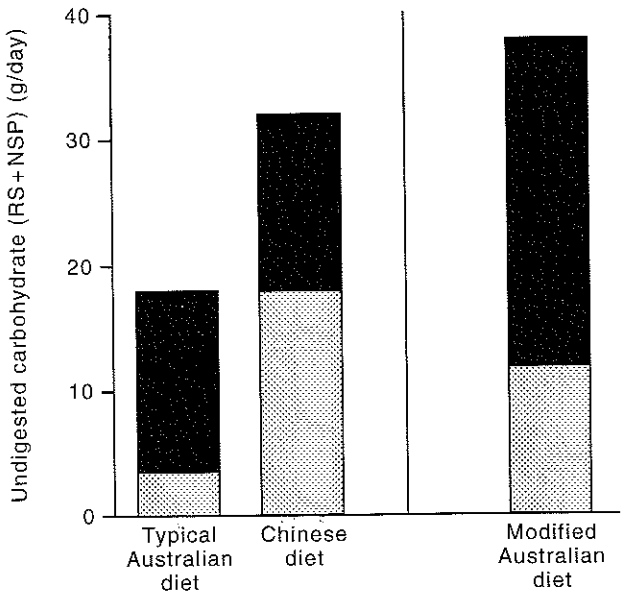

Figure 5. Comparison of resistant starch (RS) (▨) and non-starch polysaccharides (NSP) (■) excreted during a 'typical' (i.e. higher in unprocessed grains and cereals) Australian,[52] Chinese[53] and modified Australian diet.[44] The levels of RS and NSP in the Chinese diet was measured by direct laboratory analysis.[52] Excretion of RS and NSP during 'typical' and 'modified' Australian diets was measured in ileostomy effluent. The 'modified' Australian diet included additional starchy foods such as coarsely ground grains and cereals. Each diet was consumed by four healthy subjects with an ileostomy for 2 days, with effluent collected on the final day. Ileal starch excretion (i.e. RS) and ileal NSP increased significantly ($P < 0.05$) during the 'modified' Australian diet.[44]

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