

The effect of resistant starch on colon function in humans

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Starch that is resistant to human amylases forms during the cooking and subsequent cooling of some foods, and may therefore be a substrate for the bacterial flora of the colon. It is thus possible that resistant starch (RS) will affect colon function in a similar manner to non-starch polysaccharides. To test this theory, a group of eight volunteers took two diet supplements for 1 week each in a random order with a 1 week separation. One supplement comprised mainly 350 g Cornflakes/d and the other 380 g Rice Krispies/d, providing 10.33 and 0.86 g RS/d respectively. The amounts of amylase-digestible starch, non-starch polysaccharides, total carbohydrate, energy, protein and fat were balanced between the two periods by giving small amounts of Casilan, wheat bran, butter and boiled sweets. The volunteers made faecal collections during day 3 to day 7 of each period. Whole-gut transit time was calculated using the continuous method. Stool consistency and ease of defaecation were assessed by the volunteers. All episodes of flatulence noticed were recorded in a diary, along with food intake. Serial breath hydrogen measurements were made at 15 min intervals for 8 h on day 1 of each supplement. Questionnaires regarding colon function were completed at the end of each dietary period. There were no significant differences in the stool mass, frequency or consistency, ease of defaecations, transit time or flatulence experienced during the two supplements ($P > 0.05$). Significantly more H_2 (area under curve) was produced while eating Cornflakes than Rice Krispies ($P < 0.05$). The difference of 9.47 g RS/d between the two diets was over three times the calculated normal daily RS intake of 2.76 g/d. As the only significant difference observed was in the breath H_2 excretion on day 1, we suggest that either RS is rapidly and completely fermented to end-products including H_2 gas, which is subsequently excreted via the lungs and has little influence on colon function, or that bacterial adaptation removed any observable effect on faecal mass and transit time by day 3.

Starch: Hydrogen: Faeces: Whole-gut transit time: Colon

Until recently starch was thought to be completely hydrolysed in, and absorbed from, the small intestine of man because of the presence of excess quantities of salivary and pancreatic α -amylase (EC 3.2.1.1). However, a number of foods contain some starch that resists dispersion in water and hydrolysis with pancreatic amylase *in vitro* for various reasons (Englyst *et al.* 1987). Retrograded amylose is one type of resistant starch (RS) that forms when foods that have been baked or boiled are subsequently cooled (RS₃); some of the dispersed starch is thought to recrystallize and strong intermolecular hydrogen bonds are formed. Other forms of starch that resist digestion include the raw starch present in bananas, starch in unmilled grains and seeds, and retrograded amylopectin.

Starch that resists breakdown in the small intestine will pass into the large intestine where it may act in a similar manner to the unabsorbed non-starch polysaccharides of dietary fibre. Some non-starch polysaccharides reduce whole-gut transit time and increase stool output, and there are various mechanisms proposed to explain these effects. One of these proposals is that the carbohydrates act as substrate to the bacteria, stimulating bacterial growth and increasing bacterial cell mass (Stephen & Cummings, 1980) and generating fermentation end-products such as short-chain fatty acids (SCFA) and gases, which may

affect colonic motility and secretion (Fleming *et al.* 1983). Human colonic bacteria are capable of fermenting RS in *in vitro* incubations, resulting in an increase in the bacterial cell mass and production of SCFA (Englyst & MacFarlane, 1986). H. N. Englyst (personal communication) has suggested that RS in its various forms may contribute more substrate to the colonic bacteria than other polysaccharides. RS may, therefore, be very important in maintaining normal colon function, but there are no published values on the amount in the 'normal diet'. The effect of ingestion of RS on stool output and gas production in humans has never been studied before. Published investigations into the *in vivo* effects of RS are limited to a single study on the rat (Faulks *et al.* 1989). This showed that addition of between 1.32 and 1.54 g RS/d to the diet caused a slight increase in faecal output over 30 d.

The aims of the present experiment were to investigate whether RS₃ can influence colonic function in humans by monitoring whole-gut transit time, stool mass, frequency and consistency, flatus production and the ease of defaecation, and to test whether RS₃ affects breath levels of the fermentation end-product, H₂, as this may give an indication of the amount of substrate being fermented in the colon (Flourie *et al.* 1986).

Studies were carried out in normal volunteers who were asked to supplement their normal diets, with commercial breakfast cereals containing different amounts of RS₃ during two 1-week periods.

METHODS

Volunteers

We recruited eight healthy male volunteers who consumed a standard Western diet and had no history of gastrointestinal illness or bowel disturbance. Each gave his written informed consent, and the study was approved by the Ethical Committee of the Sheffield Area Health Authority.

Protocol

The study lasted 21 d in total, and was made up of two 7 d study periods, separated by 1 week. Dietary supplements were administered during the two study periods in a random order.

During one of the 1-week study periods the subjects' diets were supplemented with 350 g Kellogg's Cornflakes/d (providing 10.33 g RS₃/d). During the other period 380 g Kellogg's Rice Krispies/d was taken (providing 0.86 g RS₃/d). These amounts of cereal are equivalent to six large bowls/d. In addition to the breakfast cereals, small amounts of bran, butter, boiled sweets and Casilan (Farley Health Products Ltd, Nottingham) were consumed to balance the nutritional composition of the two supplements. The precise amounts were calculated using published data on their composition (Paul & Southgate, 1978). The amounts of non-starch polysaccharides, energy and fat provided by the two diets were balanced exactly, whilst the amounts of total starch, protein and total carbohydrate were balanced to within 1% of each other (Table 1).

The volunteers were encouraged to maintain their normal diet as far as possible but to restrict their alcohol consumption to no more than fifteen units/week and no more than four units/d (1 unit is equivalent to 0.5 pints beer, one glass of wine or one measure of spirits). It was accepted that the cereal supplements and accompanying milk and sugar would displace a certain amount of the normal dietary intake. The volunteers kept a diary of their food intake using approximate portion sizes, and they were encouraged to eat similar foods during the two study-weeks and to avoid foods known to affect their bowel habit. The approximate energy, starch and non-starch polysaccharide intakes were calculated from the diaries using food tables (Paul & Southgate, 1978).

Table 1. *The composition (g) of the daily dietary supplements for the two study periods calculated to provide a difference in intake of about 9.5 g resistant starch (RS)/d for healthy adult male volunteers*

	RS	Starch	NSP	Energy (MJ)	Protein	Fat	CHO
Diet A							
350 g Cornflakes*	10.33	262.1	2.28	5.39	30.1	5.6	298
33 g Boiled sweets	0	0.1	0	0.45	0	0	28
2.4 g Bran	0	0.3	0.88	0.02	0.3	0.1	6
2.3 g Butter	0	0	0	0.07	0	1.9	0
Total	10.33	262.5	3.16	5.93	30.4	7.6	332
Diet B							
380 g Rice Krispies*	0.86	262.1	3.16	5.79	22.0	7.5	327
9 g Casilan†	0	0	0	0.14	8.1	0.1	0
Total	0.86	262.1	3.16	5.93	30.1	7.6	327
Difference (A – B)	9.47	0.4	0	0	0.3	0	5

NSP, Non-starch polysaccharides; CHO, carbohydrate.

* Kelloggs.

† Farley Health Products Ltd, Nottingham.

During the entire 21 d the volunteers ingested fifteen small plastic radio-opaque markers at about the same time each day noting the exact time of ingestion in a diary.

During the last 5 d of both study periods the volunteers collected all stools into individual plastic bags, labelled with the time and date of defaecation. The stools were then weighed to yield the stool mass, and X-rayed to visualize the radio-opaque markers. The whole-gut transit time was calculated using the continuous method of Cummings *et al.* (1976); the number of markers excreted in each stool was fitted into an equation, along with the times of marker ingestion, to give the mean time taken for the markers to pass from mouth to anus each day.

The volunteers also noted in the diary the time each stool was passed and this allowed calculation of the stool frequency. They noted the ease of each defaecation on a visual analogue scale with the 0 and 100 points labelled 'no effort' and 'much straining'. They assessed the consistency of the resultant faeces on a scale from 1 to 8 by comparison with a set of standard descriptions and photographs, based on a linear scale of stool consistency devised by Davies *et al.* (1986). During the two study periods they noted all episodes of flatus passage per rectum in the diary.

The volunteers spent 8 h of the first day of each period in the laboratory to allow repeated measurements of breath H_2 concentration to be made. End expiratory breath samples were taken at 15 min intervals using a modified Haldane-Priestley tube, and the H_2 concentration was measured using a breath H_2 monitor (GMI, Renfrew, Scotland). The area under the H_2 curve was calculated (with no base-line correction), and this was used to give an indication of the amount of substrate fermented. The amounts and timings of the cereals and other food and drink consumed during this sampling period were repeated exactly on both occasions.

Statistics

Paired results from the two periods were compared using Wilcoxon's matched pairs rank sum test.

Table 2. *Bowel function measurements during intake of two supplements providing a difference in intake of about 9.5 g resistant starch/d by healthy adult male volunteers*
(Mean values for eight subjects)

Supplement ...	Cornflakes§	Rice Krispies§
Faecal output (kg/5 d)	0.89	0.98
Median whole-gut transit time (h)	43.4	39.5
Stool frequency (no./5 d)	5.0	5.8
Total breath hydrogen (ppm. min/8 h)	12072*	7529
Total flatulent episodes (no./week)	35	27
Mean consistency†	5.0	5.0
Mean ease of defaecation‡	28	30

* Mean value was significantly different from that for Rice Krispies: $P < 0.05$.

† Rated on scale from 1 (liquid) to 8 (hard pellets).

‡ Rated on scale from 0 (no effort) to 100 (much straining).

§ Kelloggs.

RESULTS

Faecal output

There was no significant difference between the total mass of faeces collected during the last 5 d of the two study periods ($P > 0.05$; Table 2).

There were also no significant differences in the stool frequency, the mean consistency of the faeces or in the ease of defaecations recorded by the volunteers in the diaries ($P > 0.05$; Table 2).

The questionnaires revealed that both supplements seemed to reduce the amount of faeces produced, to make the stools firmer, reduce the frequency of defaecation and to make defaecation more difficult compared with the normal bowel habit. There were no significant differences in the subjective assessments between the two study periods.

Transit time

Whole-gut transit times were not significantly different between the two periods ($P > 0.05$; Table 2).

Flatulence

There were no significant differences in the number of flatulent episodes recorded ($P > 0.05$), although when the results of all volunteers for each period were added together Cornflakes produced sixty-three more episodes than Rice Krispies. Volunteers reported that both supplements reduced episodes of flatulence compared with their normal diets.

Breath H₂

Breath H₂ excretion, measured as the area under the breath H₂ curve, was significantly higher on Cornflakes than on Rice Krispies over the 8 h measurement period ($P < 0.05$; Table 2). Although there was considerable inter-individual variation in breath H₂ levels as shown by the large standard deviations (Fig. 1), the levels were consistently higher during Cornflake ingestion.

Food intake

There were considerable differences in the background dietary intake of individuals as shown by the large ranges (Table 3). In paired analysis, however, there were no significant differences between the two periods ($P < 0.05$; Table 3).

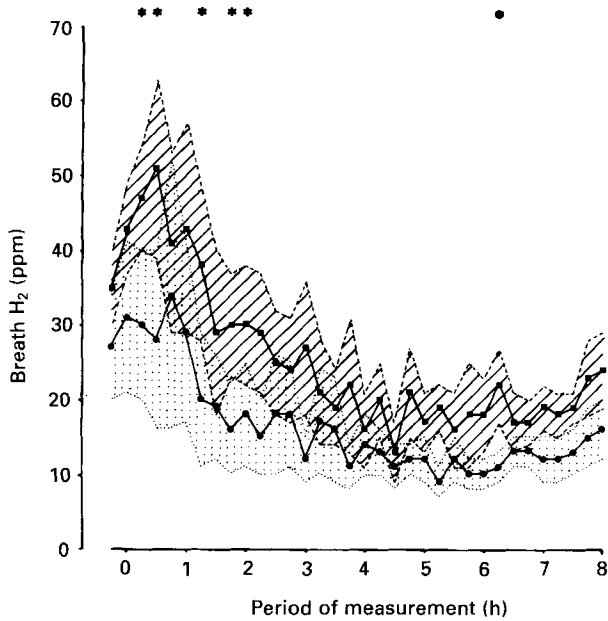


Fig. 1. Mean breath hydrogen values during 8 h on the first day of dietary supplementation with 350 g Cornflakes (Kelloggs)/d (■, with range of SE shown by cross hatching) and 380 g Rice Krispies (Kelloggs)/d (●, with range of SE shown by dapping). * Mean values were significantly different (two-tailed Student's *t* test), $P < 0.05$.

Table 3. Energy, non-starch polysaccharide and total starch intake from the background diet during intake of two supplements providing a difference of about 9.5 g resistant starch/d, and the total intake including supplement for healthy adult male volunteers

(Mean values with their standard errors)

Supplement ...	Cornflakes†		Rice Krispies†	
	Background	+ Supplement	Background	+ Supplement
Energy (MJ)				
Mean	5.02	10.95	5.04	10.97
SE	1.02	—	1.14	—
Range	1.82–9.75	7.75–15.68	1.82–10.61	7.75–16.54
Non-starch polysaccharide (g)				
Mean	14.8	18.0	14.9	18.1
SE	3.1	—	2.7	—
Range	4.4–27.1	7.6–30.3	4.4–25.1	7.6–28.3
Starch (g)				
Mean	81.2	343.7	80.7	342.8
SE	13.9	—	16.5	—
Range	31.0–132.0	293.5–394.5	31.0–156.6	293.1–418.7

† Kelloggs.

The order in which the supplements were given had no effect as there was no significant difference between any of the results when the first and second periods were compared ($P > 0.05$).

DISCUSSION

Using values for the RS content of a number of foods and food ingredients, established from the literature (Englyst *et al.* 1983; Englyst & Cummings, 1984, 1986, 1987), and the average weekly consumption of these foods (and foods prepared from the food ingredients) by the general UK population (Annual Report of the Food Survey Committee, 1985), we calculated the normal intake of RS in the UK to be about 2.76 g/d. Therefore, the Cornflake supplement should have provided over three times the normal amount of RS in the UK diet to the colonic bacteria. However, our results suggest that the difference in the quantity of RS supplied by the two supplements (9.47 g/d) is not sufficient to cause any change in faecal output, stool frequency and consistency or whole-gut transit time.

Of course it is possible that RS is less resistant to amylase digestion *in vivo* than *in vitro* (Dreher *et al.* 1984), and so less than 10.33 g/d was being supplied by the Cornflakes, or that the background 'dietary fibre' intake of up to 27.1 g/d, and dietary starch intake of up to 156.6 g/d may have masked the effect of a relatively small quantity of RS. Nevertheless, the significant difference in excretion of breath H_2 on the first day of diet supplementation suggests that a larger amount of fermentable residue was reaching the colonic bacteria from the Cornflakes. The amount of flatulence experienced was also higher during the Cornflake period, although this did not reach statistical significance.

It is possible that the RS in Cornflakes is primarily being converted to gas which is subsequently lost from the colon, rather than into SCFA or other metabolites which could be further used by the bacteria for cell growth. It has been postulated that if a readily fermentable substrate is degraded too quickly then bacterial cell yields may not increase because energetic uncoupling occurs; energy is provided at a rate which exceeds the capacity of the bacterial community to use it for biosynthesis (Hespell & Bryant, 1979). Previous studies have observed that the complex polysaccharides that are extensively fermented to gaseous end-products are usually those that are most readily degradable by the bacteria, and are also those that are not effective at increasing stool mass (Fleming *et al.* 1983; Tomlin *et al.* 1988). Flourie *et al.* (1986) found that the colonic bacteria could almost completely metabolize a colonic infusion of 50 g raw wheat starch causing no change in faecal mass, but producing a substantial increase in breath H_2 .

Since H_2 measurements were carried out on day 1 and the stool measurements on days 3–7, another possibility is that the bacteria could have adapted to the RS so that any initial change in fermentation characteristics would not necessarily be manifested in a change in stool output by day 3. The results of the rat study showed that the digestion of RS in the colon was almost complete at 97.5% after an adaptation period of only 1 d (Faulks *et al.* 1989).

In conclusion, in spite of the possible adaptation mechanisms involved, our findings do not support the view that an increase in intake of RS is likely to cause important changes in colon function.

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