Digestion and physiological properties of resistant starch in the human large bowel

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The digestion of four sources of resistant starch (RS) has been studied in twelve healthy volunteers who ate controlled diets for 15 d periods. RS from potato, banana, wheat and maize (17–30 g/d) was compared with a starch-free diet, a diet containing wheat starch that was fully digested in the small intestine, and with 18·4 g NSP from bran/d. RS increased stool wet weight by 1·6 g/d per g RS fed for potato, 1·7 for banana, 2·5 for wheat and 2·7 for maize, but this was significantly less than bran NSP at 4·9 g/g. RS was extensively digested in twenty-seven of thirty-four diet periods but five subjects were unable to break down one or two of the RS sources. Faecal N and energy excretion were increased. RS decreased NSP breakdown and RS_2 (resistant starch granules) tended to prolong transit time. All forms of RS increased faecal total short-chain fatty acid excretion. RS_2 (from potato and banana) gave greater proportions of acetate in faeces, and RS_3 (retrograded starch from wheat and maize) more propionate. We have concluded that RS_2 and RS_3 are broken down in the human gut, probably in the colon although in 26% of cases this breakdown was impaired. RS exerts mild laxative properties, predominantly through stimulation of biomass excretion but also through some sparing of NSP breakdown.

Resistant starch: Fermentation: Large bowel

The observation that some starch resists digestion by pancreatic enzymes and, thus, may reach the large intestine (Anderson *et al.* 1981; Englyst *et al.* 1982; Stephen *et al.* 1983) has important implications for human health. Colonic function, especially bowel habit, short-chain fatty acid (SCFA) production, N metabolism, bacterial activity and epithelial cell function are largely controlled by carbohydrates that enter the colon, of which the best known is NSP (British Nutrition Foundation, 1990; Spiller, 1992; Kritchevsky & Bonfield, 1995). The possibility that starch might also affect these aspects of bowel function is therefore important.

Since the original observation of resistant starch (RS), it has become clear that a variety of types of starch exist and that the rate and extent of their breakdown varies. Starch may resist digestion in the small intestine for three principal reasons; namely, its physical entrapment within a food (RS_1), the structure of the starch granules (RS_2) and retrogradation through food processing (RS_3) (Chapman *et al.* 1985; Englyst & Cummings, 1985, 1986, 1987; Wolever *et al.* 1986; Hamberg *et al.* 1989; Muir & O'Dea, 1993). On the basis of these observations a new nutritional classification of starch into rapidly digestible (RDS), slowly digestible (SDS) and resistant starch (RS) has been proposed (Englyst *et al.* 1992*a*). The rate and extent of starch digestion in the small bowel has a major influence on its physiological properties. The release of carbohydrate for absorption largely determines blood glucose and insulin responses to meals (Crapo *et al.* 1976; Jenkins *et al.* 1980; O'Dea *et al.* 1980) whilst starch that escapes digestion in the small intestine will affect large-bowel function.

There is very little published information on the fate of RS in humans and its effects on the large bowel, or on whether different types of RS have contrasting physiological properties. The present study was designed, therefore, to determine the digestibility in the gut of two examples of each of the common types of RS (RS_2 and RS_3) and to compare their effects with those of NSP from bran.

SUBJECTS AND METHODS

Subjects

Twelve healthy subjects (five female) recruited from the local community took part in the study. Their mean age was 31 (range 22–43) years, height 1·70 (range 1·58–1·83) m, weight 65·0 (range 48·1–92·0) kg and BMI 22·5 (range 18·6–28·4) kg/m^2. No subject had a history of gastrointestinal disease or had taken antibiotics within 3 months of starting the study. All subjects underwent a medical examination and full explanation of the protocol.

Study design

The study comprised the feeding for 15 d periods, in randomized order, of a series of diets that included starch-free (SF), a wheat starch that was fully digested in the small intestine (R + SDS), wheat bran (Bran-NSP), RS from potato (Potato-RS_2), RS from banana (Banana-RS_2), processed wheat starch (Wheat-RS_3) and processed maize starch (Maize-RS_3). All subjects ate the R + SDS and Bran-NSP diets but for the rest the aim was to have at least eight subjects per diet. Six dietary periods were repeated on one occasion each by five subjects after an average interval of 6 (range 0–15) months to test the reproducibility of the whole protocol. In addition, five subjects took the Potato-RS_2 at two different doses to see if there was a dose response effect in the range studied. A pilot study was undertaken at the outset but no data from this are included in the present report.

The study was conducted with subjects resident in the metabolic facility of the Dunn Clinical Nutrition Centre, Cambridge where all food was provided. No other food and no alcohol was allowed. Subjects were admitted to the Unit for an initial dietary assessment and to familiarize themselves with the routine. Dietary periods were run consecutively unless the subjects wanted a break, which was allowed. Thirty-eight of the seventy-seven dietary periods were followed by a break. Subjects were allowed to leave the Unit during the day to carry on their usual activities.

Diets

A diet comprising three 1 d menus was fed. It contained a constant level of macronutrients, with CV from day to day of less than 2%, calculated from food tables (Paul & Southgate, 1978; Englyst *et al.* 1988, 1989). In order to maintain energy balance, individual energy intakes for the volunteers were matched to BMR, calculated from body weight (Schofield *et al.* 1985), with a factor of $\times 1{\cdot}5$ to allow for total energy expenditure (World Health Organization, 1985). Necessary energy intakes, which ranged from 8 to 12 MJ/d, were calculated to the nearest MJ and diet adjusted during the first week if gains or losses of body weight occurred. The diet for a volunteer requiring 8 MJ/d comprised the foods shown in Table 1, except for substitutions necessary for some of the biscuit supplements and the SF diet (see below). All foods, apart from those indicated in Table 1, were canned or frozen from the same batch. Meat was bought in advance, cooked, defatted, weighed and deep-frozen, together with fat-free gravy. Volunteers were allowed minor variations, for example jam instead of marmalade, provided these were unchanged throughout the protocol. Increments, which were free of starch and NSP, each contained 1 MJ, and are also shown in Table 1. On day 1, for example, an individual who required 10 MJ would

Table 1. *Diet for a volunteer requiring 8 MJ/d*

Each day	Canned grapefruit 150 g Marmalade 20 g Milk (homogenized)* 120 g Biscuit supplement or substitute (Tables 2 and 3) Fiberform† 3·5 g		
	Day 1	Day 2	Day 3
Lunch	Salmon 30 g Lettuce* 100 g Tomato* 100 g Cucumber* 50 g Mayonnaise 5 g Apple* 150 g	Tuna 30 g Lettuce* 100 g Tomato* 100 g Cucumber* 50 g Mayonnaise 5 g Orange* 150 g	Corned beef 30 g Lettuce* 100 g Tomato* 100 g Cucumber* 50 g Mayonnaise 5 g Pear* 150 g
Supper	Beef 85 g Carrot 100 g Swede 100 g Gravy 60 g Peaches 100 g Ice-cream 100 g	Lamb 85 g Spinach 100 g Courgettes 100 g Gravy 60 g Mandarins 100 g Ice-cream 100 g	Chicken 85 g Runner beans 100 g Tomatoes* 100 g Gravy 60 g Apricots 100 g Ice-cream 100 g
Diet composition per d (excluding RS and NSP supplements)		Energy 8 MJ Protein 60 g Sugars 140 g RS 0·4 g	Fat 84 g‡ Starch 103 g NSP 16 g
Increments per d	Salmon/tuna/corned beef 30 g Mayonnaise 8 g Sugar 30 g (or 1 can cola drink)		
Increment composition per d	Energy 1 MJ Protein 6 g Fat 10 g Sugars 35 g		

RS, resistant starch.
* Fresh foods; all other items frozen or canned from long-term store.
† Trifyba, Tricum AB, Hoganas, Sweden.
‡ Amounts of fat shown refer to diets used for R + SDS, Potato-RS_2, and Banana-RS_2 supplements, see p. 735.

have had an extra 60 g salmon, 16 g mayonnaise and 60 g sugar or 2 cans of cola. Once energy requirements were assessed, the basic diet remained unchanged throughout the rest of the study.

Biscuit supplements

Changes in NSP, RS, and starch intake were achieved by including supplements of biscuits. The recipes for the biscuits and their composition are shown in Tables 2 and 3. The bran biscuits contained 37 g starch, and 18 g NSP mainly as Fiberform (Trifyba) provided by Tricum AB, Hoganas, Sweden, Potato-RS_2 and Banana-RS_2 biscuits contained 75 g Echo margarine, but more had to be added for palatability reasons to the bran, and to the Wheat-RS_3 and Maize-RS_3 biscuits. The additional fat was removed from the menu shown in Table 1 by substituting lower-fat milk, and reducing the amounts of mayonnaise and ice-cream. On the SF diet the biscuits were omitted and replaced with two Mars bars (140 g), 63 g double cream, and 50 g sugars as cola drink or boiled sweets. Subjects ate eight to ten biscuits daily.

Preparation of resistant starches

The wheat starch used in the making of the R + SDS and Bran-NSP biscuits was gluten-free starch. For the RS_2-containing biscuits, potato flour was obtained commercially and banana flour by freeze-drying and milling green bananas. Wheat-RS_3 was prepared by

Table 2. *Biscuit recipes**

Ingredients (g)	R+SDS	Bran-NSP	Potato-RS_2	Banana-RS_2	Wheat-RS_3	Maize-RS_3
White wheat flour	75	60	75	75	—	75
Wheat starch†	75	70	—	—	—	—
Sugar	—	26	—	—	—	—
Icing sugar	18	—	18	18	13	18
Salt	1	1	1	1	1	1
Echo margarine	75	105	75	75	100	100
RS_3 wheat flour‡	—	—	—	—	128	—
Potato flour‡	—	—	75	—	—	—
Banana flour‡	—	—	—	75	—	—
Hylon 7‡	—	—	—	—	—	75
Fiberform§	—	28	—	—	—	—

R+SDS, Rapidly and slowly digestible starch; RS_2, resistant starch granules; RS_3, retrograded starch.
* To make: mix ingredients in bowl, knead until dough forms a ball; roll out to 1/8″ thickness; cook for 8 min at 200°.
† NSP-free wheat starch.
‡ For details of resistant starch sources see pp. 735–736.
§ Trifyba, Tricum AB, Hoganas, Sweden.

Table 3. *Biscuit composition and amounts consumed**

Biscuit type...	R+SDS	Bran-NSP	Potato-RS_2	Banana-RS_2	Wheat-RS_3	Maize-RS_3
Dry matter (g/kg)	967	950	967	953	977	973
NSP (g/kg DM)	15	84	11	25	17	11
Total starch (g/kg DM)	513	371	536	510	462	471
Total RS (g/kg DM)	2	2	132	150	85	85
RS_2 (g/kg DM)	0·5	—	116	149	0	0
RS_3 (g/kg DM)	1	—	1	1	85	85
Dietary intake (g/d)						
Biscuits	210	225	210	210	205	230
NSP	3·4	18·4	2·6	5·4	2·0	2·5
Starch	102·6	79·3	107·2	102·0	90·0	105·4
Total RS	0·4	0·4	26·8	30·0	17·4	19·0
RS_2	0·1	—	26·5	29·7	0	0
RS_3	0·3	—	0·3	0·3	17·4	19·0

R+SDS, Rapidly and slowly digestible starch; RS, resistant starch; RS_2, resistant starch granules; RS_3, retrograded starch.
* For details of biscuits, see Table 2.

repeated autoclaving and cooling of the gluten-free wheat starch. Maize-RS_3 was prepared from Hylon VII maize starch (National Starch, London), which was cooked, cooled overnight and then freeze-dried. (Full details of the preparation of these materials are given by Englyst *et al.* 1996).

Stool collection

All stools passed were collected, immediately weighed and frozen at −20°. During the last 5 d of each dietary period two stools were sub-sampled and the sub-samples frozen for SCFA analysis.

Estimation of intestinal transit time and balance markers

Mean transit time (MTT) was measured using the continuous marker method (Cummings *et al.* 1976). Volunteers were given ten radio-opaque shapes with each meal (30/d). Marker type was changed every 15 d with diet, and stools collected for 5 d after the end of the study to ensure complete collection of all markers. All stools were X-rayed before sampling, and the markers in each stool counted. MTT was calculated, and marker excretion also used to correct stool weights, carbohydrate, energy and ash outputs so that results represented a 5 d collection period (Branch & Cummings, 1978). Volunteers kept a daily diary in which they recorded their weight, times of radio-opaque markers taken, stools passed and any unusual events such as diarrhoea and flatulence. In total, 450 markers (15 d × 30 markers) were given in each of seventy-seven diet periods (34650 markers). Of these 280 were not recovered. The modal number of markers not recovered per diet period was 2 and the overall average per period 3·6, giving an accuracy of faecal collections of greater than 99%.

Diet and faecal analysis

All stools from the last 5 d of each dietary period were pooled, weighed and freeze-dried to constant weight. The resultant dry samples were milled in a centrifugal mill, and used for faecal analyses. Total N was measured using an automated Kjeldahl procedure, energy was determined by an adiabatic bomb calorimeter, and ash quantified at 500° after initially burning off organic residues over a flame. All samples were analysed in duplicate.

Short-chain fatty acid analysis

Faecal sub-samples were defrosted at 4°, mixed, and 1 g was diluted 1:100 with water, centrifuged, freeze-transferred and dried (Pomare *et al.* 1985). The distillate was acidified with 100 μl 1 M-H_3PO_4, and injected onto a Pye 204 gas–liquid chromatograph fitted with a flame ionization detector and a 25 m 0·53 mm i.d. BP21 fused silica capillary column (S. G. E. Ringwood, Victoria, Australia). The column was held at 100° with He as the carrier gas at 14 psi inlet pressure (Macfarlane *et al.* 1992). All samples were analysed in duplicate, along with three calibration standards and 2-methylvalerate, which was used as an internal standard.

Diet and faecal carbohydrates

Total starch and RS were measured by the method of Englyst *et al.* (1992*a*). RS measured by this technique is starch not hydrolysed to glucose after 120 min incubation with amylolytic enzymes. Starch in faeces was measured as total starch. NSP in the diet and faeces was measured by the method of Englyst *et al.* (1992*b*).

Statistics

Systat (5.2.1) computer software (Systat, Inc., Evanston, IL, USA) was used for all data analysis. Faecal results were analysed using two-way ANOVA with subject and diet as factors. Results are presented as subject-adjusted least squares means with their standard errors. Fisher's least significant difference post-hoc test of significance was used to test effect of diet for single comparisons (Tables 4 and 5), and Tukey's post-hoc test was used for multiple comparisons (for SCFA, Table 6). Two-way ANOVA was also used to look at the effects of different types of starch grouped as RS_2 (potato and banana) and RS_3 (wheat and maize).

RESULTS

Bowel habit and transit time (Table 4)

On the R+SDS diet, which contained 103 g wheat starch and 16 g NSP stool weight was 110 g/d and MTT was 52·5 h. On the SF diet, stool weight was 107 g/d and MTT was

53·8 h; these results were not significantly different from those for the R + SDS diet. The addition of 18·4 g NSP from bran significantly increased stool weight by 83% to 201 g/d or 4·9 g stool per g NSP fed. When Bran-NSP biscuits were fed, MTT was faster at 42·8 h and stool frequency greater, though not significantly. Overall the group had stool weights within the normal range (Cummings *et al.* 1992) and responded to bran NSP in the expected way (Cummings, 1993).

The addition of RS instead of NSP to the diet also led to a significant increase in stool weight in all groups. Because the amount of RS fed varied amongst the diets, the changes in stool weight were normalized to an intake of 18·4 g NSP or RS/d and the increment in stool weight over that seen with the R + SDS diet was calculated. The changes were +46 g/d for Wheat-RS_3, +49 g/d for Maize-RS_3, +34 g/d for Potato-RS_2 and +31 g/d for Banana-RS_2. There were no significant differences amongst the RS categories even when all RS_2 and RS_3 stool outputs were compared, despite both RS_3 sources leading to greater increases in stool weight per g RS. All four increases with RS were significantly less than the change seen with Bran-NSP (+90 g/d). There was an increase in MTT with both sources of RS_2, but this was only significant for Banana-RS_2.

Stool composition (*Tables 4 and 5*)

Stool water varied from 73·0 to 77·5% amongst the diets and only Bran-NSP significantly increased this over R + SDS. DM excretion was significantly greater than for R + SDS with all diets, with the DM excretion for Bran-NSP significantly greater than all others, increasing by 17·9 g/d (69%). RS increased DM excretion by 8·6 to 10·6 g/d depending on source. Again DM excretion was not significantly different amongst RS sources, but bran produced a significantly greater change than all sources of RS.

About 75% of the increase in DM excretion with Bran-NSP was accounted for by increases in crude protein (N × 6·25), carbohydrate and minerals (ash), which together totalled 13·4 g. The other 4·5 g/d (25%) was probably increased lipid excretion (Scheppach *et al.* 1988*a*). The main effect of the RS diets was to increase N excretion, significantly so for all four supplements compared with the R + SDS diet, and carbohydrate excretion, although this was highly variable. Along with these increases there was a significant increase in faecal energy with Bran-NSP and all RS diets.

Carbohydrate excretion in faeces (*Table 5, Fig. 1*)

On the SF diet NSP intake was 16 g/d and was provided entirely by fruit and vegetable sources. Faecal NSP excretion was 3·4 g/d giving an overall digestion of these sources of 78·5%, similar to that found in many other studies (Cummings, 1984). With the additional 3·4 g/d from wheat starch, faecal NSP excretion increased by only 0·73 g/d, indicating extensive digestion (78·5%) of NSP from the cell walls of the starchy endosperm of the wheat grain. By contrast, when 18·4 g Bran-NSP was added, overall NSP digestibility fell to 57·4%. Assuming that the digestion of NSP in the basal diet remained the same, this gives a digestibility of Bran-NSP of 38·8%. When RS was added instead of NSP, overall NSP excretion increased, significantly so for Potato-RS_2 and Maize-RS_3, and digestibility fell; this fall was significant for Potato-RS_2, Wheat-RS_3 and Maize-RS_3.

RS digestion in the large bowel was very variable. Fig. 1 shows that there were considerable individual differences in RS digestibility although overall breakdown was about 80–90%. For Potato-RS_2, all subjects excreted less than 4 g starch/d in faeces except subject I, who excreted 14·9 g. For Banana-RS_2, excretion was less than 2 g/d except for subjects I (8·9 g) and J (8·2 g). However, both subjects I and J digested Wheat-RS_3 and Maize-RS_3 very effectively, whilst subject A excreted 21·4 g starch on the Maize-RS_3 diet and subjects A, D and H excreted 14·9, 18·0 and 16·2 g respectively with Wheat-RS_3. Thus whilst an individual might break down one RS source well, another source might be poorly

Table 4. *Stool output and transit time of subjects consuming diets containing wheat bran or different types of resistant starch (RS)*†

(Mean values with their standard errors for eight to twelve subjects)

	SF		R+SDS		Bran-NSP		Potato-RS_2		Banana-RS_2		Wheat-RS_3		Maize-RS_3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Wet wt (g/d)	107	13	110	9·9	201*	9·9	151*	12	161*	13	153*	12	161*	13
DM (g/d)	27·8	2·4	26·0	1·9	43·9*	1·9	34·6*	2·3	36·6*	2·4	36·4*	2·3	35·3*	2·4
Water (g/kg)	730	9·2	747	7·2	775*	7·2	756	8·6	759	9·1	755	8·6	765	9·1
Mean transit time (h)	53·8	5·0	52·5	3·9	42·8	3·9	62·5	4·7	66·4*	5·0	49·8	4·7	50·2	5·0
Stool frequency/5 d	3·7	0·5	3·7	0·4	4·7	0·4	4·6	0·4	4·9	0·5	4·2	0·4	4·4	0·5
Wet wt increment‡	—	—	—	—	90*	9·6	30	12	31	12	46*	12	49*	12
n	8		12		12		9		8		9		8	

SF, starch free; R+SDS, rapidly and slowly digestible starch; RS_2, resistant starch granules; RS_3, retrograded starch.

* Mean values were significantly different from R+SDS, $P < 0{\cdot}05$.

† For details of diets and procedures, see Tables 1, 2 and 3 and pp. 734–737.

‡ Increase over R+SDS (g/d), corrected to 18·4 g/d RS or NSP.

Table 5. *Faecal carbohydrate, nitrogen, energy and ash excretion by subjects consuming diets containing wheat bran or different types of resistant starch (RS)*†

(Mean values with their standard errors for eight to twelve subjects)

	SF		R+SDS		Bran-NSP		Potato-RS_2		Banana-RS_2		Wheat-RS_3		Maize-RS_3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
N (g/kg DM)	53·2	3·0	52·6	2·4	38·7*	2·4	54·3	2·9	54·1	3·0	45·0*	2·9	50·9	3·0
N (g/d)	1·50	0·07	1·38	0·05	1·70*	0·05	1·79*	0·06	1·94*	0·07	1·60*	0·06	1·76*	0·07
Energy (kJ/d)	607	41	551	32	907*	32	706*	39	748*	41	755*	38	714*	41
Ash (g/d)	3·55	0·15	3·30	0·12	3·93*	0·12	3·49	0·14	3·83*	0·15	3·48	0·14	3·71*	0·15
Starch (g/d)	−0·41	1·6	0·12	1·3	0·26	1·3	2·80	1·5	2·36	1·6	5·93*	2·6	3·50	1·6
RS digestibility (%)	—	—	—	—	—	—	89·1	11·4	96·6	12·4	65·0	11·4	84·1	12·4
NSP (g/d)	3·44	0·68	4·17	0·53	14·7*	0·53	5·85*	0·64	5·79	0·68	5·78	0·64	5·98*	0·68
NSP digestibility (%)	77·8	3·1	78·6	2·4	57·4*	2·4	68·6*	2·9	73·6	3·1	70·9*	2·9	68·5*	3·1

SF, starch free; R+SDS, rapidly and slowly digestible starch; RS_2, resistant starch granules; RS_3, retrograded starch.

* Mean values were significantly different from R+SDS, $P < 0{\cdot}05$.

† For details of diets and procedures, see Tables 1, 2 and 3 and pp. 734–737.

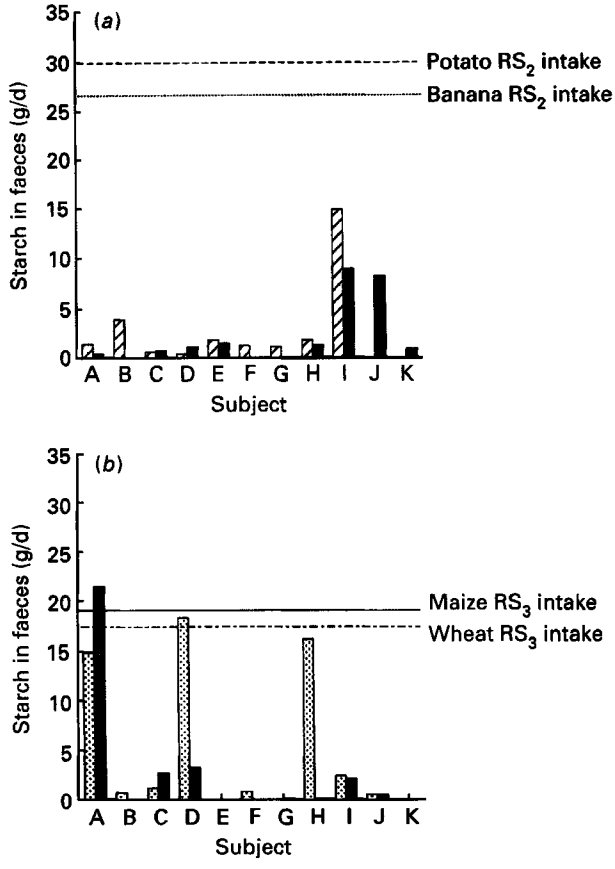

Fig. 1. Daily starch excretion in faeces by individual subjects consuming diets containing resistant starch (RS) from (*a*) banana (■) and potato (▨), and (*b*) wheat (▩) and maize (▩). Where no excretion is indicated this is because the subject did not consume that particular diet. Some RS was present in the faeces of all subjects who consumed it. Horizontal lines represent the daily intake of RS determined from *in vitro* measurements. RS_2, resistant starch granules; RS_3, retrograded amylose.

digested. There was no 'across the board' failure to digest RS by any individual. Two subjects apparently excreted more RS in faeces than was present in the diet. However, the measurement of RS in the diet reflects the average amount of starch reaching the colon in a group of ileostomy subjects (Englyst *et al.* 1996). This value can vary by up to 20% from the mean in individuals.

Faecal short-chain fatty acids (Table 6)

Mean total faecal SCFA concentrations were between 77 and 100 mmol/kg wet faeces. They were lowest for the SF and Bran-NSP diets and highest for Potato-RS_2 and R+SDS diets. Molar ratios of acetate were lower for the two sources of RS_3, significantly so compared with Bran-NSP and Banana-RS_2, while molar ratios of propionate were lower for the two sources of RS_2, significantly so compared with R+SDS and Wheat-RS_3. No consistent changes in butyrate levels were seen for RS although the molar ratio with Potato-RS_2 was significantly greater than with R+SDS, Bran-NSP and Wheat-RS_3. Banana-RS_2 significantly lowered molar ratios of branched-chain fatty acids while molar ratios of C_5 and C_6 were significantly higher for Maize-RS_3. Bran-NSP significantly lowered the total concentration of SCFA compared with R+SDS, however, it had very

Table 6. *Total faecal short-chain fatty acids (mmol/kg) and their molar ratios (% of total) in subjects consuming diets containing wheat bran or different types of resistant starch (RS)**

(Mean values with their standard errors for five to ten subjects)

	SF		R+SDS		Bran-NSP		Potato-RS_2		Banana-RS_2		Wheat-RS_3		Maize-RS_3	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
n	6		10		9		7		7		5		7	
Total	$80{\cdot}2^{ab}$	9·3	$98{\cdot}9^{b}$	7·3	$77{\cdot}1^{a}$	7·3	$99{\cdot}7^{b}$	8·2	$97{\cdot}5^{ab}$	8·2	$83{\cdot}4^{ab}$	9·7	$85{\cdot}7^{ab}$	8·2
Acetate	$55{\cdot}1^{ab}$	2·0	$55{\cdot}8^{ab}$	1·6	$57{\cdot}1^{a}$	1·6	$55{\cdot}6^{ab}$	1·8	$59{\cdot}4^{a}$	1·8	$52{\cdot}1^{b}$	2·1	$51{\cdot}6^{b}$	1·8
Propionate	$16{\cdot}8^{bc}$	1·8	$18{\cdot}8^{ab}$	1·4	$18{\cdot}4^{abd}$	1·4	$16{\cdot}0^{cd}$	1·6	$15{\cdot}0^{c}$	1·6	$20{\cdot}8^{a}$	1·8	$17{\cdot}5^{bc}$	1·6
Isobutyrate	$2{\cdot}4^{b}$	0·29	$2{\cdot}2^{b}$	0·23	$1{\cdot}9^{b}$	0·23	$1{\cdot}8^{ab}$	0·26	$1{\cdot}3^{a}$	0·26	$2{\cdot}0^{b}$	0·31	$1{\cdot}9^{ab}$	0·26
Butyrate	$15{\cdot}2^{b}$	1·3	$15{\cdot}0^{b}$	1·0	$15{\cdot}8^{b}$	1·0	$18{\cdot}4^{a}$	1·1	$16{\cdot}7^{ab}$	1·1	$15{\cdot}2^{b}$	1·3	$17{\cdot}0^{ab}$	1·1
Isovalerate	$3{\cdot}6^{b}$	0·58	$3{\cdot}5^{b}$	0·45	$2{\cdot}9^{b}$	0·45	$2{\cdot}6^{ab}$	0·51	$1{\cdot}7^{a}$	0·51	$3{\cdot}4^{b}$	0·61	$3{\cdot}3^{b}$	0·51
Valerate	$3{\cdot}3^{bc}$	0·38	$3{\cdot}0^{b}$	0·30	$2{\cdot}3^{a}$	0·30	$3{\cdot}5^{bc}$	0·33	$2{\cdot}9^{ab}$	0·33	$3{\cdot}2^{bc}$	0·40	$3{\cdot}8^{c}$	0·33
Caproate	$3{\cdot}4^{de}$	0·78	$1{\cdot}8^{b}$	0·61	$1{\cdot}6^{b}$	0·61	$2{\cdot}1^{bc}$	0·69	$3{\cdot}1^{ce}$	0·69	$3{\cdot}2^{ce}$	0·82	$4{\cdot}9^{a}$	0·69

SF, starch free; R+SDS, rapidly and slowly digestible starch; RS_2, resistant starch granules; RS_3, retrograded starch.

[a,b,c,d,e] Mean values not sharing a common superscript letter were significantly different ($P < 0{\cdot}05$).

* For details of diet and procedures, see Tables 1, 2 and 3 and pp. 734–737.

Table 7. *Repeats and mean difference between repeated diet periods*

(Mean values for five subjects who repeated a total of six diet periods: starch-free, rapidly + slowly digestible starch, bran NSP, potato resistant starch granules (RS_2), banana RS_2 and maize retrograded amylose (RS_3))

	Initial period	Repeat period	Difference	
	Mean	Mean	Mean	SD
Faecal weight (g/d)	139	136	−3·48	22·6
DM (g/d)	34·7	33·0	−1·67	2·59
DM (g/kg)	270	275	5·17	13·6
Ash (g/d)	3·13	3·29	0·19	0·46
Energy (kJ/d)	719	661	−11·4	51·4
N (g/d)	1·63	1·61	−0·13	0·19
N (g/kg DM)	48·0	50·3	2·3	6·03
Mean transit time (h)	57·7	62·9	5·27	10·9
Starch excretion (g/d)	1·95	1·30	−0·65	1·55
NSP excretion (g/d)	7·47	6·67	−0·80	2·23

little effect on the SCFA molar ratios. All RS diets and Bran-NSP increased total daily excretion of SCFA compared with R + SDS.

Dose response

Five subjects repeated the Potato-RS_2 diet period with an intake of 12 g Potato-RS_2 instead of 24 g. Mean daily stool weights (g/d) for the group were: R + SDS 109 (SE 25), Potato-RS_2 (low dose) 121 (SE 30) and Potato-RS_2 (high dose) 144 (SE 39). Wide individual differences in responses occurred but the regression of the means onto intake of Potato-RS_2 was highly significant and the increments above R + SDS were in the expected proportion (+12 and +22 g/d).

Repeatability (Table 7)

Five subjects repeated a total of six diet periods at intervals up to 15 months after the initial study period to test the reproducibility of the whole experimental design. Table 7 shows the results for all variables. Mean differences between the first and second diet periods were very small and were non-significant for all of the variables. There were no significant correlations between the interval between diet periods (in weeks) and the differences between diet periods for any variable, indicating that previous exposure to a diet had no detectable long-term effect on a subject's subsequent exposure to that diet.

DISCUSSION

This study has shown that four different sources of RS produce an increase in daily stool wet weight and DM, N and energy excretion, and that these RS sources are well digested in the large bowel of most individuals. RS_2 supplementation tended to increase transit time, but this was not seen with RS_3. All sources produced changes in colon function that were quantitatively less than an equivalent amount of NSP from wheat bran, with the exception of changes in N excretion.

It has been known for many years that some starch escapes digestion in the human small intestine (Westhuizen *et al.* 1972; Wolf *et al.* 1977; Anderson *et al.* 1981) but little attention was paid to this until it was realised that carbohydrates such as NSP that reach the colon

and are broken down by the bacteria (fermented) may have beneficial effect on large-bowel function through a variety of mechanisms (Cummings, 1983). When we first identified RS in foods (Englyst *et al.* 1982) the fraction consisted largely of retrograded amylose present in moist-heated starchy foods. Since then, however, we have shown that starch may resist digestion in the small bowel for a number of reasons and we have proposed a classification and method of analysis that categorizes RS into types 1–3 (Englyst *et al.* 1992*a*). In the present study we have determined the fate in the colon of two of these sources of RS.

Both RS_2 and RS_3 have laxative properties, as does any carbohydrate that reaches the colon. The magnitude of the effect is only modest, at 1·63 g increase in stool wet weight per g RS fed for Potato-RS_2, 1·68 for Banana-RS_2 and 2·50 and 2·66 for Wheat- and Maize-RS_3 respectively. Although it would appear that RS_3 was over 50% more effective in altering stool weight than RS_2 these differences were not significant because of great variation in responses. It would be worth pursuing this observation with further work since mechanistic differences amongst the actions of carbohydrates in the colon would have implications for health. These increases in stool weight with RS compare with 4·9 g/g for Bran-NSP. In a meta analysis of about 100 studies of stool weight changes with various NSP sources (Cummings, 1993) wheat NSP increased stool weight by 5·4 g/g on average whilst the effect of other NSP sources ranged from 1·2 to 4·7 g/g NSP. RS comes towards the bottom of this league table alongside legume NSP and pectin. Of the published reports of the effect of starch on bowel habit some report no effect, e.g. with corn flakes (Tomlin & Read, 1990), with starch infused into the caecum (Flourie *et al.* 1986), or a high-starch diet (Flourie *et al.* 1988) whilst others show an increase of 1·0 g/d per g RS (Hylon VII) (Van Munster *et al.* 1994) or report a 'significant increase' (Shetty & Kurpad, 1986*a*; Scheppach *et al.* 1988*a*). Overall it is reasonable to conclude at this stage that RS has mildly laxative properties equivalent to the less effective forms of NSP, oligosaccharides (fructo-oligosaccharides)1·3 g/g (Gibson *et al.* 1995), polydextrose 1·2 g/g (Achour *et al.* 1994) or inulin 2·0 g/g (Gibson *et al.* 1995).

What is the mode of action of RS in the colon? As the present and other studies have shown, it is largely digested in most individuals with less than 10% excreted in faeces (Van Munster *et al.* 1994; Phillips *et al.* 1995). In the present study one out of nine individuals digested Potato-RS_2 poorly, two out of eight for Banana-RS_2, three out of nine for Wheat-RS_3, and one out of eight for Maize-RS_3. This was not related to transit time since the subject who digested both Maize-RS_3 and Wheat-RS_3 poorly (A; Fig. 1) had transit times of 68·2 h on Maize-RS_3, the second longest of any subject on Maize-RS_3, and 87·7 h on Wheat-RS_3, longer than anyone else. Moreover, subject D, who digested Wheat-RS_3 least well, had a MTT of 42 h on this diet yet was able to digest Maize-RS_3 well at a MTT of 41·5 h. The explanation is more likely to be in an absence of appropriate RS-degrading bacteria in the gut. A similar phenomenon is known to occur for micro-crystalline cellulose (Betian *et al.* 1977). It is therefore possible that changes in physical structure between different starch types may make large differences in fermentability in some individuals.

In most people the mode of action of RS in the colon lies through fermentation, stimulation of bacterial growth and SCFA production. NSP affects colonic function by two mechanisms, primarily by fermentation, which stimulates biomass production and excretion (Stephen & Cummings, 1980), and secondly by water-holding in unfermented polysaccharide structures (Adiotomre *et al.* 1990). Using acarbose to impair starch digestion in a normal diet, Scheppach *et al.* (1988*a*) showed that along with an increase in faecal weight there was an increase in biomass and N excretion. In the present study N excretion increased significantly, between 0·22 and 0·56 g/d with all sources of RS, an amount that would account largely for the increase in stool wet weight observed,

41–51 g/d, assuming this was due to increased biomass and that bacteria are 6% N and 80% water. It is unlikely that RS acts in the large bowel in the same way as NSP by holding water since RS is insoluble and has no significant water-holding properties. However, the three subjects who digested Wheat-RS_3 incompletely had a greater average increase in stool weight (90 g/d) than the six who digested it well (22 g/d), a substantially greater increase in DM excretion (23·5 *v.* 4·8 g/d), but only a very small increase in N excretion (0·09 *v.* 0·31 g/d). These data indicate that the undigested RS has substantial bulking properties without apparently much effect on biomass.

An increase in NSP excretion (1·6–1·8 g/d) and decrease in NSP digestibility (5·0–10·0% less digestible) occurred when RS was added to the diet. Thus it is possible that RS exerts a sparing effect on NSP in the colon, with bacteria fermenting RS in preference to NSP. This explanation was preferred by Shetty & Kurpad (1986*a*, *b*) and Phillips *et al.* (1995) but was refuted by McBurney (1986), who cited *in vitro* evidence to show that starch did not affect NSP breakdown. In the present study exact measurements of the excretion of NSP and starch were made in all subjects. In seven diet periods where RS digestion was low (Fig. 1) there was an equivalent increase in NSP excretion in only three. Thus in a small number of RS diet periods (three out of thirty-four) there was a general change in colonic function affecting both RS and NSP digestion. In twenty-three periods, however, RS was well fermented and a fall in NSP breakdown occurred; this was significant for Potato-RS_2, Wheat-RS_3 and Maize-RS_3. Although these experiments were not designed to test the possibility, it is likely that substrate interaction occurs *in vivo* in the colon and that RS may well be fermented in preference to NSP. A small part of the laxative properties of RS may therefore be ascribed to additional NSP in faeces holding water. Such a NSP-sparing effect would constitute another mechanism to add to those already known for changes in bowel habit (Stephen & Cummings, 1980).

SCFA excretion increased with all RS and Bran-NSP diets compared with both SF and R+SDS. This change simply reflects an increased faecal output since SCFA are the principal anions in human faeces. A relationship between stool volume and SCFA output has been seen in many previous studies (Cummings, 1995).

Interpreting patterns of faecal SCFA excretion is not an accurate science. At least 95% of all SCFA produced in the colon are absorbed so faecal excretion is an insensitive guide to events more proximally in the bowel. SCFA concentrations are different between caecum and faeces (Cummings *et al.* 1987; McIntyre *et al.* 1991). Molar ratios of SCFA are probably more reliable as an indicator of dietary change than either concentration or output (Scheppach *et al.* 1988*b*). In the present study the two sources of RS_2 reduced the molar ratio of propionate, whilst RS_3 increased the molar ratio of propionate at the expense of acetate. This increase in propionate has been shown previously in rats fed with amylomaize starch (Andrieux *et al.* 1992). The only RS-containing diet to increase molar ratios of butyrate in faeces, the SCFA that most frequently has beneficial effects ascribed to it (Cummings, 1995), was Potato-RS_2. *In vitro* studies (Weaver *et al.* 1989, 1992; Gibson *et al.* 1990; Wang & Gibson, 1993) have shown a number of sources of starch, including potato (Macfarlane & Englyst, 1986), Lintners starch (Englyst *et al.* 1987) and maize starch (Weaver *et al.* 1992), to produce high molar ratios of butyrate when fermented, although few of these studies were of RS (Englyst & Macfarlane, 1986; Macfarlane & Englyst, 1986). When soluble starch and RS (Hylon VII) were compared *in vitro*, the molar ratios of the major SCFA did not differ, although there was greater production of SCFA per g soluble starch (Englyst & Macfarlane, 1986). In the present study some subjects utilized only specific types of RS, implying that different flora ferment different RS sources. Thus *in vitro* studies may show inconsistent results depending on the inocula used. Some *in vivo* studies support the role of RS in increasing butyrate (Mathers & Dawson, 1991), including that of

Van Munster *et al.* (1994), who showed this effect with maize RS (Hylon VII), and that of Scheppach *et al.* (1988*b*) who showed it by feeding acarbose; however, other studies (Flourie *et al.* 1986) have failed to show any effect of dietary RS on faecal SCFA. We have previously shown that ileal effluent from subjects on high-RS diets, when incubated *in vitro*, yields significantly higher amounts of butyrate than effluent from subjects on normal diets (Silvester *et al.* 1995). The question as to whether starch that is fermented *in vivo* produces increased amounts of butyrate cannot be answered from study of the faecal excretion of SCFA. Whilst *in vitro* studies support this possibility it is clear that the source of the inoculum is important. Unfortunately dynamic studies of SCFA production in man are difficult.

This study demonstrates that while RS has many effects on gut function, the extent of these will depend on both the sources of the RS and factors relating to the subject receiving it. Much more needs to be known, however, about the contribution of gut flora to RS utilization, and the contribution of RS to the composition of the flora.

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