Original Article

Whole cereal and legume seeds increase faecal short chain fatty acids compared to ground seeds

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We set out to compare the effect of diets containing intact seeds as food ingredients on colon function and fermentation-dependent events. Using a randomized cross over design, twelve healthy adults were recruited and required to consume an experimental diet containing intact or ground seeds for 7-days then after returning to their usual diet for 21-days to consume the second experimental diet for 7-days. All foods consumed during the experimental dietary periods were supplied by the researchers. Stools passed on three consecutive days on the usual diet prior to commencement and on days 5, 6 and 7 during each experimental diet, were collected. Outcome measures were whole gut transit time, 24 h stool output, faecal pH, particle size, and short chain fatty acid content. Seeds recovered from stools were examined by scanning electron microscopy. Nine of the twelve subjects completed all aspects of the study. Consumption of intact seeds compared to ground seeds increased 24 h faecal wet weight (mean 258g ± 123g and 170g ± 63g, respectively; P=0.005) and dry weight (78g ± 34g and 46g ± 28g, respectively; P=0.003). Whole gut transit times and moisture content of stools were not different. There was a trend for stools from the whole seed diet to be more acidic than those from the ground seed diet (pH 6.2 ± 0.3 and pH 6.6 ± 0.3, respectively; P = 0.06) and they contained more short chain fatty acids (35 ± 5.2 and 30 ± 10.5 mmol/kg, respectively; P=0.05). Large amounts of apparently whole seeds were recovered from stools, but internally the endosperm was often eroded and coated with bacteria. Intact seeds as food ingredients bring about changes to the colonic environment and to faecal composition that may reduce the risk of colon cancer.

Key Words: bacteria, colon function, electron microscopy, faecal composition, fermentation, short chain fatty acids, wholegrain, whole seeds.

Introduction

Diets high in whole grains benefit the gastrointestinal tract, reducing the risk of cancer in the pharynx and oesophagus[1] and in the stomach, colon and rectum.[2,3] In addition, in women, whole grains are associated with lower all-cause mortality.[4] The consumption of intact grain kernels also appears to elicit favorable metabolic responses,[5] which in turn may protect against colon cancer risk[6] as well as reducing the risk of Type 2 diabetes.[7]

The protective effects of whole grains against gastro-intestinal cancers have long been attributed to their content of dietary fibre. This conclusion has been supported by experimental studies indicating that fibre-rich diets mainly containing milled cereal ingredients are associated with many protective physiological effects in the lower gastrointestinal tract. These include stool bulking[8,9]; reduced intestinal transit time[10]; increased frequency of defecation [11]; and the reduction of lumenal and faecal pH.[12] Dietary fibre also sustains anaerobic bacterial fermentation resulting in the generation of short chain fatty acids (SCFA),[13] including butyrate which is considered to be protective.

Epidemiological data however has produced conflicting results. On the one hand it has been shown that dietary fibre from cereals neither prevents the development of colorectal adenoma in men[14] and women[15] nor protects against its recurrence[16] in populations eating a Western diet. On the other hand a recent European study of data from 22 countries showed that high fibre intake is associated with a 25% reduction in colon cancer risk but this could not be attributed to any particular food type (eg cereals).[36] Also, the results from a large case control study showed that fibre from particular foods (fruits and grains/ cereals) is associated in a dose dependent way with reduced risk of colon adenoma.[37] One of the factors that could help explain these inconsistencies could be the extent to which

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whole cereal grains are milled and refined before being used as food ingredients. Some have argued that many of the health benefits residing in whole grains may be lost as a result of milling.[17] For example, whole grains may be a significant source of phytoestrogens and other phytochemicals protective against cancer,[6] which may be lost during grain refining. Whilst most wholegrain food ingredients are ground prior to consumption it is possible that unmilled and intact grains may not only provide nutrient-rich fibres,[4] but also provide benefits attributable to their physical form. Firstly, the cellular architecture of intact seeds can prevent access by digestive enzymes to the endosperm that contains large amounts of starch, protein, non-starch polysaccharides and oligosaccharides which consequently pass to the colon. Here, the carbohydrate components are potential substrates for bacterial fermentation where they may generate SCFA also lowering pH.[18–21] Secondly, the physical form of the grain in itself may improve colonic function. The effect of particulate material has been demonstrated with inert plastic particles, which, if appropriately shaped to mimic wheat bran, can be as effective as wheat bran itself in improving colonic function (shortening whole gut transit, increasing stool moisture content and increasing the frequency of defecation).[22,23] We wanted to find out if the consumption of intact grains would produce effects on large bowel function that were different to ground grains as indicated by stool output, stool composition and whole gut transit times.

Subjects and methods

Subjects

Twelve healthy volunteers (four male, eight female) aged 31.2 ± 10.8 years (range 22–52 years) and with a mean body mass index of 22.8 ± 3 kg/m² (range 18.4–29 kg/m²) were recruited from the University and the general public via posted advertisements. The study was approved by the University Ethics Committee (approval number DSCH 1/97). None of the subjects had a history of gastrointestinal disorders and had not taken laxatives or antibiotics for 3 weeks prior to commencement.

Experimental design

At commencement, each subject's height and weight were measured. Subjects then maintained a 7-day weighed food record of their usual food intake. Individuals were then randomly assigned to one of two experimental diets, each of 7 days duration, during which all food and drink, including milk and sugar for use in tea and coffee, was provided. Subjects were asked to eat all of the food supplied but to return to the researchers any that remained uneaten and to refrain from eating any additional food. Following resumption of their usual diet for three weeks, subjects then consumed the alternate experimental diet for 7 days. Food was prepared in the university food laboratories and meals were individually portioned, packaged and delivered to the subjects' homes. The study was carried out with subjects living at home, but regular (at least three times per week) contact was maintained to provide support and to deliver and collect specimen containers.

Experimental diets

Each day's meals in the experimental diets were designed to supply energy equal to the seven day average of each subject's usual diet. Nutrient and energy intakes were calculated with proprietary software (Diet 4, Xyris) based on the Composition of Foods Australia database (NUTTAB 95). Tea and coffee were allowed *ad libitum*, provided that sweeteners and other additives used in these beverages were only those that were supplied. None of the subjects chose to include alcoholic drinks in the experimental diets.

During each 7-day dietary period, three 1-day menus made up of almost identical foods were fed in rotation (Table 1). To add some variety, the recipes were varied although the foods remained the same (e.g. meat was provided as steak, as stir-fried strips or as shepherds pie in the three rotations). Both test diets were identical in macronutrient composition. The calculated contribution to daily energy intake from fat was 27%, protein 22% and carbohydrate 51%. Each diet supplied 7.87g/MJ/day of dietary fibre and 17.4g/MJ/day of starch. Individual subject's energy intake during the usual diet ranged from 6.6 to 14.9MJ/day (mean 10.6MJ/day), and portion sizes in the experimental diets were adjusted accordingly.

The two diets differed only in the type of dishes that were prepared from the same foods. The whole grain (WG) diet contained grain, seed and legume components in their whole/intact form, whereas the ground grain (GG) diet contained them in a comminuted form. The grains, legumes and seeds used were linseeds; sunflower and sesame seeds; wheat grains; haricot and kidney beans; and chickpeas.

The breads for the study were baked in a large batch before the study commenced and frozen until required. The WG loaf consisted of coarse wheat flour (particle size <3mm), yeast, salt, sugar, gluten, water, improver, whole wheat grains, whole linseeds and whole sunflower seeds. The intact grains and seed were softened by boiling for 1 minute before being added to food mixtures. The GG loaf contained identical ingredients, except both the flour and additional seeds were ground to <1mm in particle size. Muesli in the WG diet comprised rolled oats, puffed rice, coarse wheat bran, peanuts, sunflower and sesame seeds, and whole wheat grains. On the GG diet, this muesli mixture was comminuted in a food processor and eaten as a porridge. Muffins contained the muesli mixture as their grain source either as purchased (WG diet) or after grinding (GG diet). Whole cooked legumes were consumed in the WG diet in a salad menu (menu 1), in a tomato-based soup (menu 2) or as part of a Shepherds Pie (menu 3) (Table 1). On the GG diet, the legumes were processed to a smooth paste and incorporated into the same recipes. All subjects indicated that they found no difficulty in complying with the experimental diets and that they did not eat any food other than that provided to them.

Faecal collections and transit measurement

On days 4,5 and 6 of each dietary period and also during the weighed intake record of their usual diet, subjects consumed at breakfast a capsule containing plastic radio-opaque markers with characteristic shapes. X-ray films of

the first stool passed after the morning of day 7 were used to count the three kinds of markers in order to estimate whole gut transit time.[24] The stools passed on days 5, 6 and 7 of the experimental diets and of the usual diet when the weighed food intake was being measured, were collected. Each bowel movement was collected in a plastic container, and immediately sealed, labelled and placed by subjects into an insulated box containing solid carbon dioxide. The frozen stools were collected within 12 hours and stored at –20°C.

Laboratory methods

Faecal samples collected over the 3 days were weighed and the mean daily stool output for each subject was calculated. Faecal samples excreted on day 7 of each dietary period (usual, GG diet and WG diet) were rapidly thawed, pooled (if there was more than one bowel motion that day for any given subject) and mixed and pH was measured using a protein-resistant glass electrode (Activon, Sydney). Duplicate 16g aliquots of this homogenate were freeze dried to determine faecal dry weight and moisture content. A further 50g portion was sieved to recover particles retained by 3mm and 1mm mesh sieves. These were examined externally and internally for adhering bacteria by scanning electron microscopy (Philips XL20, Eindhoven, Netherlands). Duplicate 2.5g aliquots of the faecal homogenate were taken for measurement of SCFA as previously described in an earlier communication.[25] All statistical tests were conducted using Microsoft Excel version 97 SR2 (Microsoft Corp, Redmond, WA) and analyzed by paired difference Student's *t*-test.

Results

Nine of the twelve subjects completed all aspects of the study. Some subjects did not take all the radio-opaque markers so that transit measurements could not be made and from others there was occasionally insufficient faecal material to complete all the stool analyses.

Usual and test diets

The subjects in this study consumed an habitual diet providing, on average, 16% energy from protein, 31% energy from fat and 49% energy from carbohydrates. On test diets, subjects obtained 21% energy from protein, 26% energy from fat and 50% energy from carbohydrates. Although dietary fibre intake was 7.8g/MJ on the test diets, which was higher than the 2.5g/MJ average recorded on the usual diets ($P<0.001$), none of the subjects reported difficulties in eating the food supplied. To demonstrate the differences in the particulate nature of the foods making up both test diets they were broken up by hand and carefully sieved using a 3mm sieve. The larger particles retained on the sieve were comprised mostly of cereal and legume seed ingredients and this material was weighed. The mass of larger food particles (>3mm) in the WG diet was 351g/10MJ food (2.63kg) whereas the GG diet contained only 31g/10MJ (2.63kg).

Faecal composition and bowel habit

Daily bowel movement remained similar to the usual pattern on both WG and GG diets (Table 2). However, the variation in daily stool output between individual subjects was large (106–562 g/day on the WG diet and 42–288 g/day on the GG diet). Compared to the usual diet, both test diets increased faecal wet weight ($P<0.01$), although the amount of dry matter excreted only

Table 1. Three day menu for whole grain (WG) and ground grain (GG) diets

Meal	Day 1		Day 2		Day 3	
	WG	GG	WG	GG	WG	GG
Breakfast	muesli [1] WG bread[2] orange juice	porridge [1] GG bread[2] orange juice	muesli [1] WG bread[2] orange juice	porridge [1] GG bread[2] orange juice	muesli [1] WG bread[2] orange juice	porridge [1] GG bread[2] orange juice
Morning tea	WG banana muffin	GG banana muffin	WG apple muffin	GG apple muffin	WG blueberry muffin	GG blueberry muffin
Lunch	WG bread[3] orange	GG bread[3] orange	WG bread[3] orange	GG bread[3] orange	WG bread[3] orange	GG bread[3] Orange
Afternoon tea	WG blueberry muffin	GG blueberry muffin	WG banana muffin	GG banana muffin	WG apple muffin	GG apple Muffin
Dinner	tomato & potato soup	tomato & potato soup	tomato soup with whole legumes	tomato soup with pureed legumes	tomato soup	tomato soup
	WG bread	GG bread	WG bread	GG bread	WG bread	GG bread
	steak	steak	beef stir-fry	beef stir-fry	shepherds pie with whole legumes	shepherds pie with pureed legumes
	whole legume salad	pureed legume dip	potato	potato		
	broccoli carrots canned peaches & yoghurt	broccoli carrots canned peaches & yoghurt	broccoli carrots canned peaches & yoghurt	broccoli carrots canned peaches & yoghurt	broccoli carrots canned peaches & yoghurt	broccoli carrots canned peaches & yoghurt

[1]Eaten with full-cream milk; [2]Eaten with butter and raspberry jam; [3]Eaten with butter and a salad (lettuce, tomato, cucumber)

Table 2. Faecal variables as influenced by usual diet, by whole grain diet and by ground grain diet

Faecal variable	Usual diet[1]	Whole grain diet[1]	Ground grain diet[1]	Subjects *N*
24h wet weight (g)	125 ± 46 (40-184)	258 ± 123.2 (106-562)[2,3]	170.5 ± 63 (42-288)[3]	11
24h dry weight (g)	43 ± 22 (9-75)	78 ± 34 (42-131)[2,3]	46 ± 28 (14.5-116)	10
Moisture (%)	67 ± 12 (40-81)	69 ± 9 (51-76.8)	74 ± 8 (60-85.7)[4]	10
Whole gut transit (h)	39.7 ± 22 (20-69)	33 ± 14 (16-54)	28 ± 9 (12-40)[4]	9
Daily stool frequency	1.5 ± 0.7 (1-2.3)	1.7 ± 0.8 (1-3.3)	1.5 ± 0.5 (1-2)	11
pH	6.6 ± 0.5 (5.8-7.5)	6.2 ±0.3 (5.8-6.8)[4,5]	6.6 ± 0.3 (6.1-7)	12

[1] mean ± SD. Range in parentheses; [2] Significantly different from the GG dietary period, $P < 0.01$ (paired-difference *t* test); [3] Significantly different from the subjects usual dietary period, $P < 0.01$ (paired-difference *t* test); [4] Significantly different from the subjects usual dietary period, $P < 0.05$ (paired-difference *t* test); [5] Significantly different from the GG dietary period, $P < 0.05$ (paired-difference *t* test)

increased significantly on the WG diet ($P<0.01$). This increase in dry weight output on the WG diet could be related to the presence of relatively intact whole grains and seeds appearing in the stools (Figure 1). Conversely, stool moisture content only increased above usual levels after the GG diet ($P<0.05$). Particles recovered from stools differed significantly according to diet consumed. Stools from the WG diet contained more of the larger size particles (>3mm) compared to those from the GG diet (8g ± 3g/100g wet weight faeces versus 2g ± 1g wet weight faeces, $P = 0.0001$). These were easily visible as apparently intact cereal grains, fragments of mushrooms, fragments of red kidney beans and coconut fibres (Fig. 1). Many of the cereal grains that were apparently intact showed, upon closer examination, that their endosperm was in fact extensively pitted and corroded (arrowed regions in Fig. 2). Electron microscopic examination revealed that these internal pitted areas were extensively covered by bacterial rods and cocci.

Faecal short-chain fatty acids

The effects of diet on faecal SCFA are shown in Table 3. The total concentration of these acids in stools from the three diets was similar, but the 24h output was significantly higher for the WG diet when compared to either the GG diet ($P=0.007$) or the usual diet ($P=0.004$). This is due to the larger mass of stools excreted by subjects when they consumed the whole grain diet (see Table 2). Butyrate concentrations were similar following consumption of both of the test diets, although again the bulkier stools of the WG diet increased the 24h output of butyrate compared to the GG diet ($P=0.07$) and the usual diet ($P=0.001$). The faecal concentrations of acetate and propionate followed a similar pattern (data not shown).

Discussion

Both experimental diets increased 24 hour stool mass. The WG diet resulted in a doubling of the amount excreted compared to the usual diet and produced an additional and statistically significant increase in stool mass compared with the same foods containing ground grain ingredients (i.e. the GG diet). This increase is not explained by differences in stool moisture content or by the weight of large undigested food particles and could be due to additional bacterial mass. This increase in bacterial mass we attribute partly to the lower digestibility of whole grain ingredients and partly to the increased secretions entering the colon as a result of accelerated small bowel transit stimulated by the physical form of the

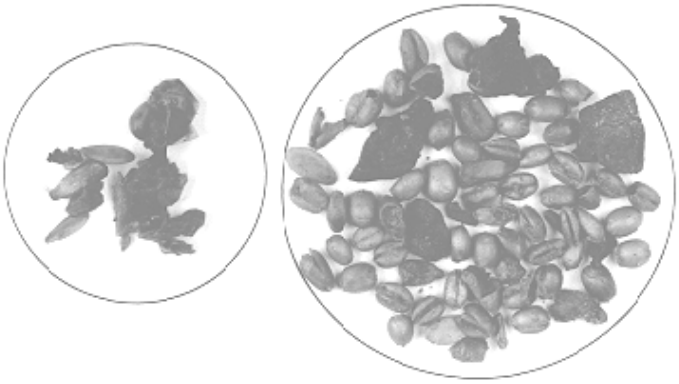

Figure 1. Particles >3mm recovered from 50g stools of a single subject collected on the seventh day of the groundgrain (left) and wholegrain dietary (right) periods

Table 3. Faecal short chain fatty acid and faecal butyrate content during usual, whole grain and ground grain diets[1]

Diet	Faecal short chain fatty acids		Faecal butyrate	
	Concentration (mmol/kg)	24h output (mmol)	Concentration (mmol/kg)	24h output (mmol)
Usual	32.9 ± 14.6	4.2 ± 2.6	9.4 ± 5.6	1.2 ± 0.8
Ground grain	29.6 ± 10.5	5.4 ± 3.3	9.6 ± 5.2	1.8 ± 1.2
Wholegrain	34.7 ± 5.2	9.3 ± 5.7[2]	11.2 ± 3.1	2.9 ± 1.4[3]

[1] Mean ± SD; N = 12 subjects; [2] Different from ground grain (P = 0.007) and from usual diet (P = 0.004)
[3] Different from ground grain (P = 0.017) and from usual diet (P= 0.001)

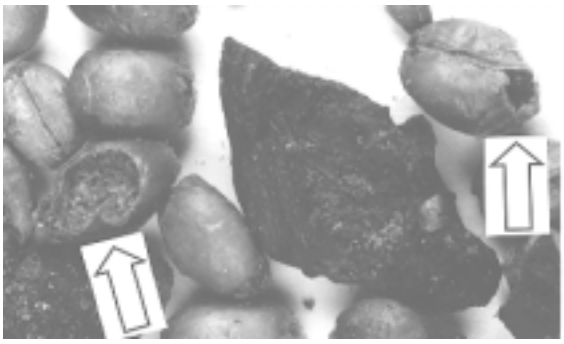

Figure 2. Photograph of grains recovered from stool showing areas of erosion as a result of bacterial digestion (arrows).

large whole grain particles.[23] Both would result in an increased supply of substrate for the colonic flora. Many studies have reported the marked faecal bulking effect of wheat bran[26] and to a lesser extent of cereal particles containing resistant starch.[18] Wheat bran particles ranging from 50µm MPS (mean particle size) to 1200µm MPS have been reported to increase faecal bulk, with the larger sizes being more laxative.[27] However, the observation that plastic particles shaped like bran and of a similar size (>1.4mm<3mm), but not plastic granules (600µm MPS), are as effective as wheat bran itself in promoting whole gut transit and faecal bulk shows that the physical form of undigested food particles may be more important than either the fermentation or water-holding capacity of fibre in controlling stool bulk.[22,23] Indeed, the concept that the rough edges of bran stimulate the colon to movement was prevalent in scientific opinion throughout the nineteenth century.[28]

The recovery of relatively intact grains and other food particles from faeces in this study show that many food particles are swallowed without significant size reduction during mastication. Since these differences persist in the colon and they affect the chemical characteristics of stools they could be important in determining health outcomes. The particle size of diets is not easily quantified and rarely measured in experimental nutrition and only a few food ingredients, such as cracked wheat, crushed wheat and whole wheat flour in the United States for example are defined in terms of their mean particle size.[29] Increased stool output is usually associated with faster whole gut transit, an effect that is also positively associated with larger particle sizes of undigested food components.[30] Because whole gut transit time is inversely correlated with stool weight,[31] we were surprised to find that transit times were significantly reduced during the GG diet compared to the usual diet, but not during the WG diet compared to the usual diet. We attributed this lack of effect on transit time of whole grains compared to ground grains to the small numbers of subjects in this study, the large inter-subject variability in mean transit times and the fact that the colonic flora might not have had time to fully adapt to the changes in diet.[32]

Stool pH and butyrate are markers of colon cancer risk[33] and we found both to be beneficially affected by consumption of a diet rich in whole grains. Others have shown that diets containing large amounts of resistant starch (RS)[18,21] also increase faecal output of SCFA and butyrate, however the combination of RS with non-starch polysaccharide (NSP) seems to more effective in promoting distal fermentation in the colon,[19,20] a region where the occurrence of malignancy is higher.[34] The cereal particles recovered from the stools in this study are rich in both RS and NSP. The fact that cereal and legume particles recovered from stools were corroded and heavily coated with bacteria indicates that they acted as both transport vehicles and as slowly fermenting substrates for colonic microorganisms and that this fermentation was continuous along the length of the colon. Not only is the physical form of food an important determinant of digestibility in the small intestine, as exemplified by physically entrapped starch (RS1),[35] it is also a determinant of fermentation-dependent events in the large bowel and affects bio-markers for colon cancer risk such as butyrate.

Our results indicate that defining whole grain foods in terms of their chemical composition does not adequately describe their physiological properties in the gut. Further work is required to provide better descriptions that include measures of physical integrity.

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