Whole-grain rye and wheat foods and markers of bowel health in overweight middle-aged men[1–3]

Graeme H McIntosh, Manny Noakes, Peter J Royle, and Paul R Foster

ABSTRACT

Background: Whole-grain cereal foods including rye have been identified as providing significant health benefits that do not occur when refined-cereal foods are ingested.

Objectives: Foods (90 g) containing whole-grain rye flour and whole-grain wheat flour were compared with low-fiber refined-cereal foods for their effects on markers of bowel health and the metabolic markers insulin and glucose.

Design: Three 4-wk interventions were undertaken in a randomized crossover design with 28 overweight men aged 40–65 y who had no history of bowel disease. Against a background intake of 14 g dietary fiber (DF), the men were fed low-fiber cereal grain foods providing 5 g DF for a total of 19 g DF/d. High-fiber wheat foods provided 18 g DF, and high-fiber rye foods provided 18 g DF, both giving a total of 32 g DF/d. Fecal samples (48-h) and fasting and postprandial blood samples were collected at the end of each period and assayed.

Results: Both high-fiber rye and wheat foods increased fecal output by 33–36% ($P = 0.004$) and reduced fecal β-glucuronidase activity by 29% ($P = 0.027$). Postprandial plasma insulin was decreased by 46–49% ($P = 0.0001$) and postprandial plasma glucose by 16–19% ($P = 0.0005$). Rye foods were associated with significantly ($P = 0.0001$) increased plasma enterolactone (47% and 71%) and fecal butyrate (26% and 36%), relative to wheat and low-fiber options, respectively.

Conclusions: High-fiber rye and wheat food consumption improved several markers of bowel and metabolic health relative to that of low-fiber food. Fiber from rye appears more effective than that from wheat in overall improvement of biomarkers of bowel health. *Am J Clin Nutr* 2003;77:967–74.

KEY WORDS Wheat, rye, dietary fiber, bowel health, glucose, insulin, butyrate, enterolactone, cereal

INTRODUCTION

Whole-grain cereal foods have been identified as providing significant health benefits that do not occur when refined-cereal foods are ingested (1–6). Rye grain is commonly consumed as whole-grain products, which are an important part of some European food cultures (6–8). Unlike wheat, which is commonly consumed in Australia, rye when ingested in reasonable amounts has potential benefits to human health that have not been examined. Compared with wheat, rye is a slightly better source of total dietary fiber (DF), is more commonly used in whole-grain food forms, and, along with cellulose, contributes more mixed linked 1→3,1→4 β-glucan and arabinoxylan (6–8). The latter fiber types are of particular interest because they are present in soluble and insoluble forms, and arabinoxylan is considered to be an optimal substrate for fermentative generation of short-chain fatty acids (SCFAs)—in particular, of butyrate in the colon (8). Butyrate at high concentrations in the colon is hypothesized to improve bowel health and lower cancer risk by several possible mechanisms (8–13).

Studies of populations consuming significant amounts of rye foods have shown health benefits, observable as reduced coronary heart disease events (14, 15), reduced risk of adult-onset diabetes (3, 4, 16, 17), reduced risk of bowel cancer, and improved bowel health, as assessed by relevant markers (5–8, 11, 12, 18–22). Whether such effects are expressed only in population groups (eg, those of Scandinavia, Germany, and Russia) in which the strong acceptance of rye foods often results in large daily intakes (100–390 g/d as rye bread or other rye foods) is unclear. More moderate intakes may have a beneficial influence as assessed by putative markers of cancer risk. The consumption of several servings of whole-grain foods per day has been recommended for increased fiber intake and improved health (5).

Functional effects of rye foods that may be beneficial to bowel health might include increased fecal bulk, the binding and effective elimination of potentially toxic metabolites, promotion of desirable fermentative activity to SCFAs (in particular, butyrate), and the release of protective components such as lignans (1, 20–23). Plant-derived lignans have been studied for their phytoestrogenic effects with regard to hormone-sensitive cancers, as potential inhibitors of breast and prostate cancers, but also with regard to colon cancer (1, 22, 23). Lignan precursors are released via microbial β-glucuronidase (EC 3.2.1.31), converted to mammalian lignans by microbial enzymes, and absorbed through the bowel wall (23–25). Plasma enterolactone concentrations are considered to provide a good measure of their generation in the colon by microbial fermentation (19, 20). Rye foods have also been

[1] From the Commonwealth Scientific & Industrial Research Organisation (CSIRO) Health Sciences and Nutrition, Adelaide, Australia.

[2] Supported by George Weston Foods Ltd and the Australian Government Food into Asia program.

[3] Address reprint requests to GH McIntosh, CSIRO Health Sciences and Nutrition, PO Box 10041, Adelaide BC, SA 5000, Australia. E-mail: graeme.mcintosh@csiro.au.

Received November 11, 2002.

Accepted for publication October 8, 2002.

TABLE 1
Daily dietary intakes from recorded diet diaries for the 3 dietary intervention periods[1]

	High-fiber rye	High-fiber wheat	Low fiber
Energy (kJ)	9563 ± 243	9919 ± 317	9989 ± 324
Protein			
(g)	104.8 ± 3.1	114.1 ± 4.7	108.4 ± 4.8
(% of energy)	17.6 ± 0.4	18.4 ± 0.5	17.4 ± 0.5
Fat			
(g)	75.0 ± 2.9	79.2 ± 3.8	78.3 ± 3.7
(% of energy)	29.0 ± 0.8	29.4 ± 0.9	28.7 ± 0.8
Carbohydrate			
(g)	278.8 ± 10.4	283.6 ± 9.9	294.9 ± 9.1
(% of energy)	49 ± 1	49 ± 1	51 ± 1
Alcohol			
(g)	12 ± 3	11 ± 3	11 ± 3
(% of energy)	4 ± 1	3 ± 1	3 ± 1
Fiber (g)	32 ± 1[2]	32 ± 1[2]	19 ± 1
Sugars (g)	84 ± 5	89 ± 6	85 ± 6
Starch (g)	173 ± 8[2]	177 ± 7[2]	199 ± 6
Cholesterol (mg)	281 ± 16	292 ± 26	278 ± 21

[1] $\bar{x}$ ± SEM.
[2] Significantly different from low-fiber treatment, $P < 0.0001$ (repeated-measures ANOVA). P value from Bonferroni's correction, $P \leq 0.05$.

shown to modulate plasma glucose and insulin concentrations, which could influence bowel cancer risk, because increased serum insulin and insulin-like growth factors (eg, immunoglobulin F) are part of a metabolic syndrome that has been associated with increased risk of colon cancer (26–29). Other breakdown products of dietary protein, such as ammonia, *p*-cresol, and phenol measured in feces and/or urine, are potentially harmful, and lesser amounts might be considered advantageous.

We undertook this study to examine the influence of < 100 g rye grain, an amount that represents a culturally acceptable level of intake in the Australian context and that might lead to clear changes in measures of bowel health function. In fiber terms, this amount represented an intake of ≈78% more than that with the low-fiber refined-cereal food option. Our specific objectives were to evaluate the effects of these amounts of whole-grain rye flour and fiber-matched whole-wheat flour and low-fiber (refined) wheat-flour foods on markers of bowel health and colon cancer risk and on postprandial glucose and insulin responses.

SUBJECTS AND METHODS

Subjects undertook 3 interventions of 4-wk duration each in a randomized crossover design. At baseline, subjects were assigned in groups of 6 on the basis of randomly generated numbers to 1 of the 6 treatment orders. The intervention diets were high-fiber rye, high-fiber wheat, and low-fiber foods.

The study protocol required subjects to visit the clinical research facility on 4 occasions for investigative procedures with an additional 3 visits for further dietary assessment and dispensing of test foods. On each study visit, subjects were weighed and a fasting (overnight) venous blood sample was taken.

Fecal, urine, and blood samples were also collected at the end of each study period. Subjects were also required to record what they ate during the final 3 d of each 2 wk. Plasma samples were stored in a frozen state (−20 °C) for subsequent analysis.

Endpoint measures were fecal weight, fecal pH, fecal SCFA concentrations (including butyrate), fecal bile acid concentrations, and fecal ammonia. Other endpoint measures were the fasting and 1-h postprandial glucose and insulin responses to a test meal with 50 g available carbohydrate as high-fiber rye, high-fiber wheat, or low fiber and the concentrations of plasma enterolactone and fecal β-glucuronidase.

Subjects

Male subjects were recruited by public advertisement. Respondents were screened on the basis of the following selection criteria: age 40–65 y; no history or presence of gastrointestinal, renal, or hepatic disease of any cause; and no current consumption of any over-the-counter medication such as laxatives or antibiotics that could interfere with the validity of the study. Exclusion criteria were the use on a regular basis (≥ 1/wk) of any form of drug therapy, medication, or supplements that may interfere with bowel function; definite or suspected personal or family history of adverse events; and hypersensitivity to rye or wheat.

Thirty-one subjects were selected and 28 subjects completed the study. Three subjects withdrew during the first week of the study because they were unable to make the necessary dietary modifications. Approval was obtained from the Commonwealth Scientific & Industrial Research Organisation (CSIRO) Health Sciences and Nutrition Human Ethics Committee, and written informed consent was obtained from all volunteers.

Diets

Subjects were provided test cereal foods for each dietary intervention. The low-fiber diet provided 19 g DF/d, including low-fiber test foods as 140 g white bread, 40 g refined-wheat crispbread (Crackerbread; George Weston Foods Ltd, Sydney, Australia), and 50 g low-fiber rice cereal (Rice Pops; Farmland Foods, Sydney, Australia). The high-fiber wheat diet provided 32 g DF/d, including wheat test foods as 140 g whole-meal bread, 40 g whole-meal wheat crispbread, and 50 g whole-wheat breakfast cereal. The high-fiber rye diet provided 32 g DF including rye test foods as 140 g whole-grain rye bread (Burgen Rye), 40 g rye crispbread (Ryvita), and 50 g whole-rye breakfast cereal (all: George Weston Foods Ltd, Sydney, Australia). Subjects were counseled by a dietitian about maintaining a moderately low-fiber background diet (ie, other than the test foods). This was to be achieved by avoiding foods high in fiber and limiting fruit to 2 servings per day and vegetables to 3 servings per day. Dietary compliance was recorded by the completion of daily checklists of test food intake. Nutrient intakes were also recorded in 3-d weighed-food records twice during each 4-wk intervention. Subjects received detailed instruction by demonstration and in writing as to the accurate documentation of food intake. The subjects' weights and diets were monitored every 2 wk by the dietitian. Nutrient intakes were calculated using relevant software (DIET-1; XYRIS Software Pty Ltd, Brisbane, Australia), a nutrient-composition database of foods based on Australian foods modified to include analysis data on the test cereal foods.

The low-fiber foods aimed to provide 6 g DF /d to the diet and ≈20% energy. The high-fiber foods aimed to provide 21 g DF/d to the diet and ≈20% energy. The rye and wheat interventions aimed to provide < 100 g/d of whole rye or wheat flour (**Table 1**). The high-fiber foods were to provide more than one-half of each

TABLE 2
Compositional analysis of foods used in the study

	Total starch	Protein	Fat	Total dietary fiber	Moisture
			% by wt		
Rye crispbread[1]					
Original	63.3	9.5	2.1	15.1	3.3
Country grain	59.8	12.3	4.3	13.5	2.4
Currant biscuit	54.5	7.4	2.1	12.0	5.9
Refined-wheat crispbread[2]	75.8	9.8	3.1	3.2	5.5
Whole-meal wheat crispbread[3]	63.3	16.6	2.9	10.2	4.4
Rye breakfast cereal[4]	53.2	9.5	3.9	14.9	0.5
Wheat breakfast cereal	59.5	11.0	2.9	11.0	1.1
Low-fiber rice cereal[5]	79.1	6.6	1.3	1.2	4.2
Rye bread[4]	33.3	13.0	5.0	5.7	36.2
Wheat bread	30.2	12.4	5.0	6.1	39.9
Low-fiber white wheat bread	35.3	12.3	4.8	2.4	40.7

[1] Ryvita; George Weston Foods Ltd, Sydney, Australia.
[2] Crackerbread; George Weston Foods Ltd, Sydney, Australia.
[3] Wholemeal crackers; George Weston Foods Ltd, Sydney, Australia.
[4] Burgen Rye; George Weston Foods Ltd, Sydney, Australia.
[5] Savings brand Rice Pops; Farmland Foods, Sydney, Australia.

subject's total DF intake per day. Nutrient compositions of the test foods are outlined in **Table 2**.

Biochemical assays and bioassays

Total DF was analyzed in all food products with the use of an enzymatic-gravimetric method, described elsewhere (30). Subjects collected all of their stools for the final 2 d on each of the 4 sampling occasions. Individual bowel movements were voided into a plastic bag placed over a toilet bowl. After the defecation, air was expelled from the bag, which was then closed with a tie, labeled with the date and time, and stored at −20 °C. For transport to the clinic, the bags were placed between 2 ice bricks in a polystyrene cooler. Subjects also collected a morning and evening spot urine sample during the 48-h sampling period. These samples were also frozen for transport to the laboratory.

At the end of each cereal food intervention, subjects underwent a 1-h meal tolerance test to assess postprandial glucose and insulin responses to that test cereal. After an overnight fast of 12 h, baseline venous blood samples were taken (20 mL) into tubes containing EDTA and (5 mL) into tubes containing sodium fluoride and EDTA, and further samples were taken 1 h after ingestion of a test cereal providing 50 g available carbohydrate, consumed with 100 mL skim milk. Blood plasma was separated by low-speed centrifugation at 1200 × *g* for 10 min at room temperature and then frozen at −20 °C until it was analyzed. At the end of each intervention period, all samples from each subject were analyzed along with quality-control samples in the same analytic run.

Plasma insulin was measured with the use of a double-antibody radioimmunoassay (Phadeseph Insulin; Pharmacia Diagnostics, Uppsala, Sweden) and RIA-CALC software and an LKB Wallac gamma counter (Wallac, Turku, Finland). Plasma glucose was measured by use of a glucose oxidase colorimetric method (Roche Diagnostics, Mannheim, Germany) on an automated analyzer (Cobas-Bio; Beckman Instruments, Fullerton, CA). Plasma lignans were assayed by H Adlercreutz at the Institute for Preventive Medicine, Nutrition and Cancer at the University of Helsinki with the use of time-resolved fluoroimmunoassay (31, 32). Fecal water for analysis of bile acids was derived by ultracentrifugation of fecal material at 150 000 × *g* for 50 min at 4 °C and then frozen at −20 °C until it was analyzed (33–35). Fecal water bile acids were analyzed by gas liquid chromatography (GC 17A; Shimadzu Corp, Kyoto, Japan) with CLASS LC10 software (Shimadzu Corp) according to a method described elsewhere (36).

Fecal SCFA concentrations were determined according to the method described elsewhere (36). Fecal β-glucuronidase activity was analyzed by a modification of a method described elsewhere (37–39). Briefly, 3 g of fresh fecal sample was homogenized in 7 mL phosphate-buffered saline. The sample was then centrifuged at 10 000 × *g* for 20 min at 4 °C, and the supernatant fluid was removed and stored at −70 °C until it was assayed. Fecal supernatant fluid (100 mL) was added to an equivalent volume of phenolphthalein:glucuronic acid conjugate reagent (Sigma Chemical, St Louis) in 0.8 mL phosphate-buffered saline, and the mixture was incubated at 37 °C for 45 min. The reaction was stopped by the addition of glycine and water added to a volume of 6 mL. Absorbance was read at 540 nm by a spectrophotometer (UV-VIS; Varian-Cary, Mulgrave, Australia). The β-glucuronidase–specific activity was expressed as U phenolphthalein released · g wet fecal $wt^{-1} \cdot h^{-1}$ against a phenolphthalein standard curve.

Fecal ammonia concentration was determined by a colorimetric technique as described elsewhere (40). Fecal phenol and *p*-cresol concentrations were assayed as described elsewhere (41).

Statistical analysis

The difference between the mean values of the variables high-fiber rye, high-fiber wheat, and low fiber was initially analyzed with the use of repeated-measures analysis of variance, with an unstructured covariance matrix. This choice of model allowed for the possibility of unequal variances and covariances among the 3 variables. Subsequent investigation of the 3 pairwise comparisons (ie, rye versus wheat, rye versus low fiber, and wheat versus low fiber) used Bonferroni's correction with significance set at $P < 0.05$. We used BMDP statistical software, Program 5V (BMDP Statistical Software, Los Angeles) for analysis.

TABLE 3
Composition of test foods consumed in each of the 3 dietary intervention periods

	Whole-grain composition	Test food	Amount of test grain	Energy	Protein	Fat	Carbohydrate	Fiber
	% by wt	*g*	*g*	*kJ*	*g*	*g*	*g*	*g*
Whole rye								
Cereal	79	50	39.5	754	5	2	36	7
Bread	21	135	28.4	1451	18	7	54	8
Crispbread	100	22	20	319	2	1	15	3
Total	—	207	87.9	2524	25	10	105	18
Whole wheat								
Cereal	79	50	39.5	770	6	1	37	6
Bread	21	135	28.4	1358	17	7	49	8
Crispbread	—	42	20	627	7	1	28	4
Total	—	227	87.9	2755	30	9	114	18
Low fiber								
Cereal (rice)	100	50	—	814	3	3	43	1
Bread (wheat)	100	135	—	1419	17	6	54	3
Crispbread (wheat)	100	42	—	674	4	1	33	1
Total	—	227	—	2907	24	8	130	5

RESULTS

Dietary intakes

Dietary intakes for each treatment are shown in Table 1. There was no difference in the subjects' energy intakes between each intervention period. Total DF intakes for rye and whole wheat did not differ from each other, but did differ significantly ($P = 0.01$) from those for the low-fiber diet. The subjects ingested 32 g DF/d with 18 g from rye foods, 32 g DF/d with 18 g from wheat foods, and 19 g DF/d with 5 g from low-fiber cereal products (**Table 3**). These were provided as 88 g of whole rye in 207 g grain foods/d, 88 g of whole wheat in 227 g grain food/d, and 227 g refined-cereal grain foods/d in the low-fiber intervention.

There was no significant difference between the body weights of subjects in the 3 intervention periods. The mean (± SEM) body weight of the 28 men was 94.6 ± 2.7 kg, and the BMI was 30 ± 0.9 at the beginning of the study. Compliance with test food intakes was excellent in 95% of subjects. Although weighed food records are not a perfect tool for assessing dietary intake in free-living subjects, the main purpose of obtaining the nutrient-intake data in this study was to ascertain relative intake, not absolute intake. In particular, we needed to show that no significant dietary changes occurred between treatments, other than those attributable to the changes in test food intake. Nevertheless, the estimated energy requirement based on the age, weight, and height of this group of sedentary men is 11.4 MJ, which is only 15% lower than our recorded intakes. We therefore felt that the food intake data for this group were valid.

Large-bowel and fecal measures

Effects of the diets on fecal values are summarized in **Table 4**. There was a significant increase in daily fecal weights on both the high-fiber rye (37%; $P = 0.003$) and wheat (27%; $P = 0.01$) diets relative to the low-fiber diet (203 g feces/d). There was a small (0.2 pH units) but significant ($P = 0.0002$) reduction in fecal pH with both high-fiber diets. This was associated with a significant ($P = 0.0001$) increase in butyrate with the high-fiber rye foods (36%) but not with the high-fiber wheat foods or the low-fiber foods. Fecal acetate concentrations did not differ significantly between treatments, whereas fecal propionate was significantly lower (12%; $P = 0.03$) with the high-fiber wheat diet than with the low-fiber diet. Fecal ammonia concentrations were significantly lower (19%; $P = 0.001$) with the high-fiber wheat diet 19%) than with the low-fiber diet. This reduction was inversely proportional to the increased bulk of feces, so that there was no difference in total output of fecal ammonia between the dietary treatments. Fecal β-glucuronidase concentration was significantly lower with high-fiber wheat (29%; $P = 0.003$) and rye (20%; $P = 0.07$) foods than with low-fiber foods. Fecal *p*-cresol concentrations were significantly lower with the high-fiber rye (37%; $P = 0.005$) and wheat (30%; $P = 0.002$) diets than with the low-fiber diet. Fecal phenol concentrations did not differ significantly between the diets (data not shown).

Bile acid concentrations showed no significant differences between treatments (Table 4). Daily fecal output of primary bile acids was significantly higher (43%, $P = 0.04$) with high-fiber rye than with low-fiber foods. As a percentage of total fecal bile acid output, primary bile acids were higher with the high-fiber rye diet (17%) than with the low-fiber diet (11%). Although mean total bile acid concentrations in fecal water were about 38% lower during the high-fiber rye and wheat diets than during the low-fiber diet, the apparent difference was not significant ($P = 0.15$). Deoxycholic acid concentrations were 16% lower for high-fiber rye and wheat foods than for low-fiber foods ($P = 0.16$). Total secondary bile acids concentrations in fecal water did not differ between the diets.

Plasma measures

Mean plasma enterolactone concentrations were significantly higher in men fed high-fiber rye than in those fed high-fiber wheat and low fiber (47% and 71% higher, respectively; $P = 0.0001$); the concentrations with the latter 2 diets did not differ from each other. The mean of baseline values for the 28 subjects at the beginning of the study was 25.6 ± 5.4 nmol/L.

Plasma insulin concentrations 1 h postprandially had significantly lower responses when high-fiber rye and wheat were fed in a standardized breakfast meal than when a low-fiber product equivalent was fed (**Table 5**). Insulin response with high-fiber rye

TABLE 4
Influence of high-fiber rye and wheat and low-fiber foods on human fecal measures for the 3 dietary intervention periods[1]

	High-fiber rye	High-fiber wheat	Low fiber
24-h fecal wt (g)	278 ± 16[2]	257 ± 21[2]	203 ± 18
Fecal pH	6.81 ± 0.06[2]	6.79 ± 0.07[2]	7.01 ± 0.08
Fecal acetate (μmol/g wet wt)	68.5 ± 3.8	65.1 ± 3.4	62.9 ± 3.3
Fecal propionate (μmol/g wet wt)	18.7 ± 1.1	16.6 ± 0.9[3]	18.9 ± 1.5
Fecal butyrate (μmol/g wet wt)	27.8 ± 2.2[4]	22.6 ± 1.5	20.4 ± 1.7
Total SCFA (μmol/g wet wt)	112 ± 7.3	104 ± 5.2	102 ± 5.7
Fecal ammonia (μmol/g wet wt)	33 ± 2.4	31.4 ± 2.7[3]	38.8 ± 3.1
Fecal β-glucuronidase ($U \cdot g$ wet $sample^{-1} \cdot h^{-1}$)	3.23 ± 0.34	2.84 ± 0.35[3]	4.03 ± 0.42
Fecal *p*-cresol (μg/g wet wt)	53.6 ± 4.7[2]	59.5 ± 5.6[2]	84.7 ± 10.1
Fecal deoxycholic acid (μmol/L)	68 ± 7.3	68 ± 12.4	82 ± 8.6
Total primary bile acids			
(μmol/L)	54 ± 6.3	50 ± 9.1	47 ± 8.6
(μmol/d)	15 ± 1.8[3]	15.7 ± 4.3	10.5 ± 2.0
Total secondary bile acids			
(μmol/L)	257 ± 56	274 ± 72	429 ± 149
(μmol/d)	70.9 ± 16	75.7 ± 20.8	84.7 ± 31.4
Total bile acids			
(μmol/L)	294 ± 60	305 ± 79	481 ± 159
(μmol/d)	84.1 ± 17.1	89.7 ± 23.4	94.2 ± 32.6
Ratio of daily primary-to-total output (%)	17	17.2	11

[1] $\bar{x}$ ± SEM, $n = 28$. SCFAs, short-chain fatty acids.
[2–4] Significantly different from low-fiber treatment (repeated-measures ANOVA): [2] $P < 0.005$, [3] $P < 0.05$, [4] $P < 0.0001$. P value from Bonferroni's correction, $P \leq 0.05$.

was 46% and that with high-fiber wheat was 49% of the low-fiber response ($P = 0.0001$). Similarly, the glucose response with high-fiber rye was 15% lower and that with wheat was 20% lower than that with the low-fiber product; both differences were significant ($P = 0.0005$). The pretreatment fasted plasma insulin concentration in the 28 subjects was 10.4 ± 1.0 uU/mL. There were no differences in the calcium, phenol, or *p*-cresol concentrations when expressed relative to the creatinine concentration in the urine samples (**Table 6**).

DISCUSSION

We have shown that, in overweight middle-aged men, moderate intakes of both high-fiber rye and wheat foods, when matched for fiber content, were equally effective in increasing the weight of fecal output and in decreasing the pH and fecal β-glucuronidase, secondary bile acids, and *p*-cresol concentrations relative to values with the intake of low-fiber foods. These markers are suggestive of improved bowel health (10–13, 35, 37–43). The rye foods were, however, more effective in increasing plasma enterolactone and fecal butyrate concentrations than were whole-wheat and low-fiber foods, which did not differ from each other. Similar observations have been reported by Grasten et al (7). Butyrate has an essential role as an energy source for the maintenance of normal colonocytes (a trophic effect), as well as being a putative anticancer agent through its ability to induce apoptosis in mutated colon cells (11–13, 44–46). Both high-fiber rye and wheat increased fecal bulking, but only rye foods significantly increased the fecal butyrate concentration. This finding is consistent with observations of other workers on the consumption of rye breads by pigs and humans (7–9, 24) where larger quantities of rye were consumed. Arabinoxylan and cellulose and resistant starch are considered important substrates for butyrate generation, and in both cereal grains, these components are well and comparably represented (6). The reason for the better response with rye than with wheat is not known, but it may have to do with the fact that arabinoxylan concentrations are higher in rye DF (3 g/100 g or 50% higher) than in wheat DF (6, 8). The other SCFAs measured were not significantly affected in this study, except for propionate, which was significantly lower in men fed high-fiber wheat. Fecal ammonia concentrations were also significantly lower for wheat than rye or low-fiber diet options. There is no obvious explanation for this difference.

TABLE 5
Influence of high-fiber rye and wheat and low-fiber foods on plasma metabolic markers for the 3 dietary intervention periods[1]

	High-fiber rye	High-fiber wheat	Low fiber
Plasma insulin, fasted (μU/mL)	6.08 ± 0.69	6.65 ± 0.78	6.83 ± 0.81
Δ Plasma insulin, fed-fasted (μU/mL)	19.6 ± 2.1[2]	20.8 ± 2.8[2]	48.9 ± 6.5
Plasma glucose, fasted (mmol/L)	5.60 ± 0.17	5.59 ± 0.13	5.64 ± 0.16
Δ Plasma glucose, fed-fasted (mmol/L)	1.35 ± 0.3[3]	0.95 ± 0.2[3]	2.42 ± 0.4
Plasma enterolactone (nmol/L)	30.9 ± 3.8[2]	21.0 ± 3.8	18.1 ± 2.7

[1] $\bar{x}$ ± SEM, $n = 28$. Δ, change in value.
[2,3] Significantly different from low-fiber treatment (repeated-measures ANOVA): [2] $P < 0.0001$, [3] $P < 0.0005$. P value from Bonferroni's correction, $P \leq 0.05$.

TABLE 6
Influence of high-fiber rye and wheat and low-fiber foods on urinary metabolic markers for the 3 dietary intervention periods[1]

	High-fiber rye	High-fiber wheat	Low fiber
Urine phenol (mg/g creatinine)	4.1 ± 0.4	4.2 ± 0.4	3.9 ± 0.3
Urine *p*-cresol (mg/g creatinine)	32.4 ± 2.4	24.5 ± 2.5	27.6 ± 2.5
Urine calcium (mg/g creatinine)	73.8 ± 7.5	83.6 ± 9.3	79.2 ± 7.4

[1] $\bar{x}$ ± SEM, n = 28.

The plasma enterolactone concentration has been proposed as a marker of large bowel health; enterolactone results from desirable microbiological fermentation in the lumen and absorption through the colon wall of mammalian lignans (1, 22, 23). Enterolactone concentrations in plasma were recently shown in a crossover study to reflect dietary change within 2 wk of the introduction of a new diet, and maximal concentrations are observed at 4 to 6 wk (47). The potential role of plasma enterolactone in cancer prevention, with particular regard to the hormone-sensitive cancers of breast and prostate but also to colon cancer, has been recognized (1, 21, 22, 45). This mammalian lignan is indeed a useful measure of the degree to which a diet provides fermentable plant lignans to the colon. Fiber-rich cereal foods, in particular rye, have been reported to be good sources, and mammalian daily urinary lignan excretion and plasma concentration have been shown to correlate with the intake of cereal fiber (9, 19, 22–25), although there are other important plant food sources as well. An inverse association of plasma lignan with plasma F_2-isoprostane reported in another study (23) signals possible antioxidant effects. Other antioxidant effects have also been reported with the consumption of whole grains in other human studies (48). The most likely interpretation for high plasma lignans is that they are a useful marker of a healthy diet, active colonic fermentation, and a healthy large bowel.

Fecal bile acid concentrations and amounts showed a higher primary bile acid component and a marginal trend to reduction in one of the secondary bile acids (deoxycholic). This finding may be interpreted as a beneficial effect to the host in terms of reduced risk of colon wall exposure to toxic secondary bile acids, and it is similar to findings of other reports on the effects on fecal bile acids of higher levels of rye food consumption (7, 17, 18, 49).

Fecal ammonia and *p*-cresol are potentially toxic components generated in the large bowel, and in this study both rye and wheat effectively lowered their concentrations relative to the low fiber control. This finding might be interpreted as indicating protection for the host (43, 44). Fecal β-glucuronidase activity was used to examine the degree to which gut microflorae are capable of deconjugating components that otherwise are in "bound" form in the feces, thereby unmasking or potentiating their toxic or mutagenic effects on the host (33, 35, 37, 39, 42, 49). Diets high in animal protein such as red meat have been shown to be associated with high activities of β-glucuronidase in feces, and in some studies that elevation was associated with increased colon tumorigenesis (35, 37, 39, 42). A reduction in the activity of that enzyme, as was seen with the high-fiber wheat diet in this study relative to the low-fiber diet, might therefore be interpreted as indicating reduced risk. It is likely that the reduced activity represents, at least to some extent, the degree to which the influence of the fecal microflora is diluted by the increased bulk effect, or it may signify an altered balance of microflora, with species beneficial to the host (50).

The reduced plasma insulin and glucose responses to rye and wheat foods at 1 h after meals are consistent with other reports of whole-grain food studies, especially rye-grain food studies (3, 16, 17, 51). For example, Leinonen et al (17) compared whole-kernel rye bread (50 g available carbohydrate) with white wheat bread in terms of postprandial glucose and insulin response in 20 healthy Finnish subjects, and they reported a 25% lower peak insulin response (P = 0.002) with the rye bread but no difference in the glucose response.

The 1-h measurement represents a limited observation, and more comprehensive testing should be undertaken before any firm conclusions are drawn. Nevertheless, it is promising that whole-grain rye and wheat foods are capable of diminishing the glycemic response in the short term. Insulin-resistant states are associated with high BMI and are part of a metabolic syndrome that has also been linked with altered risk for colon cancer (27–29). Under these circumstances, insulin and growth factors could be involved in altered bowel epithelium proliferation, thereby increasing cancer risk (29).

The cereal fiber–rich food options or whole-grain foods significantly increased total fiber intake to > 30 g/d, which has been proposed as a desirable goal for Australians in achieving health objectives (5). The fact that this change can be achieved with a substitution of whole-grain foods (< 90 g rye- or wheat-derived flour was provided as 230 g foods across all meals) for refined-grain foods in a normal diet indicates its practicability across the population. We conclude that the consumption of moderate amounts of both high-fiber rye and wheat foods, when matched for fiber, improved several markers of bowel and metabolic health relative to that of low-fiber foods. Fiber from rye appears more effective than that from wheat in the overall improvement of biomarkers of bowel health.

We acknowledge the following CSIRO Health Sciences and Nutrition staff members for their significant contributions: Roger King for executive management, Phil Leppard for statistical analysis, Jodie Avery for clinical trial coordination, and Corrina Flory, Debbie Davies, Leana Coleman, and Ben Scherer for technical input.

GM was the principal investigator, primarily involved in experimental design, interpretation of data, and writing of the manuscript. MN was the principal nutritionist, involved in experimental design and writing of the manuscript. PR was project manager, involved in all operational activities, collection and analysis of data, and writing of the manuscript. PF was consulting nutritionist and had a significant input into the dietary data collection and analysis. No author had any financial or personal interest in the company, George Weston Foods Ltd, that helped sponsor this research.

REFERENCES

1. Adlercreutz H, Carson M, Mousavi Y, et al. Lignans and isoflavonoids of dietary origin and hormone-dependent cancers. In: Waldron KW, Johnson IT, Fenwick GR, eds. Food and cancer prevention: chemical

and biological aspects. Cambridge, United Kingdom: Royal Society of Chemistry, 1993:348–52.
2. Hill MJ. Cereals, cereal fiber and colorectal cancer risk: a review of the epidemiological literature. Eur J Cancer Prev 1998;7:S5–10.
3. Slavin J, Jacobs D. Whole-grain consumption and chronic disease: protective mechanisms. Nutr Cancer 1997;27:14–21.
4. Jacobs DR, Pereira M, Slavin MJ, Marquart L. Defining the impact of wholegrain intake on chronic disease. Cereal Foods World 2000; 45:51–3.
5. McIntosh GH. Cereal foods, fibers and the prevention of cancers. Aust J Nutr Dietet 2001;58:S34–45.
6. Aman P, Nilsson M, Anderson R. Positive health effects of rye. Cereal Foods World 1997;42:684–8.
7. Grasten SM, Juntunen KS, Poutanen KS, Gylling HK, Miettinen TA, Mykkanen HM. Rye bread improves bowel function and decreases the concentrations of some compounds that are putative colon cancer risk markers in middle- aged women and men. J Nutr 2000;130:2215–21.
8. Bach Knudsen K, Johansen H, Glitso L. Rye dietary fiber and fermentation in the colon. Cereal Foods World 1997;42:690–4.
9. Glitso LV, Mazur WM, Adlercreutz H, et al. Intestinal metabolism of rye lignans in pigs. Br J Nutr 2000;84:429–37.
10. Cummings JH. Short chain fatty acids in the human colon. Gut 1981; 22:763–79.
11. Cummings J. The large intestine in nutrition and disease. Brussels, Belgium: Institut Danone, 1997.
12. McIntyre A, Gibson PR, Young GP. Butyrate production from dietary fiber and protection against large bowel cancer in a rat model. Gut 1993;34:386–91.
13. Csordas A. Butyrate, aspirin and colorectal cancer. Eur J Cancer Prev 1996;5:221–31.
14. Pietinen P, Rimm EB, Korhonen P, et al. Intake of dietary fiber and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. Circulation 1996;94:2720–7.
15. Vanharanta M, Voutilainen S, Lakka TA, van der Lee M, Adlercreutz H, Salonen JT. Risk of acute coronary events according to serum concentrations of enterolactone: a prospective population-based case-control study. Lancet 1999;354:2112–5.
16. Hagander B, Bjorck I, Asp NG, et al. Rye products in the diabetic diet. Postprandial glucose and hormonal responses in non-insulin-dependent diabetic patients as compared to starch availability in vitro and experiments in rats. Diabetes Res Clin Pract 1987;3:85–96.
17. Leinonen K, Liukkonen K, Poutanen K, Uusitupa M, Mykkanen H. Rye bread decreases postprandial insulin response but does not alter glucose response in healthy Finnish subjects. Eur J Clin Nutr 1999; 53:262–7.
18. Korpela JT, Korpela R, Adlercreutz H. Fecal bile acid metabolic pattern after administration of different types of bread. Gastroenterology 1992;103:1246–53.
19. Juntunen K, Witold M, Liukkonen K, et al. Rye bread decreases fecal concentrations of total and secondary bile acids. J Nutr 2000;130: 2215–21.
20. Juntunen K, Witold M, Liukkonen K, et al. Consumption of wholemeal rye bread increases serum concentrations and urinary excretion of enterolactone compared with wheat bread in healthy Finnish men and women. Br J Nutr 2000;84:839–46.
21. Kilkkinen A, Stumpf K, Pietinen P, Valsta LM, Tapanainen H, Adlercreutz H. Determinants of serum enterolactone concentration. Am J Clin Nutr 2001;73:1094–100.
22. Adlercreutz H. Evolution, nutrition, intestinal microflora, and prevention of cancer: a hypothesis. Proc Soc Exp Biol Med 1998;37:241–5.
23. Adlercreutz H. Cereal phytoestrogens and their association with human health. In: Liukkonen K, Kuokka A, Poutanen K, eds. Whole Grain and Human Health International Symposium. Espoo, Finland: VTT Biotechnology, 2001:23–7.
24. Adlercreutz H. Plasma enterolactone, a biomarker of fiber intake, gut bacterial activity and breast cancer risk. In: Adlercreutz H, ed. International Conference on Diet and Prevention of Cancer. Helsinki: Multiprint, 1999:S8.35–8.
25. Liukkonen K, Karppinen S, Nurmi T, et al. In vitro fermentation of dietary fiber and lignans of rye bran by human fecal bacteria. In: Liukkonen K, Kuokka A, Poutanen K, eds. Whole Grain and Human Health International Symposium. Espoo, Finland: VTT Biotechnology, 2001:89–90.
26. McKeown-Eyssen G, Epidemiology of colorectal cancer revisited; are serum triglycerides and/or plasma glucose associated with risk? Cancer Epidemiol Biomarkers Prev 1994;3:687–95.
27. Giovannucci E. Insulin and colon cancer. Cancer Causes Control 1995;6:164–79.
28. Kim YI. Diet, lifestyle, and colorectal cancer: is hyperinsulinemia the missing link? Nutr Rev 1998;56:275–9.
29. Manousos O, Souglakos J, Bosetti C, et al. IGF-I and IGF-II in relation to colorectal cancer. Int J Cancer 1999;83:15–7.
30. Prosky L, Asp NG, Furda I, DeVries JW, Schweizer TF, Harland BF. Determination of total dietary fiber in foods and food products: collaborative study. J Assoc Off Anal Chem 1985;68:677–9.
31. Adlercreutz H, Wang GJ, Lapcik O, et al. Time-resolved fluoroimmunoassay for plasma enterolactone. Anal Biochem 1998;265: 208–15.
32. Stumpf K, Uehara M, Nurmi T, Adlercreutz H. Changes in the time-resolved fluoroimmunoassay of plasma enterolactone. Anal Biochem 2000;284:153–7.
33. van Munster IP, Tangerman A, de Haan AF, Nagengast FM. A new method for the determination of the cytotoxicity of bile acids and aqueous phase of stool: the effect of calcium. Eur J Clin Invest 1993; 23:773–7.
34. de Kok TM, van Faassen A, Glinghammar B, et al. Bile acid concentrations, cytotoxicity, and pH of fecal water from patients with colorectal adenomas. Dig Dis Sci 1999;44:2218–25.
35. Glinghammar B, Venturi M, Rowland IR, Rafter JJ. Shift from a dairy product-rich to a dairy product-free diet: influence on cytotoxicity and genotoxicity of fecal water—potential risk factors for colon cancer. Am J Clin Nutr 1997;66:1277–82.
36. Topping DL, Illman RJ, Clarke JM, Trimble RP, Jackson KA, Marsono Y. Dietary fat and fiber alter large bowel and portal venous volatile fatty acids and plasma cholesterol but not biliary steroids in pigs. J Nutr 1993;123:133–43.
37. Reddy BS, Hanson D, Mangat S, et al. Effect of high-fat, high-beef diet and of mode of cooking of beef in the diet on fecal bacterial enzymes and fecal bile acids and neutral sterols. J Nutr 1980;110: 1880–7.
38. Jenab M, Thompson LU. The influence of flaxseed and lignans on colon carcinogenesis and beta-glucuronidase activity. Carcinogenesis 1996;17:1343–8.
39. Jenab M, Rickard SE, Orcheson LJ, Thompson LU. Flaxseed and lignans increase cecal beta-glucuronidase activity in rats. Nutr Cancer 1999;33:154–8.
40. Chaney A, Marvach E. Modified reagents for determination of urea and ammonia. Clin Chem 1962;8:130–2.
41. Bone E, Tamm A, Hill M. The production of urinary phenols by gut bacteria and their possible role in the causation of large bowel cancer. Am J Clin Nutr 1976;29:1448–54.
42. Salminen S, Bouley C, Boutron-Ruault MC, et al. Functional food science and gastrointestinal physiology and function. Br J Nutr 1998; 80:S147–71.
43. Birkett A, Muir J, Phillips J, Jones G, O'Dea K. Resistant starch lowers fecal concentrations of ammonia and phenols in humans. Am J Clin Nutr 1996;63:766–72.
44. Cummings J, Hill M, Bone E, et al. The effect of meat protein and dietary fiber on colonic function and metabolism. Am J Clin Nutr 1979;32:2094–101.
45. Medina V, Edmonds B, Young GP, James R, Appleton S, Zalewski PD.

Induction of caspase-3 protease activity and apoptosis by butyrate and trichostatin A (inhibitors of histone deacetylase): dependence on protein synthesis and synergy with a mitochondrial/cytochrome c- dependent pathway. Cancer Res 1997;57:3697–707.
46. Young GP, Chai F, Zalewski P. Polysaccharide fermentation, butyrate and apoptosis in the colon epithelium. Asia Pac J Clin Nutr 1999;8: S27–31.
47. Jacobs DR, Pereira MP, Stumpf K, Pins JJ, Adlercreutz H. Whole grain food intake elevates serum enterolactone. Br J Nutr 2002;88:111–6.
48. Bruce B, Spiller GA, Klevay LM, Gallagher SK. A diet high in whole and unrefined foods favorably alters lipids, antioxidant defenses, and colon function. J Am Coll Nutr 2000;19:61–7.
49. Korpela JT, Adlercreutz H, Turunen MJ. Fecal free and conjugated bile acids and neutral sterols in vegetarians, omnivores, and patients with colorectal cancer. Scand J Gastroenterol 1988;23:277–83.
50. Lidbeck A, Nord CE, Gustaffson J-A, Rafter JJ. Lactobacilli, anticarcinogenic activities and human intestinal microflora. Eur J Cancer Prev 1992;1:341–3.

The American Journal of Clinical Nutrition