

Resistant starch lowers fecal concentrations of ammonia and phenols in humans¹⁻³

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ABSTRACT We investigated the effect of resistant starch (RS) on markers of colonic protein metabolism. Eleven subjects participated in a randomized crossover study in which they consumed either high-RS (39 ± 3 g/d, $\bar{x} \pm$ SEM) or low-RS (5 ± 0.4 g/d) diets for 3 wk. All other macronutrients were kept constant. During the high-RS diet daily excretion of fecal nitrogen increased from 1.84 ± 0.15 to 2.86 ± 0.42 g/d ($P < 0.01$) and excretion of fecal phenols fell from 9.2 ± 1.4 to 5.3 ± 0.8 mg/d ($P < 0.01$). Fecal concentrations of ammonia decreased from 397 ± 33 to 278 ± 49 μ g/g ($P < 0.01$) and phenols decreased from 69 ± 8 to 39 ± 10 μ g/g ($P < 0.001$). Daily output of urinary ammonia, urea, phenols, and total nitrogen did not change significantly, but pH decreased from 6.4 ± 0.1 to 6.2 ± 0.1 ($P < 0.05$) during the high-RS period. These results suggest that RS significantly attenuates the accumulation of potentially harmful byproducts of protein fermentation in the human colon. *Am J Clin Nutr* 1996; 63:766-72.

KEY WORDS Resistant starch, protein, colon, fermentation, nitrogen, ammonia, phenols, feces, urine

INTRODUCTION

Diet plays an important role in the promotion of or protection against colon cancer and other bowel diseases. High intakes of dietary fat and/or meat are associated with increased risk (1, 2), whereas high intakes of dietary fiber appear protective (3-5). Starch is one major component of the diet that, until now, has received little research attention in relation to colon cancer. An international survey, however, recently reported a strong inverse relation ($r = -0.76$, $P < 0.001$) between consumption of dietary starch (adjusted for fat and protein intakes) and the incidence of colon cancer in 12 countries worldwide (6). In contrast, in this survey colon cancer incidence was not found to be significantly related to intake of nonstarch polysaccharides (NSPs), the major form of dietary fiber (6). These results suggest that starch intake has a protective role in colon cancer etiology.

Mechanisms for the protective effect of a high-starch diet remain to be elucidated. It seems likely that high starch consumption results in more starch reaching the colon undigested. Evidence for this hypothesis has come from studies involving subjects with ileostomies in which a linear relation was found between the amount of starch consumed and the amount escaping digestion in the small intestine (7).

The presence of this undigested or resistant starch (RS) may affect the colonic environment through its effects on fecal

bulking and bacterial fermentation. We reported recently the results of a study in which two diets, which differed greatly in amounts of RS, were fed to 11 human volunteers for 3 wk (8). The high-RS diet increased fecal output, lowered fecal pH, and increased the fecal excretion of the short-chain fatty acid (SCFA) butyrate (8).

There is evidence that butyrate may reduce the risk of malignant change in cells (9, 10), whereas population studies have shown that increases in fecal bulking (11) and lower fecal pH (12, 13) are associated with a decreased incidence of colon cancer. These results suggested that RS may have a marked effect on putative markers of colonic health comparable with those of some forms of dietary fiber.

Dietary protein may also reach the colon undigested. It has been estimated that with a typical Western diet, up to 12 g protein/d can escape digestion (14, 15). Undigested protein that reaches the colon is fermented by microflora to end products that include phenol, cresol, indoles, amines, and ammonia (16). Many of these have adverse effects. Ammonia may promote tumorigenesis by stimulating cell proliferation (17), which favors the growth of malignant cells (18). Phenols (*p*-cresol and phenol), byproducts from the metabolism of aromatic amino acids (phenylalanine and tyrosine), are known to promote skin cancer (19) and also have been implicated in bladder and bowel cancers (20-22). Individuals with ulcerative colitis were shown to have a reduced ability to remove phenols from the luminal environment through conjugation with sulfate (23). High concentrations of phenols may also exacerbate this disease (23). Urinary concentrations of both phenols and fecal ammonia were elevated in individuals consuming a diet high in meat protein (24).

Accumulation of the harmful byproducts of protein metabolism may be attenuated by the fermentation of undigested

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carbohydrate. Studies carried out in vitro (25) and in both animals (26) and humans (27) showed that the presence of fermentable carbohydrate lowers fecal ammonia concentrations. A high-fiber diet was also shown to lower urinary phenol and cresol concentrations in humans (24). It has been suggested that the presence of undigested carbohydrate stimulates rapid growth of colonic bacteria, which can then act as "nitrogen sinks" to use up existing protein and protein metabolites (eg, ammonia) for their metabolism and growth (24, 28). As a result, the luminal concentrations of these metabolites fall whereas concentrations of fecal nitrogen, indicative of the increased bacterial mass, increase (24, 28).

The interaction between undigested starch and protein in the colon has not been examined systematically in humans. Schepach et al (29) reported an increase in fecal nitrogen excretion in human volunteers after administration of an α -glucosidase inhibitor (acarbose) to deliver products of starch digestion to the colon artificially. The increase in nitrogen excretion was shown to be due to increased bacterial mass (29). This study did not examine changes in the products of protein metabolism. Animal studies, however, have reported reductions in concentrations of cecal ammonia after dietary supplementation with RS (30, 31).

The aim of this study was to examine the possibility that starch not digested in the small intestine has a significant effect on protein metabolism in the colon. This work is an extension of a previous study that described the effects of RS on fecal bulking and other fermentation-dependent events (8).

SUBJECTS AND METHODS

Subjects and study design

This investigation formed part of a larger study on the effect of RS on fecal bulk and fermentation-dependent events, which was described in detail elsewhere (8). Briefly, 11 healthy volunteers (5 males, 6 females) aged 35.5 ± 10.5 y participated in a study conducted over an 8-wk period: an initial 2 wk to quantify the baseline diet and 3 wk consuming each test diet. Subjects were randomly assigned to either the high- or low-RS diet and after 3 wk crossed over to the alternate diet. Approval for the study protocol was obtained from the Deakin University Human Ethics Committee.

Each diet had the same macronutrient composition (15% protein, 52% carbohydrate, 30% fat, and 3% alcohol) and NSP content, both soluble and insoluble (Table 1). Thus, the only dietary component that varied was the amount of RS (Table 1). RS was measured by using a slight modification (32) of an existing in vitro assay developed in this laboratory (33). The modification involved using an enzyme kit (Megazyme; Megazyme Australia Pty Ltd, North Rocks, Australia) for total starch determination. To monitor dietary intake and compliance, each subject was required to keep complete weighed-food records of all food and drink consumed for the entire study period (ie, for 8 wk). Dietary records were analyzed by using the SODA 5 database (Computer Models, Cottesloe, Australia). Because this program does not include values for soluble and insoluble NSP, this information was obtained from published food tables (34, 35) or by direct measurement as described previously (8).

TABLE 1

Macronutrient composition of the high- and low-resistant starch (RS) diets¹

Macronutrients ²	Low-RS diet	High-RS diet
Energy (kJ)	8197 \pm 549	8215 \pm 735
Protein (g)	77 \pm 4.8	82 \pm 7.5
Fat (g)	70 \pm 7.2	68 \pm 8.1
Sugars (g)	95 \pm 8.1	99 \pm 6.6
Starch (g)		
Total ³	168 \pm 13	159 \pm 14
Digestible ⁴	163 \pm 13	120 \pm 13
Resistant	5.3 \pm 0.4	38.6 \pm 3 ⁵
Nonstarch polysaccharide (g)		
Total	20.1 \pm 1	20.3 \pm 1.4
Soluble	5.3 \pm 0.3	5.5 \pm 0.3
Insoluble	14.8 \pm 1	14.8 \pm 0.6

¹ $\bar{x} \pm$ SEM; $n = 11$.

² Calculated by using SODA 5 database (Computer Models, Cottesloe, Australia).

³ Digestible starch + RS.

⁴ Total starch - RS.

⁵ Significantly different from the low-RS diet. $P < 0.01$ (Student's t test).

Dietary supplements containing RS

The RS-containing food sources were unprocessed wheat seeds or steamed and rolled wheat flakes (The Old Grain Mill, Nhill, Australia), low- (0%) and high- (85%) amylose maize kernels (Hi-maize; Starch Australasia Ltd, Lane Cove, Australia), and cooked or uncooked green banana flour (*Musca paradisiaca sapientum*). Green bananas were peeled and the banana flesh was then freeze-dried and ground. These foods were analyzed for total starch, RS, and soluble and insoluble NSP.

The high-RS diet included 158 ± 11 g ($\bar{x} \pm$ SEM, as eaten) cornbread made from high-amylose maize, 47 ± 4 g unprocessed wheat seeds coarsely milled to pass through a 3-mm sieve, and 25 ± 1.6 g raw green banana flour (particle size < 1 mm). Maize kernels used to prepare the cornbread were ground to < 1 mm diameter before use. These dietary supplements contributed 17.5 ± 1.2 , 8.7 ± 0.8 , and 12.4 ± 0.8 g RS/d for cornbread, wheat seed, and green banana flour, respectively. The amount of RS given to each subject during the high-RS diet was based on their usual energy intake, and was calculated as 4.76 mg RS/kJ. RS intake in the high-RS diet thus ranged from 26 to 50 g/d.

The low-RS diet contained 162 ± 10 g low-amylose cornbread; 44 ± 6 g steamed, flaked wheat seed (as processed by the manufacturers) milled to pass through a 3-mm sieve; and 27 ± 2.5 g cooked green banana flour (particle size < 1 mm). Maize kernels were ground to < 1 mm before incorporation into cornbread. Details of how the wheat and banana flours were cooked were given before (8, 32, 36). These low-RS dietary supplements contributed 0.8 ± 0.7 , 1.4 ± 0.2 , and 3.1 ± 0.2 g RS/d for the cornbread, wheat seed, and green banana flour, respectively. During the low-RS diet RS intakes ranged from 3 to 8 g/d.

Fecal and urine collections

In the third week of each dietary period, 24-h fecal collections were obtained and weighed over 3 consecutive days.

Fecal samples for analysis were placed on dry ice and immediately brought into the laboratory. Fecal samples were thawed quickly in a warm water bath (30 °C) and homogenized, and aliquots were frozen at either -20 or -70 °C. A 24-h urine collection was also made during the fecal collection period. The urine was kept refrigerated during collection, then weighed and frozen in aliquots at -20 or -70 °C.

Analysis of urine and feces for protein metabolites

Before measurement of urinary pH, samples were quickly thawed in a warm water bath (30 °C). Urine was mixed thoroughly and the pH was measured by using a protein-resistant glass electrode (Activon, Sydney, Australia) connected to a pH meter (Orion SA720; Linbro International, Victoria, Australia).

Urine was diluted with water and the concentration of ammonia was measured spectrophotometrically (Spectronic 20D; Milton Roy Company, Rochester, NY) (37). Ammonia concentration in feces was measured similarly after feces were diluted and centrifuged (17), filtered through number 44 filter paper (Whatman, Maidstone, United Kingdom), and diluted with water.

Urinary creatinine was measured spectrophotometrically as described by Brod and Sirota (38) by using 3 mL urine diluted 1:100. For measurement of urea, urine was diluted and incubated with urease (Sigma U1500; Sigma Chemical, St Louis) (38), and released ammonia was measured as described above (37). Total urine nitrogen content and concentrations of nitrogen in ground, freeze-dried feces (Hetovac; Heto Lab Equipment, Birkerød, Denmark) were determined by using a semi-automatic Kjeldahl apparatus (Gerhardt, Bonn, Germany) (37).

Phenol and *p*-cresol are the main phenols found in urine and feces (39). Total phenols were therefore measured as the combination of phenol and *p*-cresol, by HPLC. Before injection, urine samples were prepared according to methods described by Yoshikawa et al (40) with the following modifications: *p*-chlorophenol (BDH, Kilsyth, Australia) was added as an internal standard, diethyl ether (BDH) was used for extraction, and samples were filtered through a 0.45- μ m filter (HATF 01300; Millipore, Bedford, MA). Samples (50 μ L) were injected onto a 5- μ m C18 reversed-phase column, 250 \times 4.6 mm (Econosil; Alltech, Deerfield, IL) by using 48% methanol (HPLC grade; FSE, Homebush, Australia) and 52% phosphate buffer (0.02 mol/L, pH 4.0) (41) as a mobile phase and a flow rate of 0.7 mL/min. Detection was done at 270 nm by using a Chrom-A-Scope (Barspec, Rehovot, Israel) rapid-scanning ultraviolet detector with computerized integration software.

Feces were diluted with phosphate buffer (0.1 mol/L, pH 5.5) and the internal standard (*p*-chlorophenol) was added. Samples were then vortexed before centrifugation at 500 \times g for 10 min at room temperature, and the supernate was prepared as described above. A detailed description of the measurement of phenols in feces is given elsewhere (42); however, briefly, the supernate was mixed with hydrochloric acid and boiled for 60 min. This solution was cooled and diethyl ether added to extract the phenols, and the mixture was centrifuged again at 500 \times g for 10 min at room temperature. The organic phase was aspirated into 0.05 mol NaOH/L, and the resultant solution evaporated to dryness under a stream of nitrogen. The residue was dissolved in water, filtered through a 0.45- μ m syringe filter, and injected into the HPLC apparatus.

Statistical methods

Results are presented as mean \pm SE. Statistical analysis was performed by using the MINITAB software package (release 8; Minitab Inc, State College, PA) for paired-difference Student's *t* tests and correlations were obtained by Pearson's test. A 95% confidence limit was used to determine significant differences.

RESULTS

In this study all major macronutrient intakes were kept constant for the two diets, including fat, protein, total starch, and soluble and insoluble NSP. In addition, there were no significant differences (paired-difference Student's *t* test) between the two dietary periods in the dietary sources of the protein (ie, total, red and white meat, vegetable protein, cereal protein, and dairy protein, data not shown). The only dietary component that varied between the two diets was the amount of RS.

The high-RS diet caused a significant decrease in protein metabolites with reductions in concentrations of fecal ammonia ($P < 0.01$), phenol ($P < 0.05$), *p*-cresol ($P < 0.001$), and total phenols ($P < 0.001$) (all paired-difference Student's *t* test) (Table 2). In addition, total excretion over 24 h of *p*-cresol and total phenols decreased significantly during the high-RS diet whereas nitrogen output increased significantly ($P < 0.01$). As described previously (8), fecal output increased significantly from 138 \pm 22 g/d (wet wt) during the low-RS diet to 197 \pm 37 g/d during the high-RS diet ($P < 0.01$). Details of changes in other fecal variables (eg, pH, SCFAs, starch, and NSP) were given elsewhere (8). Our previous study found a fivefold increase in the excretion of fecal starch by individuals after the high-RS diet (8), suggesting that subjects were complying with the diets.

The daily urinary excretion of nitrogen, urea, and ammonia were all significantly greater with the high-RS diet ($P < 0.05$; Table 3). However, as shown in Table 3, the output of creatinine also increased ($P < 0.05$). Because creatinine is neither

TABLE 2
Effects of two diets differing in resistant starch (RS) content on excretion of ammonia, phenols, and nitrogen in feces¹

Fecal variables	Low-RS diet	High-RS diet
Ammonia		
(μ g/g feces)	397 \pm 33	278 \pm 49 ²
(mg/d)	55 \pm 10	45 \pm 8
Phenol		
(μ g/g feces)	4.0 \pm 0.6	3.0 \pm 0.5 ³
(mg/d)	0.5 \pm 0.1	0.5 \pm 0.1
<i>p</i> -Cresol		
(μ g/g feces)	65.0 \pm 8	36.0 \pm 10 ⁴
(mg/d)	8.7 \pm 1.4	4.7 \pm 0.8 ²
Total phenols		
(μ g/g feces)	69.0 \pm 8	39.0 \pm 10 ⁴
(mg/d)	9.2 \pm 1.4	5.3 \pm 0.8 ²
Nitrogen		
(μ g/g feces)	15.1 \pm 1.7	16.3 \pm 1.8
(mg/d)	1800 \pm 200	2900 \pm 400 ²

¹ $\bar{x} \pm$ SEM; *n* = 11.

²⁻⁴ Significantly different from the low-RS diet (paired-difference Student's *t* test): ² $P < 0.01$, ³ $P < 0.05$, ⁴ $P < 0.001$.

TABLE 3
Effects of two diets differing in resistant starch (RS) content on urine variables¹

Urine variable	Low-RS diet	High-RS diet
pH	6.44 ± 0.11	6.16 ± 0.11 ²
Urea		
(g/d)	33.0 ± 2.40	39.2 ± 3.80 ³
(g/g creatinine)	24.2 ± 1.60	25.3 ± 2.20
Phenol		
(g/d)	0.005 ± 0.00	0.005 ± 0.00
(g/g creatinine)	0.004 ± 0.00	0.004 ± 0.00
<i>p</i> -Cresol		
(g/d)	0.049 ± 0.01	0.058 ± 0.00
(g/g creatinine)	0.038 ± 0.01	0.042 ± 0.01
Total phenols		
(g/d)	0.054 ± 0.00	0.064 ± 0.00
(g/g creatinine)	0.041 ± 0.01	0.046 ± 0.01
Nitrogen		
(g/d)	12.5 ± 0.90	14.7 ± 1.60 ²
(g/g creatinine)	9.1 ± 0.40	9.3 ± 0.50
Ammonia		
(g/d)	0.90 ± 0.08	1.09 ± 0.13 ²
(g/g creatinine)	0.66 ± 0.06	0.71 ± 0.08
Creatinine (g/d)	1.40 ± 0.11	1.63 ± 0.21 ²

¹ $\bar{x} \pm \text{SEM}$; $n = 11$.

^{2,3} Significantly different from the low-RS diet (paired-difference Student's *t* test): ² $P < 0.05$, ³ $P < 0.01$.

secreted nor reabsorbed by the kidneys, its excretion is relatively constant from day to day. Creatinine output is therefore used routinely to correct for the completeness of urine collections in metabolic studies. When the results were expressed in terms of creatinine, urinary variables were found not to change significantly with intake of RS, except for urinary pH, which decreased significantly during the high-RS diet ($P < 0.05$). No significant correlation could be found between the daily output of fecal and urinary phenols (data not shown).

Correlations were found between several fecal and dietary variables (Table 4). Data are also given for fecal pH, starch, NSP, and butyrate from our previously published study of RS (8). There was a significant correlation between intake of dietary RS and starch content in feces ($r = 0.549$, $P < 0.01$, Pearson's correlation, data not shown). As shown in Table 4,

dietary RS also correlated positively with total daily fecal nitrogen output and negatively with fecal ammonia and total phenol concentrations ($P < 0.01$, nitrogen; $P < 0.05$, phenol and ammonia; Pearson's correlation).

In addition, correlations were found between output of starch in feces and indexes reflecting bacterial metabolic activity in the colon (Table 4). Fecal starch excretion was thus found to correlate positively with fecal nitrogen and negatively with fecal ammonia, phenols, combined *iso*-butyrate and *iso*-valerate content, and pH ($P < 0.05$, *iso*-butyrate and *iso*-valerate; and $P < 0.01$, other variables, Pearson's correlation). The relation between fecal starch and fecal phenol concentrations ($r = -0.50$) and that between fecal starch and fecal ammonia ($r = -0.76$) is presented in Figure 1. Other correlations between markers of protein and carbohydrate metabolism were also noted (Table 4). In particular, there was a positive correlation between fecal butyrate and fecal nitrogen ($P < 0.01$) and a negative correlation between fecal nitrogen and fecal ammonia and phenols ($P < 0.05$ and $P < 0.01$, respectively).

The ratio of fecal starch to fecal nitrogen excreted was markedly higher during the high- than during the low-RS diet (3.0 ± 0.8 compared with 0.9 ± 0.4 , $P < 0.05$; Figure 2). In addition, the ratio of fecal starch plus NSP to fecal nitrogen was 7.4 ± 0.8 with the high-RS diet and 5.1 ± 0.4 with the low-RS diet, ($P < 0.05$, data not shown).

DISCUSSION

This study showed that RS has a significant effect on the fermentation of protein in the human colon. Most notable was the effect of the high-RS diet on the excretion of the potentially harmful byproducts of protein metabolism, ammonia, and phenols. The high-RS diet resulted in a reduction in both the concentration and the total 24-h excretion of *p*-cresol. To our knowledge, this is the first diet-induced decrease in fecal phenol concentrations reported. The decreased excretion of fecal phenols over 24 h suggests that the high-RS diet influenced their metabolism.

This study did not detect a significant decrease in urinary phenols during the high-RS diet. This contrasts with previous studies that found a relation between diet and urinary phenol output (24, 43–45). For example, Cummings et al (24) showed

TABLE 4
Correlations between dietary resistant starch (RS) intake and fecal variables¹

Fecal variable	RS intake (g/d)	Fecal variable				
		Nitrogen (g/d)	Ammonia ($\mu\text{g/g}$)	Phenols ($\mu\text{g/g}$)	Starch (g/d)	Starch + NSP (g/d)
Nitrogen (g/d)	0.61 ²				0.61 ²	0.81 ²
Ammonia ($\mu\text{g/g}$)	-0.48 ¹	-0.36			-0.70 ²	-0.61 ²
Total phenols ($\mu\text{g/g}$)	-0.49 ¹	-0.59 ²	0.76 ²		-0.72 ²	-0.70 ²
pH ⁴	-0.65 ²	-0.59 ²	0.57 ²	0.58 ²	-0.82 ²	-0.84 ²
Butyrate (mmol/L) ⁴	0.45 ¹	0.56 ²	-0.49 ¹	-0.56 ²	0.80 ²	0.78 ²
<i>iso</i> -Butyrate + <i>iso</i> -valerate (mmol/L) ⁴	-0.09	-0.26	0.49 ¹	0.35	-0.45 ¹	-0.38 ¹
Wet output (g/d) ⁴	0.46 ¹	0.76 ²	-0.48 ¹	-0.61 ²	0.81 ²	0.86 ²

¹ $n = 22$ (11 subjects \times 2 diets).

² $P < 0.01$.

³ $P < 0.05$.

⁴ Data published previously (8).

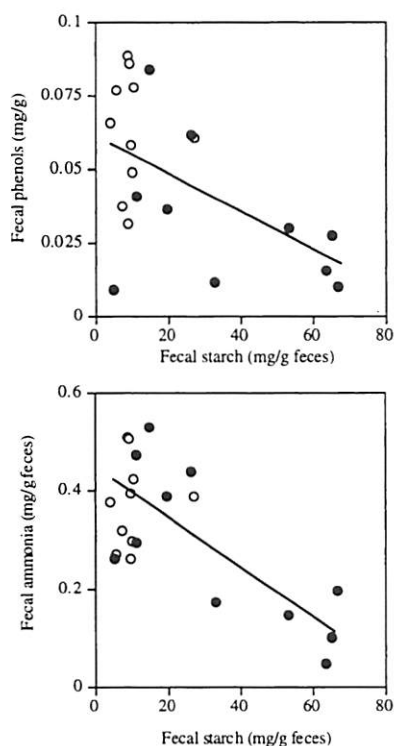


FIGURE 1. Relation between the daily excretion of starch in feces and concentrations of phenols ($r = -0.50$, $n = 22$) and ammonia ($r = -0.76$, $n = 22$). Low-resistant-starch (RS) diet (○), high-RS diet (●).

that the addition of dietary fiber to a high-protein diet reduced the excretion of urinary phenols. The reason for this discrepancy is not known; however, note that some studies have measured changes in urinary phenols spectrophotometrically (24). In contrast, we measured fecal concentrations of phenol and *p*-cresol directly by HPLC. In addition, many studies that reported changes in urinary phenol concentrations did not adequately control for both the amount and source (ie, animal or vegetable) of protein consumed during the experimental diets (24, 43–45). In the present investigation, both the amount and source of dietary protein during both study periods were kept constant.

We found no correlation between fecal and urinary phenol concentrations, which suggests that measuring changes in fecal phenols, rather than in urinary phenols, more closely reflects

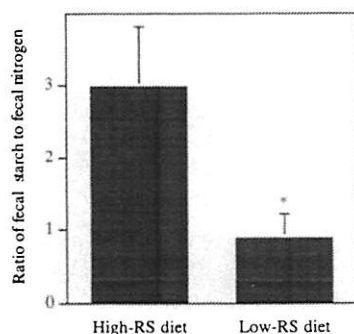


FIGURE 2. The ratio of undigested fecal starch to fecal nitrogen during the high-resistant-starch (RS) and low-RS diets. * Significantly lower than during the high-RS diet ($P < 0.05$, paired-difference *t* test, $n = 11$).

changes in the luminal environment. A significant drop in urinary pH was measured during the high-RS diet. Decreases in urinary pH were reported when wheat fiber was added to a high-protein diet (24).

We also observed a decrease in the fecal ammonia concentration during the high-RS diet. This is consistent with other studies that reported a decrease in the daily excretion of ammonia in response to fermentable NSP (25–27). It is unlikely that the reduction in ammonia observed here is due to enhanced absorption because the low fecal pH produced during the high-RS diet (8) would have trapped ammonia in the lumen in its ionized form (18). Nevertheless, the reduction in ammonia concentration could be explained by a dilution effect due to the increased stool bulk during the high-RS diet (8). However, given the results of other investigators (25–27), it is also likely that the additional carbohydrate provided here as RS stimulated the use of the ammonia for bacterial protein synthesis.

The decrease in the concentration of fecal phenols and ammonia may have important implications for the tissue health of the colonic epithelium. Phenols have been shown to be tumor promoters and have been implicated in the development of both bladder and bowel cancers (19–22). More recently, the possible involvement of phenols in inflammatory bowel disease was suggested (23). Ammonia has a range of toxic effects that suggests that it may damage the colonic epithelium. Ammonia enhances cell proliferation (17) and appears to favor growth of malignant cells in preference to normal cells (18). Ammonia is also cytotoxic to mammalian cells (18).

In contrast with ammonia and phenols, the excretion of nitrogen increased during the high-RS diet. This increase in fecal nitrogen may be due to an increase in bacterial growth, in the production of endogenous protein (eg, digestive enzymes and sloughed cells), or in malabsorption of dietary protein during the high-RS diet (15, 29, 46–49). Similar effects on the excretion of fecal nitrogen were shown in studies with fermentable NSP (47), and were attributed to increases in bacterial mass. Scheppach et al (29), using the α -glucosidase inhibitor acarbose to induce the malabsorption of starch degradation products, found that there was an increase in nitrogen excretion during acarbose treatment. They attributed this increase in fecal nitrogen to an increase in bacterial mass as measured by the increase in bacterial 2,6-diaminopimelic acid (29).

The effect of the high-RS diet on fecal excretion of nitrogen, ammonia, and phenols is consistent with the hypothesis that replicating bacteria use readily fermented carbohydrate as an energy substrate (24, 28). Our previous work showed that $\approx 80\%$ of RS fed was fermented in the colon (ie, was not recovered in the feces) (8). Because nitrogen sources are required for increased bacterial growth, the bacteria act as nitrogen sinks and reduce the fecal concentrations of ammonia and phenols while increasing fecal excretion of nitrogen. In the present study significant correlations were found between fecal carbohydrate and excretion of fecal ammonia, phenols, and nitrogen, which is consistent with there being a relation between carbohydrate fermentation and protein metabolism in the colon.

As discussed in our previous paper (8), measurement of fermentation-dependent changes in the feces may reflect events happening in the distal colon. In humans, the distal colon is the site of the most colon tumor formation (50). It

is possible that the ratio of fermentable carbohydrate (ie, RS and NSP) to protein reaching the colon undigested may influence the nature of the byproducts produced by fermentation. Thus, in this study the presence of excess undigested starch was shown to attenuate the accumulation of the potentially toxic byproducts of protein metabolism, whereas concentrations of SCFAs (including butyrate) were elevated and pH was lowered (8). It follows that the balance of undigested fermentable carbohydrate (starch and NSP) and protein (from both endogenous and exogenous sources) in the distal colon may have important implications for colon cancer risk. Western diets may need to be redesigned to allow sufficient amounts of fermentable carbohydrate to survive complete fermentation to reach the distal colon.

In conclusion, this study showed that RS had a significant effect on the accumulation of potentially harmful byproducts of protein metabolism (ammonia and phenols) in the human colon. We showed previously that RS has beneficial effects on putative markers of colonic health, including increased fecal bulk, lowered fecal pH, and increased concentrations of the SCFAs acetate and butyrate (8). Amounts of starch and protein reaching the colon undigested may have important implications for the prevention and promotion of bowel diseases, including colon cancer. Future work in this area should examine factors that can influence the proportions of dietary starch and protein reaching the colon undigested. □

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