ABSTRACT  Recent evidence suggests that resistant starch (RS) is the single most important substrate for bacterial carbohydrate fermentation in the human colon. During two 4-wk periods, 12 healthy volunteers consumed a controlled basal diet enriched with either amylomaize starch (55.2 ± 3.5 g RS/d; high-RS diet) or available cornstarch (7.7 ± 0.3 g RS/d; low-RS diet). Approximately 90% of the RS consumed disappeared during intestinal passage; increased fermentation was verified by elevated breath-hydrogen excretion. During the high-RS diet, fecal wet and dry weight increased 49% and 56%, respectively (P ≤ 0.005), whereas stool water content did not change significantly. Fecal concentrations and daily excretion of short-chain fatty acids were not different in the two study periods. During the high-RS diet, bacterial β-glucosidase activity decreased by 26% (P ≤ 0.05). Fecal concentrations of total and secondary bile acids were significantly lower during the high-RS than during the low-RS period [a decrease of 30% (P ≤ 0.05) and 32% (P ≤ 0.01), respectively, in total and secondary bile acids] whereas concentrations of primary bile acids were unaffected by RS consumption. During the high-RS diet, fecal concentrations of total neutral sterols decreased by 30% (P ≤ 0.005) and fecal concentrations of 4-cholesten-3-one decreased by 36% (P ≤ 0.05). These data suggest that RS has potentially important effects on bacterial metabolism in the human colon that may be relevant for cancer prevention. Am J Clin Nutr 1998;67:136–42.

KEY WORDS  Resistant starch, colon, fermentation, breath hydrogen, short-chain fatty acids, lipid peroxidation, fecal bacterial enzymes, neutral sterols, bile acids, humans

INTRODUCTION

It is well accepted that environmental (dietary) factors play a major part in colonic carcinogenesis. Epidemiologic studies have shown a significant positive association between fat and meat and the risk of colon cancer (1) and a reduction in risk in individuals and populations consuming high amounts of dietary fiber and vegetables (2, 3). Recently, a strong inverse association between starch consumption and incidence of large bowel cancer was shown; however, there was no significant relation with nonstarch polysaccharides (NSP) alone (4). The amount of NSP in human diets may be exceeded by the amount of indigestible starch. A substantial amount of starch escapes digestion in the small intestine, depending on physical inaccessibility, type of granule, and food processing (5). This fraction is called resistant starch (RS). Starch and dietary fiber together are the principal substrates controlling the pattern of fermentation in the colon and, thus, the metabolism of compounds like bile acids, nitrate, and enzyme activities (bacterial and antioxidant enzymes), which have been implicated in carcinogenesis. Little work has been done on the effect of starch on bowel habits, although in vitro and in vivo studies suggest that the behavior of starch is different from that of NSPs (6, 7).

The purpose of this study was to examine the effects of RS on fermentative activity and related aspects of colonic metabolism. Because of its long-term palatability, RS was integrated into common starchy products (bread, pasta, cookies, and cake).

SUBJECTS AND METHODS

Subjects and study design

Twelve healthy volunteers (five women and seven men) aged 25.2 ± 0.5 y (range: 23–28 y) and with a mean body mass index (in kg/m²) of 23.2 ± 0.9 (range: 18.0–28.9) were recruited for this clinical trial. Volunteers did not take any medication (including antibiotics or laxatives) 4 wk before or during the study. Written, informed consent was obtained from all participants and the study was approved by the Ethics Committee of the Faculty of Medicine, University of Würzburg, Germany.

During two 4-wk periods (with a washout phase of 6 wk between periods), volunteers consumed a controlled diet (47% of energy as carbohydrate, 38% as fat, and 15% as protein). Starchy foods (bread, pasta, cake, and biscuits) were enriched with a highly resistant amylomaize starch (Hylon VII; National Starch & Chemical Co, Bridgewater, NJ) during the high-RS (HRS; first period) and with an available cornstarch (Maizena; Knorr Caterplan GmbH, Heilbronn, Germany) during the low-RS (LRS; second period) period (Table 1). Breads and cakes were kept in a
cold-storage chamber or pantry for ≤2 d. Pasta and biscuits were produced twice during each study period and stored in a pantry. Pasta was boiled for 10 min shortly before consumption. The macronutrient composition of the study diet was calculated with the software package PRODI 4.2 (Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany). Values for RS were obtained by direct measurements as described below. The oral intake of dietary fiber was restricted by limiting consumption of fruit to one per day and vegetable intake to 100 g/d. Alcohol consumption was restricted to 500 mL beer or 250 mL wine/wk.

All volunteers collected their food daily from the metabolic kitchen; regular contact with the nutritionist (SH) in the study was important to ensure compliance. Volunteers ate meals at 0800, 1000, 1300, 1600, and 1900. They kept a record of abdominal complaints during the two study periods. Diarrhea, flatulence, abdominal distention, and cramps were scored on semi-quantitative scales from 1 (none) to 5 (severe).

Mean transit time (MTT) was measured from days 21 to 24 of each study period with radiopaque markers (8). From days 24 to 28 of each study period, stool was collected for quantitative assessment. Each stool was collected separately and frozen within 5 h (range: 10 min to 12 h) in the laboratory freezer. After the conclusion of each study period, 5-d stool collections were pooled without additives by using a blender and were frozen at −30°C for determination of starch, weight, pH, total fat, short-chain fatty acids (SCFAs), malondialdehyde, activity of bacterial enzymes, neutral sterols, and bile acids. Breath samples were obtained weekly during the two study periods for determination of breath-hydrogen content.

### Resistant starch in food and feces

RS was measured according to the method of Englyst et al (5), with some modifications. For analyses of food, an aliquot (50 mg) was incubated for 24 h with 2 mL acetate buffer (0.1 mol/L, pH 7) and pancreatin solution (29 U α-amylase/mg, pH 6.9, and 166.67 g enzyme/L acetate buffer; Sigma Chemical Co, St. Louis) at 37°C. After ethanol was added and after centrifugation (2200 × g for 10 min at 4°C), the pellet was washed twice with 80% ethanol and dried overnight. The dry sample used for starch determination was diluted in distilled water and 2 mol NaOH/L (1:1, by vol); after 12 h it was neutralized with 1 mol HCI/L and boiled for 30 min with heat-stable α-amylase (Sigma Chemical Co) for starch breakdown. The reaction was stopped with 0.1 mol acetic acid/L and, after centrifugation at 3000 × g for 10 min at 4°C, the supernate was used for starch determination with a glucose oxidase colorimetric kit (Boehringer Mannheim, Mannheim, Germany).

The RS in feces was measured as described above after the following preparation. A 1:10 dilution of fresh feces in distilled water was prepared and homogenized by using a stomacher (AJ Seward, London). After mucus and fat were removed by cooking with propanol and ethanol, the sample was dried overnight.

### Stool weight, pH, mean transit time, and stool fat

Stool wet weight was recorded and fecal dry weight was measured after lyophilization (Gamma IA apparatus; Christ, Osterode, Germany) of an aliquot to a constant weight. Fecal pH was measured with a microprocessor pH meter (WTW, Weilheim, Germany) in triplicate in every fecal sample before homogenization. MTT was analyzed by using the single-stool method of Cummings and Wiggins (8). Volunteers were given 20 radiopaque markers of different shapes on 3 consecutive days with breakfast. The first stool passed on day 4 was collected, the number and type of markers were assessed by X-ray evaluation, and MTT was calculated. Stool fat was analyzed according to the method of Van de Kamer et al (9).

### Fecal short-chain fatty acids

SCFAs were analyzed in thawed homogenized fecal samples by gas-liquid chromatography (HP 5890A; Hewlett-Packard, Avondale, PA) after vacuum transfer, as described previously (10). A minor modification to this method was the use of a temperature program (110–130°C). Fecal samples were prepared for vacuum transfer in < 1 h.

### Malondialdehyde

Malondialdehyde, a volatile carbonyl resulting from oxidative break down of polyunsaturated fatty acids, was measured to estimate the extent of lipid peroxidation in the colon. Malondialdehyde was analyzed in dry fecal samples in duplicate with an HPLC device equipped with a fluorescence detector. Briefly, aliquots of dry feces were diluted (1:30) with phosphate buffer (0.1 mol/L, pH 7.4). After centrifugation at 200 × g for 5 min at 4°C, 1 mL stool solution was mixed with butylated hydroxytoluene (final concentration, 10 µmol/L; Sigma Chemical Co), 0.7 mL 20% trichloroacetic acid (Merck, Rahway, NJ), 1 mL 100 mmol thiobarbituric acid/L (Merck), and 10 µL 30 mmol buty lated hydroxytoluene/L. The reaction was run at 95°C for 15 min and centrifuged at 3200 × g for 10 min at 4°C; the supernate was used for HPLC. An aliquot was applied to an HPLC column (RP C18 Capcellpak, 4.6 x 150 mm, 5-µm particle size; Shiseido, Tokyo). A mobile phase of 50 mmol potassium phosphate/L in methanol (pH 5.5) was used. Fluorescence was measured at 513-nm excitation and 550-nm emission. Malondialdehyde concentrations were calculated by using a standard curve (malondialdehyde-diethy lacetate; Merck) and expressed as nanomoles malondialdehyde per gram dry fecal weight. This method is considered more specific than colorimetric analysis of thiobarbituric acid—reactive substances (11).

### Fecal bacterial enzymes

For determination of bacterial enzyme activities, aliquots of frozen feces were diluted 1:10 (β-glucuronidase and β-glucosidase) or 1:5 (sulfatase and nitroreductase) with prereduced phosphate-buffered saline (pH 7.0) under anaerobic conditions. The

| TABLE 1 Composition of the high- (HRS) and low-resistant-starch (LRS) diets6 |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                 | HRS diet        | LRS diet        | Available energy (MJ/d) | 10.2 ± 0.26  | 10.2 ± 0.26  | Protein (g/d) | 89.3 ± 3.24  | 84.9 ± 3.17  | Fat (g/d) | 100.3 ± 2.99  | 99.8 ± 2.90  |
| Carbohydrate                   | 285.5 ± 7.39  | 289.4 ± 7.35  | Available starch (g/d) | 175.5 ± 6.23  | 196.6 ± 6.05  | Disaccharides (g/d) | 73.3 ± 2.25  | 67.2 ± 2.80  | Monosaccharides (g/d) | 36.7 ± 3.76  | 25.6 ± 2.65  | Resistant starch (g/d) | 55.2 ± 3.50  | 7.7 ± 0.27  | Dietary fiber (g/d) | 11.2 ± 0.35  | 9.8 ± 0.33  |
|                                 |                 |                 | Available energy (MJ/d) | 10.2 ± 0.26  | 10.2 ± 0.26  | Protein (g/d) | 89.3 ± 3.24  | 84.9 ± 3.17  | Fat (g/d) | 100.3 ± 2.99  | 99.8 ± 2.90  |
| Carbohydrate                   | 285.5 ± 7.39  | 289.4 ± 7.35  | Available starch (g/d) | 175.5 ± 6.23  | 196.6 ± 6.05  | Disaccharides (g/d) | 73.3 ± 2.25  | 67.2 ± 2.80  | Monosaccharides (g/d) | 36.7 ± 3.76  | 25.6 ± 2.65  | Resistant starch (g/d) | 55.2 ± 3.50  | 7.7 ± 0.27  | Dietary fiber (g/d) | 11.2 ± 0.35  | 9.8 ± 0.33  |

6 SEM.

2 Does not include energy from dietary fiber or RS.
Fecal β-glucuronidase activity was assayed in triplicate by the method of Goldin and Gorbach (12), with slight modifications. The enzyme reaction was run at 37°C in a total volume of 2 mL composed of 0.2 mL fecal extract, 0.2 mL phenolphthalein glucoside (0.01 mol/L; Sigma Chemical Co), and 1.6 mL acetate buffer (0.1 mol/L, pH 4.5). After 0, 15, 30, 45, and 60 min of incubation, the reaction was stopped by adding a 1:1 mixture of glycine buffer (0.1 mol/L, pH 12) and distilled water; the phenolphthalein released was measured at 540 nm in a spectrophotometer (Milton Roy Co, Rochester, NY).

Fecal β-glucosidase activity was assayed under similar conditions by using 0.01 mol p-nitrophenyl glucoside/L (Sigma Chemical Co). The p-nitrophenol liberated was measured photometrically at 405 nm. Fecal sulfatase activity was assayed by using 0.1 mmol p-nitroacetochol sulfate/L (Sigma Chemical Co). Readings of liberated p-nitroacetochol sampled after 0, 30, 60, 90, and 120 min were taken at 405 nm. Fecal nitroreductase activity was measured in triplicate according to the method of Wise et al (13). All enzyme activities were calculated for the linear reaction range by using standard curves for each indicator (phenolphthalein, p-nitrophenol, p-nitroacetochol, and m-aminoazobenzoic acid), and expressed as milligrams or micrograms of liberated indicator per hour per gram of fresh feces.

Fecal neutral sterols and bile acids

Fecal bile acids and neutral sterols were measured in dried feces by gas-liquid chromatography according to the method of Reddy et al (14), with some modifications (15). Briefly, neutral sterols were analyzed quantitatively as trimethylsilyle derivatives by using a gas chromatograph (HP 5890II; Hewlett-Packard) equipped with a DB1 megabore column (15 m, 0.53-mm internal diameter; J&W Scientific, Folsom, CA) and a flame-ionization detector. Neutral sterols were identified with use of known standards. Concentrations were calculated by using multilevel calibration (HP 3365II Chem-Staion; Hewlett-Packard).

Bile acids were analyzed quantitatively as methyl-trifluoroacetyl derivatives with a gas-liquid chromatograph (HP 5890II; Hewlett-Packard) equipped with a DB17 megabore column (15 m, 0.53 mm internal diameter; J&W Scientific). Bile acids were identified by comparison with standard reference peaks and concentrations were calculated relatively to the internal standard. Standards were obtained from Sigma Chemical Co, Serva (Buchs, Switzerland), and Steraloids (Wilton, NH).

Breath hydrogen

End-expiratory breath hydrogen was measured with a selective electrochemical cell (exhaled hydrogen monitor; GMI Medical, Renfrew, Scotland) calibrated with a 96.8-ppm standard gas. Breath samples were collected after subjects had fasted overnight at 1-h intervals for 14 h on days 7, 14, 21, and 28 of each study period. The area under the curve (AUC) of hydrogen concentration compared with the time curve was calculated.

Statistics

Values are given as means ± SEMs. Nonparametric Wilcoxon rank-sum tests for paired data were used for comparisons between the HRS and LRS diets (WINSTAT 3.1; Kalmia Co, Cambridge, MA). AUC was calculated with WINSTAT by using the trapezoidal rule.

RESULTS

Subjects and study design

Mean body mass index in the two study periods did not differ. Evaluation of the bowel-symptom questionnaire showed no significant difference between the HRS and LRS diet periods. In neither study period did volunteers report diarrhea. Mean scores for other symptoms were slightly but not significantly higher during the HRS than during the LRS period: flatulence, 1.38 and 1.10; abdominal distention, 1.18 and 1.04; and abdominal cramping, 1.02 and 1.00. Therefore, food enriched with a highly resistant amylomaize starch was well tolerated.

Resistant starch in food and feces

Fecal starch excretion increased significantly with consumption of the HRS diet. A huge difference between oral intake and fecal excretion of starch was observed; during the HRS and LRS diets, 89% and 88%, respectively, of the RS consumed was fermented in the colon. Two volunteers, however, excreted high amounts of RS during both the HRS (32.2 and 31.0 g/d) and LRS (5.1 and 4.2 g/d) periods (Figure 1).

Breath hydrogen

Breath-hydrogen exhalation data are shown in Figure 2. On days 7 and 28, the AUC was significantly higher during the HRS than during the LRS period. Mean breath-hydrogen concentrations (ppm) rose gradually during the day and peaked in the evening (data not shown). In the two subjects with high fecal RS excretion, end-expiratory hydrogen concentrations were not significantly different from those in other volunteers.

Stool weight, pH, mean transit time, and stool fat

The HRS diet caused a significant increase in fecal wet (49%) and dry weights (56%) but there were no significant differences in fecal water content (%) during the two diet periods. There was a tendency toward a lower fecal pH (NS) and a longer MTT during the HRS than during the LRS period (Table 2). Stool fat excretion...
increased by 23% after consumption of the HRS diet (2.9 ± 0.4 g/d) compared with that after the LRS diet (2.3 ± 0.3 g/d) (*P* ≤ 0.071).

**Fecal short-chain fatty acids**

Total concentrations and daily excretion of SCFAs were not influenced significantly by RS consumption (Table 3). However, fecal propionate concentrations decreased significantly during the HRS compared with the LRS diet (10.8 ± 0.6 compared with 14.6 ± 1.3 μmol/g wet wt; *P* ≤ 0.05). Compared with the LRS diet, the HRS diet produced no changes in the molar distribution of acetate, isovalerate, or valerate, and slight, nonsignificant alterations in the molar ratios of propionate, isobutyrate, and butyrate.

**Malondialdehyde**

Malondialdehyde concentrations were not influenced significantly by RS consumption (HRS: 17.1 ± 1.1 nmol/g dry wt; LRS: 16.9 ± 1.1 nmol/g dry wt). Significant changes in daily fecal excretion of malondialdehyde between the HRS and LRS periods were observed (524.4 ± 42.4 and 348.6 ± 38.2 nmol/d, respectively; *P* ≤ 0.005).

**Fecal bacterial enzymes**

Fecal β-glucosidase activity decreased significantly during the HRS period compared with the LRS period (1.7 ± 0.5 compared with 2.3 ± 0.7 mg·h⁻¹·g⁻¹, respectively; *P* ≤ 0.05). However, there were no significant differences between the HRS and LRS periods in the activities of fecal bacterial β-glucuronidase (0.80 ± 0.23 and 0.97 ± 0.28 mg·h⁻¹·g⁻¹, respectively), sulfatase (22.82 ± 6.59 and 34.75 ± 10.03 mg·h⁻¹·g⁻¹, respectively), and nitroreductase (181.12 ± 52.28 and 148.23 ± 42.79 μg·h⁻¹·g⁻¹, respectively). For daily stool excretion, the only significant change between the HRS and LRS periods was in total nitroreductase activity (17.3 ± 2.6 and 9.4 ± 1.6 mg·h⁻¹·g⁻¹, respectively; *P* ≤ 0.05).

**Fecal neutral sterols and bile acids**

Fecal concentrations of neutral sterols during both test diets are shown in Table 4. During the HRS diet, there were significantly lower stool concentrations of coprostanol, cholesterol, 4-cholesten-3-one, campesterol, stigmasterol, β-sitosterol, and, consequently, total neutral sterol concentrations. No significant differences were found in the daily excretion of total neutral sterols between the HRS and LRS diets (815.1 ± 51.9 and 805.7 ± 65.5 mg/d, respectively).

Fecal concentrations of total bile acids decreased significantly during the HRS compared with the LRS period (5.4 ± 0.6 and 7.7 ± 0.8 mg/g dry wt, respectively; *P* ≤ 0.05). This result was mainly accounted for by a significant decrease in the secondary bile acids deoxycholic acid (38%) and lithocholic acid (33%) (Figure 3). The concentration of secondary bile acids also decreased significantly during the HRS period compared with the LRS period (4.83 ± 0.60 and 7.14 ± 0.76 mg/g dry wt, respectively; *P* ≤ 0.01); the percentage of secondary bile acids was unchanged. Noteworthy was the slight, nonsignificant increase in isolithocholic acid during HRS compared with LRS consumption (12.37 ± 2.46% and 8.32 ± 0.75%, respectively). Concentrations of primary bile acids were not significantly affected by RS consumption (HRS: 0.45 ± 0.07 mg/g dry wt; LRS: 0.58 ± 0.05 mg/g dry wt), nor were the molar ratios (HRS: 8.63 ± 1.29%; LRS: 7.93 ± 0.76%). No significant changes in daily excretion of total bile acids were found between the HRS and LRS diets (176.8 ± 28.0 and 164.4 ± 22.9 mg/d, respectively).

**TABLE 2**

Effect of resistant starch (RS) on fecal bulk, pH, and mean transit time in healthy volunteers consuming either a high-RS (HRS) or low-RS (LRS) diet¹

<table>
<thead>
<tr>
<th></th>
<th>HRS diet</th>
<th>LRS diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight (g/d)</td>
<td>111.4 ± 11.50</td>
<td>74.7 ± 6.18²</td>
</tr>
<tr>
<td>Dry weight (g/d)</td>
<td>32.2 ± 3.18</td>
<td>20.6 ± 1.68²</td>
</tr>
<tr>
<td>Fecal water (%)</td>
<td>70.5 ± 1.11</td>
<td>72.0 ± 1.04</td>
</tr>
<tr>
<td>pH</td>
<td>6.89 ± 0.10</td>
<td>7.03 ± 0.08</td>
</tr>
<tr>
<td>Mean transit time (h)</td>
<td>68.3 ± 3.92</td>
<td>57.9 ± 5.35</td>
</tr>
</tbody>
</table>

¹* ± SEM; *n* = 12.

²Significantly different from the HRS diet, *P* ≤ 0.005 (Wilcoxon test for paired data).

**DISCUSSION**

This trial showed that it is feasible to enrich normal bakery products with a highly resistant amylomaize starch. The quality and taste of these products did not differ from those of usual products and volunteers could eat them without gastrointestinal side effects. In another intervention study with RS (16), the same highly resistant amylomaize starch was given as native powder in yogurt or water but we integrated this RS into normal starchy
foods to ensure long-term use of the RS diet. Higher amounts of starch were excreted in the feces during the HRS diet than during the LRS diet but ≈90% of the starch consumed was fermented in the colon. However, we observed a large interindividual variation in starch excretion among the volunteers. Similar variations in starch excretion were described by Phillips et al (17). In both study periods, two subjects excreted high amounts of starch compared with other volunteers. When fresh fecal samples from these two subjects were incubated with a highly resistant amylomaize starch or Lintner’s starch, a slow pattern of fermentation was observed in batch cultures (SU Christl, personal communication, 1996). Similarly, Cummings et al (7) found that starch fermentation was reduced in 25% of healthy volunteers; in their view, this may have been due to differences in the capacity of the microflora to degrade RS.

The amounts of starch detected in feces during this study were higher than those reported by van Munster et al (16) after supplementation with 28 g RS from native amylomaize starch. Similar starch excretion in feces was described by Phillips et al (17) after administration of 39 g RS in corn bread or supplements. The differences between the studies may be explained by differences in the physical structure of the different types of starch, differences in food processing, or changes in fermentation activity in some individuals (7).

Hydrogen exhalation of the volunteers was higher during the HRS than during the LRS diet. Assessment of hydrogen exhalation, a noninvasive technique, can provide semiquantitative estimates of active carbohydrate fermentation in the colon (18, 19). It is not possible to make exact quantitative estimates because the proportion of exhaled hydrogen is variable and considerable amounts of hydrogen are used up during methanogenesis or sulfate reduction (20). Alternative pathways of hydrogen metabolism may also explain why end-expiratory hydrogen concentrations were not different between subjects who excreted high amounts of RS in feces.

As shown in this and other studies, RS increased fecal wet and dry weight (7, 16, 17). Previously, we showed a higher excretion of nitrogen and bacterial mass when starch malabsorption was induced by an α-glucosidase inhibitor (acarbose) (18). It is likely that this mechanism also accounted for higher fecal bulk in this trial, especially because RS does not have a remarkable water-holding capacity. Increases in stool weight have a dilution effect on potential carcinogens, and epidemiologic studies have shown a reduced risk of colon cancer under these circumstances (2). Another indication of enhanced bacterial proliferation might have been the slight increase in fecal fat, 70% of which may be attributable to bacterial mass.

MTT increased slightly during the HRS compared with the LRS period. Previously, we found significantly longer MTTs during α-glucosidase-inhibitor–induced starch malabsorption compared with control conditions (18). MTT is usually inversely related to stool weight (21) but there is some evidence that this relation exists only if MTT is high initially (22). Cummings et al (23) showed that the higher the initial MTT, the bigger the shortening of MTT during fiber supplementation. In this trial, MTTs in the volunteers were initially low and increased during the HRS diet to within the normal range.

Mean pH did not differ significantly between the HRS (6.89) and LRS periods (7.03). This was also shown by van Munster et al (16). On the other hand, Phillips et al (17) found a decrease in pH with an RS diet. In accordance with the study by Cummings and MacFarlane (24), the present study found that pH values were lowest in the proximal colon because of high production rates of SCFAs. Because of the absorption of SCFAs during passage through the colon, pH values increased progressively toward the distal colon (pH 7.0). Consequently, differences in proximal colonic pH between HRS and LRS may not be measurable in the feces.

In accordance with the study by Grubben et al (25), the present study showed no differences in SCFA concentrations or excretions between the two diet periods. Slight alterations in molar ratios of propionate, isobutyrate, and butyrate were observed during the HRS period. Because of the high rates of SCFA absorption (≈95–99% of SCFA produced), it is difficult to use feces for detection of alterations in SCFA production rates (26). However, when fresh fecal samples were incubated with amylomaize starch or Lintner’s starch in batch cultures, marked differences were observed in the molar ratios of acetate to propionate to butyrate. Fecal samples obtained during HRS consumption yielded higher production rates of butyrate than those obtained during LRS consumption (SU Christl, personal com-
munication, 1996). This finding may be important with regard to the adenoma-carcinoma sequence, in which butyrate may have protective effects at various stages (27).

Fecal malondialdehyde concentrations were not significantly different between the study periods; however, because of a higher stool mass, malondialdehyde excretion was enhanced in the HRS period. Measurement of thiobarbituric acid-malondialdehyde complexes by using HPLC has a high sensitivity but we could not exclude the possibility that other authentic complexes formed of carbohydrates were also measured (28). In this trial, the amount of potentially reacting carbohydrates was higher during the HRS than during the LRS diet period; consequently, this disturbance could have been the reason for missed differences between study periods.

Thampi et al (29) examined the influence of coconut fiber on lipid peroxidation in rats. They found decreased malondialdehyde concentrations as well as increased sucroplase dismutase and catalase activities in distal colonic tissue during fiber supplementation compared with a fiber-free diet. Possibly, dietary fiber influenced the amount of lipid peroxidation by enhancing the activity of antioxidant enzymes; this hypothesis needs further evaluation. The potential importance of oxidative stress in carcinogenesis is highlighted by two findings: a human colon cancer cell line (Caco-2) had lower sucroplase dismutase and catalase activities than colonic epithelial cells (30) and malondialdehyde concentrations were enhanced in colon tumor tissue compared with macroscopically normal tissue (31). There is no unequivocal evidence that changes in concentrations or excretion of malondialdehyde may be relevant in colonic carcinogenesis in humans.

Fecal bacteria enzymes are related to the risk of colon cancer because they release potential chemical carcinogens, eg, aglycons (32). Fecal bacterial enzyme activity can be influenced by substrate induction and by a changed composition of the bacterial flora (12). Johansson et al (33) found a decrease in fecal bacterial enzyme activity with a lactovegetarian diet whereas Gorbach and Goldin (34) did not find changes related to fiber supplementation. Mallett et al (32) found lower β-glucosidase activity with both bran and pectin supplementation than with control diets but lower β-glucuronidase activity only with pectin supplementation. In the present study, a decrease in β-glucosidase activity was found during the HRS diet, without an effect on total activity in the daily stool excretion. The reason for this finding could have been the prevalence of RS, which has no β-glucosidic linkages. Consequently, synthesis of enzymes required for RS fermentation may down-regulate the synthesis of other enzymes, eg, β-glucosidase needed for NSP breakdown (17). It is not known whether enhanced excretion of nitroreductase (with activity per gram of stool unchanged) is important with regard to the adenoma-carcinoma sequence in the human colon.

The concentrations and total amounts of several neutral sterols were decreased during the HRS diet but daily excretion was unaffected. A dilution effect due to bulkier stool is the most likely explanation; this was shown previously with wheat bran and cellulose supplementation and α-glucosidase-inhibitor-induced starch malabsorption (15, 35) but not with corn bran supplementation (36). For most neutral sterols, it is not clear whether these compounds play a role in carcinogenesis. However, the decrease in 4-cholesten-3-one concentrations during the HRS diet may be important because Suzuki et al (37) showed its cytotoxic capacity using the colonic nuclear aberration assay. It will have to be shown in further studies whether this effect seen in animal experiments is relevant to humans.

Significantly lower total concentrations of fecal bile acids were observed during the HRS diet than during the LRS diet. This finding was described by others (35, 36) after interventions with wheat bran and cellulose but not with corn bran supplementation or during starch malabsorption (15, 36). Because it was found that populations at high risk of colon cancer had high fecal concentrations of total bile acids (38-40), this result may be important with regard to strategies for preventing colon cancer. The reduction in total bile acids was accounted for mainly by a decrease in the secondary bile acids deoxycholic acid and lithocholic acid, which are regarded as colon tumor promoters (41). In comparison, wheat bran and cellulose supplementation and induced starch malabsorption also decreased secondary bile acids; corn bran intervention was followed by a decrease in fecal deoxycholic acid concentrations (15, 35, 36). It is assumed that, in addition to a dilution effect, this finding may also have been due to diminished synthesis of secondary bile acids in acidic milieu. Enhanced production of SCFAs in the proximal colon lowers pH, whereby bacterial 7α-dehydroxylase is inhibited (43).

In many epidemiologic studies, consumption of foods rich in complex carbohydrates is associated with a reduced risk of adenoma and carcinoma formation in the colon. However, it is difficult to recommend a high-fiber diet to the general public. Under these circumstances, enrichment of common starchy foods with RS may represent a viable alternative. In this study, the effects induced by RS were not principally different from those induced by NSP in other trials. A previous finding of enhanced butyrate excretion after glucosidase inhibition with acarbose (18) was not reproduced by RS intake. Major findings were an increased stool bulk and decreased fecal concentrations of secondary bile acids and 4-cholesten-3-one (possible cocarcinogens). With regard to tumorigenesis, a reduction in concentrations seems to be more important than a reduction in the total amount excreted. Controlled long-term experiments would be desirable to answer the question of whether dietary changes have a role in colon cancer prevention.

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