Effect of Starch Malabsorption on Fecal Bile Acids and Neutral Sterols in Humans: Possible Implications for Colonic Carcinogenesis

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ABSTRACT

Epidemiological and experimental studies indicate a strong association between an elevated colon cancer risk and increased fecal excretion of secondary bile acids, neutral sterols, and prolonged gastrointestinal transit time. Starch malabsorption, on the other hand, has been reported to be a possible protective factor in colon carcinogenesis. To study the impact of starch malabsorption on these parameters, 12 healthy volunteers consumed a diet rich in starch for two 4-week periods. During a double-blind crossover trial they received the α-glucosidase inhibitor acarbose (BAY g 5421) in one of the study periods and placebo in the other. During acarbose treatment stool wet weight increased by 68%, stool dry weight by 57%, and gastrointestinal mean transit time by 30%. Fecal concentrations (mg/g dry weight) of the neutral sterols coprostanol, coprostanone, campesterol, 4-cholesten-3-one, and β-sitosterol decreased by 36.8, 48.7, 42.1, 34.6, and 39.4%, respectively, under acarbose. Concentrations of the major secondary bile acids, deoxycholic and lithocholic acid, decreased by 59.9 and 52.2%, respectively. In spite of an increased stool weight, also daily excretion (mg/day) of these two bile acids was lower under acarbose (47.9 and 36.6%, respectively) compared to placebo, whereas excretion of the main primary bile acid, cholic acid, rose from 22.58 mg/day to 379.80 mg/day during the acarbose period. The changes in fecal bile acid and neutral sterol excretion found during acarbose treatment may explain a protective effect of starch malabsorption on colon cancer development.

INTRODUCTION

Cancer of the colon is a major neoplastic disease in the industrialized countries of Northern America and Europe (1). Various epidemiologic and experimental studies have indicated that certain dietary factors, particularly a high intake of fat and a low intake of dietary fiber, play important roles in the development of colon cancer (2–5). In the search of diet-dependent luminal compounds that might act as colonic carcinogens or cocarcinogens, Hill et al. (6, 7) found increased concentrations of fecal bile acids, especially of the major secondary bile acid deoxycholic acid, in both colon cancer patients and populations at high risk of the disease. This finding has been confirmed by Reddy and Wynder (8) who reported elevated fecal concentrations also for lithocholic acid in high-risk populations. Furthermore, it could be shown that rectal instillation of the secondary bile acids deoxycholic or lithocholic acid increase tumor incidence in carcinogen-treated rats indicating their role as cocarcinogens (9, 10). Nuclear aberration assays of colonic mouse epithelium have been carried out to assess components of human feces as possible carcinogens. Using this assay, a cell-damaging effect has recently been reported for 4-cholesten-3-one (11), a neutral 3-ketosteroid that has been found in significantly higher concentrations in subjects at high risk for colon cancer compared to controls (12).

Whereas a high intake of dietary fat (high-risk Western diet) increases fecal bile acid and neutral sterol contents, the consumption of a diet enriched with dietary fiber leads to a decrease in fecal concentrations of acid and neutral sterols (13). This effect is mainly caused by the bulking capacity of fiber which chemically consists of nonstarchy polysaccharides and lignin. Thornton et al. (14) proposed that “superefficient” starch absorption by the small bowel, leading to a lower colonic carbohydrate content, may be another risk factor for colon cancer. In a study on patients with colon adenomas they found that the percentage of malabsorbed starch was considerably lower in patients (5.3%) than in healthy controls (10.9%). Recently, it could be shown that starch malabsorption increases fecal bacterial mass and stool weight (15) which in turn might affect fecal acid and neutral sterol excretion.

In the present study the malabsorption of starch or starch degradation products was induced by the α-glucosidase inhibitor acarbose (BAY g 5421). This substance is primarily used as an antidiabetic agent delaying starch digestion and, thus, glucose absorption from the small intestine (16). As a side effect, this drug also increases carbohydrate content in the colon. It was the objective of this study to investigate the potential effects of starch malabsorption on fecal steroid excretion and colonic function.

MATERIALS AND METHODS

Study Design. Twelve healthy volunteers (6 men, 6 women; ages 23–31 years) participated in the study. In a double-blind crossover design the test persons received the α-glucosidase inhibitor acarbose (BAY g 5421) or placebo in randomized order for 4 weeks. A controlled basal diet outlined below was given during the two 4-week periods with an interval of 4 weeks between the study periods. In randomized order, acarbose or placebo was administered with three daily meals in increasing doses from week 1 to week 3 for the purpose of adaptation (1st week, 50 mg three times a day; 2nd week, 100 mg three times a day; 3rd and 4th weeks, 200 mg three times a day). The volunteers did not receive any medication, especially antibiotics, for at least 4 weeks before and during the trial.

Typical German meals, prepared by the dietetic kitchen of the University Hospital, were given as a controlled basal diet. The same meals were supplied during both study periods in a cycle of 8 days. In percentage of caloric intake the diet consisted of 50% carbohydrates, 15% protein, and 35% fat. Energy intake was 10.46 MJ/day for men and 7.53 MJ/day for women. The source of carbohydrates was mainly bread, noodles, potatoes, rice, vegetables, and fruits. Fat and protein sources were pork and beef meats, cheese, vegetable oil, and butter. Dietary fiber intake was 18 g/day for males and 14 g/day for females. The starch moiety of carbohydrates was 56% (17). The volunteers consumed each meal under the supervision of one of the authors (C. F.) in our metabolic suite.

Written informed consent has been obtained from the study participants. They were all found to be in good health at a prestudy physical examination. Pregnancy was excluded in the six female volunteers. The study has been approved by a joint ethical committee of the Department of Medicine, University of Wuerzburg, and Bayer Pharma, Wuppertal, Federal Republic of Germany.

Five-day stool samples were collected from day 24 to day 28 of each study period and immediately frozen at −20°C. Stool wet weight was noted and dry weight was measured after freeze-drying with a Gamma

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IA apparatus (Christ, Osterode, Federal Republic of Germany). Oroanal MTT was assessed using radiopaque pellets by the single-stool method according to the method of Cummings and Wiggins (18).

### Analytical Methods

Fecal neutral sterols and bile acids were quantitated in the freeze-dried 5-day stool samples of each study period by GLC. A technique described by Reddy et al. (19) with slight modifications was used throughout. Solvents were obtained from Merck, Darmstadt, Federal Republic of Germany, and standards from Steraloids, Inc., Wilton, NH, unless otherwise indicated. Aliquots of 1 g freeze-dried stool were saponified with 2 ml of 10 N NaOH in 38 ml of 90% ethanol:water (3:1) by refluxing on a heater (Electrothermal, Southend, United Kingdom) for 1 h. Four mg of 5-a-cholestanate (neutral sterol) and nordeoxycholic acid (bile acid) were added to every sample as internal standards at the beginning of the analysis. The nonsaponifiable fraction containing neutral sterols was extracted with $6 \times 50$ ml of hexane. The pooled extracts were concentrated in an evaporator (Buechi, Flawil, Switzerland) and transferred quantitatively to a test tube with 10 ml chloroform. The aliquot intended for GLC (200 µl of this extract) was evaporated to dryness under a stream of nitrogen, silylated with 10 ml chloroform. The aliquot intended for GLC (200 µl of this fraction containing neutral sterols was subjected to further hydrolysis according to the method of Cummings and Wiggins (18). For the analysis of free bile acids, the aqueous phase (free of neutral sterols) from the saponified sample was subjected to further hydrolysis with 2 ml of 10 N HC1 and 25 ml of water, and after acidification of the samples to pH 2 with 5 ml of HC1 and 25 ml of 85% phosphoric acid, it was centrifuged at 2500 ×g for 15 min. The aqueous phase was used for the GLC analysis.

### Statistical Analysis

Values are given as mean ± SEM. The non-parametric Wilcoxon rank sum test for paired data was used throughout for comparisons. P < .05 was considered significant.

### RESULTS

**Stool Weight and Mean Transit Time.** The average stool wet weight during the 5 days of stool collection was 122.4 ± 17.9 g/day in the control period compared to 200.0 ± 32.8 g/day in the acarbose period ($P < 0.01$). Stool dry weight was also lower in the control phase (37.2 ± 2.9 g/day) than in the acarbose phase (58.2 ± 6.1 g/day; $P < 0.005$). MTT was significantly prolonged under acarbose (55.9 ± 3.3 h) compared to the control period (43.0 ± 5.1 h; $P < 0.05$).

**Fecal Neutral Sterols.** Fecal concentrations and daily excretion of various neutral sterols are summarized in Table 1. During acarbose treatment decreased concentrations could be seen for total neutral sterols and, in particular, for coprostanol, coprostanone, campesterol, 4-cholesten-3-one, and β-sitosterol. No significant differences were found in the concentrations of cholesterol, stigmasterol, and cholestan-3ß,5α,6ß-triol. Mean daily excretion was not significantly different under acarbose, neither for total neutral sterols nor for any individual neutral sterol. Cholestanone and cholesterol were detectable in only five subjects during the acarbose (2.15 ± 1.40 and 1.10 ± 0.26 mg/g, respectively) and placebo periods (0.07 ± 0.05 and 0.97 ± 0.17 mg/g, respectively). Therefore, no statistical test was carried out on these two neutral sterols.

**Fecal Bile Acids.** The fecal bile acid pattern is shown in Table 2. A significant decrease in concentration and mean daily excretion of the fecal secondary bile acids deoxycholic acid and lithocholic acid was found under acarbose. In contrast, daily excretion of the main primary bile acid, cholic acid, was increased during the acarbose period. The changes in mean daily excretion of secondary and primary bile acids are illustrated in Fig. 1.

**Side Effects of Acarbose Treatment.** The severity of abdominal symptoms was recorded by each volunteer and classified semiquantitatively as mild, moderate, marked, and severe according to the system of Lasser et al. (20). In the 4th week of the acarbose period, flatulence was present in all 12 subjects and increased during the acarbose period. The changes in mean daily excretion of secondary bile acids deoxycholic acid and lithocholic acid was found under acarbose. In contrast, daily excretion of the main primary bile acid, cholic acid, was increased during the acarbose period. The changes in mean daily excretion of secondary and primary bile acids are illustrated in Fig. 1.
(10 moderate, 2 marked) and abdominal discomfort was reported by 5 of 12 volunteers (4 mild, 1 moderate), whereas during the control period these symptoms occurred in 6 (5 mild, 1 moderate) and 3 (2 mild, 1 moderate) subjects, respectively. Mild diarrhea was present in 2 cases during both study periods. Severe side effects were not noted by any volunteer, nor have there been any dropouts due to the reported abdominal symptoms.

DISCUSSION

In this study starch malabsorption was induced by administering the α-glucosidase inhibitor acarbose (BAY g 5421) to subjects receiving a basal diet rich in starch. It has been shown previously that at high acarbose doses starch or starch degradation products escape small intestinal digestion and enter the colon (21). Increased bacterial carbohydrate fermentation during acarbose treatment has been demonstrated by measuring end-expiratory breath hydrogen which was four times higher compared to placebo (22).

Due to the increased carbohydrate content in the colonic lumen, stool wet weight rose by 63% and dry weight by 57% during acarbose treatment in this study. Such a stool "bulking effect" is well documented for fibrous foods with high cellulose and lignin contents (e.g., wheat bran) (13), but it is also described for readily fermentable dietary fiber substances like guar gum or pectin. Cummings et al. (23) reported an increase of fecal wet weight by 20 and 40%, respectively, when 20 g/day of guar gum or apple pectin were added to the diet.

Usually, there is an inverse relationship between stool weight and MTT (24). This negative correlation, however, occurs only if MTT is high initially. In contrast to this, dietary fiber tends to increase MTT if it is initially low. In this study MTT was decreased as a result of acarbose administration, mean daily excretion remained rather unchanged due to a higher stool weight during the acarbose period. Concerning bile acid excretion, however, acarbose-induced starch malabsorption led to a fundamental change of the fecal bile acid pattern (Fig. 1). Interestingly, not only concentration but also daily excretion of the secondary bile acids, deoxycholic and lithocholic acid, decreased under acarbose, whereas daily excretion of the main primary bile acid, cholic acid, increased during acarbose treatment. Since total bile acid excretion was not significantly different in both study periods, this effect cannot sufficiently be explained by a dilution effect due to increased stool bulk. In the light of a recent study showing that starch malabsorption increases fecal bacterial mass (15), the observed shift from secondary to primary bile acids might be explained by an altered bacterial degradation of the primary bile acids cholic and chenodeoxycholic acid. A more acidic pH level in the proximal colon as a result of higher concentrations of short-chain fatty

<table>
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<tr>
<th>Bile acids</th>
<th>Fecal concentration (mg/g dry wt)</th>
<th>Fecal excretion (mg/day)</th>
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<tbody>
<tr>
<td></td>
<td>Acarbose</td>
<td>Placebo</td>
</tr>
<tr>
<td>Deoxycholic acid</td>
<td>1.53 ± 0.36&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.82 ± 0.74</td>
</tr>
<tr>
<td>Lithocholic acid</td>
<td>1.51 ± 0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.16 ± 0.63</td>
</tr>
<tr>
<td>Isolisothic acid</td>
<td>1.26 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.97 ± 0.39</td>
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<tr>
<td>Cholic acid</td>
<td>5.18 ± 1.97</td>
<td>0.74 ± 0.35</td>
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<tr>
<td>Chenodeoxycholic acid</td>
<td>0.57 ± 0.11</td>
<td>0.77 ± 0.27</td>
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<tr>
<td>Ursodeoxycholic acid</td>
<td>1.11 ± 0.52</td>
<td>0.43 ± 0.30</td>
</tr>
<tr>
<td>6-Ketolithocholic acid</td>
<td>0.17 ± 0.11</td>
<td>0.32 ± 0.08</td>
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<tr>
<td>7-Ketodeoxycholic acid</td>
<td>1.12 ± 0.40</td>
<td>0.59 ± 0.18</td>
</tr>
<tr>
<td>12-Ketolithocholic acid</td>
<td>0.12 ± 0.01</td>
<td>0.29 ± 0.11</td>
</tr>
<tr>
<td>5α-Cholic acid-3β-ol</td>
<td>1.24 ± 0.20</td>
<td>1.61 ± 0.22</td>
</tr>
<tr>
<td>3-Ketocholic acid</td>
<td>0.47 ± 0.21</td>
<td>0.50 ± 0.30</td>
</tr>
<tr>
<td>Total</td>
<td>11.68 ± 2.48</td>
<td>13.37 ± 1.83</td>
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<sup>a</sup> Mean ± SEM.<br><sup>b</sup> P < 0.01.<br><sup>c</sup> P < 0.05.
acids found during starch malabsorption (22) would decrease the activity of bacterial 7α-dehydroxylase, an important enzyme for the degradation of primary to secondary bile acids (27). In this respect, information about the accurate luminal pH level of the cecum and ascending colon would be of great interest. Samples from this part of the colon are, however, difficult to obtain. Since fecal pH may not accurately reflect intracolonic pH (28), no attempt was made to measure pH from fresh feces obtained. Since fecal pH may not accurately reflect intracolonic for the degradation of primary to secondary bile acids (27). In the activity of bacterial 7α-dehydroxylase, an important enzyme with different fiber and starch intake. The daily carbohydrate changed. An increase in stool weight was also noted in this

44%, respectively; whereas lithocholic acid remained un

decreased from 22% to 13%; the proportion of cholic acid and

the results of the present study. Starch malabsorption may also affect bile acid conjugation which may have contributed to the observed changes in bile acid excretion. Due to alkaline hydrolysis of bile acid conjugates, the laboratory method used in the present study gives only information about the amount of free bile acids. These unconjugated bile acids, however, constitute the largest proportion of the total fecal bile acids excreted by humans (29).

Ghoos et al. (30) measured bile acid excretion in 51 subjects with different fiber and starch intake. The daily carbohydrate intake, estimated by a dietary questionnaire, was 1035 kcal/day in the low-fiber group (n = 25) and 1109 kcal/day in the high-fiber group (n = 26) with starch contents of 68 and 91%, respectively. In accordance with our data, the high-fiber group had a significantly higher stool weight, but fecal bile acid excretion was also higher compared to the low-fiber group. Since only total bile acids were determined using an enzymatic method, the authors could not give any information about the specific bile acid pattern. Recently, the effect of lactulose ingestion (60 g/day) on duodenal bile composition has been investigated (31). Being fermented in the colon in a fashion similar to that of starch, lactulose causes a fall of the pH level in the proximal colon (32). After 4 and 12 weeks of treatment with lactulose, the deoxycholic acid pool in duodenal bile samples decreased from 22% to 13%; the proportion of cholic acid and chenodeoxycholic acid increased from 36% to 43% and 40% to 44%, respectively; whereas lithocholic acid remained unchanged. An increase in stool weight was also noted in this study. Although no data on fecal bile composition were reported, the observed changes in duodenal bile are in line with the results of the present study.

To date, there are only few epidemiological data available on the relationship between starch intake and colon cancer. However, the finding of a superefficient starch absorption in patients with adenomatous polyps (14) and the low colon cancer rates in India and Japan, countries with a high intake of starchy foods (rice) (1, 33), should be emphasized in this context. In a recent study from Burma it was shown that carbohydrate malabsorption from rice occurred in 38.5% of young adults (15–39 years) and in 50% of older adults (40–70 years) (34). As pointed out by Cummings and Bingham (35), “starch may be the crucial protective dietary component” in colon carcinogenesis. The results of this study, particularly the decreased concentration of the potential (co)carcinogen 4-cholesten-3-one and the diminished fecal excretion of deoxycholic and lithocholic acid, known promoters of colon carcinogenesis, may explain how starch malabsorption may protect against colon cancer.

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