Metabolic Epidemiology of Colon Cancer: Effect of Dietary Fiber on Fecal Mutagens and Bile Acids in Healthy Subjects

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ABSTRACT

Because of potential significance of fecal mutagens and secondary bile acids in the pathogenesis of colonic cancer and of inverse association between dietary fiber and colonic cancer risk, the effect of dietary wheat and rye fiber on fecal mutagenic activity and bile acid levels was studied in 15 healthy young volunteers who were consuming high-fat/moderately low fiber diets and excreting high levels of fecal mutagens and bile acids. Each participant provided two 24-h stool specimens and a 3-day diet record while consuming their normal diet (control). All subjects were then asked to consume their normal diet plus 11 g of supplemental fiber per day in the form of whole grain bread for 4 weeks. During the last week of diet intervention, each subject provided two 24-h stool specimens and a 3-day dietary record. Fecal samples collected from both periods were analyzed for bile acids and for mutagens using Salmonella typhimurium strains TA98 and TA100 with and without microsomal activation. The concentration of fecal secondary bile acids was significantly lower during the fiber supplemental period in all subjects. Fiber supplementation also inhibited the fecal mutagenic activity in TA100 and TA98 with and without microsomal activation. Thus, the increased fiber intake in the form of whole wheat and rye bread may reduce the production and/or excretion of fecal mutagens and decreases the concentration of fecal secondary bile acids in humans.

INTRODUCTION

Cancer of the colon is the major neoplastic disease that strikes men and women in Western countries, including North America, with high frequency (1–3). Epidemiological and experimental studies suggest that dietary factors, particularly a high intake of total fat and saturated fat and a relatively low intake of certain dietary fibers, play important roles in colonic cancer (4–11). However, a recent Hawaiian prospective study suggests a strong inverse relationship between dietary saturated fat and colonic cancer and a weak positive association between rectal cancer and intake of saturated fat (12). The reason for this discrepancy might have been the fact that some of these studies did not take into consideration other confounding variables such as dietary fiber, since these food items have been associated with a reduced risk for colonic cancer in populations consuming diets high in fat (6, 7). For example, recent studies comparing populations in rural and urban areas in Finland, Denmark, and Sweden and urban populations in metropolitan New York indicated that one of the factors contributing to the low risk of colonic cancer in rural Finland, Denmark, and Sweden appears to be high dietary fiber intake, mainly whole-grain cereals and bread (7, 12), although other factors, including high calcium and lactose intake also should be considered (13, 14). A strong negative association was reported between regional colonic cancer mortality within the United Kingdom and consumption of total dietary fiber (15). These studies suggest that certain fibers reduce the risk of colonic cancer development in populations consuming diets high in fat.

The search for genotoxic compounds (carcinogens) in the colonic or fecal constituents of populations with diverse incidence rates for colon cancer was encouraged when a number of laboratories demonstrated the presence of mutagens (presumptive carcinogens) in the stools of humans (16–20). These studies have revealed that prevalence of fecal mutagens is higher in populations that are at high risk for the development of colonic cancer and consuming diets high in fat and low in fiber than in low-risk populations. An important finding was that of Hirai et al. (21) and Gupta et al. (22) who noted that the mutagenic activity of feces of certain donors appears to be due to a type of compound termed as fecapentaene, (s)-3-(1,3,5,7,9-dodeca-pentaenoyloxy)-1,2-propanediol, produced by anaerobic bacteria and active on Salmonella typhimurium strain TA100 without S9 activation.

Other studies indicate that the concentrations of fecal secondary bile acids, particularly deoxycholic and lithocholic acids which have been shown to act as colonic tumor promoters (23), were higher in populations that consume high-fat/low-fiber diets and are at high risk for colonic cancer than in populations that consume low-fat/high-fiber or high-fat/high-fiber diets and are at low risk for colonic cancer (12, 24, 25). Such a relationship is further supported by experimental studies in which rats fed diets high in wheat bran or low in fat excreted low concentrations of secondary bile acids (23).

While there is an inverse association between dietary whole-grain cereal fiber and colonic cancer risk (11, 19), the study reported here was designed to investigate the effect of supplemental dietary whole-grain cereal fiber on fecal mutagens and bile acids in healthy individuals consuming high-fat/moderately low-fiber diets and excreting high levels of fecal mutagens and bile acids. In this study, we present evidence that supplemental dietary fiber inhibits the fecal mutagenic activity as well as reduces the concentrations of fecal secondary bile acids.

MATERIALS AND METHODS

Study Population and Diet Intervention. In our previous study, individuals of both sexes from Helsinki (20 males and 30 females) with a mean age of 45 ± 4 (SE) years who met the following criteria were studied: (a) they were not on any special diets other than consumed traditionally in their area, (b) they had no history of antibiotic treatment for the 3 weeks prior to stool collection, and (c) they had no gastrointestinal diseases or surgical resection of partial or total stomach or intestine (19). These individuals were drawn as a subsample from an ongoing study of a random population group (19). They were middle income white collar workers with more than a high school education. The subjects of our previous study were initially contacted by letter and telephone. They were interviewed by a nutritional epidemiologist and diet histories were recorded. The questionnaire, which included background medical history and information about dietary practices, was administered to each subject. Nutrition analysis using 3-day food records indicated that males and females on the average per day consumed about 2900 and 2300 calories, 108 and 88 g protein, 128 and 99 g fat, 309 and 259 g carbohydrate, 21.5 and 18.3 g total dietary fiber, and 11.5 and 8.9 g total fiber from bread, respectively (19). The fecal output
were of highest grade purity and purchased from American Scientific Products, Edison, NJ; Fisher Scientific Co., Pittsburgh, PA; Supelco, Bellefonte, PA; and Sigma Chemical Co., St. Louis, MO. Prefilters and filters were from Millipore Corp., Bedford, MA and Aroclor 1254 (polychlorinated biphenyl) was from Analabs, North Haven, CT.

Mutagenicity Assay. All samples were lyophilized and analyzed individually. Preparation of S9 from Aroclor-induced male Sprague-Dawley rats was as described (29). The methods used for the extraction of fecal samples for mutagenic activity have been described previously (29). Approximately 10 g of lyophilized fecal sample were mixed with 10 ml of 0.1 n NaOH and extracted 3 times with 100 ml 1:1 (v/v) hexane:peroxide-free diethyl ether. The pooled extract was filtered through Whatman No. 1 filter paper. The filtrate was evaporated to dryness, suspended in 10 ml of hexane:ether (1:1, v/v) and divided into 2 equal parts. One part was mixed with 5 ml pentane and passed through a Sep-Pak silica cartridge (Waters Associates, Medford, MA) and the eluant collected (fraction 1). The Sep-Pak cartridge was then eluted with 10 ml of 1:1 (v/v) hexanether (fraction 2) and finally with 10 ml of equal parts of hexanether and acetonitrile (fraction 3). Fraction 1 exhibited considerable toxicity and no activity in the Ames assay system even at very low concentrations (29). Fractions 2 and 3 were pooled, dried under nitrogen, and suspended in dimethyl sulfoxide for mutagenicity assay.

Each fecal extract was assayed for mutagenicity by standard experimental procedures developed by Ames et al. (30) and McCann et al. (31) using S. typhimurium strains TA98 and TA100 with and without S9 activation. These procedures are routinely being done in our laboratory. Three dilutions of each fecal extract equivalent to approximately 50, 100, and 200 mg of dry feces were tested in duplicate for mutagenicity. Samples were considered active only when the mutagenic response to the fecal extract was dose related and were considered positive for the presence of mutagenic activity if the mutagenic ratio was equal to or greater than 3 (19). The mutagenic ratio is the number of histidine-positive revertant colonies on the test plate divided by the number of histidine-positive spontaneous revertant colonies on control plates for each dose tested in each assay.

Each experiment also contained positive controls for checking the activity of the microsomal system (S9) and mutability of TA98 and TA100 using 2-aminofluorene, sodium azide, and 2-nitrofluorene. Whenever the positive controls did not confirm its validity, the experiments were repeated.

Analysis of Fecal Bile Acids. Aliquots of fecal samples intended for bile acids analysis were thawed, weighed, and homogenized in an equal volume of (w/v) normal saline. Five-g aliquots in duplicate were used for the analysis of bile acids by gas chromatographic methods that are routinely being used in our laboratory (32). Bile acids were analyzed as trifluoroacetyl derivatives on a 3% QF-1 column with helium as the carrier gas.

Statistical Analysis. The data were analyzed statistically by Student’s t test.

RESULTS

Nutrient analysis using 3-day food records collected before and at the end of fiber supplementation period is summarized in Table 2. As might be expected, the intakes of total calories, Table 2 Dietary nutrient intake of healthy subjects during the control and supplemental fiber periods

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control diet</th>
<th>Supplemental fiber diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males (8)*</td>
<td>Females (7)</td>
</tr>
<tr>
<td>Energy, kcal/day</td>
<td>2656 ± 255</td>
<td>2405 ± 330</td>
</tr>
<tr>
<td>Total protein</td>
<td>96 ± 5</td>
<td>94 ± 15</td>
</tr>
<tr>
<td>Total lipids</td>
<td>120 ± 15</td>
<td>113 ± 29</td>
</tr>
<tr>
<td>Total carbohydrates</td>
<td>261 ± 32</td>
<td>235 ± 23</td>
</tr>
<tr>
<td>Total dietary fiber</td>
<td>14 ± 2</td>
<td>17 ± 2</td>
</tr>
<tr>
<td>Cholesterol, mg/day</td>
<td>427 ± 34</td>
<td>474 ± 37</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of participants.
* Significant at P < 0.01.
* Significant at P < 0.05.

® Numbers in parentheses, number of participants.
® Mean ± SE.
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Activity in tester strain TA 100 without S9 activation was inhibited. The data were expressed as the number of histidine-positive revertant colonies per plate and as mutation ratio. The mutagenic activity in tester strain TA 100 without S9 activation was inhibited in 10 of 13 individuals during the period of fiber supplementation, whereas no effect of dietary fiber was observed in 3 subjects. In TA 100 with S9 activation, fiber supplementation reduced the mutagenic activity in 6 of 10 subjects. Six of 8 subjects exhibited a reduced mutagenic activity in TA 98 without S9 activity, whereas 4 of 5 subjects showed an inhibition of mutagenic activity in TA 98 with S9 activation. In general, the fecal mutagenic activity was inhibited in all tester strains during the period of fiber supplementation compared to control diet period.

The fecal bile acid composition is summarized in Table 3. Previous studies from this and other laboratories have shown that the fecal mutagenic activity was higher in populations who consumed a control diet and supplemental fiber diet. Conditions were as in legend to Fig. 1. The fractions were tested for mutagenicity using S. typhimurium TA 100 with (+S9) and without (−S9) activation. The mutagenic response was expressed as mutation ratios which are the number of histidine-positive revertant spontaneous revertant colonies on control plates divided by the number of histidine-positive revertant colonies on test plates for each dose tested in each assay.

Table 3 Fecal bile acid excretion in healthy subjects during the control and supplemental fiber periods

<table>
<thead>
<tr>
<th>Bile acids</th>
<th>Control diet (15)</th>
<th>Supplemental fiber diet (15)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mg/g dry feces</td>
</tr>
<tr>
<td>Cholic</td>
<td>0.24 ± 0.06</td>
<td>0.28 ± 0.05</td>
</tr>
<tr>
<td>Chenodeoxycholic</td>
<td>0.30 ± 0.05</td>
<td>0.22 ± 0.04</td>
</tr>
<tr>
<td>Deoxycholic</td>
<td>3.62 ± 0.81</td>
<td>1.81 ± 0.41</td>
</tr>
<tr>
<td>Lithocholic</td>
<td>3.24 ± 0.60</td>
<td>1.41 ± 0.42</td>
</tr>
<tr>
<td>12-Ketolithocholic</td>
<td>0.50 ± 0.31</td>
<td>0.20 ± 0.10</td>
</tr>
<tr>
<td>Other</td>
<td>1.30 ± 0.19</td>
<td>0.68 ± 0.16</td>
</tr>
<tr>
<td>Total</td>
<td>9.20 ± 0.83</td>
<td>4.60 ± 0.63</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of participants.
* Mean ± SE.
* Significantly different from supplemental fiber diet, at P < 0.05.

Fig. 1. Mutagenic activity (histidine-positive (HIS+) revertants/plate, or histidine-positive revertants/200 mg dry feces) of fecal extracts from healthy subjects consuming control diet and supplemental fiber diet. Fecal samples were extracted with hexane:ether (1:1), passed through the Sep-Pak silica cartridge and eluted with hexane:ether (1:1) and equal parts of hexane ether and acetone. The fractions were tested for mutagenicity using S. typhimurium TA 100 with (+S9) and without (−S9) microsomal activation. Three doses of fecal extracts equivalent to 50, 100, and 200 mg dry feces in duplicate were used in each test. The spontaneous revertants averaged 115 for TA 100 without S9 and 120 for TA 100 with S9.

Fig. 2. Mutagenic activity (histidine-positive (HIS+) revertants/plate, or histidine-positive revertants/200 mg dry feces) of fecal extracts from healthy subjects consuming control diet and supplemental fiber diet. Conditions were as in legend to Fig. 1. The fractions were tested for mutagenicity using S. typhimurium TA 98 with (+S9) and without (−S9) activation. The spontaneous revertants averaged 24 for TA 98 without S9 and 32 for TA 98 with S9.

Fig. 3. Mutagenic activity (mutation ratio) of fecal extracts from healthy subjects consuming a control diet and supplemental fiber diet. Conditions were as in legend to Fig. 1. The fractions were tested for mutagenicity using S. typhimurium TA 100 with (+S9) and without (−S9) activation. The mutagenic response was expressed as mutation ratios which are the number of histidine-positive revertant colonies on the test plate divided by the number of histidine-positive revertant spontaneous revertant colonies on control plates for each dose tested in each assay.

DISCUSSION

Previous studies from this and other laboratories have shown that the fecal mutagenic activity was higher in populations who...
are at high risk for colonic cancer development than in low-risk populations (16–20, 29, 33). The mutagens detected by TA100 with and without S9 activation occurred more frequently in a high-risk Hawaiian population and those detected by TA100 and TA98 without S9 activation were higher in South African urban whites, a high-risk population for colonic cancer than in the South African urban and rural blacks, a low-risk population for colonic cancer who are consuming a low-fat, high-fiber diet (16–18). The vegetarian Seventh-Day Adventists, who are at low-risk for colonic cancer development and consuming diets moderately high in fat and high in fiber, as well as lacto-ovo and strict vegetarians were found to excrete lower levels of fecal mutagens than the non-vegetarians consuming a high-fat, low-fiber diet (20, 33). We also demonstrated that the concentration and percentage of fecal samples exhibiting mutagenic activity were higher in the metropolitan New York population who are at high risk for colonic cancer and in the urban Helsinki (Finland) population, a medium-risk group compared with people in rural Kuopio (Finland), a low-risk population for colonic cancer (19, 20). The fact that the average daily intake of protein and fat was the same in the 3 groups, but the intake of fiber in the form of whole-grain cereal and whole-grain bread was highest in Kuopio, followed by the Helsinki and New York metropolitan areas (19, 20) suggests an inverse relationship between the amount of fiber intake and the colonic mutagenic activity.

Previous studies on the dietary manipulation of fecal mutagens produced mixed results. Dion et al. (34) demonstrated that supplementary ascorbic acid and a-tocopherol decreased the fecal mutagenic activity in healthy individuals consuming a Western diet, whereas Wilkins et al. (35) could not detect any inhibitory effect of vitamins C and E on fecal mutagens in healthy individuals. The current study was undertaken to determine the effect of supplemental fiber on fecal mutagenicity. In the present study, the intake of total protein and fat was the same during the control and fiber supplementation periods, but the fiber intake in the form of whole-grain bread was higher during the fiber supplementation period compared to control diet period. The inhibitory effect of dietary fiber on fecal mutagenicity observed in this study may not be ascribed to a simple dilution due to a high fecal bulk since the mean fecal mutagenic activity was decreased by 2.5- to 4.5-fold and the stool bulk was increased by about 35%. With respect to the nature of fecal mutagens that are found in the present study, it has been shown that the production of certain fecal mutagens such as fecapentaene in the colon and subsequent excretion depends on the presence of certain precursors, which may be produced by gut microflora or be a product of mammalian metabolism, be a dietary component, or a product of interaction of all three (36). Sugimura et al. (37) and others (38) demonstrated the presence of S9-activated fecal mutagens in cooked foods. Since most of the mutagens formed in the cooking process require S9 activation, and the fecal mutagens thus far tested are also active with S9 activation, the source of S9-activated mutagens in the present study may be of dietary origin. It is also possible that interactions of constituents of food with gut microflora would provide chemical species possessing mutagenic activity (39).

Dietary fiber affects gut microflora by modifying both the metabolic activities and the composition of the colonic microflora (40, 41), which in turn alters the breakdown products of non-starch polysaccharides as well as dietary fat and protein in the gut. The amount and type of breakdown products produced by the microflora in the gut will depend partly on the intake of certain dietary fibers and other nutrients. Thus, it is possible that the intake of dietary fiber modifies the formation of certain types of mutagens in the colon and/or inhibits the activity of mutagens in the colon.

In addition to differences in fiber intake, dietary intake of cholesterol was lower during the period of fiber supplementation compared to control diet period. There is evidence that increased cholesterol intake increases the excretion of bile acids (42) which have been shown to stimulate the production of mutagens in human stool specimens that are active in TA100 without S9 activation (43). Therefore, it is possible that decreased intake of cholesterol during the fiber supplementation period might be associated with a reduction in certain fecal mutagens.

The available information does not establish what role, if any, these fecal mutagens may play in colonic cancer development. Although the population studies suggest that the populations with a high risk for colonic cancer excrete high levels of fecal mutagens compared to populations with a low risk for colonic cancer (16–19), the excretion of fecal mutagens was the same in patients with colonic cancer and in controls (44) and low in patients with adenomatous polyps compared to controls (45). In the latter study, autopsy specimens were analyzed for mutagenic activity (45). While these results do not support the suggestion that mutagens are involved in the origin of colonic cancer, it is possible that the fecal mutagenic activity was measured too late in the disease process (44) and that postmortem changes resulted in the disappearance of any mutagenic activity in the stool (45).

Although there is no direct evidence that these fecal mutagens may play a role in human colonic cancer, a recent study indicates that fecapentaene-12, a mutagen produced in colonic contents (21, 22), has been shown to act as an alkylating agent (46). In view of these considerations, we should not rule out the possibility that mutagens which are consistently found in high incidence in colonic contents are most likely candidates as potential colonic carcinogens.

We and others have previously demonstrated that the fecal secondary bile acids were higher during the period of high-fat intake when the fiber intake was kept constant (25). The results of the present study indicate that high dietary fiber, namely whole-grain bread intake, as well as a reduction in cholesterol intake decreased the fecal excretion of secondary bile acids. These results are in agreement with earlier observations in humans that indicate that dietary wheat bran decreased the concentration of fecal bile acids (47, 48). The present study also augments various other studies that have emphasized the importance of colonic secondary bile acids in colonic carcinogenesis by providing data concerning the inhibitory effect of dietary fiber on the concentration of colonic bile acids that have been shown to play a role in colonic tumor promotion.

The mechanism by which dietary fiber affects the concentration of fecal bile acids is unclear. Pomare and Heaton (49) demonstrated a decrease in deoxycholate in the bile acid pool of individuals fed a high wheat bran diet, suggesting a change in individual bile acid pool sizes in response to dietary fiber. Modification of hormonal status by dietary fiber, as has been observed with insulin, could alter the amount of each bile acid synthesized (50). Dietary fibers not only alter the distribution of cholesterol on lipoproteins, which could, in turn, affect the amount and type of bile acids synthesized and subsequently excreted into the gut, but also affect the absorption and enterohepatic circulation of bile acids (50). Also, we cannot rule out the possibility that increase in stool bulk in consequence to high dietary fiber results in a dilution of fecal bile acids, thereby decreasing their concentration in the stool. It is also possible that reduced intake of cholesterol in the current study might
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explain the reduction in fecal bile acids. In addition, dietary fiber can also alter the activity of gut microflora (40) which in turn influences the metabolism of bile acids independent of the above effects. The net effect of dietary fiber in decreasing the concentration of fecal bile acids may be due to one or more of the above mechanisms.

We realize that the small sample size of the present study has its limitations, but in the light of this and other studies, it is suggested that total dietary fiber, particularly whole-grain cereals and bread, may reduce the production and/or excretion above effects. The net effect of dietary fiber in decreasing the fiber can also alter the activity of gut microflora (40) which in vitro influences the metabolism of bile acids independent of the above mechanisms.

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