Biochemical Epidemiology of Colon Cancer: Effect of Types of Dietary Fiber on Fecal Mutagens, Acid, and Neutral Sterols in Healthy Subjects

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ABSTRACT

Several epidemiological studies suggest an inverse relationship between fiber intake and colon cancer risk. Animal model studies indicate that this inhibitory effect depends on the source of dietary fiber. Because of the potential significance of certain colonic mutagens and secondary bile acids in the pathogenesis of colon cancer, the effect of types of supplemental fiber on fecal mutagens and bile acids was studied in human volunteers. Seventy-two healthy individuals consuming high-fat/moderately low-fiber diets were screened for fecal mutagenic activity using the Ames Salmonella typhimurium/microsomal assay system. Twenty-one of them were found to excrete high levels of mutagens, and 19 of them were recruited for the diet intervention study. All participants provided two 24-h stool specimens and a 4-day food record while consuming their normal (control) diet. They were then asked to consume the control diet plus 10 g of dietary fiber from wheat bran, oat fiber, or cellulose for 5 wk. After each fiber period, they were asked to consume their control diet. At the end of each fiber and control diet period, each subject provided two 24-h stool specimens. Stool samples were analyzed for bile acids and mutagens using the Ames strains TA98 and TA100 with or without S9 (microsomal) activation. The concentrations of fecal secondary bile acids (deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid) and of fecal mutagenic activity in TA98 and TA100 with and without S9 activation were significantly lower during the wheat bran and cellulose supplementation periods. Oat fiber supplementation had no such effect on these fecal constituents. Thus, the increased fiber intake in the form of wheat bran or cellulose may reduce the production and/or excretion of mutagens in the stools and decrease the concentration of fecal secondary bile acids in humans.

INTRODUCTION

Cancer of the colon is one of the major neoplastic diseases that affects 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 a relatively low intake of certain dietary fibers, are of importance in its etiology (4–9). In certain populations the consumption of diets high in total fat and high in dietary fiber, fibrous foods, and certain whole-grain foods has been associated with a reduced risk for colon cancer (7–10). A strong negative association was also reported between regional colon cancer mortality within the United Kingdom and consumption of total dietary fiber (11). Although case-control studies on the relationship between the dietary fiber and colon cancer provided conflicting results (12), a recent case-control study in Melbourne, Australia, indicated that high-fiber foods are associated with a reduced risk for colon cancer (13).

Several studies in laboratory animal models have demonstrated that dietary cellulose and wheat bran inhibit chemically induced colon carcinogenesis (12, 14, 15), whereas corn bran and oat bran have no such inhibitory effect (16, 17), suggesting that the protective effect of dietary fiber depends on the nature and source of fiber in the diet. The discrepancy in human case-control studies on the relationship between dietary fiber and colon cancer risk might, at least in part, be explained on the basis that the comparison of fiber intake between the cases and controls was based on either total dietary fiber intake, vegetable and fruit intake, or fiber-containing foods rather than the types of fiber.

Several studies indicated that the concentrations of fecal secondary bile acids, particularly lithocholic acid and deoxycholic acid, which have been shown to act as promoters for colon cancer (18), were higher in populations who are at high risk for colon cancer and consuming high-fat/low-fiber diets than in populations who are at low risk and consuming high-fat/high-fiber diets (10, 19, 20). With regard to genotoxic compounds (carcinogens) in the colonic or fecal constituents, a number of laboratories demonstrated that the presence of mutagens (presumptive carcinogens) in the stools is higher in populations who are at high risk for colon cancer development and consuming diets high in fat and low in fiber than in low-risk populations (21–25). These studies also suggested that the mutagenic activity of feces of certain donors appears to be due to a type of compound termed as fecapentaene (26–28). Although there is no direct evidence that these fecal mutagens may play a role in human colon cancer, recent studies indicated that the mutagens, which are present in fried foods and also have been identified in the stools of humans, have been shown to induce colon and mammary tumors in laboratory animal models (29–32).

In view of an inverse relationship between dietary whole-grain cereal fiber and colon cancer risk, and the potential significance of fecal mutagens and secondary bile acids in the pathogenesis of colon carcinogenesis, a series of studies on the effect of types of fiber on fecal constituents was conducted in healthy subjects. In one such study, the concentrations of fecal secondary bile acids and fecal mutagenic activity were reduced in healthy subjects given wheat and rye fiber as a supplement (33). The study reported here was designed to investigate the effect of types of fiber and fiber fraction, namely wheat bran, oat fiber, and cellulose, on fecal mutagens and bile acids in healthy subjects consuming high-fat/moderately low-fiber diets.

MATERIALS AND METHODS

Study Population. Our previous studies demonstrated that about 25% of healthy subjects on a Western diet show significant levels of fecal mutagenic activity (25, 34). In order to identify individuals excreting significant levels of mutagens for the diet intervention study, 88 healthy subjects were recruited for the study from the New York metropolitan area. They were contacted initially by letter and telephone. Subsequently they were interviewed by a nutritionist to complete a dietary and medical history questionnaire and to obtain informed consent. Of 88 persons who were interviewed and eligible, 72 subjects (57 women and 15 men) agreed to participate in the study. Informed consent was obtained from all volunteers. The mean age was 36 ± 11 yr, and mean number of yr of schooling was 17.4 ± 2.7. The eligibility criteria also
included the following: (a) they were not on any special diets other than consumed traditionally; (b) they had no history of gastrointestinal diseases, surgical resection of partial or total stomach or intestine; and (c) they had no antibiotic treatment for the 3 wk prior to stool collection.

Twenty-four-h stool samples were collected for 2 days from each volunteer. Individuals who exhibited high fecal mutagenic activity (mutation ratio of ≥3) in at least one strain of the Ames Salmonella system were recruited for the diet intervention study.

Dietary Data Collection. A questionnaire which included background medical history data and dietary practices was administered to each subject. The frequency questionnaire, which contained 62 items, was comprised of food groupings or individual foods. To assess usual dietary intake in the recent past, subjects were asked to report the number of times per day, week, or month that the food items were consumed on the average. Any item consumed less than once a month was considered to be not consumed. The usual portion size consumed was determined by asking subjects to report their typical portions in comparison to standard food measuring utensils or to food models simulating real food.

In order to obtain accurate information on what was eaten before and during the days of stool collection, a 4- to 5-day dietary record was obtained from each participant. Most of the dietary records included 1 or 2 weekend days. This included the recording in weight or in household measurements of all food consumed during that period. Detailed instructions on food measurement were given to each participant, and a dietary scale for weighing foods was provided. Completed food records were carefully reviewed with each participant by a trained nutritionist. Clarification with added descriptive and quantitative information was obtained as needed.

Nutrient Analysis of Food Records. Food records were coded and analyzed for nutrient content by the United States Department of Agriculture nutrient data (35) were used for analysis with added total dietary fiber values from Paul and Southgate (36), Anderson et al. (37), and Lanza and Butrum (38). An independent laboratory analyzed the fiber content of the fiber-supplemented foods, using the Association of Official Analytical Chemists method (38). Those fiber values were added to the database and used in the analysis of food records completed during the study period.

Diet Intervention Study. Of 72 individuals who participated in the mutagen screening study, 34 subjects exhibited significant levels of mutagenic activity in one or more strains of Ames Salmonella typhimurium. Twenty-one subjects who agreed to participate were recruited for the diet intervention study. Two individuals dropped out at the beginning of the study and 19 subjects completed the study. All subjects were interviewed by a nutritionist, and experimental protocols were explained to each participant. A 4-day dietary record was obtained before the diet intervention study, and 24-h stool samples were collected for 2 days from each volunteer. The subjects were randomly divided into three dietary groups and were given about 10 g per day of one of the supplemental dietary fibers (micrograde roasted hard red wheat bran, Williamson’s Better Basics oat fiber, or Solka-floc BW-400 FCC or BW-200 FCC cellulose) in the form of bread, pasta, or muffins, which were prepared by a commercial source. About 126 g per day of fiber-supplemented foods were required to supply 10 g of the supplemental fibers. The volunteers were advised how to incorporate the fiber foods into their normal diet. They were also advised not to change their dietary habits during the study period. At the end of 5 wk on the high-fiber dietary regimen, 24-h stool samples for 2 days and a 4-day dietary record were obtained. A 4-wk period of normal diet without supplemental fiber followed the intervention. At the end of those 4 wk, 24-h stool samples for 2 days and a 4-day dietary record were obtained. This sequence was continued until completion of the three types of dietary fiber regimen. All participants were contacted frequently to discuss possible problems concerning adherence to the diet protocol and acceptance of the fiber-supplemented foods. They exhibited no evidence of metabolic or other disturbances.

Compliance is a great concern in every diet intervention study. In the present study, several steps were taken to increase the compliance to fiber supplementation. The selection of test subjects was based on their genuine willingness to participate in the entire diet intervention program. The participants were not required to change their normal food habits except to consume a high-fiber supplement in addition to their normal diet. In order to monitor the compliance, each subject was interviewed frequently during the study period. The fiber food portions given to the subjects were counted at delivery, and the amount of any remaining fiber food at the end of each fiber period was recorded.

Collection of Stool Specimens. Methods for collection, transportation, and processing of stool specimens have been described previously (24). Fresh fecal samples were collected in sterile plastic cups and placed immediately into a container with dry ice. Specimens were maintained in a frozen state until processing. Individual 24-h fecal specimens collected for 2 days from each volunteer before and at the end of each fiber period were handled and processed similarly. All samples were coded so that the technicians analyzing the samples were unaware of the origin of the specimens. Fecal samples collected for 2 days from each volunteer were pooled and mixed by kneading. An aliquot of 15 g was saved for bile acid and neutral sterol analysis.

The remaining sample was processed for mutagen assay as described (33).

Chemicals and Bacterial Strains. S. typhimurium strains TA98 and TA100 were obtained from the laboratory of Dr. Bruce Ames, University of California, Berkeley, CA. Oxoid nutrient broth was purchased from Oxoid, Columbia, MD; bacto agar, from Difco Labs, Detroit, MI. All media were prepared as described previously (25, 34). All organic solvents used in this study were of highest purity and purchased from Fisher Scientific Co., Pittsburgh, PA. Aroclor 1254 was purchased from Analabs, North Haven, CT, and nitrofluorene, 2-aminofluorene, sodium azide, glucose 6-phosphate, NADP, and ampicillin were from Sigma Chemical Co., St. Louis, MO. Prefilters and filters were from Millipore Corp., Bedford, MA.

Mutagenicity Assay. All samples were lyophilized and assayed individually. Approximately 10 g each of the stool sample were extracted with 3 × 10 ml of hexane:peroxide-free diethyl ether (1:1, v/v). The methods for the extraction of stool specimens for mutagenic activity have been previously described in detail (25, 34). Each fecal extract was assayed for mutagenicity by standard experimental procedures developed by Ames et al. (39) and McCann et al. (40) using S. typhimurium strains TA98 and TA100 with and without S9 activation. Preparation of S9 from Aroclor-induced male Sprague-Dawley rats was as described previously (34). Three dilutions of each fecal extract (0.025- to 0.1-m aliquots of dimethyl sulfoxide solution) equivalent to approximately 50, 100, and 200 mg of dry feces were tested in duplicate for mutagenicity. Each experiment contained positive controls for checking the activity of the S9 system and stability 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. Fecal extracts were considered active only when the mutagenic response was dose related and if the mutagenic ratio, which is the number of histidine-positive revertants in the test plate divided by spontaneous revertant colonies, was equal to or greater than 3.

Fecal Bile Acids and Neutral Sterols. Five-g aliquots of stool specimens in duplicate were used for the analysis of neutral sterols and bile acids by gas chromatographic methods that are routinely used in our laboratory (33, 41). The neutral sterols were analyzed quantitatively as trimethylsilyl derivatives on a hybrid column packed with 5% Carbowax and 5% polyethylene glycol. Neutral sterols were analyzed as trifluoroacetyl derivatives on a 3% QF-1 column.

Statistical Analysis. All laboratory data were analyzed statistically using Student’s t test or Duncan’s multiple-range test following analysis of variance. Dietary intake was analyzed using analysis of variance and Scheffe’s multiple comparison procedure, following univariate analysis. Statistical analyses of the food frequency screening questionnaire were conducted at various levels of sophistication. Initially, all food frequency items were converted to consumption in number of times per month. Descriptive statistics were then compiled to show mean, median, quantities, coefficients of skewness and kurtosis, and standard deviation for each food frequency question. For a variable with marked skewness, the distribution was trichotomized, and the data were
grouped to form a contingency table. $\chi^2$ analysis was used to test for a significant difference in frequency of consumption of foods in the mutagenic compared to the nonmutagenic group.

Food item consumption per month was then converted to a weighted amount by multiplying the number of times consumed monthly by the standard portion sizes and by an adjustment factor signifying the participant’s usual portion size. Univariate analysis was performed on the weighted amount of the variables. Discriminant analysis was carried out to screen the potential discriminatory food categories to identify potential predictors of mutagenicity. Five food types with highest discriminating power were selected for incorporation into a multiple logistic analysis with mutagenicity (positive or negative) as the response variable and food categories as the predictor variables. None of these results showed a significant difference between the two groups.

RESULTS

The diet analysis from a 5-day record from each volunteer indicates that all participants were consuming a mixed-Western diet (Table 1). The diet analysis also indicates that the individuals exhibiting high fecal mutagenic activity were not different from those showing little or no mutagenic activity with respect to major macronutrients, namely fat, protein, carbohydrates, and fiber (Table 1). However, all subjects excreting high levels of mutagens in the feces were consuming less than 10 g of total neutral detergent fiber from cereals and dietary fiber from bread.

Table 2 summarizes the mean values of his° revertants/plate. In general 47% of the total individuals tested showed positive mutagenic activity in their stools (mutation ratio $\geq 3$) in at least one tester system. The percentage of total samples exhibiting positive mutagenic activity in at least one tester system was as follows: TA100 with S9 activation, 19; TA100 without S9 activation, 31; TA98 with S9 activation, 18; and TA98 without S9 activation, 7. Sixty-five percent of positive samples were mutagenic in TA100 without S9 activation, followed by 41% in TA98 with S9 activation, and 31% in TA98 without S9 activation (not shown in the table).

Nutrient analysis of 4-day food records obtained before and at the end of the fiber supplemental period and 2-day stool wet weights recorded during these periods are summarized in Table 3. The dietary intake of total calories, fat, protein, and other micronutrients was not significantly different between the control and fiber supplemental periods. As expected, the dietary intake of total fiber was higher during the supplemental periods than during the control periods. The mean daily fresh stool output was significantly higher during the fiber supplemental periods as compared to their respective control diet periods. However, there were no significant differences in fecal output among the cellulose, oat fiber, and wheat bran supplemental periods.

Table 4 shows the fecal neutral sterol excretion which includes cholesterol and its microbial metabolites, namely coprostanol, coprostanone, cholestanol, and cholestanone. A significant decrease in the concentration of fecal neutral sterols—individual and total—was observed in subjects during the cellulose and wheat bran diet periods when compared to their respective control diet periods. The concentrations of cholesterol, coprostanone, cholestanol, and cholestanone were increased, and the conversion of cholesterol into its microbial metabolites was decreased during the period of oat fiber supplementation compared with its control diet period. Changing from supplemental cellulose and wheat bran diet to control diet (follow-up control diet) increased the concentrations of neutral sterols. Changing from the supplemental oat fiber diet to the control diet decreased the concentrations of cholesterol, coprostanone, and cholestanol and increased the concentration of cholestanone.

The fecal bile acid composition is recorded in Table 5. The primary bile acids which are synthesized in the liver are cholic acid and chenodeoxycholic acid, and their microbial secondary metabolites are deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid. The excretion of secondary bile acids—deoxycholic acid, lithocholic acid, 12-ketolithocholic acid—and total bile acids was lower during the cellulose and wheat bran periods compared with their respective control diet periods. On the other hand, the concentrations of deoxycholic acid, lithocholic acid, and 12-ketolithocholic acid were unaffected during the oat fiber period. Interestingly, the concentration of fecal bile acids was also not affected when the subjects changed from the oat fiber diet to their follow-up control diet. Changing the diets from cellulose and wheat bran to their respective follow-up control diets significantly increased the concentration of deoxycholic acid, lithocholic acid, and total bile acids.

Figs. 1 to 4 summarize the effect of various supplemental fibers on fecal mutagenicity in each (individual) subject. Individuals having a fecal mutation ratio of 3 and above in any one of the testing systems, namely TA98 with or without S9 activation and TA100 with or without S9 activation, were recruited into this study. Of 19 individuals who showed mutagenic activity in at least one testing system, 13, 6, 4, and 5 subjects exhibited mutagenic activity in TA100 without S9 activation (Fig. 1), TA100 with S9 activation (Fig. 2), TA98 without S9 activation (Fig. 3), and TA98 with S9 activation (Fig. 4), respectively. A cut-off point of 30% or greater reduction of mutagenic activity from the control values was arbitrarily considered as inhibition. In TA100 without S9 activation, of 13 subjects, 8 and 12 individuals showed a decreased fecal mutagenic activity during the cellulose and wheat bran periods, respectively, and only one subject showed a reduction in fecal activity.

Table 1: Daily nutrient intake of healthy subjects during screening period

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Males (n = 57)</th>
<th>Females (n = 57)</th>
<th>Males (n = 34)</th>
<th>Females (n = 38)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/day</td>
<td>2,340 ± 509</td>
<td>1,768 ± 354</td>
<td>1,853 ± 373</td>
<td>1,918 ± 516</td>
</tr>
<tr>
<td>Total protein, g</td>
<td>91 ± 25</td>
<td>73 ± 20</td>
<td>73 ± 20</td>
<td>80 ± 24</td>
</tr>
<tr>
<td>Total lipids, g</td>
<td>84 ± 30</td>
<td>67 ± 20</td>
<td>70 ± 21</td>
<td>72 ± 26</td>
</tr>
<tr>
<td>Total carbohydrates, g</td>
<td>295 ± 61</td>
<td>216 ± 50</td>
<td>231 ± 58</td>
<td>234 ± 65</td>
</tr>
<tr>
<td>Total dietary fiber, g</td>
<td>17 ± 9</td>
<td>16 ± 7</td>
<td>17 ± 8</td>
<td>16 ± 7</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>354 ± 134</td>
<td>277 ± 106</td>
<td>278 ± 108</td>
<td>307 ± 123</td>
</tr>
<tr>
<td>Vitamin A, IU</td>
<td>8,870 ± 7,411</td>
<td>11,358 ± 11,546</td>
<td>11,758 ± 12,137</td>
<td>9,943 ± 9,374</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>139 ± 73</td>
<td>141 ± 94</td>
<td>148 ± 105</td>
<td>134 ± 73</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>1,091 ± 353</td>
<td>729 ± 239</td>
<td>800 ± 356</td>
<td>809 ± 250</td>
</tr>
</tbody>
</table>

* Mutagenicity, individuals showing mutation ratio greater than 3 in any tester systems; nonmutagenicity, mutation ratio less than 3 in any tester system.

S Mean + SD.
Table 2: Frequency of mutagenic activity of fecal extracts from healthy individuals consuming a mixed-Western diet

<table>
<thead>
<tr>
<th>S. typhimurium strains</th>
<th>Spontaneous revertants/plate</th>
<th>Active stool samples with mutation ratio &gt;3 (n = 34)a</th>
<th>Inactive stool samples with mutation ratio &lt;3 (n = 38)a</th>
<th>% of total sample showing mutagenic activity in at least tester system</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA98</td>
<td>24 ± 15</td>
<td>141 ± 59 (13)b</td>
<td>30 ± 16</td>
<td>18</td>
</tr>
<tr>
<td>Without S9</td>
<td>21 ± 16</td>
<td>103 ± 49 (51)</td>
<td>29 ± 15</td>
<td>7</td>
</tr>
<tr>
<td>TA100</td>
<td>104 ± 58</td>
<td>409 ± 127 (14)</td>
<td>168 ± 60</td>
<td>19</td>
</tr>
<tr>
<td>Without S9</td>
<td>101 ± 46</td>
<td>495 ± 150 (22)</td>
<td>141 ± 51</td>
<td>31</td>
</tr>
</tbody>
</table>

a Number of fecal samples showing mutagenic activity either in TA98 with or without S9 activation, or in TA100 with or without S9 activation. Mutation ratio is the number of his' revertants on the test plate containing extract from 200 mg of dry feces divided by the number of his' revertants on control plate.

b Numbers in parentheses, number of samples which are active in that particular tester system.

* Mean ± SD.

Table 3: Daily nutrient intake stool output of healthy subjects during the control and supplemental fiber period

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Control diet</th>
<th>Supplemental cellulose diet</th>
<th>Control diet</th>
<th>Supplemental oat fiber diet</th>
<th>Control diet</th>
<th>Supplemental wheat bran diet</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal</td>
<td>1,654 ± 89a</td>
<td>1,894 ± 111</td>
<td>1,685 ± 84</td>
<td>1,977 ± 115</td>
<td>1,653 ± 110</td>
<td>1,971 ± 118</td>
<td>1,679 ± 112</td>
</tr>
<tr>
<td>Total protein, g</td>
<td>78 ± 8</td>
<td>73 ± 5</td>
<td>66 ± 4</td>
<td>75 ± 6</td>
<td>86 ± 6</td>
<td>75 ± 7</td>
<td>64 ± 4</td>
</tr>
<tr>
<td>Total lipids, g</td>
<td>58 ± 4</td>
<td>75 ± 5</td>
<td>61 ± 4</td>
<td>73 ± 6</td>
<td>65 ± 6</td>
<td>76 ± 6</td>
<td>62 ± 6</td>
</tr>
<tr>
<td>Total carbohydrates, g</td>
<td>196 ± 12</td>
<td>240 ± 19</td>
<td>219 ± 15</td>
<td>245 ± 17</td>
<td>200 ± 14</td>
<td>240 ± 17</td>
<td>218 ± 20</td>
</tr>
<tr>
<td>Total dietary fiber, g</td>
<td>18 ± 3</td>
<td>28 ± 3</td>
<td>17 ± 3</td>
<td>26 ± 3</td>
<td>17 ± 3</td>
<td>29 ± 4</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>236 ± 28</td>
<td>239 ± 32</td>
<td>242 ± 25</td>
<td>304 ± 44</td>
<td>329 ± 56</td>
<td>286 ± 48</td>
<td>237 ± 31</td>
</tr>
<tr>
<td>Vitamin A, IU</td>
<td>9,740 ± 2,160</td>
<td>8,622 ± 1,604</td>
<td>12,177 ± 2,262</td>
<td>11,370 ± 2,484</td>
<td>2,031 ± 2,070</td>
<td>12,908 ± 3,440</td>
<td>7,855 ± 1,290</td>
</tr>
<tr>
<td>Vitamin C, mg</td>
<td>144 ± 23</td>
<td>126 ± 20</td>
<td>136 ± 25</td>
<td>153 ± 37</td>
<td>139 ± 38</td>
<td>165 ± 49</td>
<td>123 ± 18</td>
</tr>
<tr>
<td>Calcium, mg</td>
<td>831 ± 70</td>
<td>800 ± 76</td>
<td>752 ± 52</td>
<td>818 ± 75</td>
<td>740 ± 66</td>
<td>821 ± 78</td>
<td>734 ± 64</td>
</tr>
<tr>
<td>Stool weight, g</td>
<td>88 ± 6</td>
<td>138 ± 10c</td>
<td>80 ± 6</td>
<td>132 ± 9c</td>
<td>78 ± 9</td>
<td>124 ± 12c</td>
<td>81 ± 10</td>
</tr>
</tbody>
</table>

Table 4: Neutral sterols in healthy subjects during the control and supplemental fiber periods

<table>
<thead>
<tr>
<th>Neutral sterols</th>
<th>Control diet</th>
<th>Supplemental cellulose diet</th>
<th>Control diet</th>
<th>Supplemental oat fiber diet</th>
<th>Control diet</th>
<th>Supplemental wheat bran diet</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol</td>
<td>3.52 ± 1.1c</td>
<td>3.03 ± 2.2d</td>
<td>3.17 ± 3.1</td>
<td>14.70 ± 4.4</td>
<td>15.42 ± 7.9</td>
<td>10.06 ± 5.6c</td>
<td>14.52 ± 6.3</td>
</tr>
<tr>
<td>Coprostanol</td>
<td>17.29 ± 8.6</td>
<td>10.86 ± 8.1</td>
<td>14.92 ± 5.8</td>
<td>14.70 ± 4.4</td>
<td>15.42 ± 7.9</td>
<td>10.06 ± 5.6c</td>
<td>14.52 ± 6.3</td>
</tr>
<tr>
<td>Coprostanolane</td>
<td>2.61 ± 2.5</td>
<td>1.08 ± 1.7</td>
<td>2.40 ± 1.3</td>
<td>4.04 ± 1.2</td>
<td>2.84 ± 1.7</td>
<td>1.97 ± 2.1c</td>
<td>2.43 ± 1.1c</td>
</tr>
<tr>
<td>Cholesterolane</td>
<td>1.54 ± 0.8</td>
<td>1.14 ± 0.8</td>
<td>1.60 ± 0.4</td>
<td>2.92 ± 0.4c</td>
<td>1.89 ± 0.6</td>
<td>1.25 ± 0.8c</td>
<td>2.15 ± 0.6</td>
</tr>
<tr>
<td>Cholesterolane</td>
<td>1.22 ± 2.0</td>
<td>0.41 ± 0.3</td>
<td>1.02 ± 0.18</td>
<td>1.59 ± 1.4c</td>
<td>3.14 ± 2.2</td>
<td>1.70 ± 1.3c</td>
<td>2.84 ± 2.1</td>
</tr>
<tr>
<td>Total</td>
<td>25.78 ± 11.1</td>
<td>16.52 ± 10.7c</td>
<td>23.11 ± 10.9</td>
<td>30.82 ± 5.1c</td>
<td>27.15 ± 10.4</td>
<td>17.30 ± 7.0c</td>
<td>25.47 ± 11.6</td>
</tr>
</tbody>
</table>

Table 5: Fecal bile acids in healthy subjects during the control and supplemental fiber periods

<table>
<thead>
<tr>
<th>Bile acids</th>
<th>Control diet</th>
<th>Supplemental cellulose diet</th>
<th>Control diet</th>
<th>Supplemental oat fiber diet</th>
<th>Control diet</th>
<th>Supplemental wheat bran diet</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholic</td>
<td>0.21 ± 0.07c</td>
<td>0.12 ± 0.10</td>
<td>0.27 ± 0.16</td>
<td>0.41 ± 0.12</td>
<td>0.39 ± 0.01</td>
<td>0.40 ± 0.10</td>
<td>0.10 ± 0.10</td>
</tr>
<tr>
<td>Chenodeoxycholic</td>
<td>0.42 ± 0.08</td>
<td>0.31 ± 0.08</td>
<td>0.51 ± 0.08</td>
<td>0.29 ± 0.01</td>
<td>0.29 ± 0.03</td>
<td>0.33 ± 0.09</td>
<td>0.30 ± 0.08</td>
</tr>
<tr>
<td>Deoxycholic</td>
<td>3.98 ± 2.4</td>
<td>2.64 ± 2.1c</td>
<td>3.42 ± 2.5</td>
<td>3.24 ± 2.6</td>
<td>3.80 ± 4.0</td>
<td>1.60 ± 0.6c</td>
<td>3.41 ± 2.1</td>
</tr>
<tr>
<td>Lithocholic</td>
<td>3.28 ± 1.8</td>
<td>2.16 ± 0.9c</td>
<td>2.96 ± 2.00</td>
<td>2.46 ± 2.1</td>
<td>2.98 ± 3.2</td>
<td>1.77 ± 0.7c</td>
<td>3.01 ± 2.0</td>
</tr>
<tr>
<td>12-Ketolithocholic</td>
<td>0.46 ± 0.16</td>
<td>0.30 ± 0.09b</td>
<td>0.34 ± 0.02</td>
<td>0.29 ± 0.04</td>
<td>0.40 ± 0.02</td>
<td>0.13 ± 0.01b</td>
<td>0.40 ± 0.14</td>
</tr>
<tr>
<td>Other</td>
<td>2.38 ± 0.88</td>
<td>1.66 ± 2.0</td>
<td>1.59 ± 0.9</td>
<td>1.69 ± 0.8</td>
<td>2.48 ± 2.4</td>
<td>1.80 ± 1.9</td>
<td>2.40 ± 2.0</td>
</tr>
<tr>
<td>Total</td>
<td>10.74 ± 3.3</td>
<td>7.19 ± 3.0c</td>
<td>9.49 ± 6.4</td>
<td>8.38 ± 5.9</td>
<td>10.34 ± 8.4</td>
<td>6.03 ± 5.1c</td>
<td>9.62 ± 7.4</td>
</tr>
</tbody>
</table>

* Mean ± SD (n = 19).

* Significantly different from its control diet period, P < 0.05.

 mutagenic activity during the oat fiber period (Fig. 1). The mutagenic activity in tester strain TA100 with S9 activation was decreased in 4 of 6 individuals during the period of cellulose, in one individual during the oat fiber period, and in 4 subjects during the period of wheat bran supplementation (Fig. 2). In TA98 without S9 activation, cellulose or wheat bran supplementation reduced the mutagenic activity in all 4 subjects, whereas 2 of 4 individuals showed a reduction during the oat fiber period (Fig. 3). Three of 5 subjects exhibited a reduced mutagenic activity in TA98 with S9 activation during the cellulose period, 1 subject during the oat fiber period, and 4 subjects during the wheat bran period (Fig. 4). In general, the fecal mutagenic activity was decreased during the periods of cellulose and wheat bran supplementation compared to their
DIETARY FIBER AND COLONIC MUTAGENS

TA 100 WITHOUT S9

Fig. 1. Mutagenic activity [histidine-positive (His*) revertants/200 mg of dry feces] of fecal extracts from healthy subjects consuming control diet and fiber supplements. 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 TA100 strain without (S9) microsomal activation. Three doses of fecal extracts equivalent to 50, 100, and 200 mg of dry feces in duplicate were used in each test. The spontaneous revertants averaged 124.

TA 98 WITHOUT S9

Fig. 2. Mutagenic activity [histidine-positive (His*) revertants/200 mg of dry feces] of fecal extracts from healthy subjects consuming control diet and fiber supplements. Conditions were as in legend to Fig. 1. The fractions were tested for mutagenicity using S. typhimurium TA100 strain with (S9) microsomal activation. The spontaneous revertants averaged 132.

TA 98 WITH S9

Fig. 3. Mutagenic activity [histidine-positive (His*) revertants/200 mg of dry feces] of fecal extracts from healthy subjects consuming control diet and fiber supplements. Conditions were as in legend to Fig. 1. The fractions were tested for mutagenicity using S. typhimurium TA98 strain without (S9) microsomal activation. The spontaneous revertants averaged 18.

Fig. 4. Mutagenic activity [histidine-positive (His*) revertants/200 mg of dry feces] of fecal extracts from healthy subjects consuming control diet and fiber supplements. Conditions were as in legend to Fig. 1. The fractions were tested for mutagenicity using S. typhimurium TA98 strain with S9. Spontaneous revertants averaged 21.

DISCUSSION

Food frequency questionnaire and diet analysis from the 5-day food record from each volunteer during the screening period indicate that the total fat intake was slightly lower and fiber intake was slightly higher than the recent estimates of the United States diet (42, 43). The subjects excreting significant levels of mutagens were not different from those excreting little or no mutagenic activity with respect to intake of major macronutrients and total dietary fiber, although all individuals excreting high levels of mutagens were consuming diets moderately high in total fat and low in cereal and grain fiber. It is possible that the lack of mutagenic activity in several individuals consuming the diets moderately high in fat and low in fiber may well be due to the presence of the inhibitors of mutagens in the gut, since it has been shown that the colonic contents and fecal samples may contain inhibitors as well as enhancers of mutagens (44, 45) which contribute to overall mutagenic potential of the feces.

respective control diet periods, whereas the oat fiber supplementation had little or no effect.

Table 6 shows the average values of mutagenic activity during the fiber and baseline periods for all study subjects. A significant decrease in the fecal mutagenic activity in TA100 with or without S9 activation and in TA98 with or without S9 activation was observed during the periods of cellulose and wheat bran compared to their respective control (baseline) diet periods, whereas the oat fiber had no significant effect. Changing the diets from cellulose and wheat bran to their baseline follow-up periods significantly increased the fecal mutagenic activity in TA100 without S9 activation and TA98 with or without S9 activation. On the other hand, changing from oat fiber period to its follow-up control diet period had no effect on fecal mutagenic activity.
Previous studies on the dietary alterations of fecal mutagenic activity produced mixed results. For example, studies by Bruce and Dion (46) and Taylor et al. (47) suggest that increased dietary fat had no effect on fecal mutagenic activity (TA100 without S9 activation and on fecapentaenes, respectively). The above studies used organic solvent-extracted fecal fractions for mutagen assay. Another study using a water-extracted fraction showed an increase in fecal mutagenic activity during the period of high-fat intake in healthy subjects (48).

The present study was undertaken to determine the modifying effect of types of fiber, namely wheat bran, oat fiber, and cellulose, on fecal mutagenicity. Our results indicate that the mutagenic activity in all tester strains was decreased during the periods of wheat bran and cellulose supplementation, whereas no measurable inhibition was seen during the period of oat fiber supplementation. The results of this study and our previous study (33) are compatible with the hypothesis that not all dietary fibers reduce colonic mutagenic activity and that their modifying effect depends on the type of fiber consumed.

With regard to the nature of fecal mutagens, the fecapentaenes which are active in TA98 without S9 activation and on fecapentaenes, respectively. The present study was undertaken to determine the modifying effect of various types of dietary fiber in animal models. Dietary fibers may reduce the production and/or excretion of secondary bile acids and neutral sterols, thereby decreasing their concentration in the stool. In the case of oat fiber, there was an insignificant difference in the concentration of stool bile acids between the periods of oat fiber and control diet, but the total bile acid and secondary bile acid excretion per day were increased during the oat fiber period. Also, the concentrations of fecal cholesterol and other neutral sterols as well as the total daily output of neutral sterols were increased during the oat fiber period. One of the mechanisms suggested to explain the increased excretion of bile acids and neutral sterols during the oat fiber period is that it binds the bile acids and neutral sterols, thereby reducing the absorption and increasing the excretion (55). Therefore, the net effect of bile acid and neutral steroid concentration in the colonic contents and their daily output depends on the type of fiber consumed. Attention must be paid to the concentration of secondary bile acids in the colon because of the relationship between increased bile acid concentration and increased susceptibility to colon cancer (18).

In conclusion, the results generated from this and our earlier study (33) on the effect of dietary fiber indicate that certain dietary fibers may reduce the production and/or excretion of fecal mutagens and decrease the concentration of secondary bile acids that seemingly play a role in colon carcinogenesis. The effects of mutagen production and secondary bile acid excretion may depend on the type of fiber consumed. Studies examining the role of various types of dietary fiber in animal models indicate that dietary wheat bran and cellulose showed a protective effect in chemically induced colon carcinogenesis, whereas dietary oat fiber at a 20% level produced a mild enhancing effect (14–17).

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