High-Fat Diets and Fecal Level of Reductase and Colon Mucosal Level of Ornithine Decarboxylase, β-Glucuronidase, 5'-Nucleotidase, ATPase, and Esterase in Mice

Norman J. Temple and Shukri M. El-Khatib

ABSTRACT—In one experiment Swiss mice were maintained on a 16 or 23% fat diet (laboratory chow with added fat, principally corn oil) or on laboratory chow alone (5.5% fat). In another experiment C57BL/1 mice were given a 23% fat diet (as above) or a low-fat diet (57% laboratory chow, 1.5% corn oil, and 31% starch; 5.5% fat). Colon mucosal samples were analyzed for several enzyme activities. In Swiss mice the analyses revealed the following: 1) Ouabain-insensitive ATPase was unaltered in male mice, but it rose significantly in females fed a high-fat diet (this effect was seen when a resuspended high-speed pellet was analyzed but not seen with the initial homogenate); 2) 5'-nucleotidase activity showed a significant stepwise increase with dietary fat; 3) nonspecific esterase activity tended to rise with a high-fat diet (not significant); 4) β-glucuronidase levels were not altered by diet fat; and 5) ornithine decarboxylase levels were not altered by diet fat. In C57BL/1 mice analyses were done on ouabain-insensitive ATPase, 5'-nucleotidase, nonspecific esterase, and β-glucuronidase, but no diet effects were seen. Fecal reductase activity was measured with the use of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride hydrate. A high-fat diet did not affect the activity in C57BL/1 mice, but it caused a significant rise in Swiss mice.—JNCI 1984; 72:679-684.

Cancer of the colon is one of the most common cancers in Westernized countries. Its epidemiology strongly points to environmental factors as being almost entirely responsible (1) and to diet in particular (2). A high intake of dietary fat is a prime suspect, but evidence on this is still unclear (2-5).

The most plausible route by which the diet could determine colon cancer incidence is by its effect on fecal composition, e.g., the level of initiators and promoters of carcinogenesis (6, 7). The interactions between diet, fecal flora, fecal enzyme levels, and the chemical composition of feces are extremely complex (2, 8). However, within this general area many clues may be found concerning the etiology of the disease. For this reason we investigated fecal reductase activity with the use of INT, a synthetic electron acceptor, to explore the possibility that a high-fat diet influences this crucial area of metabolism.

Diet also affects the colon mucosa, which is, of course, to be expected if diet determines cancer risk in that organ. We believe studies of diet-induced changes in colon mucosal biochemistry to be highly relevant to this problem. For instance, Wargovich and Felkner (9) recently reported that dietary corn oil raised the activity in rat colon microsomes for the conversion of DMH (CAS: 540-73-8) to a mutagen.

It is well established that the tissue level of ODC is closely associated with the process of carcinogenesis (10, 11). Apparently, this is also true for the rat colon. In the colon, ODC is induced by bile acids (12, 13), some of which are thought to be promoters of colon carcinogenesis (2), and also by complete carcinogens (13-15). We now report our observations concerning the effect of a high-fat diet on the level of ODC in mouse colon mucosa.

We also studied enzymes associated with the plasma membrane and endoplasmic reticulum to explore the possibility that a high-fat diet enhances colon cancer risk partly by affecting membrane function. Another enzyme studied was β-glucuronidase. This enzyme may hydrolyze glucuronide conjugates of toxins excreted by the liver and may therefore reactivate carcinogens. For instance, in rat colon mucosa the enzyme is induced by an injection of DMH (16), a carcinogen that is excreted in the bile after metabolism to a glucuronide derivative (17).

MATERIALS AND METHODS

Diets and environmental conditions.—The formulation of diets is shown in table 1. Mice were fed ad libitum. They were housed in a temperature-controlled room with a 12-hour light-dark cycle.

Mice.—Swiss and C57BL/1 mice were used. Details concerning the mice are indicated in tables 2-5. All mice were from colonies maintained in our animal house.

Preparation of samples.—Mice were killed by cervical dislocation and were analyzed individually. For each of tables 2-5, mice were killed over 11-19 days in batches of usually 2 mice per diet group on each occasion. The feces was collected from the entire length of the colon and rectum. It was cooled on ice and mixed, and a sample was homogenized in a Potter-Elvehjem homogenizer (30 mg/ml of 100 mM sodium phosphate, pH 7.0). The homogenate was centrifuged.

Abbreviations used: DMH=1,2-dimethylhydrazine; INT=2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride hydrate; ODC=ornithine decarboxylase; Pi=inorganic phosphate; TCA=trichloroacetic acid.
TABLE I.—Composition of diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Control</th>
<th>F16b</th>
<th>F23c</th>
<th>F6d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chowb</td>
<td>100</td>
<td>89</td>
<td>81</td>
<td>67</td>
</tr>
<tr>
<td>Corn oilc</td>
<td>10.7</td>
<td>18.5</td>
<td>1.9</td>
<td>0.16</td>
</tr>
<tr>
<td>Oleic acidc</td>
<td>0.16</td>
<td>0.28</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Starchc</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat contentf</td>
<td>5.5</td>
<td>16</td>
<td>23</td>
<td>5.5</td>
</tr>
<tr>
<td>Nutrient/caloriesf</td>
<td>100</td>
<td>75</td>
<td>61</td>
<td>61</td>
</tr>
</tbody>
</table>

*The diets were prepared approximately once per week by mixing the ingredients with water until a soft consistency was obtained. They were stored at 4°C.

bRodent Laboratory Chow (Ralston Purina Co., St. Louis, Mo.).
c“Maxola” (Best Foods, San Juan, Puerto Rico).

E. Escolar

Calculated on a dry weight basis.

Relative content of vitamins, minerals, protein, and fiber per 100 calories.

(1,500Xg for 2 min at 4°C). The supernatant was adjusted to 0.1% Triton X-100 (to release bacterial enzymes) and was centrifuged again (24,000Xg for 11 min at 4°C). The final supernatant was used for analysis.

Part of the homogenate was centrifuged (130,000Xg for 44 min at 4°C), and the pellet was resuspended by homogenization medium as above. The resuspended pellet contained all of the particulate matter of the homogenate. All samples were stored in an ice bath, and assays were completed within 11 hours of sample preparation.

**INT reductase.**—Reductase activity of the fecal supernatant sample was measured with the use of INT as electron acceptor. INT can accept electrons from FADH2 but not from NAD(P)H. Incubations were in a final volume of 0.5 ml containing 0.2 ml supernatant sample (or homogenization medium for controls), 2.9 mM INT (Aldrich Chemical Co., Milwaukee, Wis.), and 170 mM glycine-NaOH (pH 9.5). After the mixture was incubated for 30 minutes at 37°C, the reaction was stopped by addition of 2.25 ml stopping mixture (56 mM glycine-NaOH in 1.1% TCA), giving a final pH of approximately 10.4. The tubes were then centrifuged (1,500Xg for 10 min), and the extinctions were measured at 550 nm.

**Esterase (naphthyl acetate hydrolase).**—Non-specific esterase was assayed by the measurement of 1-naphthol production from 1-naphthyl acetate in samples of initial homogenate (23). Incubations were for 30 minutes at 37°C in 30 mM barbitone sodium-HCl buffer (pH 7.4), which contained 0.45 mM 1-naphthyl acetate. ODc.—ODc was measured by the estimation of the [1H]putrescine production from [1H]ornithine on the basis of the procedure of Djurhuus (24). Colon mucosal homogenate was prepared as described above except that the homogenization medium was 50 mM sodium phosphate (pH 7.2), containing 0.1 mM pyridoxal 5-phosphate and 0.1 mM EDTA. Supernatant was used for analysis after centrifugation (90,000Xg for 10 min at 4°C).

In a volume of 112 µl was contained 87 µl supernatant sample (or homogenization medium for controls) plus 0.55 mM 1,4-diisothiothreitol, 0.22 mM pyridoxal 5-phosphate, and 50 mM sodium phosphate (pH 7.2). After equilibration for 10 minutes at 37°C, 11 µl of 1 mM L-[5-3H]ornithine (14 Ci/mol) (Schwarz/Mann, Spring Valley, N.Y.) was added. After incubation for 30 minutes at 37°C, the tubes were cooled to 0°C and 90 µl incubation mixture was transferred to P81 paper (Whatman Inc., Clifton, N.J.). The uptake of 3H]putrescine was measured by the estimation of the [3H]putrescine production from [3H]ornithine on the basis of the procedure of Djurhuus (24). Colon mucosal homogenate was prepared as described above except that the homogenization medium was 50 mM sodium phosphate (pH 7.2), containing 0.1 mM pyridoxal 5-phosphate and 0.1 mM EDTA. Supernatant was used for analysis after centrifugation (90,000Xg for 10 min at 4°C).

**β-Glucuronidase.**—Samples of initial homogenate were assayed by a procedure adapted from Reddy et al. (22). The incubation volume (0.5 ml) contained 0.25 ml sample (or homogenization medium for controls), 0.05% Triton X-100, 1 mM phenolphthalein glucuronide, and 80 mM sodium acetate (pH 4.5). After the mixture was incubated for 3 hours at 37°C, the incubation was terminated by addition of 2.25 ml stopping mixture (56 mM glycine-NaOH in 1.1% TCA), giving a final pH of approximately 10.4. The tubes were then centrifuged (1,500Xg for 10 min), and the extinctions were measured at 550 nm.

**β-Glucuronidase.**—Samples of initial homogenate were assayed by a procedure adapted from Reddy et al. (22). The incubation volume (0.5 ml) contained 0.25 ml sample (or homogenization medium for controls), 0.05% Triton X-100, 1 mM phenolphthalein glucuronide, and 80 mM sodium acetate (pH 4.5). After the mixture was incubated for 3 hours at 37°C, the incubation was terminated by addition of 2.25 ml stopping mixture (56 mM glycine-NaOH in 1.1% TCA), giving a final pH of approximately 10.4. The tubes were then centrifuged (1,500Xg for 10 min), and the extinctions were measured at 550 nm.

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**Ouabain-insensitive ATPase.**—The assay was as above except that all incubations included ouabain. Control incubations contained homogenization medium in place of enzyme sample (resuspended pellet or initial homogenate as indicated).

**5'-Nucleotidase.**—The assay procedure was as described by Murer et al. (21). The incubation volume of 0.5 ml contained 4 mM MgCl2, 4 mM 5'- or 3'-AMP, 50 mM Tris-HCl (pH 7.4), and 0.25 ml resuspended pellet. After the mixture was incubated at 37°C for 1 hour, the reaction was stopped and P1 determined as above. 5'-Nucleotidase corresponds to 5'-AMP hydrolysis minus 3'-AMP hydrolysis.

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TABLE 2.—Effect of diet on colon mucosal enzyme activities of female Swiss mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet period 1</th>
<th>Diet period 2</th>
<th>No. of mice</th>
<th>Ouabain-insensitive ATPase&lt;sup&gt;c&lt;/sup&gt;</th>
<th>5'-Nucleotidase&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Esterase&lt;sup&gt;6&lt;/sup&gt;</th>
<th>β-Glucuronidase&lt;sup&gt;6&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resuspended pellet</td>
<td>Homogenate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
<td>8</td>
<td>7.90±1.73</td>
<td>6.7±1.85</td>
<td>0.38±0.139</td>
<td>74±15.5</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>F16</td>
<td>8</td>
<td>9.45±5.27</td>
<td>7.1±1.88</td>
<td>0.51±0.221</td>
<td>94±35.4</td>
</tr>
<tr>
<td>3</td>
<td>F16</td>
<td>F16</td>
<td>8</td>
<td>13.01±4.83</td>
<td>6.9±1.80</td>
<td>0.57±0.234</td>
<td>81±15.4</td>
</tr>
<tr>
<td>4</td>
<td>F16</td>
<td>F23</td>
<td>7</td>
<td>15.16±5.23</td>
<td>7.3±1.35</td>
<td>0.85±0.453</td>
<td>95±43.8</td>
</tr>
</tbody>
</table>

<sup>c</sup>Diet consumed by mice for 7.5 mo after weaning. The same diet had been fed to the parents of the mice since weaning. Control and F16 diets contain 5.5 and 16% fat, respectively. See table 1.
<sup>d</sup>Diet consumed for 5 mo before sacrifice commencing at end of period 1. Control, F16, and F23 diets contain 5.5, 16, and 23% fat, respectively. See table 1.
<sup>e</sup>Values are means ± SD. Units are μmol/hr/mg protein. Results show no significant difference unless indicated.

GROUPS SIGNIFICANTLY DIFFERENT: 1 vs. 3 and 1 vs. 4 (both P<.02); 1 vs. 3, 4 pooled (P<.01); 1 vs. 2-4 pooled (P<.05). No significant trend was found with the use of one-way analysis of variance.

GROUPS SIGNIFICANTLY DIFFERENT: 1 vs. 4 (P<.02); 2, 3 pooled vs. 4 (P<.05). A significant trend is indicated (P<.05) by one-way analysis of variance.

TABLE 3.—Effect of diet on colon mucosal enzyme activities of male Swiss mice

<table>
<thead>
<tr>
<th>Diet period 1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Diet period 2&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of mice</th>
<th>Ouabain-insensitive ATPase&lt;sup&gt;c&lt;/sup&gt;</th>
<th>5'-Nucleotidase&lt;sup&gt;5&lt;/sup&gt;</th>
<th>Esterase&lt;sup&gt;6&lt;/sup&gt;</th>
<th>β-Glucuronidase&lt;sup&gt;6&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resuspended pellet</td>
<td>Homogenate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>11</td>
<td>15.59±5.94</td>
<td>9.26±2.95</td>
<td>57.1±14.5</td>
<td>0.62±0.224</td>
</tr>
<tr>
<td>Control</td>
<td>F16</td>
<td>10</td>
<td>16.77±5.62</td>
<td>9.32±2.79</td>
<td>53±21.5</td>
<td>0.58±0.162</td>
</tr>
<tr>
<td>F16</td>
<td>F16</td>
<td>10</td>
<td>14.91±6.09</td>
<td>9.8±3.10</td>
<td>46±19.9</td>
<td>0.73±0.130</td>
</tr>
<tr>
<td>F16</td>
<td>F23</td>
<td>9</td>
<td>16.2±5.96</td>
<td>8.9±1.86</td>
<td>45±19.2</td>
<td>0.60±0.131</td>
</tr>
</tbody>
</table>

<sup>a</sup>Diet histories as in table 2 except that after weaning diet period 1 lasted 0.5 mo followed by diet period 2, which lasted 8 mo.
<sup>b</sup>Values are means ± SD. Units are μmol/hr/mg protein. Results show no significant difference.

RESULTS

Studies on Na<sup>+</sup>,K<sup>+</sup>-ATPase and 5'-Nucleotidase

Our initial interest was Na<sup>+</sup>,K<sup>+</sup>-ATPase. Repeated attempts to accurately analyze this enzyme in mouse colon mucosa samples were unsuccessful. The problem was caused by the high level of ouabain-insensitive ATPase relative to the level of Na<sup>+</sup>,K<sup>+</sup>-ATPase. With Swiss mice ouabain-sensitive activity generally represented under 10% of the total ATPase activity. This percentage was found to be slightly higher in C57BL/1 mice.

We tested sodium dodecyl sulfate (26, 27), Lubrol PX (28), Triton X-100 (23), repeated freezing and thawing, an altered incubation time, and the addition of 1,4-dithiothreitol to the homogenization medium, but these modifications proved of no value. Therefore, in our diet studies analyses were directed at ouabain-insensitive ATPase.

5'-Nucleotidase was found to be assayable with far greater sensitivity than was possible for Na<sup>+</sup>,K<sup>+</sup>-ATPase because in resuspended pellet samples, as used here, 5'-nucleotidase represents a much higher proportion of the total (5'-AMPase) activity (mean: 54% in C57BL/1 mice and 71% in Swiss mice). Resuspended pellet rather than initial homogenate was used for routine analysis due to the presence of an enzyme activity that hydrolyzes 3'-AMP at a faster rate than 5'-AMP and which is removed in the high-speed supernatant (29).

Colon Enzyme Levels and Diet

Ouabain-insensitive ATPase activity in a high-speed pellet of colon mucosa from female Swiss mice rose with a high-fat diet (table 2). This was seen in the 2 groups where high-fat diets had been fed for 2 generations (groups 3 and 4) but not where such a diet had only been fed for a much shorter time period (group 2). It is possible, of course, that this was merely a chance observation. No such response was seen in male Swiss mice (table 3). When the initial homogenate was analyzed for ouabain-insensitive ATPase, no diet effect...
TABLE 5.—Effect of diet on colon mucosal and fecal enzyme activities of C57BL/1 mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet period 1</th>
<th>Diet period 2</th>
<th>INT reductase&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Female&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Male&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>Control</td>
<td>35.8±18.4</td>
<td>42.3±19.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>F16</td>
<td>64.0±26.0</td>
<td>59.7±17.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>F16</td>
<td>F16</td>
<td>50.4±12.9</td>
<td>60.2±30.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>F16</td>
<td>F23</td>
<td>57.6±26.4</td>
<td>51.6±16.2</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Diet histories and numbers of mice as in table 2 (female) and table 3 (male).

<sup>b</sup>Values are means ± SD. Units are nmol/hr/mg feces. (Quantity of feces refers to original feces from which the sample was extracted.)

<sup>c</sup>Results show no significant difference with the use of a 2-t-test. Significant trends were found with two-way analysis of variance for both diets (<.01) and batch variation (<.0005); see text.

<sup>d</sup>Groups significantly different: 1 vs. 2 (P<.05); 1 vs. 4 (P=.01); 1 vs. 2-4 pooled (P<.02).

was seen in either sex (tables 2, 3). In female Swiss mice mucosal 5'-nucleotidase activity appeared to manifest a dose-response relationship with diet fat (table 2). Thus, in comparison to the activity in mice fed a control diet, the activity was higher by about 42% in mice fed diet F16 (not significant) and by 123% in mice fed diet F23 (P<.02). In both male and female Swiss mice mucosal esterase activity was a little higher in mice fed diets F16 and F23 compared with the activity in mice fed the control diet (tables 2, 3). The differences were not significant. No diet-induced alterations were apparent for mucosal β-glucuronidase (tables 2, 3).

In Swiss mice of both sexes high-fat groups had a rise in fecal INT reductase of about 40-80% in comparison to the level of fecal INT reductase in mice fed the control diet (table 4). In female mice but not in males the activity fell in each diet group by roughly 50-68% between the first and last batches (see "Preparation of samples," "Materials and Methods"). The cause of this is unknown, but it may be related to the fact that females were studied at a different time of the year than males. As a result, a t-test revealed a significant trend in males only. The data for female mice were reanalyzed by two-way analysis of variance, and this analysis indicated significant differences for both diet and batch variation.

TABLE 6.—Effect of diet on ODC activity of colon mucosa<sup>e</sup>

<table>
<thead>
<tr>
<th>Diet period 1</th>
<th>Diet period 2</th>
<th>No. of mice</th>
<th>ODC&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Control</td>
<td>6</td>
<td>133.1±48.4</td>
</tr>
<tr>
<td>F16</td>
<td>F16</td>
<td>7</td>
<td>129.1±56.8</td>
</tr>
<tr>
<td>F16</td>
<td>F23</td>
<td>6</td>
<td>122.4±37.3</td>
</tr>
</tbody>
</table>

<sup>e</sup>Mice are all Swiss females.

<sup>f</sup>Diet histories as in table 2 except that diet period 2 lasted 4.5 mo.

Values are means ± SD. Units are pmol/30 min/mg protein. Results show no significant difference.

The above enzyme activities were also studied in C57BL/1 mice, but no significant effects of a high-fat diet were observed (table 5). In female Swiss mice colon mucosal ODC activity did not respond to diet fat (table 6).

DISCUSSION

The studies on Swiss mice used a different experimental strategy to those on C57BL/1 mice. The parents of most Swiss mice fed a high-fat diet also had been maintained on such a diet. The parents of all C57BL/1 mice, however, were fed only a control diet.

With Swiss mice the control (low-fat) diet differed from the high-fat diets not only in its fat level but also in its (higher) content of nutrients. Inasmuch as diets F16 and F23 were prepared by simple addition of fat to the control diet, this procedure reduced the content of vitamins, minerals, protein, and fiber per 100 calories to 75% (diet F16) or to 61% (diet F23) of the level in the control diet. This experimental design does not test fat as an independent variable, but it does resemble the human situation in Westernized countries where the high fat content will tend to be at the expense of other nutrients.

In the case of C57BL/1 mice, the low-fat diet (diet F6) resembled diet F23 in all respects except its fat and starch contents. These various dietary differences are reflected in the quantity of feces obtained. With C57BL/1 mice similar quantities of feces were present in the 2 diet groups. However, with Swiss mice only

TABLE 7.—Effect of diet on colon mucosal and fecal enzyme activities of C57BL/1 mice

<table>
<thead>
<tr>
<th>Diet&lt;sup&gt;e&lt;/sup&gt;</th>
<th>Sex</th>
<th>No. of mice</th>
<th>Ouabain-insensitive ATPase&lt;sup&gt;b&lt;/sup&gt;</th>
<th>5'-Nucleotidase&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Esterase&lt;sup&gt;d&lt;/sup&gt;</th>
<th>β-Glucuronidase&lt;sup&gt;e&lt;/sup&gt;</th>
<th>INT reductase&lt;sup&gt;f&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>F6</td>
<td>♂</td>
<td>7</td>
<td>11.64±2.19</td>
<td>11.65±2.99</td>
<td>0.253±0.066</td>
<td>54.8±19.5</td>
<td>0.201±0.082</td>
</tr>
<tr>
<td>F23</td>
<td>♂</td>
<td>8</td>
<td>12.11±2.03</td>
<td>12.17±2.93</td>
<td>0.328±0.115</td>
<td>65.5±23.1</td>
<td>0.228±0.079</td>
</tr>
<tr>
<td>F6</td>
<td>♂</td>
<td>11</td>
<td>12.53±2.36</td>
<td>7.91±0.96</td>
<td>0.318±0.170</td>
<td>74.1±15.1</td>
<td>0.768±0.480</td>
</tr>
<tr>
<td>F23</td>
<td>♂</td>
<td>10</td>
<td>12.43±2.97</td>
<td>8.15±2.10</td>
<td>0.363±0.186</td>
<td>54.0±19.7</td>
<td>0.838±0.492</td>
</tr>
</tbody>
</table>

<sup>e</sup>Mice received a control diet until 4 mo after weaning. The experimental diet was then fed for 7.5 mo. Diets F6 and F23 contain 5.5 and 25% fat, respectively.

<sup>b</sup>Values are means ± SD. Units are μmol/hr/mg protein (mucosal) or nmol/hr/mg feces (INT reductase). Groups of the same sex show no significant difference.

<sup>c</sup>Mice received a control diet until 4 mo after weaning. The experimental diet was then fed for 7.5 mo. Diets F6 and F23 contain 5.5 and 25% fat, respectively.

<sup>d</sup>Values are means ± SD. Units are μmol/hr/mg protein (mucosal) or nmol/hr/mg feces (INT reductase). Groups of the same sex show no significant difference.

<sup>f</sup>Mice received a control diet until 4 mo after weaning. The experimental diet was then fed for 7.5 mo. Diets F6 and F23 contain 5.5 and 25% fat, respectively.

<sup>g</sup>Values are means ± SD. Units are μmol/hr/mg protein (mucosal) or nmol/hr/mg feces (INT reductase). Groups of the same sex show no significant difference.

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about half as much feces was present with diet F16 and F23 than with the control diet (data not shown). For the above reasons (and also differences in the diet histories of the animals as indicated in tables 2-5), results with the two strains are not directly comparable. It is also noteworthy that the high-fat diets induced obesity in Swiss mice but not in C57BL/1 mice (Temple NJ, El-Khatib SM: Unpublished data).

We were not able to detect any significant alteration in colon mucosal ODC level in female Swiss mice. This finding contrasts with a stimulation of between 5- and 35-fold in rat colon after bile acid application (12, 13). Possibly, dietary fat produces a weak enhancement of ODC activity, which, nevertheless, is of biologic importance over a prolonged time period. The assay for ODC in virtually all other laboratories is based on the determination of \(^{14}\)CO\(_2\) release from \([1-^{14}\)C]ornithine. In reality, some of this activity may be a measure of the combined effects of enzymes other than ODC (24). The assay used here measures the conversion of \([^{3}]\)H-ornithine to \([^{3}]\)Hputrescine.

In the guinea pig small intestinal mucosa an endoplasmic reticulum localization has been demonstrated for nonspecific esterase (with the use of the same assay procedure as that used here) (30). In mouse colon mucosa, however, we found appreciable, but highly variable, quantities in the high-speed supernatant (5.7X10\(^{-6}\) g/min) (data not shown). Its precise localization in this tissue is therefore unclear. In Swiss mice, but not in C57BL/1 mice, a trend was observed for the high-fat groups to show a raised activity of the esterase, but this did not achieve statistical significance.

We failed to observe any changes in mucosal \(\beta\)-glucuronidase activity resulting from a high-fat diet. Reddy et al. (22) reported no effect on the \(\beta\)-glucuronidase activity of rat colon mucosa after feeding diets rich in beef protein and beef fat or soybean protein and corn oil (added at the expense of starch). However, these diets did increase the level of the enzyme in the rat small intestinal mucosa.

As part of our studies of changes in colon mucosa membranes induced by a high-fat diet, we attempted to measure the level of Na\(^+\),K\(^+\)-ATPase. Unfortunately, this proved impossible to perform accurately, because the proportion of the total ATPase activity that is ouabain-sensitive is about 10%, which is similar to that observed in rat colon mucosa (31) but is markedly different from that reported in other tissues. In rat brain, for instance, 40-80% of the total ATPase is ouabain-sensitive (32). Perchmin P: Personal communication.

With respect to the level of mucosal ouabain-insensitive ATPase, no significant effects of a high-fat diet were seen except in female Swiss mice where the activity was elevated in 2 groups fed a fat-rich diet. This elevated activity was seen with resuspended pellet samples but not with homogenate. The relationship of this elevation to carcinogenesis is unknown as is also whether the apparent sex effect is genuine or spurious. Ouabain-insensitive ATPase is probably mainly due to nonspecific phosphatases in lysosomes and brush borders.

With Swiss mice mucosal 5'-nucleotidase activity manifested a stepwise increase with the level of dietary fat. No dietary effect was observed with C57BL/1 mice. Possibly, this strain difference results from the experimental design as discussed above. If, however, it is genuine, it may reflect changes in the feces or may be related to the fact that a high-fat diet induces obesity in Swiss mice but not in C57BL/1 mice (Temple NJ, El-Khatib SM: Unpublished data).

Swiss mice have a much greater susceptibility to DMH-induced colon tumors than do C57BL/1 mice. This phenomenon has been found both by ourselves with the colonies of mice used here (Temple NJ, El-Khatib SM: Unpublished data) and by Evans et al. (33). This finding may be related to the apparently different responses of colon mucosa 5'-nucleotidase activity of the two strains to a high-fat diet.

In another investigation we studied the colon mucosa membranes of Swiss mice by performing density gradient centrifugation (isopyknic banding). Fractions were analyzed for \(5\)-nucleotidase. The enzyme is apparently divided between the basolateral plasma membrane and the brush border. It was observed that diets F16 and F23 significantly reduce the median density of the brush borders (29). Thus diets F16 and F23, which in some respects resemble human diets associated with a high colon cancer incidence, cause changes in the colon mucosa and at least one of its associated enzymes. We consider this area of diet-colon interaction worthy of further investigation.

In Swiss mice, but not in C57BL/1 mice, a high-fat diet resulted in a raised enzyme activity for fecal INT reductase. This possible strain difference is noteworthy in view of the above-mentioned strain difference in susceptibility to colon tumors. We do not know the significance of the altered INT reductase level. Possibly, it is associated with the ratio of anaerobic to aerobic bacteria. Hill et al. (8) reported that a high ratio was often found in human populations where colon cancer is common. Fecal INT reductase should be studied in both experimental animals and humans to clarify its possible relationship to colon carcinogenesis.

REFERENCES


