Modulation of Experimental Colon Tumorigenesis by Types and Amounts of Dietary Fatty Acids

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ABSTRACT

Epidemiological studies and laboratory animal model assays suggest that a high intake of dietary fat promotes colorectal cancer. Several in vivo and in vitro studies support the hypothesis that ω-6 fatty acids promote colon tumorigenesis, whereas ω-3 fatty acids lack promoting activity. Fat intake in the United States traditionally includes high amounts (30% of total caloric intake) of saturated fat rather than ω-6 fatty acids. Therefore, the present study was designed to compare the modulatory effects of a high-fat diet containing mixed lipids (HFML), a diet rich in saturated fatty acids (the average American diet), a diet with fish oil (HFFO) that is rich in ω-3 fatty acids, and a low-fat corn oil diet (LFCO) on the formation of chemically induced colon aberrant crypt foci (ACF) and tumors, cyclooxygenase (COX)-2 activity, and apoptosis during experimental colon carcinogenesis. At 5 weeks of age, groups of male F344 rats were fed a 5% corn oil diet (LFCO), at 7 weeks of age, rats intended for carcinogen treatment received s.c. injections of azoxymethane at a dose level of 15 mg/kg of body weight once weekly for 2 weeks. Beginning 1 day after the carcinoma treatment, groups of rats were then maintained on experimental diets containing 20% HFML or 20% HFFO. Rats were killed at 8, 23, or 38 weeks after azoxymethane treatment. Colonic ACF and tumors were evaluated histopathologically, and apoptosis was evaluated by the terminal deoxynucleotidyl transferase-mediated nick end labeling method. Colonic mucosa and tumor samples harvested at week 38 were analyzed for COX-2 synthetic activity and expression. The rats fed the HFML diet showed significantly increased total colonic ACF (P < 0.001-0.0001) with a multiplicity of ≥4 aberrant crypts/focus (P < 0.0001) compared with the effects of the HFFO or LFCO diets at week 8, 23, and 38. Interestingly, there was a 2- to 3-fold increase (≥4) in multicrypt foci in rats given the HFML diet as compared with such foci in rats fed the HFFO or LFCO diets. By week 23, the HFML diet had significantly increased the incidence of colonic tumors (30–60%) and their multiplicity (100–141%) when compared with the effects of the LFCO or HFFO diets. At week 38, the HFML diet had induced 100% colon tumor incidence and a 4-fold multiplicity of adenocarcinomas compared with the LFCO and HFFO diets. At weeks 23 and 38, a significantly lower percentage of apoptotic colonic epithelial cells were observed in the tumors of animals fed the HFML diet as compared with those fed the HFFO diet. The HFML diet caused significantly increased levels of COX-2 activity in colon tumors (P < 0.05–0.01), and these tumors had enhanced levels of COX-2 expression as compared with those in assays with LFCO or HFFO diets. These observations demonstrate for the first time that HFML diets containing high levels of saturated fatty acids (such as those in Western diets) promote colon carcinogenesis. Although the mechanisms involved in colon tumor promotion by a HFML diet are not fully known, our results indicate that the modulation of eicosanoid production via the influence on COX activity and the suppression of apoptosis may play a key role in HFML diet-induced colon tumorigenesis.

INTRODUCTION

Cancer of the large bowel is one of the leading causes of cancer deaths in both men and women in Western countries, including the United States where about 150,000 new cases of colorectal cancer and 56,000 related deaths are reported for the year 2000 (1). Diet, especially fat intake, has been regarded as the most important nutritional influence on colon cancer development (2, 3). On the basis of comparative data and case control studies in Japan and the United State in the late 1960s, Wynder et al. (4) suggested that dietary factors in general, and dietary fat in particular, play a role in the etiology of colon cancer. Since then, several epidemiological studies provided evidence for an association between the intake of dietary fat and total calories and an increased risk for colon cancer (2, 3, 5, 6). Evidence for an association between the intake of saturated fat and/or animal fat and colon cancer risk is very strong (2, 3, 5, 7, 8). An ecological study suggests that mortality data for colorectal cancer in 22 European countries, the United States, and Canada, correlate with the consumption of animal fat (9). Several case-control studies, if not all, found that a higher intake of these types of fat increases the risk of colorectal cancer development (2, 7, 10, 11). That eating a diet with high polysaturated fat content (rich in ω-3 fatty acids) may decrease the risk of colorectal cancer has been hypothesized in relation to fish and fish oil (9). Caygill et al. (12) reported an inverse correlation between fish and fish oil consumption and colorectal cancer. On the basis of epidemiological evidence from ecological and case-control studies, it is reasonable to suggest that diets high in total fat, and especially in saturated fat, increase the risk of colorectal cancers, whereas diets high in fish and fish oil reduce it.

Laboratory animal model studies in our laboratory and elsewhere have consistently provided evidence to link the colon tumor-promoting effect of dietary fat depend on both the type and amount of dietary fat (13–17). Diets rich in ω-3 fatty acids (marine oils) reduce the risk of chemically induced colon carcinogenesis compared with diets high in ω-6 fatty acids and/or saturated fatty acids. This suggests that the composition of ingested dietary fatty acids is more critical to colon cancer risk than is the total amount of fat (18–20). In addition, laboratory animal model assays have indicated that the influence of type and amount of dietary fat is exerted foremost during the postinitiation phase of carcinogenesis (15, 21). In a Phase II clinical trial of patients with colon polyps, dietary fish oil supplements have in fact inhibited cell proliferation in the colonic mucosa (22). In general, the overall evidence from studies with laboratory animals is consistent with the epidemiological data.

Thus far, progress has been made with regard to the relationship between dietary fat intake and colon cancer risk, in that we know of the tumor-promoting effects of diets rich in ω-6 fatty acids and saturated fatty acids, and the lack of such effects by ω-3 fatty acid-rich diets (23–29). However, among the sources of dietary fat, animal fat with its high saturated fatty acid content is by far the most important contributor (>60%) to the Western diet. Importantly, consumption of dietary fat in the United States and Canada consists predominately of a mixture of saturated, monounsaturated, and polyunsaturated fats, whereby the ratio of polyunsaturated to saturated fat is 0.55 (7, 30). Despite the high dietary intake of mixed lipids in Western populations, not much attention has been placed on understanding the role of such mixed-lipid diets in colon tumor promotion. A recent assay in mice demonstrated that administration of a high-fat diet simulating the...
mixed-lipid composition of the average American diet produces dysplastic lesions in the colon, indicative of tumorigenesis (31).

With regard to the mode of action of saturated fats, ω-6 PUFAs3, and ω-3 PUFAs in colon tumorigenesis, several studies indicate that diets high in lard, beef tallow, or corn oil increase the concentration of colonic luminal secondary bile acids, whereas dietary fish oil at high concentrations had no such enhancing effect (23–28). Secondary bile acids have been shown to induce cell proliferation and to act as promoters in colon carcinogenesis (32). Additionally, the high intake of dietary fat, specifically of saturated fats and ω-6 fatty acids, increases colon tumor promotion by altering membrane phospholipid turnover, releasing membrane AA from phospholipids, and affecting prostaglandin synthesis via COX enzyme (27, 28). It is noteworthy that elevated levels of COX isomers, particularly COX-2, have been observed in human colon tumors and in chemically induced colon tumors in rodents (33–35). This indicates the significance of COX enzymes in colon tumor growth. Tsuji and DuBois (36) have shown that overexpression of the COX-2 gene in colon epithelial cells leads to altered adhesion properties and resistance to apoptosis. Recent studies suggest that the regulation of apoptosis is central to tumor promotion and neoplasms (31).

ACF, which are recognized as early preneoplastic lesions, have been consistently observed in experimentally induced colon carcinogenesis in laboratory animals (45). Pretlow et al. (46), who demonstrated the occurrence of these lesions in the colonic mucosa of patients with colon cancer, suggested that aberrant crypts are putative precursor lesions from which adenoma and carcinoma develop in the colon. There is also evidence that several inhibitors of ACF development reduce colon tumorigenesis in laboratory animals (47).

In view of the significance of mixed lipids in colon carcinogenesis, and because of potential tumor-inhibitor properties of ω-3 fatty acids, the present study was designed to examine the effects of high-fat diets that contain mixed lipids rich in saturated fatty acids and to compare them with the effects of fish oil (rich in ω-3 fatty acids) during the different stages of AOM-induced colon carcinogenesis in male F344 rats. Colonic ACF and colon tumors serve as end points. In addition, we assessed the effects of these diets on colonic tumor cell apoptosis. We examined AA metabolism and expression of COX-isoforms to provide an understanding of the effects of these types of dietary fats on the modulation of morphological, cellular, and molecular events relevant to colon carcinogenesis.

**MATERIALS AND METHODS**

**Materials.** 14C- AA was bought from Amersham (Arlington Heights, IL). AOM was purchased from Ash Stevens (Detroit, MI). Precoated Silica G plastic TLC plates were purchased from Fisher Scientific Co. (Springfield, NJ), and Amberlite XAD-2, 50–70 mesh, was purchased from V. W. R. Scientific Co. (Piscataway, NJ). Weanling male F344 rats were bought from Charles River Breeding Laboratories (Kingston, NY). Fish oil was donated by the Menhaden Oil Refinery of Zapata Protein, Inc. (Reedville, VA). The ingredients of semipurified diets including mixed lipids were obtained from Dyets, Inc. (Bethlehem, PA).

**Animals and Diets.** A total of 360 male F344 rats receiving at weaning were quarantined for 7 days and then randomly assigned to one of three dietary groups (LFCO, HFFO, or HFML) of 120 animals each. Each dietary group was then divided into AOM-treated and vehicle-treated subgroups. The rats were housed three to a plastic cage with filter top in a holding room that was maintained under controlled conditions (21°C and 50% relative humidity) in a 12-h light/dark cycle. All rats were fed ad libitum, with fresh food replenished every day.

The composition of the experimental diets (Table 1) was based on a modified American Institute of Nutrition-76A diet (18). The composition of the experimental diets was adjusted so that all diets would offer the same amount of calories, protein, vitamins, minerals, and fiber (18). As indicated in Table 1, LFCO contained 5% corn oil, and HFFO contained 17% fish oil and 3% corn oil. In the HFML diet, 20% fat content was formulated using a slight modification of the American Blend Fat developed by the Institute of Shortening and Edible Oils (30). The HFML diet was formulated to simulate the fat content of the American diet with mixed lipids derived from beef tallow (16%), lard (10%), butter fat (12%), hydrogenated soy bean oil (30%), peanut oil (5%), and corn oil (27%). The diets were prepared in our laboratory three times weekly and stored under nitrogen in airtight containers in a cold room at 4°C. Aliquots of the experimental diets were analyzed for their fatty acid composition. As expected, the LFCO diet contained a high percentage of ω-6 fatty acids such as linoleic acid, whereas the HFFO diet contained high levels of ω-3 fatty acids such as docosahexaenoic acid and eicosapentaenoic acid. The ω-6 fatty acid content of the HFML diet was 8%; the ω-3 fatty acid content of HFFO diet was 32%. The level of oleic acid (ω-9) in the HFML diet was 16%, whereas the total saturated fatty acid content was 29%. HFML diets contained ω-3, 24, and 28% of saturated, monounsaturated (ω-7) and polyunsaturated (ω-6) fatty acids, respectively.

**Experimental Procedure.** The experimental design is summarized in Fig. 1. Beginning at 5 weeks of age, all rats were fed the modified American Institute of Nutrition-76A (LFCO) diet. At 7 weeks of age, the animals intended for carcinogen treatment were given s.c. injections of AOM or saline once weekly for 2 weeks at a dose rate of 15 mg/kg body weight, whereas those intended for the vehicle-treatment were given equal volumes of physiological saline. One day after the AOM or saline injection, groups of animals designated for the HFFO or HFML diets were fed their respective high-fat diets containing fish oil and mixed lipids, whereas one group continued on the LFCO diet. All rats were weighed twice monthly until termination of the assay. Food consumption was monitored at two time points for a period of 2 weeks. During the course of the study, groups of rats were killed by CO2 asphyxiation at weeks 8, 23, and 38 after the last AOM or saline treatment. After laparotomy, the entire stomach, small intestine, and large intestine were resected. The organs were opened longitudinally, and the contents were flushed with normal saline. Rats sacrificed at weeks 23 and 38 were examined for intestinal tumors and the location, number, and size of the tumors were assessed with a dissection microscope and recorded. Colon tumors with a diameter of ≥0.5 cm were cut into halves; one portion of the tumor was analyzed for apoptosis and the location, number, and size of the tumors were assessed with a dissection microscope and recorded. Colon tumors with a diameter of ≥0.5 cm were cut into halves; one portion of the tumor was analyzed for apoptosis and the location, number, and size of the tumors were assessed with a dissection microscope and recorded.

**Table 1 Composition of experimental diets**

<table>
<thead>
<tr>
<th>Diet ingredients</th>
<th>LFCO</th>
<th>HFML</th>
<th>HFFO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>20</td>
<td>23.5</td>
<td>23.5</td>
</tr>
<tr>
<td>DL-methionine</td>
<td>0.3</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>Corn starch</td>
<td>52</td>
<td>35.7</td>
<td>35.7</td>
</tr>
<tr>
<td>Dextrose</td>
<td>13.2</td>
<td>9.02</td>
<td>9.02</td>
</tr>
<tr>
<td>Alphacel</td>
<td>5</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Mixed lipid</td>
<td></td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Fish oil</td>
<td></td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Mineral mix</td>
<td>3.5</td>
<td>4.11</td>
<td>4.11</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.18</td>
<td>1.18</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.24</td>
<td>0.24</td>
</tr>
</tbody>
</table>

Table 1 Composition of experimental diets.

* Mixed lipid contains 16% beef tallow, 10% lard, 12% butter fat, 30% hydrogenated soybean oil, 27% corn oil, and 5% peanut oil.

3 The abbreviations used are: PUFA, polyunsaturated fatty acids; AA, arachidonic acid; COX, cyclooxygenase; AOM, azoxymethane; ACF, aberrant crypt foci; LFCO, low-fat corn oil diet; HFFO, high-fat corn oil; HFML, high-fat mixed-lipid diet; Tdt, terminal deoxynucleotidyl transferase.
mediated by COX. For enzyme assays, the colons were rapidly removed and rinsed in ice-cold normal saline. They were slit open longitudinally, freed from all contents, and cleaned with ice-cold normal saline. They were laid flat on a glass plate, and the tumor-free colon mucosa was scraped off with a microscope glass slide. The mucosal scrapings and portions of the tumors were quickly frozen in liquid nitrogen and stored at −80°C until analyses.

**ACF Analysis.** For the ACF analysis, the rats were killed by CO2 asphyxiation and the colons were removed from each of the 12 rats/group at each time point. The colons were flushed with Krebs Ringer solution, opened from cecum to anus, and fixed flat between two pieces of filter paper in 10% buffered formalin for ACF analysis. After a minimum of 24 h in buffered formalin, the colons were cut into 2-cm segments, starting at the anus; for the next 5–10 min they were placed in a Petri dish containing 0.2% methylene blue in Krebs-Ringer solution. They were then placed mucosal side up on a microscope slide and observed through a light microscope. ACF were counted and recorded according to standard procedures that are being used routinely in our laboratory (48). Aberrant crypts were distinguished from the surrounding normal crypts by their increased size, the significantly increased distance from lamina to basal surface of cells, and the easily discernible pericryptal zone. The parameters used to assess the aberrant crypts were their occurrence and multiplicity. Crypt multiplicity was determined as the number of crypts in each focus. They were categorized as containing up to four or more aberrant crypts/ focus. All colons were scored by one observer without knowing the identity of the agents under study; scores were checked at random by a second observer.

**Intestinal Tumors.** For histopathological evaluation, intestinal tumors harvested at week 23 (24 rats/group) and week 38 (36 rats/group) were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine histological procedures with H&E staining. The stained sections were examined for tumor types according to the classification of Pozharisski (48), which is followed routinely in our laboratory. Most of the colon tumors obtained at week 38 were invasive or noninvasive adenocarcinomas. The invasive adenocarcinomas were mostly the signet ring mucinous type, invading muscularis mucosa deep into the intestinal wall and beyond. The noninvasive adenocarcinomas were those growing outward toward the intestinal lumen and not invading the muscularis mucosa. They were usually well differentiated adenocarcinomas.

**Apoptosis.** Six-μm sections were cut from colon samples collected at various time points and mounted on slides, rehydrated, and stained using the Tdt-mediated nick end labeling method for the detection of apoptosis (49). Briefly, slides were incubated with 3% H2O2 in PBS for 5 min, rinsed, and then incubated in Tdt buffer [140 mM cacodylate (pH 7.2), 30 mM Tris, and 1 mM CoCl2] for 15 min at room temperature. Tdt reaction mixture [0.2 unit/μl Tdt, 2 μM biotin-11-dUTP, 100 mM cacodylate, 2.5 mM CoCl2, 0.1 mM DTT, and 0.05 mg/ml BSA] was added and incubated for 30 min at 37°C. After blocking with 2% BSA and incubation with avidin-biotin peroxidase complexes, the Tdt-mediated nick end labeling reaction was visualized by chromogenic staining with 3,3′-diaminobenzidine. Slides were counterstained by hematoxylin. Stained crypt epithelial cells were counted manually in a single-blind fashion.

**COX Activity.** Because COX enzyme is firmly bound to the luminal surface of the endoplasmic reticulum and nuclear envelope, the particulate fractions of the colonic mucosa and tumor samples were prepared as described previously (50). COX activities in colonic samples were assayed by using a slight modification of methods published previously (28). Protein was determined with Bio-Rad protein assay reagents (Bio-Rad Laboratories, Richmond, CA) with BSA as the standard. Analysis of COX activity was as follows. Briefly, 150 μl of the reaction mixture containing 12 μM 14C-AA (420,000 dpm), 1 mM epinephrine, 1 mM glutathione in 50 mM of phosphate buffer (pH 7.4), and 50–70 μg of microsomal protein were incubated at 37°C for 20 min, and the reaction was terminated by adding 40 μl of 0.2 M HCL. The COX-mediated metabolites of AA were extracted with ethyl acetate (3 × 0.5 ml). The combined extracts were evaporated to dryness under N2, redissolved in chloroform, and subjected to TLC on precoated TLC plastic plates. The TLC plates were developed with a solvent system containing chloroform/methanol/acetic acid/water (100/15/1.25/1, v/v/v/v) and were exposed in an iodide chamber for 5 min to visualize the standards. The metabolites of 14C-AA-derived individual eicosanoids were detected by their comigration with authentic standards. The area of each metabolite was determined in a Bioscan System 200 image scanning counter (Bioscan, Inc., Washington, DC) equipped with β-detector.

**Western Blot Analyses of COX-2.** COX-2 purified protein, which was purchased from Cayman Chemicals, was used as the electrophoresis standard. The protein was separated on 8% PAGE-gel and then electroplated on polyvinylidene difluoride membranes as described (35, 50). The method of analysis of COX-2 was as described previously (51). After blocking membranes in 5% nonfat dry milk, they were incubated with antibody to COX-2 for 1 h. The membranes were washed three times and incubated once more with secondary horseradish peroxidase-linked antirabbit IgG antibody at a final concentration of 1:2000. The membranes were developed by the enhanced chemiluminescence system and exposed to Kodak XAR5 film.
RESULTS

General Observations. As expected, the body weights of animals fed the LFCO diet were significantly lower (P < 0.01) when compared with the HFFO diet group (Fig. 2). The HFML diet did not produce any significant body weight change (P > 0.05) compared with those fed the HFFO diet (P < 0.05–0.01). Interestingly, food consumption between the LFCO, HFFO, and HFML diets was 18.0, 16.05, and 18.5 grams/day/rat, respectively. On the basis of food consumption and body weight gain, data suggest that rats in the HFML group consume a greater amount of food than those in the LFCO or HFFO groups. In vehicle-treated animals, feeding of the HFFO and HFML diets did not induce any gross changes in liver, kidney, stomach, intestine, or lungs, nor did it cause any histopathological changes in the liver or intestine that was attributable to toxicity.

ACF. ACF were assessed at weeks 8, 23, and 38 using a minimum of 12 rats/group at each time point. The rats treated with saline and fed the LFCO, HFFO, and HFML diets showed no evidence of ACF formation in the colon (data not shown). ACF were predominantly observed in the distal colons of carcinogen-treated rats at all time points. End points used in this study were the occurrence of total ACF as well as the number of multicrypt clusters (four or more) of aberrant crypts (Figs. 3 and 4). Rats treated with carcinogen and fed the HFML diet showed a significantly greater (P < 0.0001; 77%) number of total ACF/colon compared with those fed the LFCO or HFFO diet at weeks 8 and 23; at week 38 there was a significant increase in total ACF (P < 0.01) in the rats fed the HFML diet as compared with those fed the LFCO or HFFO diet (Fig. 3). The incidence of multicrypt (≥4) aberrant foci was significantly higher in the HFML diet group than in the HFFO or LFMC diet groups at weeks 8, 23, and 38 (P < 0.0001; Fig. 4). Also, the total ACF and multicrypt focal lesions were significantly increased from week 8 to week 23 in all dietary groups (P < 0.0001).

Table 2 Effect of types and amount of dietary fat on AOM-induced colon tumor incidence during different stages of carcinogenesis

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Tumor incidence&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adenocarcinomas&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 wk</td>
<td>38 wk</td>
</tr>
<tr>
<td>LFCO</td>
<td>50</td>
<td>63</td>
</tr>
<tr>
<td>HFML</td>
<td>80&lt;sup&gt;c&lt;/sup&gt;</td>
<td>100&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>HFFO</td>
<td>50</td>
<td>69</td>
</tr>
</tbody>
</table>

<sup>a</sup> Includes adenomas and adenocarcinomas.
<sup>b</sup> Includes noninvasive and invasive adenocarcinomas.
<sup>c</sup> Significantly different from LFCO and HFFO diet groups by Fisher’s exact probability test; P < 0.01.
<sup>d</sup> Significantly different from LFMC and HFFO diet groups by Fisher’s exact probability test; P < 0.0001.
<sup>e</sup> Significantly different from LFCO diet group by Fisher’s exact probability test; P < 0.02.
<sup>f</sup> Significantly different from LFCO and HFFO diet groups by Fisher’s exact probability test; P < 0.0001.
<sup>g</sup> Significantly different from LFCO and HFFO diet groups by Fisher’s exact probability test; P < 0.0002.

Tumor Incidence and Multiplicity. The results summarized in Tables 2 and 3 indicate that at week 23, animals fed the HFML diet showed a significantly higher colon tumor incidence (P < 0.05; Table 2) and multiplicity (P < 0.01; 2-fold increase; Table 3) when compared with those fed the LFCO or HFFO diet. At week 38, the incidences and multiplicities of colon tumors were also significantly higher in rats fed the HFML diet when compared with the results for rats fed either the LFCO or the HFFO diet. Importantly, rats fed the HFML diet showed 100% incidence of adenocarcinomas compared...
with incidences of 63% and 69% in rats fed the LFCO and HFFO diets, respectively. Moreover, the multiplicity of adenocarcinomas was significantly higher (P < 0.0001) in animals fed the HFML diet (an about 4-fold increase) when compared with the results for rats fed the LFCO and HFFO diets. However, there were no significant differences in the incidences and multiplicities of colon tumors between rats fed the LFCO or HFFO diets. Another important observation is that rats fed the HFML diet showed ~97% tumor incidence in the colon and only ~3% in the small intestine, whereas those maintained on the LFCO or HFFO diet showed ~80% tumors in the colon and 20% in the small intestine (data not shown).

Apoptosis. Apoptotic rates in colonic epithelium and tumors harvested at weeks 23 and 38 are summarized in Fig. 5. Diets containing LFCO, HFFO, or HFML had no significant effect (P > 0.05) on apoptotic rates in colonic epithelial cells at all time points (data not shown). However, colonic tumors harvested at weeks 23 and 38 from rats fed the HFML diet showed a nearly 50% lower apoptotic index (P < 0.001) than was observed in rats fed either the LFCO or the HFFO diets. This observation is consistent with previous studies from our laboratory and elsewhere that have shown that dietary fat influences tumor apoptosis in male F344 rats.

COX Activity and Expression. We investigated whether the increase in colon tumor incidence seen after the HFML diet is associated with the modulation of AA metabolism by COX enzyme. Administration of the HFML diet produced 472 ± 33 pmol (mean ± SE) of AA metabolites (eicosanoids), significantly higher levels of eicosanoids from AA compared with the LFCO diet in rat colon tumors harvested at week 38. To assess the modulatory role of these diets on COX-2 expression, we determined the enzyme expression in the particulate fractions of colon tumor extracts by immunoblot analysis. A representative immunoblot analysis of COX-2 expression in the colon tumors of rats fed the HFML, HFFO, or LFCO diets is shown in Fig. 6. The HFML diet clearly enhanced the expression of COX-2 in colon tumors as compared with COX-2 expression with the HFFO or LFCO diets.

DISCUSSION

The present study is part of a large-scale preclinical investigation of the effects of types and amounts of dietary fat in colon carcinogenesis. It evaluated the colon tumor-promoting effect of mixed lipids. Previous studies from our laboratory and elsewhere have concluded that the colon tumor-promoting effect of dietary fat depends on the amount of ω-6 PUFAs and saturated fatty acids, whereas, in contrast, ω-3 PUFAs (DHA, EPA) and ω-9 monounsaturated fatty acids lack colon tumor-promoting effects (14, 15, 17–21, 51). However, these studies traditionally used a single source of fat as a variable in investigating the role of dietary fat in colon carcinogenesis. Thus, these studies do not reflect the types of fat that are normally consumed in the United States and other Western countries where the risk for colorectal cancer development is high. In the current study, we have formulated the high-fat diet (HFML) so as to simulate the types of fat most often consumed in the United States. We have tested this diet for its colon tumor-promoting effect.

The data presented here demonstrate that compared with the LF CO or HFFO diets, administration of a HFML diet significantly augmented the AOM-induced colonic preneoplastic lesions, ACF. Even at early stages of colon carcinogenesis (at week 8) the HFML diet significantly enhanced not only the total number of ACF, but also affected multicrypt foci containing 4 or more AC/foci in the colon. Recent studies suggest that there is a high degree of correlation between the number of multicrypt AC foci formed and the outcome of colonic tumors at the later stages (45, 52). Thus, the present study shows that administration of the HFML diet significantly promotes the formation and growth of preneoplastic lesions in the colon.

**Table 3** Effect of types and amount of dietary fat on AOM-induced colon tumor multiplicity during different stages of carcinogenesis

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>Total tumor multiplicity (No. of colonic tumors/rat)</th>
<th>Adenomas</th>
<th>Noninvasive</th>
<th>Invasive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>23 wk</td>
<td>38 wk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LFCO</td>
<td>0.75 ± 0.12a</td>
<td>1.31 ± 0.22</td>
<td>0.13 ± 0.07</td>
<td>0.05 ± 0.17</td>
<td>0.23 ± 0.09</td>
</tr>
<tr>
<td>HFML</td>
<td>1.50 ± 0.20b</td>
<td>5.14 ± 0.34</td>
<td>0.44 ± 0.10b</td>
<td>3.80 ± 0.33bc</td>
<td>0.90 ± 0.14d</td>
</tr>
<tr>
<td>HFFO</td>
<td>0.62 ± 0.10</td>
<td>1.67 ± 0.26</td>
<td>0.23 ± 0.08</td>
<td>1.15 ± 0.24</td>
<td>0.28 ± 0.08</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

a. Includes adenomas and adenocarcinomas.

b. Includes noninvasive and invasive adenocarcinomas.

*Significantly different from LFCO diet group by Student t-test; P < 0.001.

**Fig. 5.** Modulatory effect of LFCO, HFFO, and HFML diets on AOM-induced colon tumor multiplicity during different stages of carcinogenesis.

**Fig. 6.** Modulatory effect of LFCO, HFFO, and HFML diets on AOM-induced colon tumor COX-2 expression in male F344 rats.
whereas the HFFO diet, which is rich in ω-3 fatty acids, had no such enhancing effect. Also, long-term feeding of the HFML diet for 23 and 38 weeks significantly increased the percentage of multicrypt foci. This indicates that, in addition to the amount and type of dietary fat, the duration of exposure to these fats is important. There were no previous studies enabling any comparison of the present results regarding the modulatory effect of HFML on colonic ACF. However, studies in various laboratories with the role of an HFML diet in experimental colon tumorigenesis support the results of our present study in that ω-3 fatty acid-rich diets inhibited the formation of colonic ACF (18–21, 53). It is not yet clearly understood why only very few colonic ACF will ultimately be transformed into tumors. The data from this study suggest that, unlike the LFCO or HFFO diet, a HFML diet significantly increases the conversion of ACF into tumors. Understanding the exact mechanisms involved in the transformation of colonic ACF into tumors in general, and in the presence of a HFML diet or other tumor-promoting agents, is of great interest.

The present study also demonstrates that the colon tumor-promoting effect of the HFML diet is nearly the same as that of the LFCO diet. This supports our previous investigations in which we used diets high in fish oil or low in fat (16, 18, 21). The results of the present study also demonstrate for the first time that dietary HFML strongly promotes colon carcinogenesis in a well-established colon carcinogenesis animal model. It is noteworthy that here we observed a 4-fold increase in colon adenocarcinomas in rats fed the HFML diet, compared with those fed the LFCO diet. Equally important, the HFFO diet containing 20% fat (mostly in the form of fish oil) induced fewer colon tumors than the HFML diet containing the same amount of fat mostly from mixed lipids. This reinforces that both the type and the amount of fatty acids in the diet play a critical role in colon carcinogenesis. Another important observation in the present study is that feeding of the HFML diet induced intestinal tumors mainly in the colon (97%) and a negligible number of tumors in the small intestine when compared with the effects of the HFLO and LFCO diets. This observation is very important because it reflects observations on intestinal cancer in humans where the occurrence of colonic tumors is very high in comparison with tumors in the small intestine. The precise reason for a predominance of tumors of the colon in rats fed the HFML diet is uncertain, but it is believed to be the result of promotional factors in the diet.

Several reports indicate that apoptosis has a role in the pathogenesis of colon cancer (38–42). We have observed in this investigation that feeding rats a HFML diet significantly suppresses colonic tumor apoptosis compared with feeding the LFCO or HFFO diets. Our previous studies have indicated that administration of a HFCO diet significantly suppresses apoptosis in AOM-induced colon tumors compared with a LFCO diet (44). Recently, Latham et al. (54) showed that a fish oil diet given to Wister rats significantly enhances colonic crypt apoptosis, and that it reduces carcinogen-induced colonic ACF formation. Several studies also have demonstrated that resistance to apoptosis will lead to the development of colorectal cancer (40, 53). Induction of apoptosis by a HFFO diet, as observed in the present study, can lead to diminished tumor incidence in the colon of rats. This suggests that inhibition of apoptosis contributes to increased tumor development. It is not clear how the HFML diet induces the suppression of apoptosis that leads to the high colon-tumor promotion. One speculative hypothesis is that the administration of a HFML diet modulates the levels of the colonic luminal secondary bile acids, which have been shown to promote colon carcinogenesis and suppresses apoptosis. Magnuson et al. (55) have reported that administration of bile acids leads to enhanced resistance to apoptosis in mice. It is possible that the tumor promoting effect of HFML diets is in part mediated through the bile acid-induced inhibition of apoptosis.

The present study also demonstrates that a HFML diet enhances AOM-induced expression of COX-2 and eicosanoid formation from AA, whereas the ω-3 PUFA diets in the HFFO diet inhibit the levels of COX-2. Feeding a HFML diet also significantly increases eicosanoid formation from AA via COX enzyme activity. Tsuji and DuBois (36), who have implicated COX-2 activity in the regulation of apoptosis of rat intestinal epithelial cells, have shown that overexpression of COX-2 can lead to the suppression of apoptosis. Results of our present study, in which the HFML diet elevated COX-2 expression and inhibited apoptosis and the consequent tumor burden, support this contention. Additional in-depth studies on the mechanism of action of HFML on colon tumor promotion are warranted to gain a clear understanding of the colon tumor-promoting effects of diets high in saturated fats.

In conclusion, the results of this investigation show for the first time that consuming a Western-style mixed-lipid diet (HFML) has a 4-fold higher potential to promote colon tumorigenesis than ingestion of a diet with an equivalent amount of fat containing fish oil (HFFO) or low-fat corn oil (LFCO). This observation makes a strong case for using the mixed-lipid diet (HFML), which reflects the American dietary pattern, in a broad range of preclinical efficacy studies involving chemopreventive agents. In addition, the HFML diet suppresses apoptosis and increases COX-2 activity and expression in colon tumors compared with the LFCO or HFFO diets. The exact mechanism by which the HFML diet promotes colon carcinogenesis has yet to be defined; even so, it would appear that the modulation of AA metabolism through COX activity plays a role in the suppression of apoptosis, leading to the enhancement of colonic ACF and tumors.

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