Effect of Restricted Caloric Intake on Azoxymethane-induced Colon Tumor Incidence in Male F344 Rats

Bandaru S. Reddy, Chung-Xiou Wang, and Hiroshi Maruyama

ABSTRACT

The effect of 30% caloric restriction on azoxymethane (AOM)-induced colon carcinogenesis was investigated in male F344 rats. Starting at 5 weeks of age, groups of animals were fed ad libitum a high-fat (23.5%) semipurified diet. At 7 weeks of age, all animals except the vehicle-treated groups were s.c. injected with AOM (15 mg/kg body wt, once weekly for 2 weeks). Four days after the second AOM injection, groups of animals were continued on high-fat diet and fed ad libitum (ad libitum group) whereas other groups were restricted to 70% of total calories (calorie-restricted group) consumed by the ad libitum group, but received same amounts of fiber, vitamins, and minerals. Thirty-two weeks after AOM injections, all animals were necropsied. The animals in the caloric-restricted group developed significantly fewer colon tumors and had a lower colon tumor incidence than did the rats in the ad libitum group. The size of colon tumors was also reduced in the caloric-restricted group.

INTRODUCTION

The question of whether the effect of dietary fat on carcinogenesis is due to specific action of fat or to an associated caloric effect has been raised several times and is a subject of recent discussion (1, 2). The concept of a relationship between caloric intake and carcinogenesis is more than 40 years old. The demonstration from actuarial records that overweight or obese men had a higher cancer mortality than normal or underweight men led to pioneering studies by Tannenbaum and colleagues (3-5) which indicated that caloric restriction inhibits spontaneous and chemically-induced mammary tumors and benzo(a)pyrene-induced skin tumors in mice. It has been shown that fewer tumors are observed in rats when growth is retarded by caloric deprivation (6).

Recent studies on the relationship between caloric intake and carcinogenesis indicate that high fat and high caloric intakes increase the rate of mammary carcinogenesis in rats induced by 7,12-dimethylbenz(a)anthracene or MNU (7-9). Another study suggests that mammary tumor appearance does not depend on the amount of fat in the diet per se, but rather on a complex interaction involving energy intake, energy retention, and body size (10).

The epidemiological evidence supporting total caloric intake as a risk factor for cancer and especially to cancer of the colon is slight and largely indirect. The results of a long-term prospective study indicated an evaluation of mortality due to cancer of the colon and rectum in men 40% or more overweight (11). Because this study did not provide any information on caloric intake, it was not possible to evaluate the relative importance of being overweight in comparison with total caloric intake or intake of other nutrients. A recent case-control study in Canada demonstrated a direct association between colorectal cancer and caloric intake, but not as strong as the association for intake of saturated fat (12). Another case-control study in England suggested that high energy diets rich in sugar and fat are associated with the development of colorectal cancer in humans (13).

With respect to the effect of diet restriction on colon carcinogenesis in animal models, Pollard and Luckert (14) demonstrated that MAM acetate-induced colon tumors, but not MNU-induced colon tumors are inhibited in rats given a 25% restricted chow diet. In another study a low-fat semipurified diet severely restricted in calories by 40% showed an inhibition of DMH-induced colon tumors in rats (7).

In this study, we investigated the effect of a high-fat semipurified diet fed ad libitum and of calorie-restricted diet on AOM-induced colon carcinogenesis in rats. The rationale for the selection of a high-fat diet in this study was to simulate the western diet which is high in total fat. The calorie-restricted diet was formulated so that animals on this diet consume the same amount of minerals, vitamins, and nonnutritive fiber, but consume 70% calories, as do the ad libitum-fed counterparts.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. Inbred male F344 rats were obtained as weanlings from Charles River Breeding Laboratories, Wilmington, MA. All semipurified dietary ingredients were purchased from Dyets, Inc., Bethlehem, PA, and AOM (CAS: 25843-45-2) was from Ash-Stevens, Inc., Detroit, MI.

A total of 84 male rats received at weaning were quarantined for 10 days and then randomly distributed by weight into two dietary groups of 42 animals each. Each dietary group was divided into AOM-treated (30 animals) and vehicle-treated (12 animals) subgroups and housed individually in a room maintained under controlled environmental conditions of a 12-h light-dark cycle, 50% humidity, and 22°C. Starting at 5 weeks of age, animals allotted to both dietary groups were fed a high-fat diet ad libitum and had free access to water.

The composition of experimental semipurified diets (high-fat ad libitum and 30% calorie restricted) is shown in Table 1. The composition of 30% calorie-restricted diet (70% of total calories of ad libitum diet) was adjusted so that the animals in the high-fat ad libitum group and 30% calorie-restricted group would consume the same amount of minerals, vitamins, and nonnutritive fiber. All diets were prepared in our laboratory once weekly and stored in a cold room.

Experimental Procedure. Beginning at 5 weeks of age, all animals in each dietary group were fed the high-fat diet ad libitum and continued on this diet until 4 days after carcinogen or vehicle treatment. Starting at the age of 7 weeks, animals intended for carcinogen treatment were treated s.c. with AOM for 2 weeks with a dose level of 15 mg/kg body weight, once weekly, whereas the animals intended for vehicle treatment were given an equal volume of normal saline. Before injection, AOM was dissolved in normal saline. Four days after the second injection of AOM or normal saline, all animals in the calorie-restricted group were pair-fed the calorie-restricted diet (Table 1). Animals intended for ad libitum feeding were continued on high-fat ad libitum diet. All animals were fed the experimental diets until termination of the experiment at
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32 weeks postcarcinogen treatment. Body weights were determined weekly until the animals reached 16 weeks of age and then every 2 weeks until termination of the experiment.

Both AOM- and vehicle-treated animals were sacrificed by CO₂ euthanasia as scheduled. At necropsy, all organs including intestines were examined grossly under the dissection microscope for the presence of tumors, which were confirmed by histological examination. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections were then stained with hematoxylin and eosin and examined histologically.

The data were analyzed statistically by the χ² method and Student's t test to identify differences among dietary treatments.

RESULTS

The mean daily food intake for the ad libitum group and calorie-restricted group was 13.9 and 10.1 g, respectively (Table 1). The caloric intake was 66.7 per day in the ad libitum group and 46.7 in the calorie-restricted group. The calorie-restricted group consumed about 21.3 calories from fat, 9.5 calories from protein, and 15.9 calories from carbohydrate, whereas the ad libitum group consumed about 29.4 calories from fat, 13.2 calories from protein, and 24.2 calories from carbohydrate. As expected, body weights of animals fed the calorie-restricted diets were lower than those fed the ad libitum diet in both AOM- and vehicle-treated groups throughout the study (Fig. 1).

Table 2 summarizes the AOM-induced colon tumor incidence and multiplicity in animals fed the experimental diets. There was no evidence of presence of tumors in vehicle-treated animals. The incidence (percentage of animals with colon tumors) of colon adenomas and adenocarcinomas and colon tumor multiplicity (tumors/animal and tumors/tumor-bearing animal) were significantly lower in animals fed the calorie-restricted diet. Feeding of calorie-restricted diet had no significant effect on small intestinal and ear duct tumor incidences. Histologically, adenocarcinomas of the colon and small intestine were well-differentiated frank malignant tumors that invaded across the line of muscularis mucosae. Adenomas of the colon were benign with mild or moderate epithelial atypia. Ear duct tumors were squamous cell carcinomas.

Fig. 2 summarizes the colon tumor size (diameter in centimeters). The size of colon neoplasms ranged from 0.2 to 1.5 cm in ad libitum diet group, and from 0.2 to 0.5 cm in calorie-restricted diet group. The number of tumors with size ranging from 0.2 to 0.5 cm was higher in the ad libitum diet group than in the calorie-restricted diet group. None of the animals in the calorie-restricted diet group showed colon tumors with size ranging from 0.6 to 1.5 cm.

DISCUSSION

Recent studies demonstrated that male Sprague-Dawley rats fed 25% restricted gain-based, low-fat diet had fewer MAM-induced colon tumors, but not MNU-induced colon tumors than did the animals fed the diet ad libitum, suggesting that the metabolic activation of MAM was interrupted in the rats on the restricted dietary regimen (14). Kritchevsky et al. (7) reported that the incidence of DMH-induced colon tumors was inhibited in rats fed a 40% calorie-restricted low-fat diet, although the calorie-restricted rats ingested twice as much fat as ad libitum-fed animals. The results of our present study suggest that caloric restriction at 30% induced fewer colon tumors as well as produced a reduction of the size of colon tumors when compared to feeding a high-fat diet ad libitum. In this study, the animals on the calorie-restricted diet consumed about 30%

Table 1 Composition of experimental semipurified diets and mean daily food intake

<table>
<thead>
<tr>
<th>Diet ingredients</th>
<th>High-fat ad libitum diet</th>
<th>Calorie-restricted diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Composition (%)</td>
<td>Mean daily food intake (g)</td>
</tr>
<tr>
<td>Casein, vitamin-free</td>
<td>23.00</td>
<td>3.20</td>
</tr>
<tr>
<td>dL-Methionine</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>Corn starch</td>
<td>33.75</td>
<td>4.69</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.52</td>
<td>1.18</td>
</tr>
<tr>
<td>Corn oil</td>
<td>23.00</td>
<td>3.20</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td>Dextrose</td>
<td>8.52</td>
<td>1.18</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>Vitamin mix, AIN revised</td>
<td>1.18</td>
<td>0.16</td>
</tr>
<tr>
<td>Mineral mix, AIN</td>
<td>4.11</td>
<td>0.57</td>
</tr>
<tr>
<td>Calories consumed/day</td>
<td>66.7</td>
<td>46.7</td>
</tr>
</tbody>
</table>

Fig. 1. Mean body weights of male F344 rats treated with azoxymethane or normal saline and fed a high-fat diet ad libitum or a calorie-restricted diet. The standard deviation ranged from 9.5 to 31.2 for all dietary groups.

Table 2 AOM-induced tumor incidence in male F344 rats fed a high-fat diet ad libitum or a calorie-restricted diet

<table>
<thead>
<tr>
<th>% Animals with tumors (tumor incidence)</th>
<th>Colon</th>
<th>Small intestine**</th>
<th>Ear duct</th>
<th>Tumors/animal</th>
<th>Tumors/TBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum (30)</td>
<td>83 (25)*</td>
<td>83 (25)*</td>
<td>30 (9)*</td>
<td>30 (9)</td>
<td>13 (4)</td>
</tr>
<tr>
<td>Calories-restricted (30)</td>
<td>33 (10)</td>
<td>33 (10)</td>
<td>0</td>
<td>27 (8)</td>
<td>3 (1)</td>
</tr>
<tr>
<td>Vehicle-treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum (12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Calories-restricted (12)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* All small intestinal tumors are adenocarcinomas.
** Number in parenthesis, number of animals.
*** Statistically significant from calorie-restricted diet group at P < 0.05 (χ² test and Student's t test).
**** Mean ± SD.
fewer calories from carbohydrates, protein, and fat, but consumed the same amount of vitamins, minerals, and fiber compared to animals on the high-fat ad libitum diet. Therefore, the observed differences in colon tumor incidences between the calorie-restricted group and the ad libitum group may seem to be due to total caloric restriction as well as to the restriction of calories from fat, protein, and carbohydrate. However, our previous studies demonstrate that a reduction in the intake of dietary carbohydrate (starch and dextrose) and an increase in the intake of dietary fat with no change in the intake of protein, fiber, minerals, and vitamins increased the incidence of AOM-induced colon tumors in rats suggesting that a reduction of calories from carbohydrate had no inhibitory effect but an increase in calories from fat had a promoting effect on colon carcinogenesis (15, 16). In conclusion, the present study demonstrated that caloric restriction by about 30% induced fewer colon tumors than did the high-fat diet fed ad libitum.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 2. Size (diameter in centimeters) of azoxymethane-induced colon tumors of male F344 rats fed a high-fat diet ad libitum or a calorie-restricted diet. Numbers in parentheses, number of tumors.
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