Enhancement of Pancreatic Carcinogenesis in Hamsters Fed a High-Fat Diet ad Libitum and at a Controlled Calorie Intake

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ABSTRACT

An enhancement of pancreatic cancer induced by N-nitrosobis-(2-oxopropyl)amine (BOP) was reported previously in Syrian hamsters fed high-fat diet following carcinogen treatment. The purpose of our research was to determine if this enhancement was due to the consumption of more calories by the hamsters fed the high-fat diet. Male hamsters were treated with a single injection of BOP (20 mg/kg body weight s.c.) at 8 weeks of age. One week later they started either on a low-fat diet (4.3% corn oil) or a high-fat diet (20.5% corn oil) and were fed until the end of the experiment at 92 weeks after BOP. Diets were fed either ad libitum or in a control-fed protocol. The control-fed groups had equivalent calorie intakes and were restricted slightly in comparison with the ad libitum-fed hamsters. BOP treatment reduced survival slightly but survival did not differ significantly in accordance with dietary assignment. Body weight was elevated in the hamsters fed high-fat diet ad libitum in comparison with those fed low-fat diet ad libitum. However, differences were not observed in hamsters fed low- and high-fat diets by the control-fed protocol. Pancreatic carcinogenesis was enhanced about 3- to 4-fold when hamsters were fed high-fat diet by either protocol. The degree of enhancement did not differ with the feeding regimen. However, the higher death rate with pancreatic cancer occurred earlier in the ad libitum-fed hamsters than in the control-fed hamsters.

INTRODUCTION

We found a 5- to 6-fold increase in pancreatic cancer induced by BOP3 in hamsters fed diets containing 20.5% corn oil compared with hamsters fed 4.3% corn oil following BOP treatment (1, 2). This enhancement by high-corn oil diets fed ad libitum was observed at control protein levels (18 g/386 kcal) and high protein levels (36 g/385 kcal), but not at low protein levels (9 g/386 kcal) (2). The increased tumor yield in high-fat groups (1, 2). This enhancement by high-corn oil diets fed ad libitum in comparison with those fed low-fat diet ad libitum. However, differences were not observed in hamsters fed low- and high-fat diets by the control-fed protocol. Pancreatic carcinogenesis was enhanced about 3- to 4-fold when hamsters were fed high-fat diet by either protocol. The degree of enhancement did not differ with the feeding regimen. However, the higher death rate with pancreatic cancer occurred earlier in the ad libitum-fed hamsters than in the control-fed hamsters.

STUDIES ON THE RELATIONSHIP BETWEEN CALORIE INTAKE AND CARCINOGENESIS

Studies on the relationship between calorie intake and carcinogenesis have demonstrated a striking inhibition of tumorigenesis at sites such as mammary glands (3), colon (4), skin (5), and pancreas (6) in diet-restricted animals (reviewed in Ref. 7). Investigations into the interaction between calorie intake and dietary fat intake on mammary carcinogenesis suggest that the influence of dietary fat on carcinogenesis in part may be attributed to elevated calorie intake in rodents fed high-fat diets (8).

This study aims to determine if the increased pancreatic cancer in hamsters fed high-fat diet was due to the elevated calorie intake by these animals.

MATERIALS AND METHODS

Male Syrian hamsters were obtained from the Eppley Institute colony (Uneni:SYR) at 6 weeks of age. The average body weight was 79 ± 1 (SE) g, and they were fed the control semipurified diet shown in Table 1 for the first 2 weeks of the experiment. Eight-week-old hamsters were randomized into the 8 treatment groups shown in Table 2. They were treated once with 20 mg BOP/kg body weight (s.c.) in physiological saline or saline alone. One week later they were given their assigned dietary treatment. In the ad libitum-fed groups, hamsters were allowed free access to the low- or high-fat diet for the remainder of the experiment. For training in equivalent calorie consumption, the control-fed groups were given the assigned diets in a pair-fed pattern for the first 20 weeks following BOP treatment. The low-fat diet and control-fed hamsters were maintained on the diet 8 h/day for the first week of this period. Then the diet was provided for 16 h/day. Their food intake was measured and the distribution of their calorie consumption was fed to the high-fat/control-fed hamsters such that there were high-consumption high-fat hamsters paired to high-consumption low-fat/control-fed hamsters and low-consumption high-fat/control-fed hamsters paired to low-consumption low-fat/control-fed hamsters. At 20 weeks after BOP treatment it became apparent that the high-fat/control-fed hamsters were not consuming all of their allotment and that some of the low-fat/control-fed hamsters were losing weight. For these reasons we allowed the control-fed groups unlimited access to food. Their intake was monitored daily. If the distribution of calorie consumption by the high-fat and control-fed hamsters exceeded their low-fat/control-fed diet counterparts, the high-consuming hamsters in this high-fat group were identified and fed reduced allotments for 1 week to retrain them to consume fewer calories. Less than 10% of the hamsters in the high-fat/control-fed group required retraining.

Hamsters were housed in temperature (21 ± 2°C)- and humidity (40 ± 5%)-controlled facilities with a light-dark cycle of 12-h/12-h and 10 air exchanges/h. Hamsters in the ad libitum- and control-fed groups were caged in groups of five and singly, respectively, in corncob bedding (Bed-O-Cobs; Anderson Cob Co., Maumee, OH).

The experimental low-fat and high-fat diets are shown in Table 1. The diet termed "low-fat" actually contains a control level of dietary fat. However, to avoid confusion with the control feeding protocol, we are calling this diet low-fat. We used these diet formulations in previous studies with Syrian hamsters (1, 2), in which the diets improved survival and were useful in demonstrating the enhancement of pancreatic carcinogenesis in hamsters fed a high-fat diet. These diets are formulated so that protein, vitamin, mineral, and fiber intake remains constant per kcal. Thus, with hamsters consuming equivalent calorie allotments, the only differences in intake will be increased calories from fat and decreased calories from carbohydrates in hamsters fed high-fat diets in comparison with those fed low-fat diets.

Hamsters were killed when moribund or at week 94 of the experiment when only 40% of the hamsters remained alive. Pancreases were removed, fixed in Bouin's fixative, and stained with hematoxylin and eosin following extraction with petroleum ether in a Soxhlet extractor and evaporation of the solvent. Protein was determined by the micro-Kjeldahl procedure (10).

Carcasses of hamsters killed at the end of the experiment were ground and lyophilized and carcass lipid and protein were determined. Lipid was determined gravimetrically following extraction with petroleum ether in a Soxhlet extractor and evaporation of the solvent. Protein was determined by the micro-Kjeldahl procedure (10).

The abbreviation used is: BOP, N-nitrosobis(2-oxopropyl)amine.

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The hamsters in the non-BOP-treated groups and 29% of the hamsters in the BOP-treated groups had survived ($P < 0.01$ by $\chi^2$ analysis). Among non-BOP-treated hamsters and BOP-treated hamsters on the control fed protocol, survival was extended slightly (15%) in the hamsters fed high-fat diet in comparison with those fed low-fat diet (Table 5). These dietary effects on survival were not statistically significant.

The percentage of body lipid and protein (Table 6) was not influenced by feeding protocol and, therefore, the data were pooled across this variable. Carcass lipid was elevated in hamsters fed the high-fat diet following BOP treatment, but differences were not observed in the saline-treated hamsters. Carcass lipid and protein were reduced in the carcinogen-treated hamsters but only the lipid reduction was statistically significant.

Feeding the high-fat diet increased pancreatic carcinogenesis in both ad libitum- and control-fed hamsters as shown in Table 5. These data were age adjusted to correct for the possibility that some of the effect of high-fat diet on pancreatic cancer was due to an effect of dietary fat improving longevity (Table 7). The results of this analysis demonstrated that the excess of cancers in the high-fat groups occurred from weeks 60 to 79 in ad libitum-fed hamsters and from weeks 80 to 94 in control-fed hamsters. This observation indicates that hamsters developed more tumors when fed high-fat diet but that the excess death rate of hamsters with cancer was delayed when the diets were control fed. Further, the earliest adenocarcinoma was observed in an ad libitum-fed hamster at 42 weeks, whereas in control-fed hamsters such lesions were not seen until after week 70. The average survival of hamsters with carcinoma was increased in control-fed hamsters given high-fat diet in comparison with ad libitum-fed hamsters given high-fat diet (Table 5), but the difference was not statistically significant. Of the observed carcinomas, 19% were carcinoma in situ, 19% were intraductal carcinoma, 17% were microcarcinoma, and 45% were adenocarcinoma. The distribution of carcinoma type was not significantly influenced by dietary protocol.

BOP-treated hamsters had fewer ductular adenomas in the low-fat/control-fed group in comparison with all other BOP-treated groups (Table 8). Age adjustment of these data demonstrated that this relationship was statistically significant in comparing hamsters in these groups killed from weeks 60 to 79 or from week 90 to week 94. Non-BOP-treated hamsters developed fewer adenomas. Acinar cell nodules developed only in the hamsters fed high-fat diet ad libitum (12% incidence, 0.8 acinar cell nodule/effective animal).

Hamsters fed high-fat diet by either feeding protocol following BOP treatment also had elevated incidences of pancreatic ductular hyperplasia (51% in hamsters fed high-fat diet and 28% in those fed low-fat diet; $P < 0.02$) and dysplasia of ductal cells (19% in high-fat groups and 6% in low-fat groups; $P < 0.05$). In addition, both carcinogen- and non-carcinogen-treated hamsters fed high-fat diet had more pancreatic islet hyperplasia (7%) in comparison with hamsters fed low-fat diet (1%; $P < 0.05$).

Galbladder and pancreatic lesions, which were influenced by the feeding protocol but were not influenced by dietary fat level or BOP treatment, are shown in Table 9. Control-fed hamsters had higher incidence of gallbladder hyperplasia, pancreatic fibrosis, and pancreatitis but lower incidence of gallbladder inflammation and pancreatic lipomatosis. Gallbladder and common duct polyps were observed in 43 and 28%, respectively, of the BOP-treated hamsters and 4 and 1%, respectively, of the non-BOP-treated animals. Gallbladder adenocarcinomas were found in 4% of the BOP-treated hamsters. None of these lesions were altered significantly by the dietary treatments.
DISCUSSION

In the present experiment, pancreatic ductal/ductular carcinogenesis was enhanced in hamsters fed high-fat diets irrespective of calorie consumption. In particular, ad libitum consumption of the high-fat diet was accompanied by consumption of more calories and elevated body weight whereas control-fed hamsters avoided the calorie allotments and did not differ in body weight. The increased pancreatic cancer rate in hamsters fed high-fat diet was observed among hamsters dying between weeks 60 and 79 in ad libitum-fed groups and among hamsters dying between weeks 80 and 94 in control-fed hamsters. The delay in the latter group could have been due to an increase in the latency of pancreatic cancer in the control-fed hamsters or to the aggressiveness of cancers in the ad libitum group. Inasmuch as hamsters in the control-fed groups were dying during weeks 60–79 with pancreatic cancer but cancer rates were not significantly associated with dietary fat (Table 7), it seems more likely that the latency of the tumors was increased in some hamsters in the control-fed/high-fat group.

Studies from other laboratories have suggested that calories more profoundly modified breast and colon carcinogenesis than dietary fat levels. For example, Boisoneaout et al. (13) compared ad libitum consumption of low- and high-fat diets with the restricted high-fat diet fed at the equivalent net energy value consumed by the low-fat group. Net energy value was calculated from values published earlier (14). Their results showed that restricting the high-fat diet to a net energy value equivalent to that consumed by the ad libitum low-fat group inhibited 7,12-dimethylbenz(a)anthracene-induced mammary carcinogenesis. Klurfeld et al. (15) observed that restricting high-fat diets significantly inhibited mammary and colon tumorigenesis and suggested that calorie intake was probably more important than fat intake per se in determining cancer rates. However, these studies used diets containing about 13% fat in the high-fat-restricted group, a level which was not effective in the enhancement of pancreatic cancer in the control-fed hamsters or to the aggressiveness of cancers in the ad libitum group. Inasmuch as hamsters in the control-fed groups were dying during weeks 60–79 with pancreatic cancer but cancer rates were not significantly associated with dietary fat (Table 7), it seems more likely that the latency of the tumors was increased in some hamsters in the control-fed/high-fat group.

Table 7 Age-specific rates of pancreatic adenocarcinoma in BOP-treated hamsters fed low- and high-fat diets by ad libitum or control-fed protocol*

<table>
<thead>
<tr>
<th>Type of feeding</th>
<th>Experimental wk</th>
<th>Low-fat</th>
<th></th>
<th>High-fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad libitum</td>
<td></td>
<td>No. of hamsters dying</td>
<td>Incidence (%)</td>
<td>No. of CRC/EAN</td>
</tr>
<tr>
<td>40–59</td>
<td>3</td>
<td>33</td>
<td>0.33</td>
<td>2</td>
</tr>
<tr>
<td>60–79</td>
<td>11</td>
<td>0 (A)</td>
<td>0.00 (C)</td>
<td>8</td>
</tr>
<tr>
<td>80–94</td>
<td>10</td>
<td>10</td>
<td>0.20</td>
<td>23</td>
</tr>
<tr>
<td>Control-fed</td>
<td></td>
<td>No. of hamsters dying</td>
<td>Incidence (%)</td>
<td>No. of CRC/EAN</td>
</tr>
<tr>
<td>40–59</td>
<td>2</td>
<td>0</td>
<td>0.00</td>
<td>10</td>
</tr>
<tr>
<td>60–79</td>
<td>15</td>
<td>20</td>
<td>0.27</td>
<td>14</td>
</tr>
<tr>
<td>80–94</td>
<td>13</td>
<td>8 (A)</td>
<td>0.15 (C)</td>
<td>14</td>
</tr>
</tbody>
</table>

* Results of \( \chi^2 \) tests within each row using age adjustment according to the method of Peto (12); A < B, C < D (P < 0.05).

* CRC, carcinoma; EAN, effective animal.
ment of colon cancer in rats (16). Similar studies on pancreatic carcinogenesis have not been reported previously. Our results suggest that the enhancement of pancreatic cancer in hamsters fed high-fat diet is due to more than an effect of calorie consumption.

One difference in the results from mammary and colon carcinogenesis studies and our investigation on pancreatic carcinogenesis is the length of the studies. The current investigation extended until nearly one-half of the non-carcinogen-treated hamsters had died. As indicated above, the results suggest that the pancreatic carcinomas generally developed earlier in the ad libitum-fed hamsters than in the control-fed groups. In comparison, the studies on mammmary and colon carcinogenesis ended when the dietary fat effect was expected in the ad libitum-fed groups. Thus, it is possible that cancer would have developed in the restricted high-fat animals in the mammary and colon carcinogenesis studies if the animals had been allowed to live longer.

Also, the hamsters in the present investigation actually were trained to consume fewer calories, then monitored and held to that consumption rather than be provided less food than would be consumed by ad libitum animals. In addition, we conducted similar training on low-fat and high-fat groups.

This experiment, in contrast to studies on the mammary carcinogenesis model, noted the absence of a profound influence of dietary fat or calorie intake on body composition. The absence of such an effect in our study may be due to conducting this measurement only at the end of the experiment. It would be useful to perform the measurement earlier in the experiment to address this issue adequately.

From this experiment and previous experiments on dietary fat effects on pancreatic carcinogenesis, it appears that high-fat diets enhanced pancreatic carcinogenesis only in the experiments that used a single dose of 10 or 20 mg BOP/kg body weight and were extended for at least 80-90 weeks following BOP treatment. We observed no influence of dietary fat in studies of the influence of dietary selenium (17) or dried cabbage (18) in control and high-fat diets in which a dose of 40 mg BOP/kg body weight was used and surviving hamsters were killed at 40–50 weeks following BOP treatment. The dependence of dietary modulation on carcinogen dosage is so often observed that it must be appreciated in designing nutrition and cancer studies.

REFERENCES
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